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Migrants and allergy: a new view of the atopic march

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Summary
Atopy is the result of the influence of environmental factors on genetically predisposed individuals. Migration flows represent an interesting model to study the possible reciprocal roles of genes and environment. In this review the following issues influencing the development of allergic sensitization and/or atopic disorders in migrants will be investigated: 1) ethnicity, genetic polymorphisms and risk of atopy; 2) double faceted effects of parasitic infestations; 3) biodiversity loss and industrial progress. Moreover, an extensive revision of the literature about the relationship between the migratory status and allergy development is provided.

Introduction

Atopy march is usually interpreted as the progression of the clinical manifestations of atopic diseases through the different ages (1). Even if this concept is world-wide accepted and truly demonstrated by several papers (2), since a couple of decades the significance of the 'march' of atopy could be interpreted in an alternative way, that means the progression and widespread of atopic disorders through the world because of the expansion of formerly considered wellness-related diseases in "in march-populations". Migration flows in association with the progressive amelioration of life conditions in several countries with a previous subsistence economy, seem to be the new gateway of 'westernization'. Thus, movement of populations in a South-North and East-West gradient together with the shift from rural to industrialized economy along with the GDP gradient represent an interesting model to approach how the environment can modulate the genetic background. It is generally assumed that exposure to new allergens is the necessary pre-requisite to develop sensitization and eventually allergic disease, because immune recognition of what was previously unknown is essential to develop memory, although of a non virtuous functional phenotype. However, in addition to the individual predisposition based on genes eventually shared with the personal ethnic membership, several external factors may influence this initial process of immune recognition. Actually, atopy also 'marches' along with climate changes and consequent spread of new allergens in previously 'untouched' countries. At the same time, climate-affecting emissions might enhance allergenicity of environmental proteins. Finally, biodiversity is impoverished that means not only the reduction of animal or vegetable variety, but also modification (and decrease) of our privileged relationship with the 'old friends' hosted inside our bowel, at the surface of our skin or respiratory tract.
In this review these new concepts about the 'march' of atopy will be considered.

The 'march' of allergy: genetics and migration

Atopy might be interpreted as the result of the influence of environmental factors on a genetically predisposed individ-

ual. Twin studies have offered the best evidence of the heritability of this predisposition, with almost 80% concordance in monozygotic twins versus 20% in dizygotic ones (3). On the other hand, the higher prevalence of allergy in developed, rather than less affluent, countries may account for the influence of environmental factors (4). Which one of these two factors exhibits the prominent impact is still unknown (5). The study of migration flows may be a way to provide a more in-depth knowledge on this issue. In particular, it may help to replicate the concept of "atopy march", that is the age-related progression of atopic disorders.

Differences in the rate of atopic disorders have been demonstrated among different ethnic groups but the impact of genetics might be underestimated because genome-wide association studies (GWAS) have been mainly focused on individuals of European ancestry (5). Atopic dermatitis (AD) has been extensively studied from a genetic point of view by GWAS approach and best represents a clear example of that underestimation. Suppression of filaggrin (FLG) in keratinocytes has been associated with skin barrier deficiencies and early-onset AD in Europeans (6). The higher prevalence of FLG mutations observed in Northern Europeans could indeed favor the penetration of UV-B rays with the consequence of more vitamin D3 synthesis or increased immunity towards infectious diseases, such as tuberculosis and plague (5). Similar FLG mutations have not been found in other ethnicities, but black children living in the United Kingdom show a 6-fold higher risk of AD when compared to the local population (7) and Chinese immigrated children have a 2-fold higher annual incidence of AD than local Australian population (8). Surprisingly, no *FLG* mutations were found in subjects of African descent who share the same rate of FLG level deficiency with Europeans (9). On the contrary, mutations in the *FLG2* gene, closely related to *FLG*, have been demonstrated in African Americans with AD but not in Europeans, but native Africans were not included in this study (10). These *FLG2* mutations are eventually completely different from those found in subjects of Chinese ancestry which, in turn, are even different from those found in Japanese people. Nevertheless, differences in the prevalence of atopy exist in different ethnicities (5). Studies from the United States highlight that African ancestry seems to be a risk factor for atopy (11). Disease-associated single-nucleotide polymorphisms (SNPs) may account for these disparities. Actually, SPNs variants in the *IL4*, *IL4* receptor and *IL13* coding genes, known to be strictly related to the type 2 immunity, were found to be more frequent in African subjects than in Europeans (48% vs 12%, respectively) (12), possibly representing an evolutionary footprint. It has been hypothesized that the *IL4* 589 variant (C>T) (rs2243250) may be associated with a lower risk of malaria infection (13) as well as the Gln551Arg (rs1801275) (14) and Ile50Val (rs1805010) (15) variants of the *IL4 receptor* coding gene and C-1112T (rs1800925) (16) variant of the *IL13* gene may confer increased resistance to parasitic infestation as skewing immunity towards the type 2 response is more protective.

