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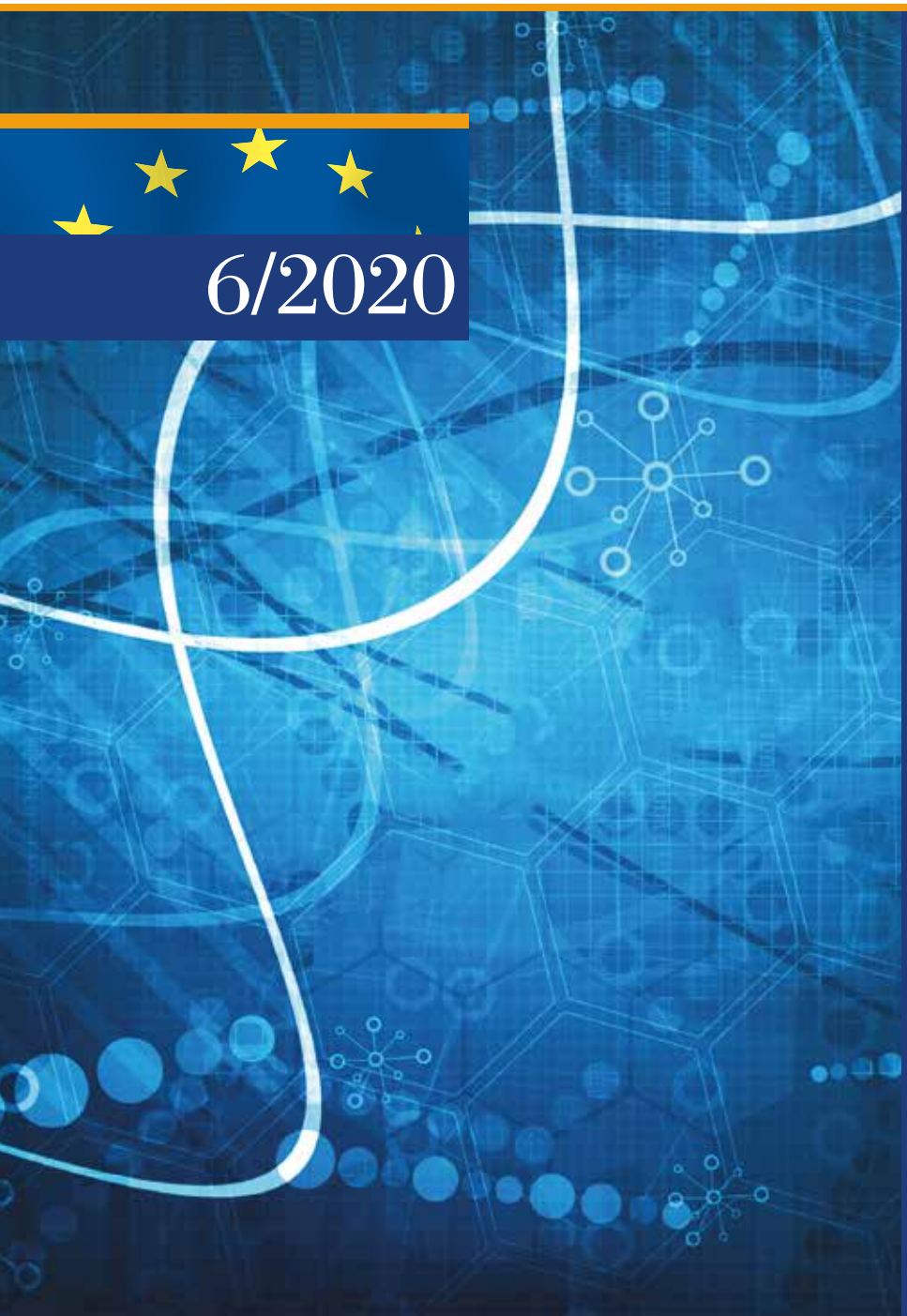

6/2020

Allergy diagnostics: where are we going?

Subcutaneous and sublingual allergen-specific immunotherapy: a tale of two routes

Comparison of the performance of Skin Prick and ISAC Tests in the diagnosis of allergy

Are currently available biomarkers useful to discriminate CSU patients not controlled by low dose omalizumab maintenance therapy?

Primary Immunodeficiency Disorders in children with Non-Cystic Fibrosis Bronchiectasis

Does maternal diet during pregnancy influence clinical and laboratory characteristics of infantile-onset atopic dermatitis?

The show must go on. The impacts of SARS-CoV-2 pandemic on cutaneous allergology and patch testing

Angiotensin Converting Enzyme-2 (ACE2) Receptors, asthma and severe COVID-19 infection risk

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Table of Contents

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Allergy diagnostics: where are we going?

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The implementation of the EU directive (2001/83/EC) regarding allergens for both in vivo testing and for allergen specific immunotherapy is leading to a worrying deprivation of allergy diagnostics. The directive states that "[..] no medicinal product (including allergens for in vivo tests) may be placed on the market of a Member state unless a marketing authorization has been issued by the competent authorities […]" (1). This is certainly a theoretically correct approach pointing to an increase in quality and safety of marketed products. The dark side of the moon is however that, in view of the stricter quality requirements and of the elevated costs associated with the updating of existing licenses, allergen producing companies do no longer find it convenient to market extracts for in-vivo testing of less common airborne allergens and of foods. In fact, to be economically convenient, these products should be sold at such a high price that most doctors, hospitals or health care systems would not buy them. So, we are left with a dropping number of extracts of most common airborne allergens and with a short list of food extracts for in-vivo diagnostics. Another important point will be the legal status of fresh foods when they are used for in-vivo diagnostic purposes. Should an apple or a fresh shrimp be considered as a medicinal product as soon as they enter a hospital or a medical office to be used for prick-prick testing? Fresh foods represent the simplest, cheapest, most sensitive, and most rapid way to diagnose hypersensitivity to a certain source, and in some cases, they represent inalienable diagnostic means. The interesting study by Scala (2) and co-workers that appears in the present issue of *European Annals of Allergy and Clinical Immunology* compares the ISAC test with the skin prick test and finds discordant results in 20-30% for pollen allergens, 25% for dust mites, between 7-25% animal dander and between 14-33% for foods. Clearly, comparing a multiplex test based on allergen molecules conceived for component-resolved diagnostic with commercial whole allergen extracts may lead inevitably to detect some discrepancies. However, in real life the two methods should be considered more as complementary than as in contraposition. On one hand, limiting allergy diagnostics to the use of currently available allergenic molecules (either as singleplex or as multiplex) may lead to catastrophic mistakes. One example is shrimp allergy. To date, a very limited number of shrimp allergens is available on the marked despite in certain countries shrimps represent the second cause of primary food allergy among adults and contain a large number of allergenic proteins many of whom are still not characterized (3,4). In such a situation, the risk of getting a false negative result from the in-vitro test is quite high and a not experienced doctor might be tempted to consider the patient as "non allergic" despite a clear-cut history of shrimp allergy. Many other examples of this type might be given. On the other hand, extracts and fresh food-based skin tests are poorly standardised and variable in terms of allergens composition, thus leading to diagnostic errors as well. For instance, in the Scala *et al.* paper, ISAC testing identified

from 22% to 26% more cases than skin prick tests in peach and nuts hyper-sensitivity (2).

Progress in molecular biology and in the characterization of allergen molecules has led to the development of potent diagnostic instruments for in-vitro diagnosis. However, these are not perfect as they do not (and, arguably will never) contain all allergenic proteins. As a consequence, these instruments should be complemented by in-vivo tests (either commercial extracts or fresh material) possibly containing all the allergens of that specific source. In this view, allergy specialists are those who should lead this new phase of allergy diagnostics, preserving in vivo tests from too strict legislations on one side and improving diagnostic accuracy and allergens availability of in vitro tests on the other side.

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Subcutaneous and sublingual allergen-specific immunotherapy: a tale of two routes

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Key words
Allergen immunotherapy; respiratory allergy; subcutaneous route; sublingual route; efficacy; safety.

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Summary
Specific immunotherapy is the only treatment acting on the causes and not only on symptoms of respiratory allergy. It was first introduced as subcutaneous immunotherapy (SCIT) with the aim to induce immunological tolerance to the administered allergen(s). In the 1980s, sublingual immunotherapy (SLIT) was developed, mainly to improve the safety, which was a critical issue at that time.
This article reviewed the available literature, including a large number of randomized controlled trials, meta-analyses, and real-life studies as well, on the outcomes of SCIT and SLIT concerning the treatment critical issues of the two routes, that are efficacy, safety, cost-effectiveness, and compliance to treatment.
The efficacy of SCIT and SLIT is similar in respiratory allergy, providing, based on the induction of typical changes in the immunologic response, an early control of symptoms that steadily increases during the treatment and its efficacy lasts after the recommended duration of three years. Such results are the reason why SCIT and SLIT have economic advantage over symptomatic drugs.

Introduction

Allergen immunotherapy (AIT) is defined as "the repeated administration of specific allergens to patients with IgE-mediated conditions for the purpose of providing protection against the allergic symptoms and inflammatory reactions associated with natural exposure to these allergens" (1). This outcome is obtained by the ability of AIT to induce a number of cellular and humoral effects that result from the shift from the Th2 lymphocyte dominated pattern, typical of the allergic response, to the Th1 pattern associated to tolerance, and from the generation of T regulatory cells (2,3). As the fine mechanisms of AIT are being revealed, the need to identify reliable biomarkers predicting the response to treatment is an emerging issue (4,5). The quality of allergen extracts used in AIT is crucial for inducing immunological changes and providing clinical success. This concerns both the initial injective route of administration, *i.e.* subcutaneous immunotherapy (SCIT) and the subsequently introduced sublingual immunotherapy (SLIT). The development of these two forms of AIT was quite different, because SCIT was initiated more than a century ago as a merely empirical treatment of hay fever (6), while SLIT was introduced only in the 1980s, when the knowledge on pathophysiology of allergy and mechanisms of AIT became clear (7). In this review, meta-analyses were preferred to evaluate the effectiveness and safety of SCIT and SLIT, while large-scale trials performed for registration from the regulatory agencies were used for the latest generation of SLIT products. Also real-life studies on very large patients population were considered.

The milestones in the development of allergen immunotherapy

The injective route was introduced in 1911 although the pathogenesis of allergic disease was not fully elucidated until the discovery of IgE antibodies in the 1960s (8), which clarified the mechanisms of allergy and the immunologic modifications achieved with AIT (9,10). Despite this, safety issues emerged in the 1980s, when fatal reactions to SCIT from the UK (11) and USA (12) were described. Nowadays it has been clarified that

the actual risk factor for fatal reactions to SCIT is the presence of uncontrolled asthma at the time of the allergen extract injection; in fact, fatalities become extremely rare when patients with uncontrolled asthma are excluded from this treatment (13). However, in the 1980 this factor was not yet acknowledged, thus the development of other routes of administration was deemed essential. Therefore, the oral route and the local nasal route were investigated, although these types of administration were reconsidered due to the high allergen doses required for efficacy and the repeated nasal reactions to the administered allergen, respectively (14). In fact, oral immunotherapy remains under investigation as a treatment for food allergy but non for respiratory allergy, while local nasal immunotherapy was abandoned. Indeed, the development of SCIT and SLIT, as summarized in **table I**, proceeded through stages of evolution showing similarities and differences. The current advance is the ongoing registration of immunotherapy products, based on the fulfillment of criteria from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. In fact, until recently all allergen extracts were classified as named patients products (NPP), that are defined by their production through at least one industrial process based on individual medical prescription, not responding to the above criteria. Some NPP were registered at local level, with no acknowledgement by other nations. Nowadays, guidelines for product registration from the European Medicines Agency are available regarding production and quality issues (15) and the adherence to such guidelines has already resulted in internationally registered products, which will be discussed below.

Table I *- Stages of evolution of SCIT and SLIT.*

	SCIT	SLIT
Birth	1911	1986
First controlled trial	1966	1986
First meta-analysis	1995	2003
First real-life studies on large populations	2015	2017
Products registration	Ongoing, thus far registered products only in single nations	International registration for grass pollen and dust mites tablets; ongoing for ragweed pollen tablets

Similarities of SCIT and SLIT

Efficacy of SCIT

After decades of open studies on SCIT, the first placebo-controlled trials were commonly based on small populations of patients, often not higher than 30-35 subjects, about 50% of whom treated with placebo. Such low numbers obviously exposed the results to the statistical risk of stochastic observations. Similar problems concerned SLIT after its introduction. The risk of poor statistical power was managed by using meta-analysis, which has the crucial advantage to aggregate the information and thus to achieve a higher statistical power and a more solid point estimate than obtained from any individual study. Still, meta-analysis also has the limit to be biased by the methods of search and selection of studies of the investigator, incomplete data, and the kind of data analysis (16). An appropriate way to address this limit is to use the Cochrane approach, which was specifically designed to reduce investigator-related bias (17). **Table II** shows the main results from meta-analyses of randomized double-blind, placebo-controlled trials (DBPC) of SCIT in patients with allergic asthma or allergic rhinitis (18-23). In particular, Abramson *et al.* performed from 1995 to 2010 three Cochrane meta-analysis on asthma. The latest meta-analysis update included 88 trials, 42 of them concerning SCIT with dust mites, 27 with pollens, 10 with animal dander, 2 with latex, 2 with molds, and 6 with multiple allergens, using the standardized mean difference (SMD) as analysis parameter (21). A significant improvement in asthma symptom scores was found (SMD -0.59, 95% CI -0.83 to -0.35) and it would have been necessary to treat 3 patients with SCIT to avoid one deterioration in asthma symptoms and to treat 4 patients to avoid one patient requiring increased drug treatment. SCIT significantly decreased both allergen specific and non-specific BHR (20). Based on the level of evidence, no further Cochrane meta-analysis was performed, while other meta-analyses addressed aspects such as efficacy in asthmatic patients treated with dust mite extracts (23), or the outcome of SCIT compared with pharmacotherapy (22). For the latter, besides the usual SMD, also by the relative clinical impact (RCI), which is the percentage reduction in TSSs and TNSSs obtained with active treatment compared with placebo was used, with a significantly better result for SCIT than for mometasone (-31.7% ± 16.7%, $p < 0.00001$) and montelukast (6.3% ± 3.0).

Efficacy of SLIT

The first meta-analysis on the efficacy of SLIT on allergic rhinitis was performed in 2015 on 22 randomized trials (24). A significantly higher efficacy of SLIT *versus* placebo was found, as assessed by an SMD of -0.42 for symptom scores (p=0.002) and -0.43 for medication scores (p=0.00003). Due to the relatively low numbers, the authors were not able to detect differences in patient subgroups defined by age and the kind of allergen,

Table II *- Efficacy of SCIT.*

Authors (ref.)	Year	Study	Population	Results
Abramson *et al.* (18)	1995	Cochrane meta-analysis	20 randomized double-blind placebo controlled trials on patients with allergic asthma	Symptomatic improvement, reduction in medication scores and in bronchial hyper-reactivity
Abramson *et al.* (19)	2003	Cochrane meta- analysis	75 randomized double-blind placebo controlled trials on patients with allergic asthma	Reduction of asthma symptoms and use of asthma medications, improvement of bronchial hyper-reactivity
Calderon *et al.* (20)	2007	Cochrane meta- analysis	51 trials on patients with allergic rhinitis	Reduction of medication scores and symptoms scores
Abramson *et al.* (21)	2010	Cochrane meta- analysis	88 randomized double-blind placebo controlled trials on patients with allergic asthma	Reduction of asthma symptoms and use of asthma medications, improvement of bronchial hyper-reactivity
Matricardi *et al.* (22)	2011	Review	Meta-analyses with 5 or more randomized, double-blind, placebo-controlled trials of SCIT or antisymptomatic treatment in patients with SAR	reduction in drug consumption, preventive effect on asthma and new sensitizations, antisymptomatic effect starting as early as the first season after treatment onset.
Lu *et al.* (23)	2015	Meta-analysis	19 randomized double-blind placebo controlled trials on patients with allergic asthma	Reduction of asthma symptom scores and asthma medication scores

but this was possible in subsequent analyses. **Table III** reports the main results of further meta-analyses on allergic rhinitis or asthma (24-34). The most recent meta-analyses included both SCIT and SLIT trials. In particular, the meta-analysis endorsed by the European Academy of Allergy and Clinical Immunology, including 61 SCIT trials and 71 SLIT trials demonstrated significant reductions in AR symptom (SMD -0.53), medication (SMD -0.37) and combined symptom and medication scores (SMD -0.49) with active treatment, with no significant difference between SCIT and SLIT (32). The guidelines from the European Academy of Allergy and Clinical Immunology (EAACI), based on the level of evidence stated that in patients with AR both SCIT and SLIT can be recommended (35). However, in a meta-analysis comparing SCIT and SLIT in patients with allergic asthma SCIT improved quality of life and decreased allergen-specific airway hyperreactivity, while SLIT did not reach such outcome (33).