Several other genes have been related to atopic disorders in their whole (*IL5, TSLP, FOXP3, IL10, IFNG, CCL11, CCL26, FCER2, CD4, IGHG4, RNASE2, RNASE3, KCNE4*), but studies on their mutations in different ethnicities are still lacking (5).

The 'march' of allergy: parasites and migration

As stated above, these mutation differences might be explained by a balanced selection process of atopic-related genes as the result of multiple environmental factors, such as the pressure of pathogens (17). In this context, parasitic infestations have acquired a major interest during the last few years. Parasitic infestations can positively or negatively influence the development of atopy by respectively stimulating or suppressing the immune response. Factors claimed for this dual activity are the type of helminth, the concentration levels, the time of exposure and the genetic susceptibility of the host (17).

Few clear examples of this "Janus" activity of parasite infestation on allergy are present in the literature. A first example has been represented by the Falascia people, an Ethiopian ethnic group of Jewish faith. In 1984 and 1991 two immigration waves brought almost 30,000 Ethiopians to Israel (18). At their arrival, most of them presented parasitic infestation and very high IgE levels, with no symptoms of allergy or asthma (19). The entire population of immigrants received anti-parasite treatment and re-evaluation 3-years later found a 11% prevalence of allergy, mainly allergic rhinitis and asthma. In addition, in an Ethiopian cohort that lived in Israel for more than 8 years, the prevalence of asthma was higher than in the local population itself (20). Similarly, a regular anti-helminthic treatment of Venezuelan children was associated with the increase of atopic sensitization to house dust mite from 17% to 68% after a follow-up period of two years (21). Another study conducted in Ethiopia on 12,876 individuals, found that the presence of parasitic infestation independently reduced the risk of wheeze-onset in already atopic subjects (22).

On the other side, these data were not confirmed in other studies carried out in the Tropics, where urbanization, Western lifestyle and a great range of infections including heavy parasitic infestation are present at the same time (17). As an example, in Ecuadorian children a 12-month anti-helminthic treatment did not promote any allergic sensitization during the follow-up period (23). Moreover, and intriguingly, a study in China demonstrated that infestation with *A. lumbricoides* was a risk factor for the development of asthma and aeroallergen sensitization (24). Nonetheless, it is important to underline that the molecular basis of the high prevalence of sensitization to common allergens when assessed with specific IgE levels among population living in helminth-endemic areas can be due to cross-reactive

carbohydrate determinants (CCDs), complex N-glycans on plant and invertebrate glycoproteins, also present on parasites surfaces. This has been demonstrated analyzing molecular IgE profile on ImmunoCAP-ISAC in the serum of Indonesian children with virtually absent clinical allergy, but extremely high prevalence (65% to 85%) of sensitization to common airborne and food allergens (25). Although many epidemiological studies demonstrated that ascariasis is a risk factor for atopy and asthma as cross-reactive with house dust mite tropomyosin (26), basic research clearly demonstrated that helminths have immunomodulatory effects, playing a protective role against the development of allergy, through the induction of interleukin-10 and TGF-beta, the expansion of peripheral regulatory T cells and the production of high level of IgE and IgG4 by plasmacells (17,27,28) (**Figure 1**).

From an evolutionary prospective, parasitic products are able to downregulate T-cell receptor–MHC interactions favoring a Th2 response in the same way as long-lasting exposure to low-dose allergens with low affinity for the T-cell receptor activates the type 2 response in allergy (29). Actually, baseline levels of total IgE are usually high in immigrants from less developed countries (18). On this favored type-2 background, any change in lifestyle and habits and/or the exposure to new allergens instead of continuous exposure to parasite products, may make immigrants even more susceptible to atopic disorders than the local population itself. Indeed, in Western countries, pollutants, dietary changes and different socioeconomic factors in the absence of infectious stimuli may be able to redirect immunity towards the inappropriate type 2 response to allergens (**Figure 1**). This hypothesis is strengthened by the demonstration of a direct proportion between rate of sensitization and time of residence in the new country (30-32).

Inclusion of different ethnicities in cohort studies and clinical trials as well as a clear definition of what the term atopy means (symptoms versus simple sensitization), would be key to defining the real impact of genetics, gene-environment connections and parasitic infestations on atopy development.

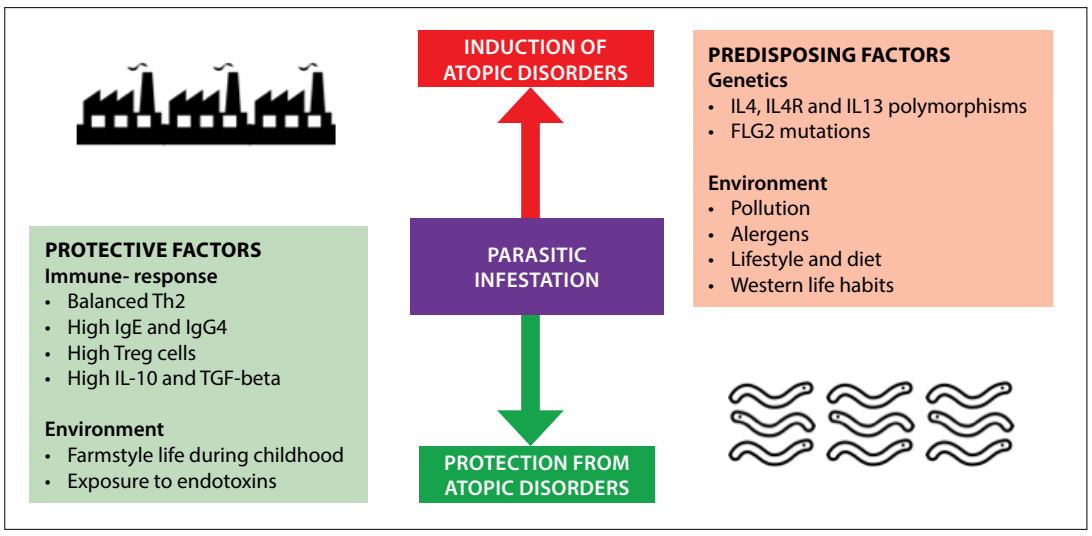
The "migration" of allergy: role of environmental factors

While it remains irrefutable that genetic predisposition has its weight, it is now widely accepted how genetics *together* with environmental exposure are key to shaping the immune system, especially during early life. Environmental exposure is in fact necessary to promote development and progression of allergic diseases (33). In 1958 Sherman stated that "Sensitization is never found to those allergens whose distribution precludes the exposure of the patient" (34). At that time, scientists were just beginning to deal with the consequences of imported ragweed pollen (*Ambrosia*) from America to Europe (35), and the sensitization that shortly followed exposure to an *Ambrosia*-naive population. The same could be witnessed the other way round, as described early on by Hughes in a series of 60 patients who immigrated to Canada, and developed sensitization to ragweed after at least one season of contact to the weed pollen (35).