As hinted above, the need to fulfill the rigorous process of registration as pharmaceutical therapies required by regulatory agencies resulted in the performance of large trials on the new SLIT products in standardized tablets. The recent preparations for SLIT in tablets of grass pollen extract were the first to be assessed. A pre-requisite was the inclusion of patients' populations much larger than commonly used previously, to make unlikely casual observations and thus making meta-analysis unnecessary. Actually, the two trials on the 1-grass tablets and the 5-grass tablets included 855 and 628 adults, respectively (36,37). In the trial on the 1-grass (*Phleum pratense*) tablets patients were randomized to receive sublingually 2500, 25,000, or 75,000 SQ (Standard Quality)-T (the units used by the producer to measure allergen activity) or placebo once daily for a mean duration of 18 weeks. The average rhinoconjunctivitis scores during the grass pollen season were reduced by 16% and the medication use was reduced by 28% for the grass tablet 75,000 (p=0.047) compared with placebo. A significant improvement in rhinoconjunctivitis QoL scores (p=0.006) and in the number of well days (p=0.041) were observed (36). In the trial on 5-grass tablets three dosages for once daily administration were tested, namely 100, 300 and 500 IT (Index of Reactivity) and compared with placebo. SLIT was started 4 months before the expected grass pollen season and continued throughout the season. The 300-IR and 500-IR doses significantly reduced the mean rhinoconjunctivitis total symptom score (3.58 ± 3.0, p=0.0001 and 3.74 ± -3.1, p=0.0006, respectively) compared with placebo (37). The 5-grass tablets were also evaluated in 278 pediatric patients (aged 5-17 years) with rhino-conjunctivitis receiving 300-IR once-daily or placebo, with the same time horizon of the study in adults. The SLIT treated group showed a mean improvement in rhinoconjunctivitis total symptom score of 28% compared with placebo, this being significantly better (p=0.001). Significant differences between active and placebo treatment were also found concerning the rescue medication score and the propor-

Table III *- Efficacy of SLIT.*

Authors (ref.)	Year	Study	Population	Results
Wilson *et al.* (24)	2005	Meta-analysis	22 randomized trials	Reductiopn in symptom scores and in medication scores
Penagos *et al.* (25)	2006	Meta-analysis	Pediatric patients with allergic rhinitis	Reductiopn in symptom scores and in medication scores
Calamita *et al.* (26)	2006	Cochrane meta-analysis	Patients with allergic asthma	Reduction in asthma severity
Penagos *et al.*(27)	2008	Meta-analysis	Pediatric patients with allergic asthma	Significant reduction in symptom scores and medication scores
Compalati *et al.* (28)	2009	Meta-analysis	8 trials on patients with allergic rhinits and 9 trials on patients with allergic asthma	Decrease in symptoms in both groups of patients
Di Bona *et al.* (29)	2010	Meta-analysis	Patients with seasonal rhinoconjunctivitis	Reduction in symptoms and medication use with a higher efficacy in adults than children
Radulovic *et al.* (30)	2010	Cochrane meta-analysis	49 trials on patients with allergic rhinitis	Reduction in symptom scores and medication scores
Normansell *et al.* (31)	2015	Cochrane meta-analysis	52 studies on patients with allergic asthma	Need for the introduction of validated scales and important outcomes for patients and decision makers
Dhami *et al.* (32)	2017	Meta-analysis	Comparison between SCIT and SLIT	No significant difference between SCIT and SLIT, reduction in AR symptoms and medication scores
Dhami *et al.* (33)	2017	Meta-analysis	Comparison between SCIT and SLIT	SCIT decreases allergen specific airway hyper-reactivity and improves QoL, while SLIT does not reach such outcome
Huang *et al.* (34)	2019	Meta-analysis	6 trials on SCIT and SLIT with dust mites extracts for the treatment of allergic rhinitis	No significant difference between SCIT and SLIT

tion of days using rescue medication throughout the pollen season (p=0.0064 and p=0.0146, respectively) (38). Both 1-grass tablets and 5-grass tablets trials clearly demonstrated the need to administer high allergen doses to achieve clinical efficacy. Of interest, in a post-hoc analysis of data from the 5-grass tablets trials, Devillier *et al.* found that the magnitude of efficacy was higher in patients with more severe symptoms during the grass pollen season (39).

In 2014, the first trial on the efficacy of dust mite SLIT tablets was published. The trial included 604 patients (aged more than 14 years) with mite-induced rhinitis and mild-to-moderate asthma, who were randomized to receive 1, 3, or 6 SQ dust mite tablets or placebo. The primary endpoint was the use of inhaled corticosteroid (ICS), which was adjusted at baseline and the end of one year of treatment to the lowest dose giving asthma control. The 6 SQ dose reduced the daily ICS dose by a mean difference of 81 μg compared with placebo (p=0.004). The most common adverse events were local oral reactions, with a higher rate and severity for 3 and 6 SQ-dust mite than for 1 SQ-dust mite and placebo (40). This outcome resulted in the important goal to take account in the 2017 update of the Global Initiative on Asthma (GINA) guideline of dust mite tablets as add-on therapy in mite allergic adult patients who have asthma exacerbations despite ICS treatment, with a FEV1 value of at least 70% of predicted (41).

The dust mite tablets standardized in IR were evaluated instead as a treatment for mite-induced AR. From a group of 509 participants, patients were randomized to receive once daily 500 IR tablets, 300 IR tablets or placebo for one year, with a year of follow-up after the end of treatment. The two SLIT doses of 500 and 300 IR significantly reduced AR symptoms, as measured by the Average Adjusted Symptom Score (AASS), compared to placebo, by 20.2% (p=0.0066) and 17.9% (p=0.015), respectively. With both active treatments the efficacy was maintained

during the one-year follow-up with no treatment (42). Further support for effectiveness of AIT was provided by real-life (also called real-world) studies. The value of these studies is to verify if the results obtained in controlled trials are reproduced in patients managed in routine clinical practice. In fact, the rigid inclusion criteria make unlikely the admission of such patients to trials, making uncertain their clinical response. The first paper mentioning the term real-life for AIT dates back to 2004 (43). Since then, more than 20 real-life studies on the effectiveness of SCIT and SLIT were published and recently reviewed (44). In the present review, only the studies on very large population of patients will be considered. In the study by Zielen *et al.* 2851 patients treated with grass pollen SLIT tablets and 71,275 control patients were analysed by a retrospective multiple regression analysis of data from a German prescription database over a period of 7 years. Changes over time in symptomatic drug consumption after SLIT withdrawal, use of medications for asthma, and time of asthma onset in patients with AR were used as indicators. A significant difference in favour of SLIT was detected for all comparators. In particular, the use of symptomatic drugs for AR compared to the pre-treatment period was 18.8% lower ($p < 0.01$) in SLIT treated patients than in controls, the asthma medication use decreased by an additional 16.7% (p=0.004) after treatment withdrawal in SLIT treated compared with controls, and the onset of asthma was less frequent (odds ratio 0.696, p=0.002) in SLIT treated patients than in controls (45). Another large real-life study used the same German prescription database and a time horizon of 2-6 years on a retrospective cohort of 9001 patients treated with SCIT or SLIT for birch pollen-induced AR and asthma and 45,005 patients treated only with symptomatic drugs as controls. AIT was performed by different birch or other *Betulaceae* (hazel, alder) pollen extracts, administered by natural pollen SCIT, allergoid preparations for SCIT and SLIT drops. At the end of a 6 years follow-up, the results of multiple-regression analysis showed that the rate of patients no longer taking symptomatic drugs for AR was 65.4% of AIT treated patients *vs.* 47.4% of controls ($p < 0.001$). Concerning asthma, the rate of patients using no longer using anti-asthmatic drugs was 49.1% of AIT treated patients *vs.* 35.1% of controls ($p < 0.001$). The evaluation of the risk of developing asthma in patients with only AR was significantly lower in AIT treated *vs.* controls (odds ratio 0.83, p=0.001) (46). The most recent real-life study analyzed the prescription fulfilment data collected from French retail pharmacies from 2012 to 2016. Using linear regression analyses, 1099 patients who had received at least two prescriptions of grass pollen SLIT tablets for at least two consecutive years were compared with 27,475 control patients who had received only symptomatic medications. A 50% decrease in the use of symptomatic AR medications was observed, compared with a 30% increase in the control group without age matching ($p < 0.0001$ *vs.* SLIT) and a 20% increase in the control group with age matching ($p < 0.0001$ *vs.* SLIT). Regarding asthma prevention, during the follow-up 1.8% of SLIT-treated patients and 5.3% of controls initiated asthma treatment. A lower risk of drug dispensing for new onset asthma was observed in SLIT treated *vs.* controls, by 62.5% without age matching (p=0.0025) and by 63.7% with age matching (p=0.0018). The authors concluded that prescription of grass SLIT tablets lessens the dispensing of AR and asthma medications in real life (47).

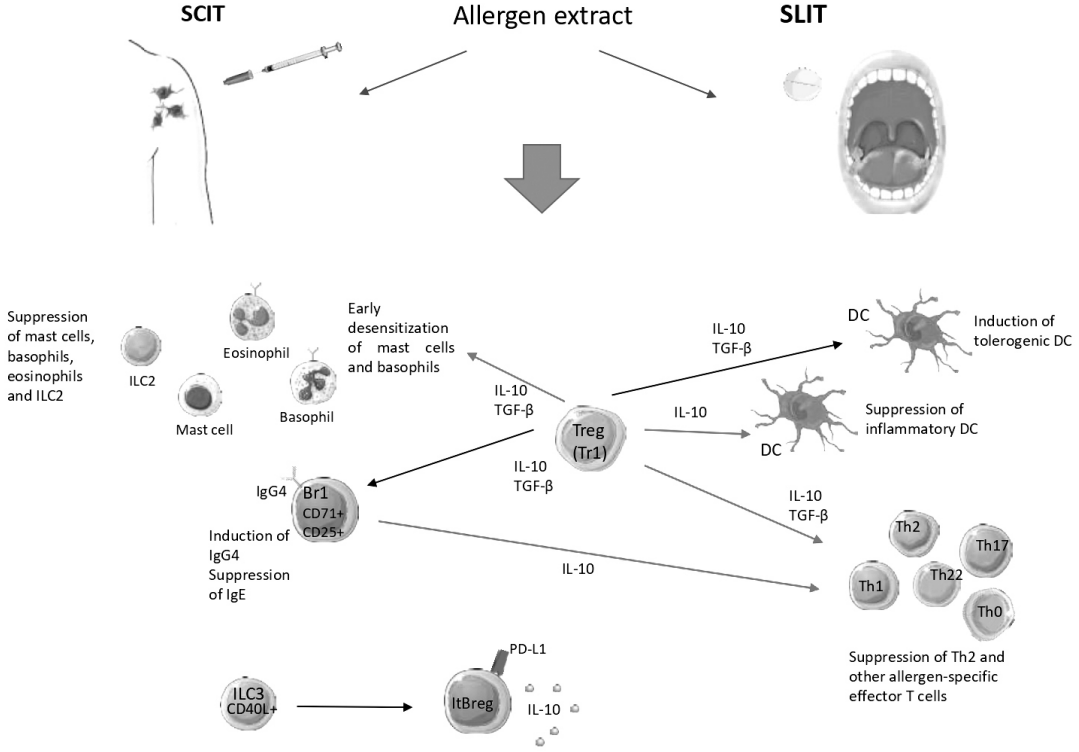
Immunological mechanisms of AIT

The exclusive disease-modifying outcome of AIT is due to its immunological effects on IgE-mediated allergic diseases. Successful AIT can in fact restore immune tolerance to allergens, by the inhibition of the early and late allergic response, the induction of allergen-specific regulatory T (Treg) and B cells (Breg) and the increase in production of anti-inflammatory cytokines, such as IL-10 and TGF-β (2,3). It is known that in allergic diseases the dis-regulation of Th2 response induces an increased release of cytokines such as IL-4, IL-5 (which stimulate the IgE class switch of B cell), and IL-13 (48). IgE exposed on the cellular membrane of mast cells and basophils bind the specific allergens, thus inducing the degranulation and the release of preformed mediators, such as histamine and leukotrienes, and generating the allergic response. IL-5 has a major effect on eosinophils by activating them and prolonging their survival rate, in addiction it stimulates the growth of B cell (49). These immunologic effects of AIT result in a long-lasting tolerance to the specific allergen, by decreasing the number and the activation of eosinophils and mast cells, and modulating the response and the activity of T and B cells, reducing the production and the release of IgE while increasing IgG4, a subclass of blocking antibody which compete with IgE for binding with the allergens, causing the reduction in activation of basophils and B cells. Although routes, doses of allergen and site of administration differ between SLIT and SCIT, the final effect in the modulation of the immune network is the same: the induction of peripheral tolerance mediated by Treg, which modulates the activation and the survival of peripheral immune cells by the release of anti-inflammatory cytokines such as IL-10 with suppression of IgE production and inhibition of B cells proliferation (50). **Figure 1** summarizes the main mechanisms of action if SCIT and SLIT. They result in the persistence of the clinical effects after stopping the treatment and the preventive capacity to interfere with the natural history of AR, and particularly the development of asthma. As to the first effect**,** a number of long-term studies, often following an initial trial, showed that treatment durations longer than three years resulted in prolonged maintenance of AR symptoms control over time (51). A recent review analyzed the evidence for long-term effects of SCIT and SLIT assessed

through placebo-controlled, randomized clinical trials including a follow-up of at least 1 year after treatment withdrawal. The data suggest the evidence on the adequacy of 3 years of either SCIT or SLIT in providing allergen-specific tolerance maintained for at least 2-3 years after treatment stopping (52). This observation supports the need to avoid a duration shorter than 3 years.

In addition, there is good evidence on the ability of both SCIT and SLIT to prevent the progress from AR to asthma. Following initial investigation on small groups of patients, the preventive allergy treatment (PAT) study enrolled 183 children with grass and/or birch pollen allergy undergoing SCIT or drug treatment, assessing the development of asthma by clinical evaluation. After 3 years of SCIT, in the period of follow-up performed for up 5 years a significantly less frequent onset of asthma was found in comparison with drug-treated children (odds ratio 2.68 (1.3-5.7)) (53). Several other studies, also addressing SLIT, confirmed such outcome, as recently reviewed (54). Indeed, no such evidence was achieved for the AIT capacity to prevent new sensitizations. This modification of the evolution of atopy was initially suggested by Des Roches *et al.*, who followed-up for 3 years a group of 22 children monosensitised to dust mites and treated with SCIT, comparing them to 22 matched non SCIT treated children. All 22 non-treated children developed new sensitivities *vs.* only 10/22 SCIT treated children ($p < 0.01$), suggesting that AIT in children monosensitised to dust mites can prevent new sensitizations (55). However, this outcome is far from clear, as demonstrated by the contrasting results of subsequent studies (56). A recent meta-analysis on 18 studies including 1049 children and 1057 adults concluded that low evidence supports the ability of AIT to prevent the onset of new sensitizations, the highest benefit being reported in small studies with a shorter follow-up. Still, the authors commented that high quality trials could change this estimate (57).

Figure 1 *- Mechanisms of action shared by SCIT and SLIT. Sublingual and subcutaneous immunotherapy. In SCIT the allergen contacts the antigen presenting cells (APC) of the skin while in SLIT APC involved are those in the sublingual mucosa. Both SCIT and SLIT induce immunotolerance. The induction of Treg results in early desensitization of mast cell and basophil and their suppression, together with eosinophils. IL-10 and TGF beta induce immunoglobulin class switch, with a decrease of IgE and induction of IgG4. Th2 response and cytokines result suppressed too. Inflammatory dendritic cell (DC) are suppressed in favor of tolerogenic DC.*



Practical application of AIT

The practice patterns of SCIT in Europe and US are significantly different, being mostly based on single allergens in Europe, while including an average of 8 allergens in US (58). Nelson analyzed 13 studies based on simultaneous administration of two or more unrelated allergens published from 1961 to 2007. Only few studies were well-designed and powered DBPC trials, and direct comparison between single-allergen and multiple-allergen was rarely performed. The author concluded that multiple-allergen can be effective but claimed for further investigation (59). Concerning SLIT, Calderon *et al.* studied the published evidence on the efficacy of the two strategies in polysensitized patients. According to the results of post-hoc analyses of DBPC trials, the efficacy of SLIT with grass-pollen tablets was found to be similarly effective in monosensitised and polysensitized patients. The authors also analyzed a number of studies from Europe evaluating SLIT in polysensitized patients using only the most clinically important allergen in two thirds of patients and a mix of two allergens in the remaining patients: a significant improvement in symptom and medication scores was achieved. Calderon *et al.* concluded that "multi-allergen immunotherapy in polysensitized patients needs more supporting data from large clinical trials to validate it as a treatment option" (60). A Workshop organized by the National Institute of Allergy and Infectious Diseases (NIAID) and the Agency for Healthcare Research and Quality (AHRQ) was aimed at developing trial concepts able to improve the use and effectiveness of AIT for respiratory allergy. One of the task to be addressed by the Expert groups is "to propose a study design to compare the effectiveness and safety of AIT by using 1 or a few allergens *versus* all or most allergens to which a patient is sensitized" (61). It is to be hoped that this initiative can definitively clarify this issue. As far as the schedules to perform SCIT are concerned, conventional protocols require a build-up phase followed by a maintenance dose at monthly interval, but short protocols are also available (62). For example, for pollen allergy a short course of four injections of tyrosine-absorbed allergoids enhanced with the adjuvant monophosphoryl lipid A was demonstrated to be effective and safe in children, adolescents and adults with grass and tree pollen allergy (63) and its use is likely to increase in the event of regulatory approval.

Cost-effectiveness of AIT

The long-term efficacy of AIT after its stopping is the one of the greatest pharmaco-economic advantage of this therapy. In fact, the progressive improvement of symptoms after AIT is paralleled to the decrease in the use of symptomatic drugs. In the first study on SCIT, the analysis of cost-effectiveness was based on the simple calculation of the monetary expenditure for patients treated with SCIT and symptomatic drugs compared with patients treated only with drugs. Such studies, evaluating mostly SCIT but also SLIT, were reviewed in 2008 by Berto *et al.*, who concluded that AIT, in both routes of administration, may be beneficial to the healthcare systems, because of the better clinical outcome at a cheaper cost or additional benefit at an acceptable extra cost (64). In the ensuing years, the appropriate tools for cost-effectiveness evaluation, such as quality-adjusted life-years (QALYs) and incremental cost effectiveness ratio (ICER) (65) were increasingly used for AIT. In a more recent analysis, 24 studies on health economics of AIT, performed in Northern, Central and Southern Europe and in North America, were reviewed by Hankin and Cox. Nine studies were on SCIT, 10 on SLIT and 5 on both (66). Only one early study comparing the costs for SCIT to costs for drugs in patients with asthma exacerbated by seasonal ragweed exposure failed in detecting a cost-effectiveness, because reduced medication costs were counterbalanced by the costs of immunotherapy (67). However, the study duration was limited to two years of SCIT, thus lacking the post-treatment effect which is now known to enhance the economic advantage of AIT. All the other studies provided, according to Hankin and Cox, "compelling evidence for the cost saving of AIT, whether delivered subcutaneously of sublingually, over symptomatic drug treatment". As far as the comparison between SCIT and SCIT is concerned, most of the analyzed studies reported cost savings favoring SLIT. After this review, two studies comparing SCIT to SLIT were published. Verheggen *et al.* used QALYs in a time horizon of 9 years (3 years of AIT) to compare the cost-effectiveness of SCIT by a mix of injectable allergoid products to that of SLIT by 5-grass tablets in patients with grass pollen AR. A cost-utility ratio of the 5-grass tablet *vs.* the market mix of injectable allergoids of €12,593 per QALY in the base case analysis was found. Compared to the allergoid mix the likelihood of the 5-grass tablet to be the most cost-effective treatment was predicted as 76% at a willingness-to-pay threshold of €20,000 (68). Different results were reported by Brüggenjürgen *et al.* in a study involving three nations (Austria, Spain and Switzerland) and comparing SLIT with 5-grass tablets to a single allergoid and to drug treatment. Both SCIT and SLIT were dominant in the health economic aspects compared to pharmacological symptomatic therapy, while the comparison SCIT-SLIT showed lower total costs of SCIT *vs.* SLIT for the three nations (€1,368 *vs.* €2,012, €2,229 *vs.* €2,547, and €1,901 *vs.* €2,220 for Austria, Spain and Switzerland, respectively). Also, higher cost-effectiveness in term of QALYs (SCIT=8.02, SLIT=7.98 QALYs, symptomatic therapy=7.90) (69). In the systematic review by Meadows *et al.* both SCIT and SLIT were found to be cost-effective when compared with standard treatment from about 6 years (threshold of £20,000-30,000 per QALY), but the authors

claimed further research to establish the comparative effectiveness of SCIT compared with SLIT (70).