The concept of biodiversity

Biodiversity is defined as the variability among living organisms from all sources, including *inter alia*, terrestrial, marine and oth-

Figure 1 *- The "Janus" activity of parasitic infestation on the development of allergic diseases.*



Factory and worm icons made by Freepik, available from www.flaticon.com

er aquatic ecosystems and the ecological complexes of which they are part. This includes diversity within species, between species and of ecosystems, a definition provided at the Convention on Biological Diversity in 1992. As a fact, the concept that biodiversity loss could lead to disease was introduced only recently in 2011, when the connection between two global megatrends, biodiversity loss and inflammatory diseases, was eviscerated (36). Although the neonate immune system has been vastly regarded as immature, it has been recently shown that strong antigenic stimuli can indeed induce efficient protective Th1 responses similarly to adults (37). The expression of cell activation markers, such as inducible T cell co-stimulator ligand (ICOS-L) and regulator markers, such as programmed death ligand 1 (PD-L1), has been found on dendritic cells of the neonatal lung in rats, implying the capacity of taking up antigens and processing them with a fine regulation of the immune response (38). On this basis, environmental exposure may exert an enormous impact on the immune system from an extreme early age onwards. This concept was evident from early on, when in 1989 the 'hygiene hypothesis' theory was introduced, stating that increased early-life exposure to infections and larger family size lead to a decreased risk of allergic disease development (39). Over a decade later, the 'old friends' hypothesis was proposed, where an explanation for the increase in allergic diseases was linked to the loss of symbiotic relationships with beneficial parasites and bacteria (1,40). Just a year later, in 2005, the 'microflora-microbiota' concept was introduced, blaming a reduced microbiome diversity for altered epithelial and immune cells (41). Indeed, numerous cohort studies dating back from 2001 to 2016, carried out in Europe and Australia, have shown that alterations in the gut microbiota during infancy and early childhood are associated with allergic disease (42-44). We now know that our intestinal tract is loaded with up to 10^{14} microbes (45) and data from metagenomic sequencing showed that every individual gives hospitality to at least 160 species of bacteria with a total number of bacterial species, identified in a sample of 124 Europeans, between 1000 to 1150 (46). Diminished early life exposure to the environmental microbiota could be responsible for priming the naïve immune system towards a Th2-predominant state, thus increasing the risk of developing allergic disorders. Indeed, microbiota has been claimed to modulate immune-responses through a so-called metabolic control (e.g. the action of short-chain fatty acids), being able to promote T regulatory cells and release hormones thus reducing the expression of pro-inflammatory cytokine (47,48).

Noteworthy, not only the number but also the variety of commensals seems to be relevant to prevent or favor allergies. Along with the 'biodiversity hypothesis', it is now accepted that "contact with natural environments enriches the human microbiome, promotes immune balance and protects from allergy and inflammatory disorders", as written by Haahtela T. in a recent review on this topic (49). In this view two layers of biodiversity are identified: the *outer* (soil, natural waters, plants and animals), and the *inner layer* (gut, skin, airways). Biodiversity was further defined into three categories: macro-, micro- and genetic-diversity, the macro-diversity being the only easily observed, but the all three necessary for a global balance. Studies investigating the role of macro-diversity are important for understanding the delicate but intricate relationship between the inner-outer layer balance. In 1998 the Karelia Allergy Study began to substantiate the increase of the allergy incidence in the Finnish population (50). To this end a number of interesting studies were set up to evaluate the Finnish and Russian Karelia populations, living in adjacent areas of northern Europe, geoclimatically similar but socio-economically distinct. A once united population faced severe separation: the Russian population living in a small-scale agricultural lifestyle, while the Finnish population started to urbanize (51). Comparing the occurrence of asthma and allergy across the border, sensitization was low in both populations in individuals born at the time of the War. However, a linear increase was observed for allergy only in the Finnish younger generations (52). Another study carried out on children and adolescents from Finland and Estonia came to the same conclusion: the greener the environment, the lower the risk of allergy (53). These documented allergy gaps all presented in a relatively short period of time and between genetically close, if not identical, populations, supporting the view that not all can be explained by the genome, but rather in the change of lifestyle and environment (49).