Differentiation of SCIT and SLIT

Treatment safety and patients' adherence are the main differences between SCIT and SLIT, as shown by literature.

Safety of SCIT and SLIT

Safety is the major difference between the two AIT routes. As described above, the reports of fatal reactions to SCIT in the 1980s (11,12) was a critical issue, which resulted in a limitation of SCIT use and also in the search of alternative routes of administration. SLIT appeared an effective treatment for patients with respiratory allergy with a better safety profile than SCIT, as demonstrated by the absence of fatalities even in the case of consumption of massive dosages. There is a case report of a patient who, after discontinuing SLIT for several months, took all the previously unused doses at once developing an anaphylactic reaction, but surviving (71). Of interest, in the first systematic review on SLIT safety, no difference in the risk of adverse reactions was detected comparing low dose and high dose SLIT products, whereas the dose dependence of SR to SCIT is well known (72). Nevertheless, a risk of anaphylactic reactions in particular circumstances cannot be excluded, as shown by Nolte *et al*., who analyzed the use of epinephrine in 2408 patients recruited in trials on SLIT tablets with various allergens. A global number of 32 epinephrine administration was necessary (10 for 1-grass pollen, 9 for ragweed pollen, and 13 for dust mite tablets). The reactions treated with epinephrine were not life-threatening. The authors concluded that epinephrine use in SLIT tablets is uncommon, typically occurring in the initial phase of treatment (73). A particular issue concerns the use of protocols directly starting with the maintenance dose, with no build-up, in patients admitted to SLIT because of previous SR to SCIT. In fact, two cases anaphylaxis after the first SLIT dose were described, influencing the subsequent removal of such admission criteria to SLIT (74). Actually, in the most recent US practice parameter update on SLIT it is stated that the first dose of SLIT tablets must be administered in the physician's office and that each patient must be prescribed injectable adrenaline to be used in case of need according to the instructions received (75). This behavior remains mandatory for SCIT, although there was a significant decrease of anaphylactic reactions to SCIT after the identification of uncontrolled asthma in the day of the allergen injection as the major risk factor (13). In a recent EAACI endorsed overview of systematic reviews on AIT, for SCIT about 4% of systemic reactions were graded as severe (with no fatality), compared with 2% of such reactions with SLIT (76).

Adherence to SCIT and SLIT

Adherence to prescribed therapies is a general problem in medicine, as highlighted by Cutler and Everett, who estimated that as many as half of all patients do not adhere to their prescription-medication regimens, with a relevant impact on medical health costs, corresponding to more than $100 billion spent each year on avoidable hospitalization (77). The first studies that analyzed adherence to AIT involved patients treated with SCIT. The direct administration of the allergen extract by the physician (or trained nurses) theoretically should ensure good compliance and adherence, but rates of compliance of approximately 50% were reported instead, both in adults (78) and children (79), the inconvenience from frequents injection and the lack of reimbursement being reported as the major cause of SCIT discontinuation (80). More favorable results were observed using less demanding schedules for injections, with a raise of compliance from 62% to 88%, provided optimal doses of allergen extracts were administered (81-83). Recent studies substantially confirmed such figures. In a real-life study, the patients who received a SCIT prescription for AR and/or asthma in 2009-2011 were contacted in 2014 and asked about the completion of at least the 3 years of SCIT. A close relationship between allergists and their patients during SCIT and the follow-up period was warranted, resulting in an overall rate of 87.3% of patients considered adherent (84). In a retrospective chart review of SCIT patients in US between 2003 and 2016, compliance to treatment was evaluated using analysis of variance to compare mean compliance between payer groups. Linear regression showed that age, duration of SCIT therapy and asthma status were not related to the percentage of missed doses, while payer status was statistically predictive of missed doses ($p=0.02$). In fact, Medicaid patients missed 34.2% of doses, followed by Medicare (24.4%), commercial insurance (19.9%) and HSN in Massachusetts (18.5%). The authors concluded that in patients referring to an urban tertiary care setting serving a low-income population, compliance to SCIT was generally high but lower in the Medicaid population (85). As to SLIT, the first studies on compliance and adherence were optimistic, reporting rates of 80-90% (86). However, in 2010 an investigation based on the prescription data from SLIT products manufacturers revealed that only 13% was still under treatment after three years, *i.e.* that duration needed to provide the persistent effect of SLIT over time (87). Given the lack of adherence to SLIT, researchers focused on methods to improve it, demonstrating the importance of patient education and accurate monitoring during the treatment, while the effect of technology-based tools, including online platforms, social media, e-mail, and short message service by phone, is under evaluation (86). In the latest years, studies comparing the compliance and adherence of SCIT and SLIT were published. In a retrospective

analysis of a community pharmacy database from The Netherlands involving 6486 patients initiating AIT between 1994 and 2009, 2796 patients were treated with SCIT and 3690 were treated with SLIT. Globally, only 18% of patients (23% for SCIT and 7% for SLIT) completed the required duration of treatment. A premature discontinuation was influenced by the kind of prescriber (a longer persistence was found for patients of general practitioners compared with patients referring to allergists and other medical specialists) by single allergen *vs.* multiple allergens AIT, by lower socioeconomic status and younger age. The authors claimed for an urgent need to further define the potential barriers for compliance to AIT (87). A study from Spain assessed the effect on compliance of allowing patients to select the route of AIT. Patients were divided into two groups, the study group being formed by patients who chose the route of administration, while control group included patients for whom their physician decided the route. Before starting AIT, all patients received an educative session on the benefits and risks of the treatment, with an additional session in the active group informing about specific characteristics of SCIT and SLIT. After 6 months, 24 of 204 patients in the active group (11%) and 22 patients of 103 in the control group (21%) had stopped AIT, this difference being significant (p=0.02). In the active group no significant difference in compliance was detected between those who preferred SCIT or SLIT, whereas in the control group the number of withdrawals was significantly higher for SLIT than for SCIT (p=0.05) (88). The rate of compliance was much better than in the Dutch study, but the far shorter observation period (6 months) must be taken into account. A further comparison was done in a retrospective review of 384 patients treated with SCIT or SLIT for at least two years at a tertiary care otolaryngology and allergy practice. SCIT compliance was defined according to injections schedules defined as excellent or good, while for SLIT compliance excellent or good rating was defined according to the number of vials refilled within the expiration date. Excellent or good compliance rates were found in 83.7% of SCIT patients and in 65.5% of SLIT patients. Limiting the analysis to excellent compliance rates, a significant difference ($p > 0.05$) was detected in favor of SCIT (89). The latest study used questionnaires to retrospectively analyze the compliance among 236 patients with AR with or without asthma initiating AIT in 2009 or 2010. The compliance rates after 3 years were 58.7% in SCIT treated and 11.6% in SLIT treated patients. The most common causes of non-compliance were the frequency of injections and the duration of treatment, for SCIT, the inconvenience (to take the allergen extract everyday) and the improvement without treatment for SLIT (91). Globally viewing the results of these studies, it is apparent that, although there is margin of improvement in SCIT compliance, prescribers must be committed to greatly improving SLIT compliance, which is very lacking.

Conclusions

AIT has unique characteristics among treatments for respiratory allergy, that rely on its ability to induce immunological tolerance to the specific allergen. Currently, two routes of administration are available, namely SCIT and SLIT, that share a number of patterns but differ for others. In particular, a large number of meta-analyses support the efficacy of both routes, without clear evidence of superiority over one another, as acknowledged by recent position papers (92) and analysis by opinion leaders (93). Also, comparable cost-effectiveness was found, especially when the treatment is stopped after adequate duration, moreover the clinical effects persists, with great advantage over symptomatic drug therapy. However, since several NPPs are still present on the market alongside the registered products, a clarification is needed on the existence of different brands and the fact that the indication of an AIT should take into account the quality of the product.

This makes it mandatory to inform prescribers that specific products, rather than AIT, SLIT or SCIT, should be considered as a whole, based on the fact that among the many different products available, for some of them the effectiveness has been demonstrated, while for others it has not.

As far as safety and compliance are concerned, there are differences between SCIT and SLIT. The former has substantially reduced the risk of severe SR following the acknowledgement of the importance of uncontrolled asthma in favoring such reactions, but the safety profile of SCIT is inferior when compared with SLIT. The opposite occurs with compliance, all recent studies reporting rates of compliance much lower for SLIT. This warrants commitment by physicians to improve the outcome, as recently reviewed, by "better patient education at the beginning of treatment, sharing with patients the decision on which type of immunotherapy to select and showing sincere interest in their treatment concerns" (94).

Future perspective

At the time AIT was introduced (6), all medications were available only in galenic formulation, and in fact AIT was firstly administered through galenic type preparations, obtained directly from the allergenic source, for example grass pollen. In the following decades, the progress in drug development was not mirrored by similar technological improvements in AIT, that, despite an undeniable evolution (94) remained long based on NPP, *i.e.* allergen extracts prepared for single patients, firstly for SCIT and later also for SLIT, and the quality of these products was uneven. Currently, the need for new products to fulfill the requirements of the regulatory agencies to achieve approval and license to commerce prompted a significant improvement of quality and resulted in major advances, such as the inclusion of SLIT tablets for mite-induced allergic asthma in the GINA

guidelines for allergic asthma (41). Moreover, according to Nolte and Maloney, the high allergen concentrations administered with SLIT tablets may silence reactive T cells via anergy/deletion. This process reverses the immune deviation of weakly primed Th2 responses to inhalants towards, typical of allergic subjects, possibly resulting in long term protection against atopic sensitization (93). According to a document from the Agency for Healthcare Research and Quality, the effects of SCIT and SLIT on asthma are not superimposable (95). Actually, the recent EAACI guidelines on AIT for mite-induced asthma gave the recommendations for the different allergen products for AIT. Mite tablets were recommended for adults with controlled or partially controlled asthma, while SCIT was recommended for adults and children and SLIT drops were recommended for children, provided products with demonstration of efficacy by DBPC trials are used and asthma is well controlled (96). Still, in children AIT remains underused and its evidence is challenging due to heterogeneity among studied populations, selection of potential responders, products and outcomes (97), as well as for recognizing optimal schedules of administration (98). The identification of efficacy biomarkers able to predict or monitor the AIT efficacy in early stage is likely to improve the treatment outcome of AIT (99), by avoiding its use in potentially poor responders while supporting the adequate duration in potential responders. In the near future, new advances in AIT are likely, including further methods of administration by intralymphatic or epicutaneous route, which are already supported by several studies, use of recombinant allergens (and hypoallergenic variants), as well as of T- and B-cell peptide approaches (100). However, the effectiveness of these new approaches still has to be compared with the latest generation of SLIT tablets, which are acknowledged as reference products for AIT.

Finally, regardless the route of administration, high quality trials are warranted to demonstrate the preventive effects of AIT, which was reported in some studies, on the development of new sensitizations and allergic comorbidities with a progressive multiorgan involvement (101).

Executive Summary

- AIT is the only treatment for respiratory allergy which works on the causes of allergy and not only on symptoms.
- The first method of AIT was SCIT, followed by SLIT in the 1980s. The two routes of administration are now considered of comparable efficacy and produce a similar cost-effectiveness, particularly when the treatment is stopped after appropriate duration.
- Some outcomes show difference between SCIT and SLIT, concerning safety, which is better for SLIT, and treatment compliance, which favors SCIT.
- The new generation of SLIT tablets has achieved a significant advance in quality, that resulted in important accomplishments, such as the inclusion in the GINA guidelines as a therapy for dust-mite induced asthma.

Conflict of interests

The authors declare that they have no conflict of interests.

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Comparison of the performance of Skin Prick and ISAC Tests in the diagnosis of allergy

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Key words

Skin Prick test; microarray test; allergic disease; dermatological disease; food allergy; singleplex; multiplex.

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Summary

The recent European Union and Italian regulations in the matter of in vivo test could strongly impact on current diagnostic approach, increasing the usage of in vitro tests in daily clinical practice.

We evaluated 506 patients with both skin prick test and a microarray system (ImmunoCAP ISAC 112). The overall evaluation between ImmunoCAP® ISAC vs SPT showed a moderate agreement (k=0.509, 95% C.I. 0.480–0.540, SE: 0.016) considering both aeroallergens and food allergens. When we considered the concordant results (double-positive plus double-negatives), the agreement ranged from 69% to 80% for pollen allergens, between 74% and 76% for dust mites, and between 74% and 93% for animal epithelia. In the case of food allergens, the accordance was pretty lower, accounting values ranging from 67% to 86%. ISAC testing identified from 22% to 26% more cases than SPTs in peach and nuts hyper-sensitivity. In 2.8% of the control group, the ISAC-test failed to detect an allergy sensitization caused by dust mite, shrimp, Anisakis, or seed storage proteins.

Multiplex testing is more than a promising tool for more precise and comprehensive profiling of allergic patients and can be considered as a second-line approach, after the anamnesis, in the diagnosis of allergic diseases.

Introduction

In the classical inductive allergic diagnostic workup (Top-down approach), based on the patient-reported history, several tests can be performed to confirm or exclude possible causes of sensitization (1). Classically, the first line investigation is represented by extract-based skin testing, usually using a panel of biological sources, chosen following the current guidelines (2). Furthermore, in vitro singleplex tests with extract-based analytes are commonly prescribed as a sort of confirmatory evaluation of the *in vivo* testing and the single components are performed for an in-depth analysis. Several multiplex systems have been recently developed, allowing the evaluation of hundreds of distinct components at the same time and in the same patient. Such an in vitro test could detect a comprehensive profile of IgE sensitization (3). Due to higher costs, in most allergy units in Italy ISAC test is offered to the patient as a private test and therefore is currently prescribed only in selected situations or in case of complex diagnoses (4).

In 2001 a directive (2001/83/EC) of the European Parliament stated that "[..] no medicinal product (including allergens for in vivo tests) may be placed on the market of a Member state unless a marketing authorization has been issued by the competent authorities […]" (5). Recently this directive was implemented in Italy and several determinations have been published in the GAZZETTA UFFICIALE establishing the trading denial for many allergenic products for in vivo tests and immunotherapy in patients suffering from environmental or food allergies.

Given the likely downsizing soon of traditional in vivo in favor of a predominantly in vitro diagnostic assessment, we retrospectively evaluated a large cohort of patients to verify the amount

of loss (or gain) in diagnosis precision obtained with a comprehensive proteomic approach instead of a classical multistep evaluation utilizing skin prick testing (4,6,7).

Materials and methods

The observational controlled study cohort was enrolled at the outpatient Allergy Unit of IDI-IRCCS in Rome, a National Reference Center for Allergic and Dermatological diseases. Demographic details together with clinical data (food-related reactions, respiratory and dermatological symptoms) were recorded using the TD-Synergy® Laboratory Information System (Siemens Healthcare Diagnostics, Muenchen, Germany) and a customized electronic database.
The study received ethical approval from IDI-IRCCS Ethical Committee (496/1).

Patients

Patients aged 18 years and over, born in Central or Southern Italy presenting with a history of adverse reactions to foods, allergic rhinitis, bronchial asthma and/or atopic dermatitis were recruited between January and December 2019.
The case group consisted of 256 patients (males: 135, mean age 34 ± 17; range 18-69), having a clear reactivity to one or more biological sources currently spotted in the ImmunoCAP ISAC. Clinical categorization was as follows: [Food Allergy, FA] history of symptoms unequivocally suggestive of adverse reaction to a suspected plant food-derived trigger(s), including urticaria and external angioedema, laryngeal angioedema, respiratory difficulty and/or pre-syncope/syncope in the last 6 months; [Respiratory Symptoms, RS] symptoms of rhino-conjunctivitis and/or bronchial asthma only.
The control group comprised 250 adults (males: 115, mean age 33 ± 16; range 18-72) with negative results after the ImmunoCAP ISAC test, despite a patient reported a history of chronic urticaria (64%), atopic dermatitis (28%), or vasomotor rhinitis (19%).

Diagnostic assays

Skin Prick Tests

All subjects underwent Skin Prick Tests (SPT) to a series of glycerinated aeroallergen and food extracts (either from Stallergenes, Antony, France or ALK Abelló, Horsholm, Denmark from), and positive and negative control solutions (histamine hydrochloride 10 mg/mL and diluent) on the volar forearms. The inhalant panel included pollen from a grasses mixture (*Phleum pratense*, *Lolium perenne*, *Poa pratensis*), *Artemisia vulgaris*, *Parietaria judaica*, *Plantago lanceolata*, olive, birch, hazel, oak, cypress, plane trees, *Dermatophagoides pteronyssinus*, and *farinae*, dog, cat, and horse dander, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus mixture*, latex, and cockroach. The panel of food allergens, all available as extracts 1:20 w/v, included *Anisakis simplex*, shrimp, peanut, walnut, and hazelnut. Peach extract (ALK Abelló) was chosen as a marker for nsLTP sensitization and birch pollen as a marker for pollen food syndrome related to PR-10 proteins (8,9). SPTs were performed using sterile stainless steel standardized lancets (Stallergenes) by the same operator, and taken at 15 min, using standardized techniques according to international guidelines (10).

Serum analysis

A semi-quantitative allergen microarray assay was used to determine the individual participant's specific IgE sensitization to 112 allergen components in triplicate, measured using the Immuno Solid-phase Allergen Chip (ImmunoCAP ISAC 112) microarray system platform according to the manufacturer's instructions (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE values were expressed in ISAC standard units (ISU), with values of 0.3 ISU or greater considered positive.
For the specific purpose of comparing allergenic molecule IgE prevalence to extract based SPT evaluation, single molecular results from each distinct biological source or panallergen subset were pooled together as follow: grasses (Cyn d 1 + Phl p 1 + Phl p 11 + Phl p 12 + Phl p 2 + Phl p 4 + Phl p 5.0204 + Phl p 6 + Phl p 7); cypress (Cup a 1 + Profilin + Polcalcin); mugwort (Art v 1 + Art v 3 + Profilin + Polcalcin); plane tree (Pla a 1.dic + Pla a 2.dic + Pla a 3.dic + Profilin + Polcalcin); birch tree (Betv 1 + Bet v 2 + Bet v 4); oak tree (Bet v 1 + Bet v 2 + Bet v 4); pellitory (Par j 2 + Profilin + Polcalcin); olive tree (Ole e 1 + Ole e 7 + Ole e 9 + Profilin + Polcalcin); *Dermatophagoides pteronyssinus* (Der p 1 + Der p 10 + Der p 2 + Blot5 + Pen m 2); *Dermatophagoides farinae* (Der f 1 + Der f 2 + Der p 10 + Blo t 5 + Pen m 2); *Alternaria* (Alt a1 + Alt a 6); *Aspergillus* (Asp f1 + Asp f 3 + Asp f 6); cat dander (Fel d 1 + Fel d 2 + Fel d 4); dog dander (Can f 1 + Can f 2 + Can f 3 + Can f 5); horse (Equ c 1 + Equ c 3); latex (Hev b 1 + Hev b 3 + Hev b 5 + Hev b 6.01 + Hev b 8.0204); *Blattella* (Bla g 1 + Bla g 2 + Bla g 5 + Bla g 7); *Anisakis* (Ani s 1 + Ani s 3); peach (Pru p 1 + Pru p 3); shrimp (Pen m 1 + Pen m 2 + Pen m 4); hazelnut (Cor a 1.0101 + Cor a 1.0401 + Cor a 8 + Cor a 9); peanut (Ara h 1 + Ara h 2 + Ara h 3 + Ara h 6 + Ara h 8 + Ara h 9 + Profilin); walnut (Jug r 1 + Jug r 2 + Jug r 3); LTP (Ara h 9 + Art v 3 + Cor a 8 + Jug r 3 + Ole e 7 + Pla a 3 + Pru p 3 + Tri a 14); Profilin (Bet v 2 + Hev b 8.0204 + Mer a 1 + Phl p 12); Polcalcin (Bet v 4 + Phl p 7).

Statistical analysis

All data were analyzed using the SPSS/PC + statistical package for statistical evaluation (SPSS, version 15, Chicago, IL). The TD-Synergy Laboratory Information System was used to search

and collect demographic (age and gender), clinical and laboratory data for Allergy Clinic patients who attended the outpatient Allergy clinic and underwent specific IgE testing.
Each variable of interest obtained with SPTs or the microarray system was dichotomized (as negative or positive), and the degree of relationship between the categorical variables studied was analyzed using the Pearsons' χ^2 or Fisher's exact test when indicated.
Inter-rater agreement between SPT and ImmunoCAP ISAC was calculated for qualitative outcomes (positive-negative); Cohen's kappa coefficient (k), positive and negative agreement were assessed for every single extract based on skin prick test (SPT) result and molecule considered. As conventionally assumed, kappa results have been interpreted as follows: k: ≤ 0 no agreement, 0.01-0.20 none to slight, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial and 0.81-1.00 almost perfect agreement (11).

Results

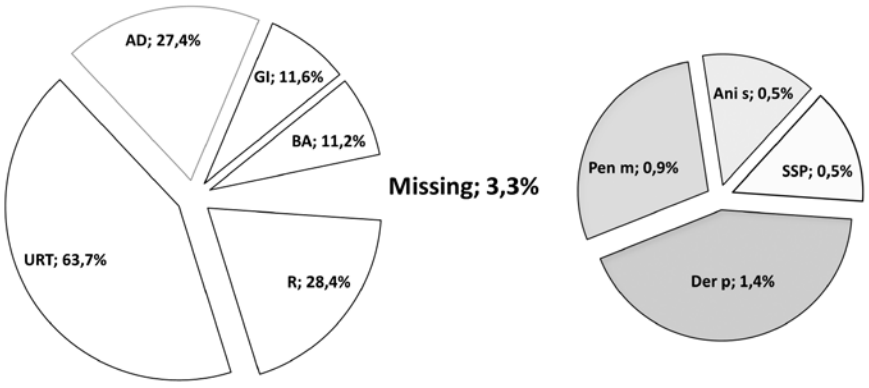
Skin prick tests

Grass pollen allergens (67,6%), house dust mites (52,9%), and cypress pollen allergens (52,8%) were the top-ranking reactivity to aeroallergens recorded in the case group. Peanut (29,8%), peach (17,6%), and walnut (16,4%) represented the most frequently positive result in food SPT evaluation.
Respiratory symptoms were significantly associated with hyper-reactivity to SPTs *vs* birch pollen (p=0,02; OR=2,67; 95% CI=1,13-6,30), grasses (p=0,01; OR=2,24; 95% CI=1,20-4,19), cypress tree (p=0,02; OR=2,04; 95% CI=1,10-3,81), and plane-tree R (p=0,03; OR=2,56; 95% CI=1,02-6,41).
Oral allergy syndrome (OAS) occurrence was associated with a positive SPT to hazelnut (p < 0.001; OR=4,71; 95% CI=2,28-9,75), birch (p=0,001; OR=3,04; 95% CI=1,47-6,28), and mugwort pollen (p=0,001; OR=3,06; 95% CI=1,49-6,25). Mugwort reactivity was also linked to the occurrence of severe reaction (SR) to food (p=0,01; OR=2,18; 95% CI=1,22-3,91).
Seven out of 250 subjects in the control group, despite the negative results after the ISAC testing, had a positive SPT to dust mites in 3 cases (1.4%), to shrimp in 2 patients (0.9%), to Anisakis in one subject (0.5%), and peanut in one participant (0.5%) (**figure 1**).

Microarray

The ISAC results showed a profile of sensitization comparable to what recorded with STP. Grass pollen allergens (56,6%), cypress molecules (54,7%), and house dust mite (48,4%) component reactivity were the most commonly observed among the inhalant molecules tested, whereas 39,3% of the food reactive patients had a positive test to peanut allergens, 38,3% to peach components, and 31,2% to walnut molecules.
RS occurrence was strictly associated with ISAC test reactivity to molecules belonging to mugwort Art v 1 (p=0,03; OR=3,20; 95% CI=1,09-9,38); cypress pollen Cry j 1 (p=0,01; OR=2,25; 95% CI=1,16-4,35) and Cup a 1 (p < 0,001; OR=2,55; 95% CI=1,34-4,84), and grasses Cyn d 1 (p < 0,001; OR=2,57; 95% CI=1,35-4,90) and Phl p 1 (p < 0,001; OR=2,53; 95% CI=1,33-4,79).
Severe Reactions (SR) to food were strictly linked to molecular reactivity to the 2S Albumin from Brazilian nut Ber e 1 (p=0,01; OR=9,27; 95% CI=1,12-76,48), nsLTPs from peanut Ara h 9 (p < 0,001; OR=5,79; 95% CI=2,79-12,01); mugwort Art v 3

Figure 1 *- Clinical data of patient in the control group (Urt: chronic spontaneous urticaria; R: Rynithis; AD: Atopic Dermatitis; GI: gastro-intestinal symptoms; BA: Bronchial Asthma; Pen m: Shrimp; Ani s: Anisakis simplex; SSP: Seed Storage protein; Der p: dust mite). The extract reactivity of patients not detected by ISAC test ("missing") is reported on the right.*



(p < 0,001; OR=3,38; 95% CI=1,75-6,52); hazelnut Cor a 8 (p=0,00; OR=4,49; 95% CI=2,14-9,42); walnut Jug r 3 (p < 0,001; OR=4,34; 95% CI=2,30-8,19); plane tree Pla a 3 (p < 0,001; OR=4,18; 95% CI=2,21-7,90); peach Pru p 3 (p < 0,001; OR=5,32; 95% CI=2,89-9,80), and wheat Tri a 14 (p=0,03; OR=2,71; 95% CI=1,06-6,98). OAS was significantly associated with PR10 molecules from birch Bet v 1 (p=0,01; OR=2,23; 95% CI=1,17-4,23); apple Mal d 1 (p=0,01; OR=2,52; 95% CI=1,26-5,03); peach Pru p 1 (p=0,03; OR=2,15; 95% CI=1,06-4,36), and hazelnut Cor a 1.0401 (p=0,03; OR=2,15; 95% CI=1,06-4,36).

In addition, we verified SPT results in subjects with panallergen reactivity. As shown in **figure 2**, a significantly higher number of profilin reactive participants had a positive test to grasses, mugwort, birch, and hazel trees. Polcalcin sensitized individuals were more likely to be reactive to all kinds of pollen allergen, except for the olive tree. nsLTP hyper-sensitivity was associated with an increased occurrence of mugwort, plane tree, and pellitory STP reactivity. PR10 population showed an increased amount reactivity to birch, hazel and oak trees.

SPT and Microarray comparison

The comparison between SPT outcomes and ImmunoCAP ISAC evaluation is detailed in **table I**. The overall evaluation between ImmunoCAP® ISAC *vs* SPT showed a moderate agreement (k=0.509, 95% C.I. 0.480–0.540, SE: 0.016) considering both aeroallergens and food allergens.

Among the inhalant allergens, no agreement (k ≤ 0) was observed for cockroach and *Aspergillus*, slight agreement for latex (k=0,096), a fair agreement for oak tree (k=0,235), plane tree (k=0,321), dog dander (k=0,329), and pellitory (k=0,404), moderate agreement for grasses (k=0,410), horse (k=0,416), olive tree (k=0,425), cypress (k=0,436), house dust mite [Der f (k=0,494) and Der p (k=0,515)], birch tree (k= 0,501) and mugwort (k=0,549), whilst a substantial agreement was found only in the case of cat dander (k=0,613) and alternaria (k=0,761).

The overall agreement for food allergens resulted in slight to fair agreement comparing extract-based ST and molecular components. Particularly, no agreement was found for shrimp allergens, slight agreement for walnut (k=0,170), and fair agreement for hazelnut (k=0,229), peanut (k=0,238), peach (k=0,317), and Anisakis (k=0,380).

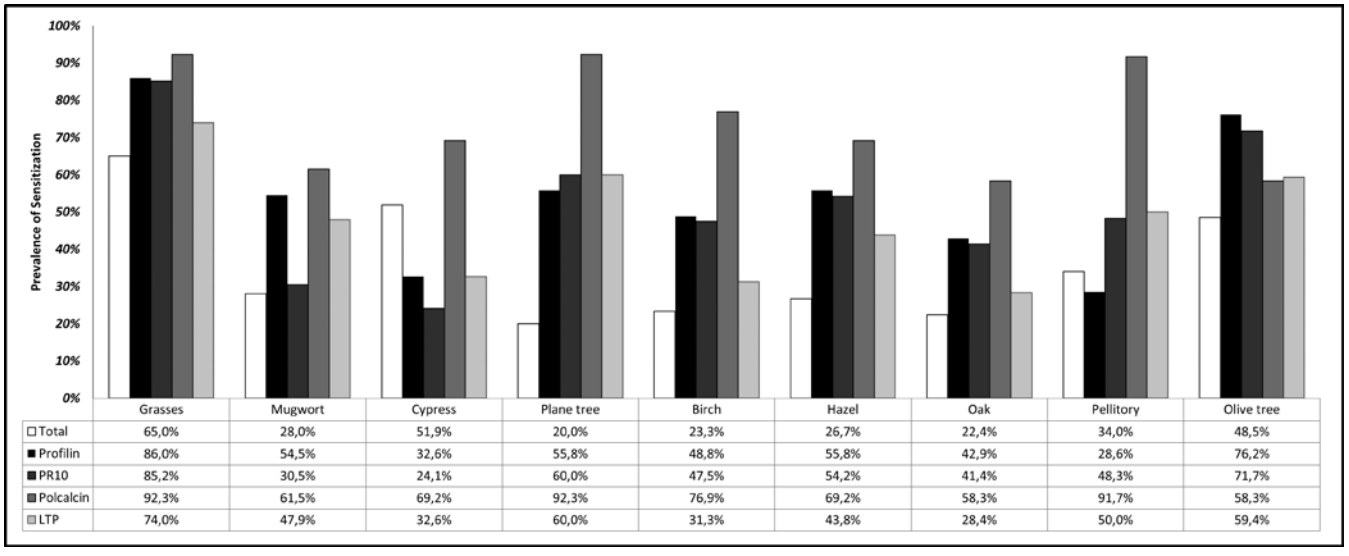
In **figure 3** the prevalence of component recognition profiles, in SPT reactors and not, is shown. Interestingly patients SPT-positive to grasses, pellitory, and olive tree showed a significantly higher prevalence of Phl p 1, Par j 2 and Ole e 1 IgE recognition, respectively, than the patients SPT-negative. In the case of pellitory-of-the-wall, significantly higher occurrence of Polcalcin recognition was achieved in SPT reactors, whilst patients with negative to skin testing showed a higher occurrence of Profilin recognition.

Discussion

Our data indicate that, in the majority of cases, only a moderate concordance among SPT and ISAC-test was found, whereas, in the case of food allergens, the concordance was even lower.

When we considered the concordant results (double-positive plus double-negatives), the agreement ranged from 69% to

Figure 2 *- Prevalence of sensitization to pollen, as evaluated by means of SPT, in panallergen reactors. *p < 0.01.*

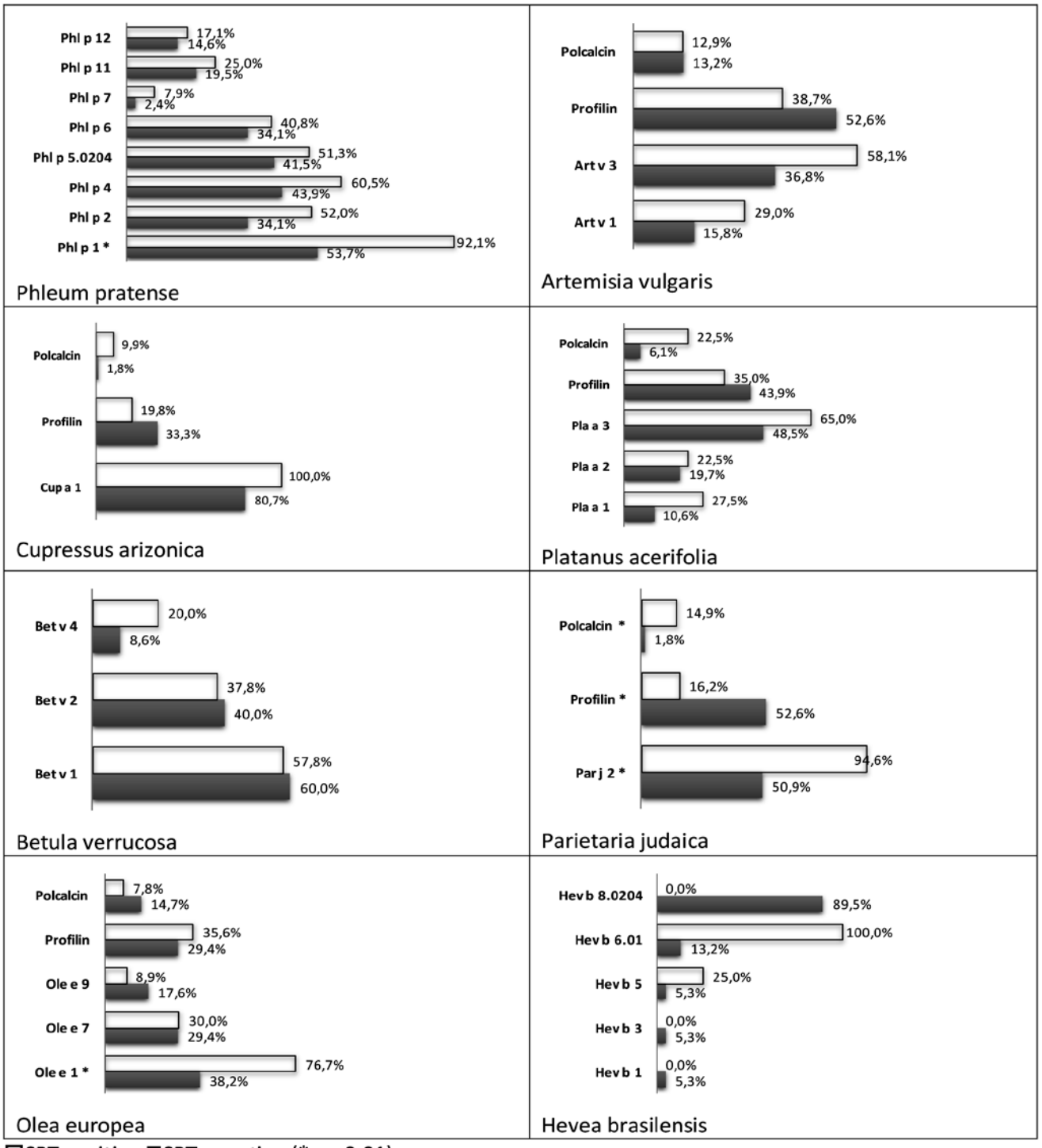


	Grasses	Mugwort	Cypress	Plane tree	Birch	Hazel	Oak	Pellitory	Olive tree
Total	65,0%	28,0%	51,9%	20,0%	23,3%	26,7%	22,4%	34,0%	48,5%
Profilin	86,0%	54,5%	32,6%	55,8%	48,8%	55,8%	42,9%	28,6%	76,2%
PR10	85,2%	30,5%	24,1%	60,0%	47,5%	54,2%	41,4%	48,3%	71,7%
Polcalcin	92,3%	61,5%	69,2%	92,3%	76,9%	69,2%	58,3%	91,7%	58,3%
LTP	74,0%	47,9%	32,6%	60,0%	31,3%	43,8%	28,4%	50,0%	59,4%