Urbanization infallibly results in the loss of biodiversity and increased air pollution alongside improved socioeconomic conditions and better sanitation infrastructure, which in turn positively correlate with an increased incidence of atopy. In a large birth cohort in New Zealand, exposure to green areas and diverse vegetation has recently demonstrated to prevent from asthma development (54). However, these results have been not so clearly replicated and showed variations by region. In a study involving 2,472 children participating in the ongoing INfancia y Medio Ambiente (INMA) cohort located in Spain, higher residential surrounding greenness and higher proximity to green spaces were overall negatively associated with wheezing in the Euro-Siberian region. At the same time, in the Mediterranean region, higher residential proximity to green spaces was associated with a reduced risk for bronchitis (55). Opposite results on allergy risk were reported in a study based on individual data from Swedish (BAMSE), Australian (MACS), Dutch (PIAMA), Canadian (CAPPS and SAGE), and German (GINIplus and LISAplus) birth cohorts, involving a total of 13,016 individuals, which examined cohort-specific and combined associations of residential greenness with allergic rhinitis and aeroallergen sensitization. Here, residential greenness appeared to be associated with childhood allergic rhinitis and aeroallergen sensitization with the effect varying by location (56).

Inner layer modifications are also being associated with various adverse health effects. It was recently confirmed that alterations in the composition of the gut and skin microbiota as effect of reduced biodiversity are associated with various inflammatory conditions such as asthma, allergic and inflammatory bowel diseases (IBD), type 1 diabetes, and obesity (57), due to different immunopathologic mechanisms but all showing how tolerance mechanisms can rapidly become impaired in microbe-poor environments. These concepts has been acquired into the 2013 World Allergy Organization (WAO) Statement, where the loss of macro-diversity is associated with shrinking of micro-diversity, which is in turn associated with alterations of the indigenous microbiota (58). Their proposal highlighted an exponentially inverse relationship between biodiversity and the asthma/allergic rhinitis incidence: as biodiversity plummeted to record-lows, the incidence of allergic diseases sky-rocketed.
Finally, several epidemiological studies with large evidence suggested that exposure to high microbial loads in early life, such as a farming lifestyle, the presence of older siblings, and pet ownership, may be protective against atopy by shifting the immune milieu back towards a healthy Th1/Th2 balance (59).

Rural-Industrial Gap

As stated above, the phenotype of allergic diseases is heavily influenced by environmental exposure (33). When discussing the concept of biodiversity and its impact on the regulation of the immune system throughout an individual life, there is no better model to study than that of the consequences of immigration and/or emigration, a phenomenon which has lead hundreds of thousands across the world to become exposed to a complete new indoor and outdoor environment. Immigration and emigration to industrialized areas and the adoption of a more Western lifestyle has quickly led to behavioral, environmental, and dietary changes as the process of migration exposes immigrants to changes in socioeconomic, cultural, psychological, and, notably, environmental factors (60). The prevalence of diseases can greatly vary in settings with different socioeconomic conditions but also between regions, countries, and centers within a city or country (61). Studies have shown disparities in health and disease indices between migrant and native populations living in the same geographical location, especially regarding allergy (62). Comparisons between rural versus non rural microbial exposure in children in studies from all over the world, such as PARSIFAL and GABRIELA, or from studies conducted in Russia, China, Finland and more, have all come to one halting conclusion: early bacterial exposure carries a protective role in exposed individuals (52,63,64). One of the most striking study comparing microbial exposure in Amish and Hutterite children, respectively living on traditional or on large modern communal farms, showed a very low in the former and a significantly higher asthma incidence in the latter ones (5% vs 23%, respectively) (65). In this case, the importance of the origin and nature of bacterial exposure was highlighted, in fact it is supposed that the environmental protecting factor in Amish people consists of the higher endotoxin levels in household airborne dust. A Korean study involving 13,11 subjects divided into 3 groups according to the degree of urbanization (urban, semirural and rural) of the area where they resided, showed a positive correlation between sensitization to allergens (in particular house dust mite) and degree of urbanization (urban 17.2%, semirural 9.8% and rural 6.0%) (66).
The environmental and behavioral changes deriving, as an example, from urbanization, time spent indoors or antibiotic use result in increased exposure to air and traffic pollution, fungi, infectious agents, tobacco smoke, and other early-life and lifelong risk factors for the development and exacerbation of asthma and allergic diseases (33). Not surprisingly, migrants from rural areas moving to urban areas in developed countries actually show lower risks of allergic diseases compared to native urban residents.
This rural-urban gradient was well demonstrated also in a Danish study in which 1,236 male participants were divided into four groups depending on area of upbringing (city, town, rural area and farm) and assessed for allergy sensitization by skin prick tests (SPT) and specific serum IgEs towards inhalant allergens, clearly showing how exposure to a less urbanized childhood was associated with a lower risk of allergic sensitization and disease as an adult (67). Along with this first observation, a large body of evidence from Switzerland, Austria, Finland and Germany suggested that living on a farm and having contact with livestock is associated with protection against atopy, hay fever and asthma. The prevalence of atopy and hay fever has been reported to be reduced by between 31% and 69% in farmers' children (68–70). However, whether the presence of livestock or just agricultural farming is protective, still remains unclear. In a pioneering study from Italy, exposure to oro-fecal and food-borne microbes was inasmuch able to prevent the development of atopy (71). Moreover, a study carried out on Australian children found allergy protection depending on the type of farming (72). In another study carried out in China, rural children exposed to farming and higher endotoxin levels had decreased asthma risk compared with urban children (64). Although childhood farm-living have a lifelong protective effect on the prevalence of allergic rhinitis, it was also shown that an increasing prevalence of this disease goes hand in hand with the increasing degree of urbanization regardless of previous farm exposure (70). A cross-sectional study in the Chinese city of Suzhou observed that migrants from the countryside had lower rates of asthma and allergic symptoms compared to the local population (73). Interestingly enough, migrant children had higher rates of asthma compared to their parents, highlighting once again the critical role of early-life environmental