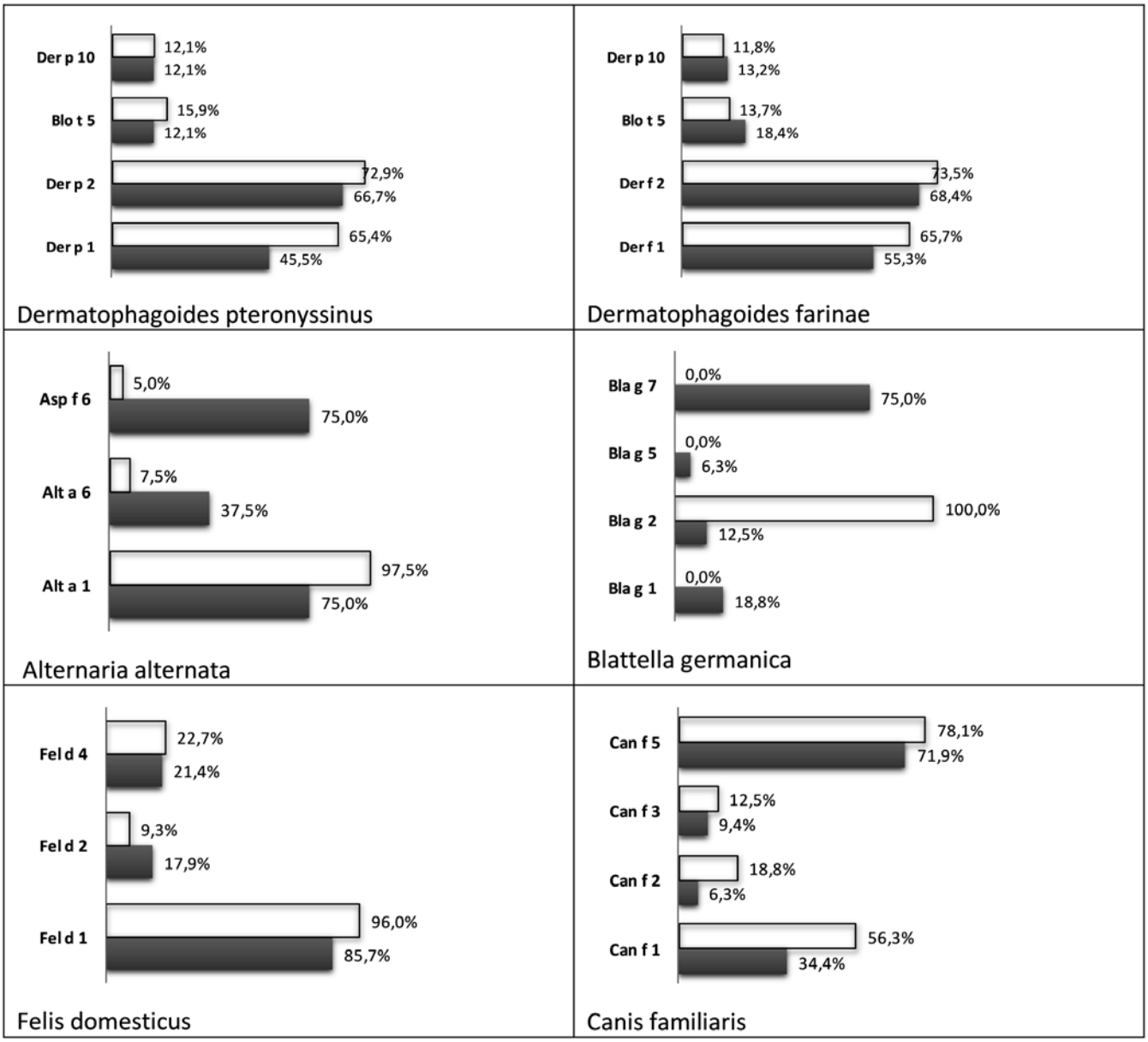
Table I *- ImmunoCAP ISAC® vs skin prick test frequency reactivity comparison.*

		ISAC / ST				Cohen's kappa coefficient	Pearsons' $\chi 2$	Significance (p)
		+/-	-/+	+/+	-/-			
1	**Grasses**	16,0%	8,2%	59,4%	16,4%	0,410	44,727	*p: 2,27^{-11}*
2	**Cypress**	22,4%	5,1%	47,6%	24,8%	0,436	55,302	*p: 1,03^{-13}*
3	**Mugwort**	15,2%	5,6%	24,8%	54,4%	0,549	78,657	*p: 7,39^{-19}*
4	**Plane tree**	26,6%	4,4%	16,1%	52,8%	0,321	33,415	*p: 7,44^{-09}*
5	**Birch tree**	13,9%	6,4%	17,9%	61,8%	0,501	65,147	*p: 6,95^{-16}*
6	**Oak tree**	11,6%	14,3%	8,8%	64,9%	0,235	14,592	*p: 6,78^{-04}*
7	**Pellitory**	22,2%	7,8%	28,8%	41,2%	0,404	45,671	*p: 1,40^{-11}*
8	**Olive tree**	13,4%	15,4%	35,4%	35,8%	0,425	46,039	*p: 1,16^{-11}*
9	**Der p**	12,8%	11,3%	41,6%	34,2%	0,515	68,223	*p: 1,46^{-16}*
10	**Der f**	14,8%	10,5%	39,7%	35,0%	0,494	63,177	*p: 1,89^{-15}*
11	**Alternaria**	3,2%	4,4%	15,9%	76,6%	0,761	146,231	*p: 1,16^{-33}*
12	**Aspergillus**	6,0%	4,8%	0,0%	89,2%	ns	ns	*ns*
13	**Cat dander**	10,9%	7,4%	29,3%	52,3%	0,613	96,641	*p: 8,31^{-23}*
14	**Dog dander**	12,7%	12,7%	12,7%	61,8%	0,329	27,148	*p: 1,88^{-07}*
15	**Horse**	3,2%	3,6%	2,8%	90,4%	0,416	43,396	*p: 4,47^{-11}*
16	**Latex**	15,1%	2,4%	1,6%	81,0%	0,096	4,082	*p: 4,33^{-02}*
17	**Blattella**	6,4%	2,0%	0,4%	91,2%	ns	ns	*ns*
18	**Anisakis**	4,7%	7,9%	5,1%	82,3%	0,380	31,827	*p: 1,69^{-08}*
19	**Peach**	25,0%	4,2%	13,4%	57,4%	0,317	30,508	*p: 2,37^{-07}*
20	**Hazelnut**	26,0%	6,0%	11,2%	56,7%	0,229	14,63	*p: 1,31^{-04}*
21	**Peanut**	22,3%	12,6%	17,2%	47,9%	0,238	12,735	*p: 3,59^{-04}*
22	**Walnut**	23,7%	7,4%	8,4%	59,1%	0,170	7,926a	*p: 1,90^{-02}*
23	**Shrimp**	9,8%	4,7%	1,4%	84,1%	ns	ns	*ns*

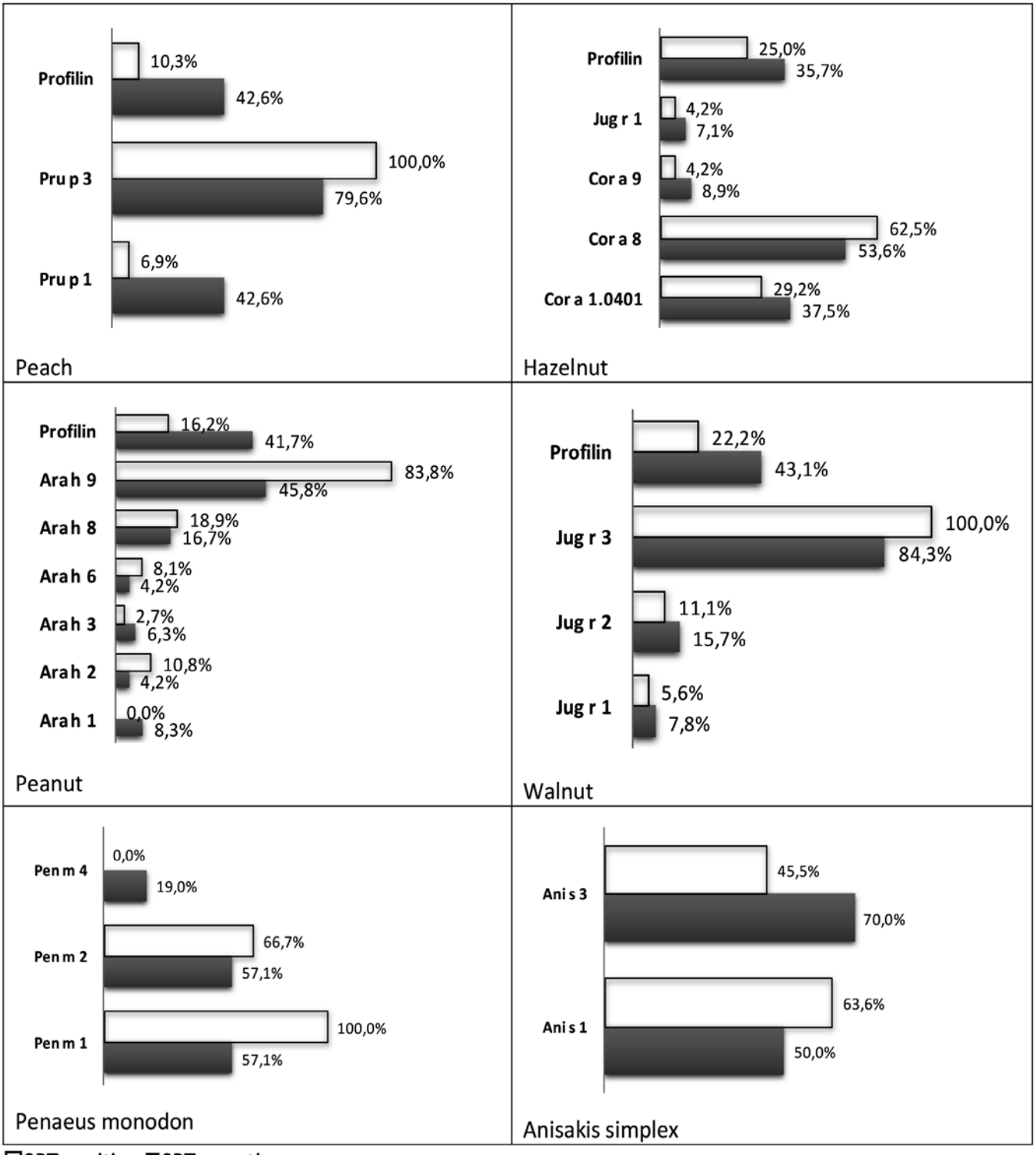
Figure 3 *- Prevalence (%) of component recognition profiles in patients detected (white bar), and not detected by skin prick test (grey bar) for pollen allergen (A), dust mite, mould, and animal dander extracts (B), and food allergen (C). *p < 0.01.*



B


Der p 10
12,1%
12,1%
Blo t 5
15,9%
12,1%
Der p 2
72,9%
66,7%
Der p 1
65,4%
45,5%
Dermatophagoides pteronyssinus
Der p 10
11,8%
13,2%
Blo t 5
13,7%
18,4%
Der f 2
73,5%
68,4%
Der f 1
65,7%
55,3%
Dermatophagoides farinae
Asp f 6
5,0%
75,0%
Alt a 6
7,5%
37,5%
Alt a 1
97,5%
75,0%
Alternaria alternata
Bla g 7
0,0%
75,0%
Bla g 5
0,0%
6,3%
Bla g 2
100,0%
12,5%
Bla g 1
0,0%
18,8%
Blattella germanica
Fel d 4
22,7%
21,4%
Fel d 2
9,3%
17,9%
Fel d 1
96,0%
85,7%
Felis domesticus
Can f 5
78,1%
71,9%
Can f 3
12,5%
9,4%
Can f 2
18,8%
6,3%
Can f 1
56,3%
34,4%
Canis familiaris
SPT positive
SPT negative

C


Profilin
10,3%
42,6%
Pru p 3
100,0%
79,6%
Pru p 1
6,9%
42,6%
Peach
Profilin
25,0%
35,7%
Jug r 1
4,2%
7,1%
Cor a 9
4,2%
8,9%
Cor a 8
62,5%
53,6%
Cor a 1.0401
29,2%
37,5%
Hazelnut
Profilin
16,2%
41,7%
Arah 9
83,8%
45,8%
Arah 8
18,9%
16,7%
Arah 6
8,1%
4,2%
Arah 3
2,7%
6,3%
Arah 2
10,8%
4,2%
Arah 1
0,0%
8,3%
Peanut
Profilin
22,2%
43,1%
Jug r 3
100,0%
84,3%
Jug r 2
11,1%
15,7%
Jug r 1
5,6%
7,8%
Walnut
Pen m 4
0,0%
19,0%
Pen m 2
66,7%
57,1%
Pen m 1
100,0%
57,1%
Penaeus monodon
Ani s 3
45,5%
70,0%
Ani s 1
63,6%
50,0%
Anisakis simplex
SPT positive
SPT negative

80% for pollen allergens, between 74% and 76% for dust mites, and between 74% and 93% for animal epithelia. Surprisingly, the highest level of concordance was achieved by mould extracts (range from 89% to 92%), probably due to the elevated frequency of double negative results.

As reported in **table I**, the detection rate of ISAC is frequently higher than SPTs. Overall, in the assessment of the reactivity to pollen allergens, ISAC identified about 10% more cases than SPT. It is worth noting that the higher detection rates by ISAC were observed in the case of plane-tree (22%), cypress tree (17,3%) and *Parietaria* (14.4%). Previous studies have shown that certain pollen extracts such as pellitory and cypress tree lack Profilin either because this allergens is scarce in these sources or because it is a different isoform (12), and this could be the reason why several patients are better recognized by a specific molecular approach than after the usage of extracts possibly lacking relevant components. In the past, the usage of a pollen Profilin-enriched extract could overcome this caveat, but unfortunately, such a device is no more available in the market, due to the well known regulatory restriction about the usage of such product. Riccardo Asero *et al*. demonstrated that pollen extracts could significantly inhibit IgE reactivity to rBet v 4, whilst only grass pollen extract could inhibit rPhl p 7 IgE reactivity, as a further demonstration of the importance of a molecular approach for a better patient evaluation (13).

A similar result has been obtained in previous studies. Singleplex and multiplex systems showed comparable specificity and sensitivity in detecting grass and cypress pollen hyper-reactivity (14), or pollen (grass and birch) and animal dander (cat) allergy (15). In our cohort, a moderate agreement was found between SPT and ISAC for all these biological sources and a higher agreement for cat dander sensitization recognition.

In the case of food allergens, the accordance was pretty lower, accounting values ranging from 67% to 71%. ISAC testing identified from 22% to 26% more cases than SPTs in peach and nuts hyper-sensitivity, in partial disagreement with previous studies where SPT and ISAC tests showed comparable results in the detection of patients with allergy to nuts (16).

On the other hand, it has been suggested that ISAC test can help in about 20% of cases, to identify the culprit allergen responsible for "idiopathic" anaphylaxis, particularly when the patient-reported history, SPT, and singleplex tests have not revealed the cause the adverse reaction (17). However, it is important to underline that, whatever the method, the presence of an IgE sensitization is only evidence of sensitization that should be correlated with the clinical history before drawing any conclusion.

Panallergen reactivity affected SPT outcome. Interestingly plane tree (18) and mugwort (19) sensitization were strictly related to FA and not to RS in the study group, as previously suggested (20). It is worthy of note that other mugwort pollen allergens, not fully identified yet, other than Art v 3 may be relevant as a food allergen, such as a 60 kDa molecule isolated in mugwort extract, highly homologous to the fennel Api g 5 (21). As expected, Polcalcin recognition was associated with increased occurrence of pollen reactivity, and PR10 reactivity with positive SPT to trees belonging to the Fagales order (8,22).

In 2.8% of the control group, the ISAC-test failed to detect a food allergy sensitisation caused by dust mite, shrimp, *Anisakis*, or seed storage proteins. It is widely known that in the case of house dust mite, the ISAC system could evaluate only molecules belonging to group -1, -5, -10 and, indirectly measured by Pen m 2 (23), group -20, whereas there are several other allergens not included in the ISAC platform, such as Der p 5, Der p 7, Der p 11, and Der p 23 (24). Der p 23 was recently added in the latest version of ISAC (ISAC 122e) (25,26).

On the other hand, it is extremely important to check Der p 1 or Der p 2 reactivity in dust mite-positive patients before to prescribe allergen-specific immunotherapy, since it has been demonstrated that only this subset of patients seems to respond more properly to SIT (27).

Similarly, in the case of shrimp allergy, only three molecules can be evaluated (Tropomyosin, Arginine Kinase, and Sarcoplasmic Ca++ Binding) out of about 14 distinct components currently identified, but not still available for diagnostic purposes (28). Therefore shrimp allergy diagnosis still represents a challenge for clinical allergologists.

In the case of seed storage protein, out of about 90 molecules registered in the IUIS/WHO database, only 13 components are available on the ISAC platform. Therefore, it is conceivable that in several cases a diagnostic approach based on the currently available molecules could not be sufficient for a comprehensive investigation (29). It is worth noting that Cor a 14 has been recently implemented in the most recent version of the ISAC (ISAC 112e), enhancing the diagnostic power of the test.

In conclusion, soon the recent European Union and Italian regulations in the matter of in vivo test could strongly impact on current diagnostic approach, increasing the usage of in vitro test in daily clinical practice. Multiplex testing is more than a promising tool for more precise and comprehensive profiling of allergic patients.

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Conflict of interests

Enrico SCALA has received consultant arrangements and speakers' bureau participation from Stallergenes and Thermo Fisher Scientific.

The rest of the authors declare that they have no conflict of interests.

Author contributions

ES designed and completed the study, and GM organized and oversaw in vivo analysis. ES wrote the manuscript which was reviewed and amended by LC, IB, and DV.

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Are currently available biomarkers useful to discriminate CSU patients not controlled by low dose omalizumab maintenance therapy?

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Key words
Chronic Urticaria; omalizumab; biomarkers; IgE; D-dimer; therapy.

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Summary
In patients with Chronic Spontaneous Urticaria (CSU), low dose omalizumab maintenance therapy is effective in about one half of complete, fast responders to the drug. Omalizumab 150 mg/month was given as maintenance therapy to 21 patients with a history of severe CSU showing a complete (UAS7=0) response to the dose of 300 mg/month. After 2 months of such regimen, patients were divided into controlled (n=14; UAS7=0) and not controlled (n=7; UAS7 > 10) and ESR, CRP, total IgE, and D-dimer were measured. The two groups did not differ in any of the biomarkers considered, nor in disease duration or in pre-treatment UAS7 score. The study confirms that it is possible to halve the dose of omalizumab without any loss of efficacy in a subgroup of patients with CSU but that none of the currently available biomarkers is able to predict which patients will lose disease control following omalizumab dose reduction.

Introduction

Omalizumab has become an essential part of the treatment of chronic spontaneous urticaria (CSU). In patients with severe disease that are unresponsive to antihistamine treatment at any dosage, omalizumab at a monthly dose of 300 mg has been recommended as a safe and effective third line treatment (1). Omalizumab induces a rapid drop in UAS7 levels in about 70% of cases (the so-called fast responders) and a slower but equally good response over 3-4 months in further 15% of patients (slow responders); in contrast the remaining 15% seem refractory to the treatment (2).

Due to the current national Regulatory Agency (AIFA) rules, in Italy it is not possible to treat CSU patients with omalizumab for > 1 year (11 administrations are licensed in total: a first course of 6 monthly 300 mg doses followed by 5 further doses after a stop of no less than 2 months in case of relapse) with no possibility to resume the treatment in case of further relapses (3). This situation has prompted to look for alternative therapeutic strategies aiming to prolong the duration of the treatment as much as possible. Recently, this allergy center belonging to the GA2LEN-UCARE network proposed to pursue omalizumab treatment at the reduced dosage of 150 mg/month as maintenance in patients who had shown a complete response to the drug (*i.e.*, UAS7=0) at 300 mg/month (4). In that study, about one-half of the patients undergoing this regimen showed an ongoing excellent response, while in the other half the dose appeared to be insufficient and symptoms started again, albeit with lesser intensity than before the start of omalizumab treatment (4). In recent years several biomarkers have been detected for chronic spontaneous urticaria: some, like D-dimer plasma levels, are associated with a severe disease (5) that is unresponsive to antihistamine (6), while others such as total IgE are predictive of the response to omalizumab (7-9). In the present

study these and other biomarkers were measured and compared in two subgroups of CSU patients responding differently to omalizumab 150 mg/month as maintenance treatment with the aim to investigate their prognostic value.

Methods

Twenty-one patients (M/F 7/14; mean age 49.4 years, median 51 years) with severe CSU (baseline UAS7 > 30) were enrolled. All of them had shown a rapid and complete (UAS7=0) response to omalizumab at the dose of 300 mg/month. After an informed written consent was obtained, the maintenance dosage of the drug during the second course of treatment was halved (*i.e.*, 150 mg/month were given) in order to prolong the therapy period. ESR, CRP, plasma D-dimer, and total IgE were measured after two months at the reduced dose regimen. Based on their clinical response, patients were classified as fully controlled (*i.e.*, persistence of UAS7=0) or insufficiently controlled (appearance of wheals with or without angioedema; *i.e.*, UAS7 > 10). Disease duration in months and thyroid autoimmunity were considered as well.

Clinical results compared by Chi-Square Test with Yates' correction. Probability values less than 5% were considered statistically significant.

Results

Table I shows the clinical findings in the study population. Fourteen patients continued to show a complete control of the disease despite the dose reduction of omalizumab, whereas 7 showed a relapse of the disease whose severity did nonetheless never reach the levels preceding the start of omalizumab treatment. The two subgroups did not show any difference in any of the analyzed parameters. A marked increase in total IgE from

Table I *- Clinical features of the study population.*

		Baseline Data							Omalizumab			
Patient	**Sex**	**Age**	**DD**	**ESR**	**CRP**	**Atopy**	**Thyroid**	**D-dimer**	**IgE**	**ESR**	**CRP**	**D-dimer**	**IgE**
1A	M	50	24	Neg	Neg	Neg	Neg	312	nd	Neg	neg	170	459
2A	M	59	60	Neg	Pos	POS	Neg	588	251	Neg	Pos	746	438
3A	F	37	88	Neg	Neg	POS	Neg	3764	nd	Neg	neg	365	256
4A	F	47	36	Neg	Neg	Neg	Neg	450	nd	Neg	neg	250	97
5A	F	22	48	Neg	Neg	POS	POS	443	372	POS	neg	230	2251
6A	F	47	18	Pos	Pos	Neg	POS	1200	nd	Neg	neg	437	62
7A	M	65	18	Neg	Pos	Neg	Neg	1815	nd	Neg	Pos	541	523
1B	M	60	200	Neg	Neg	Neg	POS	6063	256	Neg	Neg	350	565
2B	F	58	3	Neg	Neg	Neg	Neg	315	490	Neg	Neg	320	876
3B	F	28	6	Neg	Neg	POS	Neg	514	18	Neg	Neg	294	52
4B	F	29	16	Neg	Neg	Neg	Neg	263	181	Neg	Neg	251	263
5B	M	67	180	Neg	neg	Neg	Neg	622	51	Neg	Neg	380	136
6B	F	66	36	Neg	Neg	Neg	Neg	985	20	POS	Neg	392	133
7B	F	35	150	Neg	Neg	POS	Neg	1500	nd	Neg	Neg	181	422
8B	F	39	4	Neg	Neg	Neg	Neg	397	308	Neg	Neg	340	583
9B	M	69	7	Neg	Neg	Neg	Neg	402	148	Neg	Neg	200	391
10B	F	70	2	Neg	Neg	Neg	Neg	370	68	Pos	Neg	310	169
11B	F	31	48	Neg	Neg	Neg	Neg	446	76	Neg	Neg	360	237
12B	M	55	2	Neg	Neg	POS	Neg	502	392	Neg	Neg	189	951
13B	F	53	24	Neg	Pos	POS	Neg	2520	nd	POS	pos	520	322
14B	F	51	49	Neg	Neg	Neg	POS	1158	24	Neg	Neg	291	134

Legend: Patients: A not controlled by Omalizumab 150 mg/month; B: well controlled by Omalizumab 150 mg/month.
DD: disease duration (months); D-dimer levels are expressed as ng/ml; Total IgE: cut-off 100 UI/ml.
POS: positive; Neg: negative; nd: not done.

baseline levels was recorded in all patients; in contrast, D-dimer plasma levels had dropped to normal levels in 19/21 patients and to borderline levels in the remaining two. ESR and CRP were normal in virtually all cases. The prevalence of thyroid autoimmunity was similar in the two groups as was the disease duration and the severity of the disease at the start of omalizumab treatment. Full blood counts showed a reduced number of basophils (basopenia) in all cases before the beginning of omalizumab treatment (first course) but were not controlled again after the start of the treatment.

Discussion

Previous studies showed that D-dimer plasma levels are elevated in a proportion of patients with chronic spontaneous urticaria and decrease dramatically according to the clinical response to treatment (10). This study fully confirmed this finding, as in all patients showing very elevated D-dimer plasma levels before starting anti-IgE treatment D-dimer dropped within the normal range during the treatment. Theoretically it was conceivable that in some patients the loss of clinical control was associated with an increase in D-dimer levels (10) but this event did not occur, possibly because these patients were in effect omalizumab responders (albeit undertreated) and did not develop any resistance to the drug (11).

Total IgE baseline levels are frequently slightly elevated in patients with CSU, especially in those who respond promptly to omalizumab (7-9). Omalizumab administration eventually leads to an increase in total IgE levels while reducing their free fraction due to the prolongation of their half-life, and such increase may last for more than one year after stopping the treatment (12). Since fast omalizumab responders represented the whole population enrolled in the study it is not surprising that total IgE levels were frequently elevated before omalizumab treatment and increased in all cases under anti-IgE therapy. Theoretically, it could be hypothesized that patients whose disease was no longer controlled by 150 mg/month of omalizumab showed higher mean total IgE levels than persistent full responders but, again, this was not the case, possibly because total IgE that are measured in serum reflect only partially the IgE fraction bound to effector cells. Finally, that blood basophils count is inversely related with disease activity is well known (13). This was observed also here, as all patients showed basopenia when omalizumab treatment was started. Unfortunately, since circulating basophils numbers were not re-measured during the treatment with anti-IgE, whether patients not responding or responding to 150 mg of omalizumab as maintenance therapy showed differenced in basophils counts remains unclear.

Thus, the present study confirms that it is possible to halve the dose of omalizumab without any loss of efficacy in a large subgroup of CSU patients showing an excellent response to the full dose of the drug but also shows that none of the currently available biomarkers of efficacy or severity is able to predict which patients will lose the control of the disease following omalizumab dose reduction.

Conflict of interests

The author declares that he has no conflict of interests.

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Primary Immunodeficiency Disorders in children with Non-Cystic Fibrosis Bronchiectasis

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Key words

Non-cystic fibrosis bronchiectasis; bronchiectasis; respiratory infections; common variable immunodeficiency; combined immunodeficiency; RASGRP1.

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Summary

Introduction. *Primary immunodeficiency diseases (PID) are common in patients with non-cystic fibrosis bronchiectasis (NCFB). Our objective was to determine ratio/types of PID in NCFB.*

Methods. *Seventy NCFB patients followed up in a two-year period were enrolled.*

Results. *Median age was 14 years (min-max: 6-30). Male/female ratio was 39/31; parental consanguinity, 38.6%. Most patients with NCFB (84.28%) had their first pulmonary infection within the first year of their lives. Patients had their first pulmonary infection at a median age of 6 months (min-max: 0.5-84), were diagnosed with bronchiectasis at about 9 years (114 months, min-max: 2-276). PID, primary ciliary dyskinesia (PCD), bronchiolitis obliterans, rheumatic/autoimmune diseases, severe congenital heart disease and tuberculosis were evaluated as the most common causes of NCFB. About 40% of patients (n=16) had bronchial hyperreactivity (BH) and asthma. Twenty-nine patients (41.4%) had a PID, and nearly all (n=28) had primary antibody deficiency, including patients with combined T and B cell deficiency. PID and non-PID groups did not differ according to gender, parental consanguinity, age at first pneumonia, age of onset of chronic pulmonary symptoms, bronchiectasis, presence of gastroesophageal reflux disease (GERD), BH and asthma ($p > 0.05$). Admission to immunology clinic was about 3 years later in PID compared with non-PID group($p < 0.001$). Five patients got molecular diagnosis, X-linked agammaglobulinemia (n=2), LRBA deficiency (n=1), RASGRP1 deficiency (n=1), MHC Class II deficiency (n=1). They were given monthly IVIG and HSCT was performed for three patients.*

Conclusions. *PID accounted for about 40% of NCFB. Early diagnosis/appropriate treatment have impact on clinical course of a PID patient. Thus, follow-up in also immunology clinics should be a routine for patients who experience pneumonia in the first year of their lives and those with NCFB.*

Introduction

Bronchiectasis is a chronic pulmonary disease of the conducting airways. It produces persistent productive cough, recurrent respiratory infectious exacerbations, and irreversible bronchial dilatation in children and adults. Two different types of bronchiectasis are defined according to the pattern of the lesion, diffuse and focal (1). Focal bronchiectasis is usually associated with bronchial obstruction, such as aspiration of foreign body, that leads to infection (2). Diffuse bronchiectasis is more often found in association with underlying disorders such as cystic fibrosis (CF), primary immunodeficiencies (PID), primary ciliary dyskinesia (PCD), and recurrent aspiration syndromes (3). Bronchiectasis is often a consequence and a complication of recurrent, uncontrolled respiratory infections and inflammation. In many studies, acute, severe or recurrent pneumonia is the

most common cause (4,5). Subsequent acute or chronic damage in the conducting airways results in a significant physical and social morbidity (6). The diagnosis depends on radiological imaging of the typical changes in addition to clinical findings. Chest X-ray is sometimes insufficient to make the clinicians reach the diagnosis. Thus, a high-resolution computed tomography (HRCT) scan is the gold standard diagnostic procedure.
Nowadays, with early immunization and the widespread use of antibiotics in childhood, acute post-infectious damage is likely to be less relevant (7). However, especially in the countries which the consanguineous marriages are relatively frequent, chronic damage due to hereditary diseases of respiratory system, such as cystic fibrosis, PID and PCD is common cause of bronchiectasis (8).
PIDs are among the frequent causes of non-cystic fibrosis bronchiectasis (NCFB) (9). Bronchiectasis is seen as a common long-term complication especially in patients with primary antibody deficiency (PAD) (10,11). With a detailed history, physical examination and laboratory analysis, it is not difficult to detect the underlying immunological etiology. Our objective was to identify the ratio of underlying PID in patients with NCFB, and also evaluate the characteristic clinical, microbiological or radiological features in patients with and without PID.

Methods

In a two year period, 87 patients who were diagnosed with NCFB in pediatric chest disease department and referred to pediatric immunology department were retrospectively evaluated. Seventy patients come to the control visits in each department routinely. However, 17 out of 87 were lost follow-up.
Patients' clinical parameters such as age, gender, parental consanguinity, age at diagnosis, age at onset of infections were recorded from the files. The diagnosis of bronchiectasis was confirmed with a HRCT scan in each of the patients. Cystic fibrosis was excluded in the patient cohort via sweat chloride test and in some of them by mutation analysis in chest disease department (12). After exclusion of CF, patients underwent investigations for the common etiologies of bronchiectasis which included nasal nitric oxide (NO) test (n=64), gastroesophageal reflux scintigraphy (n=64), pulmonary function tests with spirometry (n=58) and flexible bronchoscopic evaluation (n=65).
Diagnosis of PCD was based on presentation of the characteristic clinical phenotype, nasal NO results, the presence of ciliary ultrastructural defects (visualized by electron microscopy), and the presence of abnormal ciliary function (as determined by video microscopy).
Bronchiolitis obliterans (BO) was defined as the presence of mosaic pattern in chest X-ray in addition to the history of respiratory symptoms which developed after a severe pulmonary infection, and the findings of obstructive airway disease which does not respond to bronchodilator therapy in respiratory function test.
Evaluation for possible immunodeficiencies included complete and differential blood counts, serum immunoglobulin levels (n=70), and lymphocyte subgroups (n=37), serum complement hemolytic activity (CH50) (n=39), nitroblue tetrazolium test (NBT) (n=50) and pneumococcal antibody response (n=26). European Society of Immunodeficiency and Pan-American Group for Immunodeficiency (ESID and PAGID) criteria was used for the diagnosis of PID (13). Selective IgA and selective IgM deficiency are diagnosed according to the ESID criteria (14). Secondary hypogammaglobulinemia is excluded by history, absence of renal, gastrointestinal and cutaneous protein loss, and other drug or disease related causes (15). Urinary analysis for proteinuria was done, total protein and albumin values were measured in all the patients with hypogammaglobulinemia. After exclusion of the secondary causes, some of the patients may be classified as idiopathic primary hypogammaglobulinemia (IPH), or undefined/unclassified hypogammaglobulinemia (16,17).
Sputum (n=64) and bronchoalveolar lavage (BAL) (n=65) results of the patients were also recorded to determine the microbiological etiology. The final diagnosis of the patients with NCFB were recorded after the follow-up period. All patients with NCFB were grouped according to having PID or not according to their final diagnosis (PID and non-PID).
The study is approved by the Institutional Review Board.

Statistical analysis

SPSS 18.0 was used for the statistical analysis. One-way ANOVA analysis was used for analysis of more than two groups. Pearson's correlation coefficient was used to evaluate correlation of two variables.

Results

Characteristics of Patients with Non-Cystic Fibrosis Bronchiectasis

The mean age was 14.23 ± 4.72 years (median: 14 (6-30)). Out of 70 patients, 39 (55.7%) were male, 31 (44.3%) were female. Parental consanguinity ratio was 38.6%. The patients had their first pneumonia at a median age of 6 months (0.5-84). Most patients (84.28%) had their first pulmonary infection within the first year of their lives. The median age of onset of chronic pulmonary symptoms (chronic cough, growling *etc*.) was about two years (24 months (0.5-276)). The median age at diagnosis of bronchiectasis was about 9 years (114 months (2-276)).
Out of 70 NCFB patients, 46 (60%) experiences other infections, such as tonsillopharyngitis, sinusitis, otitis media. Bronchial hyperreactivity was shown in 26 (37.14%) out of 58 patients by pulmonary function test. Gastroesophageal re-

flux disease (GERD) was shown in 12 (18.46%) out of 65 patients by scintigraphy. Lipid laden macrophages were detected in BAL in 18 (30.5%) out of 65 patients who were evaluated by bronchoscopy. The congenital heart disease found in one of the NCFB patients was ventricular septal defect and high venosum ASD. The patient had also pulmonary hypertension. The NCFB patients are grouped as PID (n=29, 41.4%), and non-PID (n=41, 58.6%).

Totally 18.6% (n=13) of NCFB patients, 31.7% of non-PID had the diagnosis of PCD, and among them two patients (15.4%) had Kartagener's Syndrome. One (complement deficiency) out of all PID group were associating with hypogammaglobulinemia (common variable immunodeficiency, combined immunodeficiency, agammaglobulinemia, *etc*. (**table I**)). Bronchial hyperreactivity and asthma (n=16, 39%), PCD (n=13, 31.7%), GERD (n=9, 21.9%), and BO (n=3, 7.3%) associate with non-PID (**table I**). Other associated diseases are rheumatic/autoimmune diseases (n=2, 4.9%), tuberculosis (n=2, 4.9%), and severe congenital heart disease (n=1, 2.4%) (**table I**).

The two groups did not differ according to gender, the age at first pneumonia episode, age of onset of chronic pulmonary symptoms, parental consanguinity, presence of BH and asthma, GERD, and frequency of infections (**table II**). Also, the age of diagnosis of bronchiectasis did not differ between groups

Table I *- Classification of PID and non-PID causes of NCFB.*

PID Group (n= 29 (41.4%))		**Non-PID Group (n= 41 (58.6%))**	
CVID	9 (30.9%)	Unidentified	13 (31.7%)
Combined immunodeficiency	6 (20.6%)	Asthma and bronchial hyperreactivity	16 (39%)
Selective IgA deficiency	4 (13.8%)	Primary ciliary dyskinesis	13 (31.7%)
IPH	3 (10.3%)	Gastroesophageal reflux	9 (21.9%)
Selective IgM deficiency	3 (10.3%)	Brochiolitis obliterans	3 (7.3%)
XLA	2 (6.9%)	Rheumatic/Autoimmune disease	2 (4.9%)
Hyperimmunoglobulin M syndrome	1 (3.4%)	Tuberculosis	2 (4.9%)
Complement deficiency	1 (3.4%)	Congenital heart disease	1 (2.4%)

CVID, common variable immunodeficiency; XLA, X linked agammaglobulinemia; Ig, immunoglobulin; idiopathic primary hypogammaglobulinemia, IPH.

Table II *- Characteristics of patients with PID and non-PID.*

	Non-Cystic Fibrosis Bronchiectasis		
Characteristics	**PID (n=29)**	**Non-PID (n=41)**	**p**
Gender (M/F)	19/10	20/21	0.16
Parental consanguinity	44.8%	34.1%	0.17
Age at first pneumonia*	9.1±13.9 / 6 (0-72)	9.4±13.7 / 6 (0-84)	0.83
Age at onset of chronic pulmonary symptoms *	43.6±53.7 / 24 (0-276)	31.8±35.9 / 18 (0-120)	0.23
Age at diagnosis of bronchiectasis*	139±64.3 / 132 (12-276)	124.4±62.9 / 108 (2-224)	0.22
Age at referral to Immunology department (year)	16.8±5.1 / 16 (10-30)	12.4±3.4 / 13 (6-20)	**<0.001**
Gastroesophageal reflux	4 (14.8%)	9 (24.3%)	0.69
Bronchial hyperreactivity and asthma	10 (38.5%)	16 (50%)	0.93
Frequent infections	16 (55.2%)	24 (58.5%)	0.61
Isolation of microorganism in sputum	13 (44.8%)	21 (51.2%)	0.59
Isolation of microorganism in BAL	9 (34.6%)	22 (66.7%)	**<0.014**
Lobectomy	2 (6.8%)	3 (7.3%)	0.53

* months.

Median (min.-max.) and mean (±standard deviation) ages are given in the table.

(108 months (2-224) in non-PID, and 132 (12-276) in PID) (p=0.223). The admission to immunology clinic in PID was 13 years (6-20), however it is 16 years (10-30) in non-PID (p < 0.001) (**table II**). Totally five patients underwent left-sided lobectomy, two was in PID, other two was in the group of unidentified causes of non-PID, one was PCD. The two PID patients who underwent lobectomy were diagnosed with Combined Immunodeficiency (CID) and common variable immunodeficiency (CVID). The patient with CVID developed amyloidosis and died of a severe pneumonia and respiratory failure (18). Genetic tests were not performed routinely to the NCFB patients. However in the follow-up, five patients got molecular diagnosis; X-linked agammaglobulinemia (BTK defect) (n=2), LRBA deficiency (n=1), RASGRP1 deficiency (n=1) (19), MHC Class II deficiency (n=1). They were given monthly IVIG, and HSCT was performed in three patients (with RASGRP1 deficiency, MHC Class II deficiency, and LRBA deficiency). All tranplanted patients are alive and well.

Microbiology

The sputum microbiology was positive in 55% (33) out of 60 patients (*Hemophilus influenza* (*H. influenza*) in 25 (75,75%), *Streptococcus pneumonia* (*S. pneumonia*) in 14 (42.4%), *Candida albicans* (*C. albicans*) in two (6%), group A *beta hemolytic streptococcus* (GAS*)* in two (6%), *Pseudomonas aeruginosa* (*P. aeruginosa*) in two (6%) patients), multiple agents (*H. influenza*, *S. pneumonia, Moraxella catarrhalis* (*M. catarrhalis*)*, Hemophilus parahemolyticus* (*H. parahemolyticus*)*,* GAS*, C. albicans, P. aeruginosa*) in 11 (33.3%) patients.
BAL microbiology was positive in 56.9% (n=37) (*H. influenza* in 59.46% (n=22), *S. pneumonia* in 21.62% (n=8), *H. hemolyticus*, *Hemophilus agnus* (*H. agnus*)*, H. hemolyticus, Hemophilus aphrophilus* (*H. aphrophilus*)*, Hemophilus segnis* (*H. segnis*)*, H. parainfluenza, M. catarrhalis, P. aeruginosa, Stenotrophomonas maltophilia* (*S. maltophilia*) were each isolated in 1 (2.7%) patients), multiple agents (*H. influenza*, *S. pneumonia*, *S. maltophilia*) in 4 (13.5%) of the patients.

The non-PID and PID group did not differ according to the ratio of sputum culture positivity (p=0.59). Increased ratio of positive BAL culture was recorded in younger patients than older ones (p=0.019). Nine patients (34.6%) in PID, 22 patients (66.7%) in non-PID had positive BAL culture, the difference was statistically significant (p=0.014). The results of microbiological analyses in groups are given in **table III**.

Radiology

According to HRCT results, most affected areas were recorded to be right middle and left lower lobe. Diffuse involvement was seen in 41.43% (n=29) of the patients, diffuse right lung involvement in 15.71% (n=11), diffuse left lung involvement in 7.14% (n=5), isolated left lower lobe involvement in 17.14% (n=12) of the patients, isolated right lower lobe involvement in 2.8% (n=2), left or right upper lobe involvement in 5.7% (n=4) patients. The involvement in PID and non-PID groups is given in **table III**. There was no statistical difference (p > 0.05).

Discussion

Bronchiectasis is still one of the most common causes of childhood morbidity and mortality (20-22). Main causes are infections, immunodeficiencies, congenital and genetic disorders, aspirations (23). Pulmonary infections account for 17-20.6% of bronchiectasis cases (24,25).
Underlying etiology is not identified in 14.2-37% of children (24,25), and in 35-50% of adults with bronchiectasis (26,27). Undiagnosed PID may be partly responsible for the development of unidentified bronchiectasis. The overall prevalence of brochiectasis in CVID is found as 34% (28) and 62.3% (29) in different series. The diagnosis of PID is generally made at the irreversible state when the disease progressed into the end-stage respiratory disease/failure (30). Physicians usually believe that the PID presents in childhood, and neglect PID especially in adulthood. The median age on admission to immunology clinic was about 3 years later (p < 0.001) in PID group. One of the

Table III *- Comparison of the microbiological agents and radiological involvement in PID and Non-PID Groups.*

		PID	Non-PID
Microbiological agents	**Sputum**	*H. influenza, S. pneumonia, P. aeruginosa, K. pneumonia, C. albicans*	*H. influenza, S. pneumonia, P. aeruginosa, M. catarralis, C.albicans*
	BAL	*H. influenza, S. pneumonia*	*H. influenza, S. pneumonia, P. aeruginosa, M. catarralis, H. parainfluenza, H. parahemolyticus*
Radiological involvement (HRCT)		Diffuse, left lower, bilateral lower lobes, right middle and right lower lobe, left total	Diffuse, left lower, right lower, bilateral lower lobes, right upper, and right middle lobes

several reasons of this delay may be due to the later admission of PID patients to the primary physician due to the insidious symptoms and other systemic problems. PID could vary greatly in clinical course, and the presentation of patients are not only with infectious diseases, but with allergy, autoimmunity, inflammation, lymphoproliferation/malignancies. With the increase in awareness and the definition of new PID disorders, PID are becoming one of the most common causes of NCFB. Complete blood count and determination of serum immunoglobulin levels were suggested as the baseline immunological tests in guidelines (31). Immunodeficiencies account for 10-34% of the childhood bronchiectasis (32,33), and among them, antibody deficiencies were common disorders leading to NCFB (34,35). In our study, PID accounts for about 40% of NCFB, and about 90% was primary antibody deficiency (**table I**). CVID (27.6%) was the most common PID. This high ratio of PID may be due to the routine follow-up of patients in an immunology clinic. Detailed evaluation with not only the suggested baseline tests, but other tests during the follow-up period, such as lymphocyte subset analysis, CH50 and NBT tests were performed to some of the patients. In the study of Reisi *et al.*, PIDs associated with bronchiectasis were CVID, XLA, HIGM and Hyperimmunoglobulin E syndrome (29). The ratio of bronchiectasis was found to be 62.3% in CVID, and 43% in XLA patients.

In our study, about 40% of NCFB patients had BH and asthma, and about 1/5 had GERD. Although GERD, asthma and BH, were common in non-PID group, each of them was evaluated as associations, rather than a cause. Bronchiectasis could result in BH, as it leads to airway obstruction, increased bronchial secretions and consequently to increased incidence of pulmonary infections. On the other hand, BH could exacerbate the symptoms of bronchiectasis (36). BH and asthma may associate with PID (29,37). In our series this ratio is about 40%, nearly the same as the ratio in all NCFB patients. Asthma and BH may also associate with PCD, BO and GERD. The presence of BH and asthma and the presence of recurrent infections did not differ in PID and non-PID groups in the present study. These data show that bronchiectasis should be evaluated as a multifactorial disease. Infections, BH/Asthma, PID and PCD may be present in the same patient, and it is not easy to determine accurately the most important reason of bronchiectasis. So, the treatment should be individualized.

Non-typeable *H. influenza* and *S. pneumonia* are the main bacterial pathogens in children with bronchiectasis and predominates in all age groups (38,39). BAL culture results in NCFB shows that most common pathogens are *H. influenza* (47%), *S. pneumonia* and *M. catarrhalis* (40). Data about microbiological agents in NCFB patients with PID is scarce. In a retrospective study, PID ratio was 8.6%, and in half, *H. influenzae, S. aureus, S. pneumoniae and C. parapsilosis* were isolated (41). In our study, most frequently isolated microorganisms were *H. influenza* and *S. pneumonia* both in sputum and BAL. The ratio of microorganism isolation in BAL culture was significantly increased in young and non-PID patients ($p < 0.05$), possibly due to frequent use of antibacterial agents in chronic cases.

Although bronchiectasis tend to appear in upper lobes in CF, it generally locate to the basal segments of lower lobes in children with NCFB (42). Patients with hypogammaglobulinemia was demonstrated to have lower/middle lobe and lingular segment bronchiectasis (43). In our study, most affected areas were right middle and left lower lobe in all NCFB patients. Lower, especially left lower lobe and diffuse involvement were seen mostly in PID group.

In our study, underlying etiology is not identified in about 1/3 of NCFB patients. PID, PCD, BO, rheumatic and autoimmune diseases and tuberculosis were evaluated as causes. Asthma and BH were evaluated as associations.

An important finding was that most patients (84.28%) had their first pulmonary infection within the first year of their lives. As far as we know, this study is the first study which compares the PID and non-PID NCFB. Patients with PID would highly benefit from an early diagnosis and appropriate treatment (44,45). Earlier immunoglobulin replacement therapy and antibacterial prophylaxis will decrease the infectious episodes, preventing the progress of bronchiectasis in patients with primary antibody deficiency. Thus, follow-up in also immunology clinics should be a routine for NCFB patients, and also for patients who experience pneumonia in the first year of their lives.

Conflict of interests

The authors declare that they have no conflict of interests.

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Does maternal diet during pregnancy influence clinical and laboratory characteristics of infantile-onset atopic dermatitis?

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Key words

Atopic dermatitis; diet; pregnancy; primary prevention; peripheral eosinophila; serum total IgE.

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Summary

Background. *Prenatal environmental factors are suggested to be implicated in the dramatic increase in atopic dermatitis (AD) in recent years. The aim of this study was to investigate the possible associations between pregnant woman's diet and clinical and laboratory variables of AD in offsprings.*

Methods. *A cross-sectional study was performed in children 3-36 months of age with infantile-onset AD. Maternal dietary habits during pregnancy were evaluated in terms of the usual intake of dairy foods, eggs, red meat and poultry, fish, fruits and vegetables.*

Results. *One hundred pairs of mothers and their children with AD were included. A higher serum total IgE and peripheral eosinophila in children were associated with a lower maternal egg intake during pregnancy. Except for a strong trend toward significance of correlation between fish consumption and the lack of atopic multimorbidity, no relationships were revealed between clinical variables of child's AD (the age of onset of AD, its severity, atopic multimorbidity) and the mother's dietary habit.*

Conclusions. *Our preliminary findings suggest that maternal egg intake during pregnancy might be a factor influencing laboratory markers of atopy in offsprings. Prospective cohort studies are needed to confirm and clarify this relationship.*

Introduction

Atopic dermatitis (AD) is an important public health problem all over the world. This chronic, relapsing, intensely pruritic, inflammatory condition of the skin, in 60% of cases starts during the first 12 months of life and affects 20% and 35% infants and small children (1). Most children with infantile-onset AD develop other atopic diseases such as food allergies, asthma or allergic rhinitis later in life (2). Apart from genetics, environmental exposures, such as a pregnant woman's diet, are often considered to be possible factors in the development of AD and the so-called "atopic march" in children (2). Prescription of an antigen avoidance diet (including the exclusion of cow's milk, eggs, and peanuts) to a high-risk women during pregnancy, however, did not reduce atopic morbidity (3). A Western diet seems to have a causative effect, while the Mediterranean diet characterized by high intake of fruits, vegetables and fish is usually recommended as a healthy dietary pattern (4). Nonetheless, the link between prenatal dietary factors and AD is not well established.

The aim of this study was to examine the clinical and laboratory characteristics of the infantile-onset AD in relation to the maternal dietary habit during pregnancy.

Materials and methods

A cross-sectional study was carried out in mothers and their children with AD recruited consecutively at the Paediatric Dermatology Outpatient Clinic in Bielsko-Biała, Poland. Ethical approval from the Research and Ethics Committee of the Regional Medical Chamber in Bielsko-Biała and individual informed consent from each mother were obtained before the study was conducted. Participants had to meet the following inclusion criteria: children aged between 3 months and 36 months, AD diagnosed clinically according to the Hanifin and Rajka classi-

fication (5), onset of AD symptoms within the first year of life, effective communication with mother. Other concomitant skin disorders in children, such as seborrheic dermatitis, psoriasis or ichthyosis were considered as exclusion criteria. Clinical data were obtained by face-to-face interviews and from the medical records. The severity of AD was evaluated according to the Rajka and Langeland grading (6), with the parameters of extent, course, and intensity. Depending on the score, the following disease subgroups were distinguished: mild (3.0-4.0), moderate (5.0-7.0) and severe AD (8.0-9.0). Coexistent allergic diseases like food allergy, asthma or allergic rhinitis were diagnosed by a physician. All children underwent blood investigations to determine their eosinophil count and serum total IgE (tIgE) levels. The number of eosinophils in peripheral blood samples was determined with the XS-1000i by automated blood haematology analyser 5 diff (Sysmex, Kobe, Japan). Serum tIgE level was measured using ELFA (Enzyme Linked Fluorescent Assay) technology (Vidas, bioMerieux SA, Lyon, France). All samples were tested in duplicate.

Data on maternal demographics (age at delivery, place of residence, educational level) and dietary habit during pregnancy were collected using a self-administrated standardized questionnaire, combining questions and statements already reported in the literature (7,8). The following categories of foods were examined: dairy foods (milk, cheese and yogurt), eggs, red meat and poultry, fish, fruits and vegetables. The questionare concerned their usual intake with frequencies defined as: "never or less than once a month", "1-3 times a month", "once a week", "2-4 times a week", "5-6 times a week", "once a day", "2-3 times a day", "4-5 times a day" or "6 or more times a day".

Statistical analysis of obtained data was performed with Excel and Statistica v.12 (StatSoft Inc., Tulsa, Oklahoma, USA). To create continuous variables and calculate descriptive statistics, categories of intake frequency for the different food items were converted to "times a day", "times a week" or "times a month" depending on the food item. Data were not found to be normally distributed, Kruskall-Wallis's test, U Mann-Whitney's tests and Kendall's rank correlation were therefore used when necessary to assess the relation of each food item on the AD clinical and laboratory variables. In all cases, $p < 0.05$ was defined as statistically significant.

Results

A total of 100 mother-child dyads participated in this study. The majority of them gave birth to a child at age between 20 and 29 years (n=65), lived in urban areas with fewer than 100.000 people (n=72) and the greater number of mothers had tertiary education (n=76). Forty-seven of children were under 12 months old and 52 were males. AD began before the age of 3 months in 25 of the children, between the ages of 3 to 6 months in 38, between the ages of 6 to 9 months in 17, and between the ages of 9 to 12 months in 20 of the children. The median total objective AD severity score of the study subjects was 5.0 (IQR: 4.0-6.0). Forty-six of children had mild, 41 moderate and 13 severe AD. Atopic multimorbidity — coexistent allergic diseases like food allergy, asthma and/or allergic rhinitis (alone or associated) — was present in 33 of the cases. Seventy-seven of children had a positive family history of atopy. Fifty-nine of children had raised peripheral eosinophil counts (> 5%), in 47 they ranged from 6 to 10.0%, in 9 from 11 to 20%, and in 3 they were greater than 20%. In 83 of children tIgE levels were higher than 60 UI/mL, in 75 they ranged from 61 to 100 UI/mL, in 4 from 101 to 1000 UI/mL, in 6 from 1001 to 2000UI/mL, and in 2 they were greater than 2000 UI/mL. **Table I** presents the associations between clinical and laboratory characteristics of AD and maternal dietary habit during pregnancy. An inverse relationship between child's serum tIgE ($t=-0.24$; $p < 0.05$), eosinophilia ($t=-0.19$; $p < 0.05$) and the maternal egg intake during pregnancy was the only statistically significant association. A higher maternal consumption of fish during pregnancy had a non-significant tendency towards a negative relationship with the appearance of atopic multimorbidity ($Z=1.74$; 0.081).

Discussion

Our study found no association between maternal dietary habit during pregnancy and the clinical variables of children with AD, but the value of serum tIgE levels and blood eosinophil counts were lower in children if their mothers consumed more eggs during pregnancy. The correlation between severity of AD and the serum tIgE as well as eosinophilia is well known (9). Infantile-onset AD seems to be associated with a higher total serum IgE level and high eosinophilia, suggesting that the disease possibly leads to more allergic sensitization and resulting in more severe eczema (10,11). Baïz *et al.* (4) showed that preconceptional and gestational exposure of potential allergens, including egg allergens, may be beneficial for allergic rhinitis prevention in offsprings. In addition, they found that high consumption of eggs before pregnancy was inversely associated with the risk of wheezing, which may be an allergic disease symptom (4). There is also some evidence that a higher maternal intake of fruits, vegetables, fish, peanut, milk and wheat, during pregnancy may be associated with a lower risk of atopic diseases in children (4,12). Conversely, a significant positive association was found between meat intake during the preconceptional period and a risk of AD, wheezing and allergic rhinitis (4).

In the current research, except for a strong trend toward significance of correlation between fish consumption and the lack of atopic multimorbidity, none of the other food categories considered for analyses were linked with the clinical characteristics of AD. The study has several important limitations. First, the

Table I *- Associations of clinical and laboratory characteristics of infantile-onset atopic dermatitis with the maternal dietary habit during pregnancy.*

	H	Age of atopic dermatitis onset		Rajka & Langeland Score		Atopic multimorbidity		Eosinophil count	Serum total IgE level
		P*	H	P**	Z	P**	Kendall's τ statistics		
Frequency of food intake	Dairy foods	2,634	0,621	3,385	0,184	0,63	0,528	-0,01	-0,12
	Eggs	6,930	0,140	1,766	0,414	1,19	0,233	**-0,19**	**-0,24**
	Red meat or poultry	1,309	0,860	1,259	0,533	0,35	0,728	0,05	0,00
	Fish	5,453	0,244	3,824	0,148	1,74	0,081	0,02	-0,06
	Fruits and vegetables	2,893	0,576	1,884	0,390	0,79	0,430	-0,10	-0,10

*p values obtained by Kruskal-Wallis test; **p values obtained by Mann-Whitney U test; The values given in bold are p < 0,05.

use of self-administrated questionnaire to assess food intake can lead to errors as it may be subject to memory bias. The time since the delivery varied between participants, therefore some women had difficulty assessing their intake and more specific assessment of consumption data was not feasible. However, the aim of this research was to explore the overall dietary habit (frequency of intake of groups of foods) rather than details of dietary intake (portion sizes were not defined), which may have less of an impact on the availability of the information. To simplify the analysis, the questionnaire covered only important foods: dairy foods, eggs, red meat and poultry, fish, fruits and vegetables, and the results were related to these individual foods. Low correlation coefficients were found, so a clinically relevant connection could not be demonstrated. Moreover, this was a retrospective cross-sectional study and a control group was missing, thus causality could not be confirmed. Alternative mechanisms could be at play here.

Knowledge of factors determining disease severity in small children with AD is of great importance for preventive measures and optimizing care. Current findings indicate a balanced and diverse diet during pregnancy without restrictions (3). However, further studies in cohorts of pregnant women or infants are needed to match the proper strategies in this issue (3).

Conclusions

Our results suggest that maternal egg intake during pregnancy might be a factor influencing serum tIgE level and eosinophila in infantile-onset AD. Additional research is warranted for a better understanding of this relationship.

Conflict of interests

The authors declare that they have no conflict of interests.

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The show must go on. The impacts of SARS-CoV-2 pandemic on cutaneous allergology and patch testing

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Dear Editor,

While public health campaigns against SARS-CoV-2 pandemic are underway to promote frequent hand washing as one of the ancillary strategies to reduce transmission of the newly identified virus, we are seeing a rise in the frequency of hand dermatitis, many of which are predominantly irritative in nature. While irritative contact dermatitis due to the use of Personal Protection Equipment (PPE) and frequent hand sanitizing has been under the mediatic spotlight we must not forget the significant impact social isolation measures may have on our allergic contact dermatitis patients, who may find themselves deprived of the diagnostic work-up or clinical follow-up necessary for their improvement, during this crisis.

It is well established that allergic contact dermatitis carries a significant impact on quality of life (1). It is also known that the diagnosis of this condition is often delayed and that patch tests are fundamental for allergen identification and avoidance, in many cases (2). This may be particularly important in occupational contact dermatitis in healthcare professionals, where early identification of the culprit allergen may reduce the risk of sick leave, bacterial infection due to skin barrier dysfunction and inadequate use of PPE.

The ongoing SARS-CoV-2 outbreak has impacted clinical practice worldwide. Due to contingency measures, most outpatient appointments and diagnostic procedures are being postponed to reduce patient exposure to high risk environments. The current contingency plan has made patch testing difficult to carry in hospital setting, as two to three visits to a hospital in a week's time carries a significant risk for viral exposure. However, denying patch testing to our patients also takes a toll on their health and quality of life.

Not only will these patients lack the differentiated opinion of an expert in cutaneous allergology, which could provide them

with the answers and recommendations they need to control their dermatosis, they will also tend to aggravate during this period where frequent washing and disinfection with alcohol based antiseptic solutions may constitute a further aggression to their already sensitized skin. This will inevitably contribute to the perpetuation of the skin barrier disruption-allergen penetration-inflammatory response cycle that characterizes allergic contact dermatitis. This worsening may be disproportionately higher in certain occupations where frequent hand washing is mandatory, as is the case of healthcare professionals.

Furthermore, patch testing is important in the study of patients with severe forms of cutaneous drug eruptions, such as Drug Rash with Eosinophilia and Systemic Symptoms (DRESS), Acute Generalized Exanthematous Pustulosis and even in Stevens-Johnson and Lyell syndromes. Patch testing is also crucial when the suspected culprit drug may be fundamental for current treatment of comorbid diseases (*e.g.* Anticonvulsants) or when future implications for drug choices for common conditions are significant *(e.g.* Antibiotics). While the overall sensitivity of patch testing may not be high, it remains one of the most informative tests to date for culprit drug identification, as lymphocytic transformation tests aren't easily accessible and show equally limited sensitivity in most instances, and oral challenge proves carry significant risks when the original dermatosis was severe (3,4). The current constrains may compromise culprit drug identification, which may pose significant hindrances in those instances where patients are taking several different drugs on a daily basis. Paradoxically, these patients with several comorbidities where patch testing may impact clinical management the most tend to be the most vulnerable to severe COVID-19, which would recommend against hospital visits during a pandemic crisis.

Despite all difficulties, it is our duty to provide support to our patients during these challenging times. In the largest tertiary teaching hospital in Portugal, one of the most severely afflicted countries by this outbreak, we have adopted some strategies to help our patients.

All patients referred to our Cutaneous Allergology appointments are being assessed through teleconsultation. At present our main goals are the identification of those patients which need immediate presential assessment and providing all patients with both oral and written recommendations on how to reduce skin aggressions while keeping sanitary measures.

Those cases where patch testing is paramount (about 5% of all scheduled patients for testing in our center), as is the case of severe drug eruptions, are still being assessed and tested. We try to conduct these appointments in physical spaces separate from those dedicated to COVID-19 patients. Ready-to-use test panels or preparing Finn chambers in advance to patient visit may reduce the duration of initial patch test appointment and contribute towards lower potential viral exposure for patients. We are also reducing the number of hospital visits for patients undergoing patch testing, as patients are being advised to remove the panels at D2 and take appropriate pictures at this timepoint, which are then assessed by the dermatologist at the D4 reading. With these simple measures we can make sure that essential care is being provided to those who need it the most while improving the quality of life of patients who can be assessed when normal clinical activity resumes. Patch testing can be postponed safely in most instances, but it must not be cancelled altogether during this pandemic crisis, as it may provide information with profound implications on the clinical management of some patients.

Conflict of interests

The authors declare that they have no conflict of interests.

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Angiotensin Converting Enzyme-2 (ACE2) Receptors, asthma and severe COVID-19 infection risk

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Key words
Asthma; severe asthma; infection; COVID-19; ACE2; TMPRSS2; SARS-CoV-2.

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Abbreviations
ACE2: Angiotensin converting enzyme-2
BAL: Bronchoalveolar lavage
CDC: Centers for Disease Control and Prevention
COVID-19: Coronavirus disease
COPD: Chronic obstructive pulmonary disease
HBECs: Bronchial epithelial cells
IL: Interleukin
SARS-CoV-2: SARS-coronavirus 2
TMPRSS2: transmembrane protease serine 2

Highlights

- SARS-CoV-2 uses the ACE2 receptors for host cell entry.
- ACE2 receptor gene expression in airways seems to be similar in asthma and health.
- Asthma does not seem to be a risk factor for severe COVID-19.
- T2 high inflammation, inhaler steroid use may have an impact on ACE receptor gene downregulation.
- ACE2 receptor gene expression may vary in central and peripheral airways.
- ACE2 receptor gene expression may differ in various asthma endotypes and some subgroups such as smokers may have more risk for COVID-19.

To Editor,

Coronavirus disease 2019 (COVID-19), was first detected in Wuhan, China, and it has since spread to most countries around the world. A recent metanalysis including studies from China have shown that preexisting chronic disease including hypertension, diabetes, cardiovascular and respiratory system diseases is one of the main risk factors for mortality in adult COVID-19 inpatients (1). When severe and non-severe COVID-19 patients are compared, higher risk of respiratory system disease with the odds ratio of 2.46 has been detected (1).

Asthma is one of the most common chronic respiratory diseases in the world and it is possible that asthma-related factors may influence susceptibility to COVID-19 or infection severity. So, there is a great concern about the effect of asthma on COVID-19 morbidity: Centers for Disease Control and Prevention (CDC) stated that people with moderate to severe asthma may have an increased risk for COVID-19 and infection may lead to an asthma attack, pneumonia, or acute respiratory disease. On the other hand, ARIA-EAACI statement on Asthma and COVID-19, reported that asthma does not seem to be a risk factor for severe COVID-19 (2). Because surprisingly a low rate of asthma has been reported in patients having COVID-19 (3,4). In Zhang *et al*. study including 140 community-infected COVID-19 patients, asthma or other allergic diseases were not reported by any of the patients (3). Although the prevalence of asthma in China was 4.2% and allergic rhinitis in Wuhan was 9.7%, allergy or asthma was not detected as a risk factor for COVID-19 infection (3). In another article from Wuhan, Li *et al*. reported the prevalence of asthma as 0.9% in 548 patients with COVID-19 and the asthma rate did not differ between severe and non-severe COVID-19 cases (4). Available data is limited, there is no concrete evidence that asthma is a risk factor for COVID-19 and it needs further investigation whether asthma itself or the treatments used in asthma modification have a protective or causal effect on COVID-19 severity.

The link between ACE receptors and COVID-19

Angiotensin Converting Enzyme-2 (ACE2) receptors mediate the entry of SARS-coronavirus 2 (SARS-CoV-2) into target cells via its structural spike glycoprotein (S), and the spike protein of SARS-CoV-2 is primed by transmembrane protease serine 2 (TMPRSS2) which allows fusion of viral and cellular membranes into host cells (5). ACE2 receptors are expressed in the heart, vessels, gut, lung, kidney, testis, and brain (6). Binding of SARS-CoV-2 to ACE2 receptors markedly down-regulates ACE2 receptors which have protective biological effects on human body (6). With the loss of the protective effect of these receptors interstitial fibrosis, endothelial dysfunction, enhanced inflammation, oxidative stress and increased coagulation can be seen (6). It is interesting to note that severity of the COVID-19 disease is associated with several conditions which may have ACE2 deficiency such as older age, male gender, hypertension, diabetes, or cardiovascular disease.

Complex interplay between asthma, ACE2/TMPRSS2 receptors, Th2 high inflammation, inhaler corticosteroids and COVID-19

ACE2 deficiency and its association with asthma is not clear yet. Peters *et al*. investigated differences in ACE2 and TMPRSS2 gene expression in sputum cells of 330 asthma patients and 79 healthy controls (7). Gene expression of ACE2 and TMPRSS2 was found similar in asthmatics and healthy controls. Among asthma patients, higher expressions of ACE2 and TMPRSS2 were observed in males, African Americans, and patients with diabetes mellitus. Interestingly, use of inhaled corticosteroids was associated with lower expression of ACE2 and TMPRSS2 after adjustment for asthma severity.

This is an important study giving clues about possible factors explaining the low prevalence of asthma among COVID-19 patients. Asthma itself or the use of inhaled steroids may have protective effect against SARS-CoV-2 infection. However, there are some limitations. ACE2 receptors are particularly expressed in type 2 pneumocytes which have an effective role on triggering a cascade of inflammation in the lower respiratory tract. Sputum may not reflect the amount of ACE2 receptors in the lower respiratory tract including type 2 pneumocytes. Many of the inhaler steroids have less peripheral airway deposition and so inhaler steroids might not effect ACE2 expression of type 2 pneumocytes. Smoking status of the patients and asthma endotypes (eosinophilic or neutrophilic, atopic, non-atopic) were not provided in Peters *et al*. study.

Radzikowska *et al*. analyzed ACE2 and TMPRSS2 gene expression in primary Human Bronchial Epithelial Cells (HBECs), bronchial biopsies and bronchoalveolar lavage fluid of healthy children/adults and adult asthma and COPD patients (8). They did not see any significant difference in ACE2 expression in HBECs or bronchial biopsy between control, asthma and COPD patients. However, ACE2 expression in bronchial biopsies found to be higher in smokers. TMPRSS2 expression were high in HBECs of asthmatic patients. These results suggest that smoking status have an enhancing effect on ACE2 expression which may have a positive impact on the entry of SARS-CoV-2 into lung cells and negative impact on COVID-19 severity. Even though TMPRSS2 was found similar in asthmatics and healthy controls in Peters *et al*. study, Radzikowska *et al*. observed higher expression of TMPRSS2 in asthmatic airways. The difference between two study may be related with the use of different samples for the analysis of

Table I *- ACE2 and TMPRSS2 expression in nasal epithelial cells, sputum and bronchial biopsy, and human bronchial epithelial cells in asthma.*

	Nasal Epithelial Cells					**Sputum**							**Bronchial Biopsy**						**HBEC**					
	ACE2			TMPRS2		ACE2			TMPRS2				ACE2			TMPRS2			ACE2			TMPRS2		
	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity	mRNA	Protein	Activity
Peters et al[8]	NA	NA	NA	NA	NA	NA	↔	NA	NA	↔	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Radzikowska et al[9]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	↔	NA	NA	↔	NA	NA	↔	NA	NA	↑	NA	NA
Sajuthi et al[10]	↓	NA	NA	↑	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

↓ = decreased; ↑ = increased; ↔ = no significant difference; NA = not analysed; ACE2:Angiotensin converting enzyme-2; BAL: Bronchoalveolar lavage; HBECs: Bronchial epithelial cells: TMPRS2: Transmembrane protease serine 2.

ACE2 expression: sputum in Peters *et al.* study *versus* bronchial biopsy in Radzikowska *et al*. study. It might also be related to the heterogeneity of asthma endotypes such as eosinophilic (Th2-high) asthma which is most likely seen in atopics or neutrophilic (Th2-low) asthma which is most likely seen in smokers.

Asymptomatic nasal carriage of COVID-19 is more common in children and children mostly seems not to have a severe COVID-19 infection. Therefore, to understand the association between ACE receptors and childhood asthma which is mainly eosinophilic or Th2 high endotype may help to understand the possible link between Th2 high asthma endotype and COVID-19 infection severity. Sajuthi *et al*. used nasal airway transcriptome and network co-expression analysis to identify the cellular and transcriptional factors in COVID-19 infectivity (9). They used a children cohort including 695 subjects with asthma and healthy controls between the ages of 8 and 21. They mainly focused on ACE2 and TMPRSS2 expression. They found that interleukin (IL)-13 mediated Th2 high inflammation had a major role in ACE2 downregulation and TMPRSS2 upregulation. Th2-low and Interferon-high individuals were found to express high level of ACE2. They also showed that ACE2 was expressed in secretory cells and ciliated cells while TMPRSS2 was expressed by all epithelial cell types (9). The results of this study can be interpreted as Th2 rich inflammation may have protective role against COVID-19 by causing ACE2 downregulation. Virus behavior may be different depending on ACE2 and TMPRSS2 expression variations in different part of the airways such as nasal and peripheral airways. TMPRSS2 may have more effective role in nose compared to ACE2. ACE2 and TMPRSS2 expression in nasal epithelial cells, sputum and bronchial biopsy, and human bronchial epithelial cells in asthma is summarized in **table I**.

In conclusion, it is not yet to be proved that asthma is a risk factor for COVID-19 infection. Whether there is a link between asthma and COVID-19 infection remains to be determined. The heterogeneous nature of asthma may cause interindividual variation in COVID-19 infection immunology. More clinical studies focusing ACE2 and TMPRSS2 expression in central and peripheral airways are warranted to understand the role of individual factors such as atopy, obesity and smoking habit and treatment related factors such as inhaled/systemic steroid use in different asthma groups (mild/severe, Th2-high and Th2-low asthma) for the risk of COVID-19 morbidity.

Conflict of interests

The authors declare that they have no conflict of interests.

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