factors in the pathogenesis of allergic disorders. The concept of rural-industrial gap is somehow even clear in less affluent countries, where urbanization is relatively lower when compared to Western countries. Early studies conducted in South-East Asia in the 1980s, highlighted the higher prevalence of asthma in the cities (74,75). A study carried out in Mongolia in 2005 identified that the geographical distribution of allergic diseases was directly proportional to the level of urbanization of the area (76). Regarding Africa, a pioneering study in 1979 showed for the first time a greater prevalence of asthma in children of Xhosia ethnicity grew up in Cape Town when compared to peers residing in villages (3.17% versus 0.14%) (77). Subsequent studies in Kenya and Ethiopia broadly confirmed the urban-rural distribution of allergies (78,79). Similar data have been found in Latin America, with a prevalence of atopic dermatitis, allergic rhinitis and asthma up to 4-times higher than in the rural cohort (80). An interesting industrial-rural distribution for allergic-related diseases has been shown in several studies (81,82) carried also in Canada and USA.

Evaluating *when* and *what* happens when rurally-born and grown individuals move to a more industrial area is an intriguing point. A study conducted in Denmark demonstrated a decrease in the risk of allergic sensitization as well as rhinitis, and allergic asthma in adulthood with decreasing degree of an urbanized childhood (67). An Italian study better documented this concept in migrants from Albania. In spite of the low prevalence of allergic diseases in the country of origin, the prevalence of sensitization to local allergens and nasal symptoms increased in a time-dependent manner once migrants moved to the new urban setting (Italy)(83): more time passed, the higher the incidence of sensitization. Moreover, age at the time of migration does influence the risk of atopy and the rate of allergy acquisition (60). Subsequently, it was showed how children below the age of 4 years at the time of migration to Italy had a higher prevalence of rhinitis, atopic dermatitis, food allergy and allergic sensitization compared to children who migrated after the age of four (84). *Viceversa*, in this cohort an older age at the time of migration was associated with a shorter time to the onset of allergic symptoms from migration.

Migrants also offer a view on the environmental dynamics to the development of allergic diseases. It is clear that first-generation immigrants have a lower allergy prevalence compared to the native population, second-generation immigrants have a higher allergy prevalence compared to first-generation immigrants and second-generation immigrants with 2 foreign-born parents have a lower allergy prevalence than those with only 1 foreign-born parent (85-87). A clear correlation between immigration and insurgence of allergic symptoms was well shown in a study where first-generation immigrants acquired the sensitization profiles and allergic disease prevalence of the host country (88). These data were confirmed in another cross-sectional Italian study, involving 21 allergy units in Italy, where the onset of allergic respiratory symptoms were shown to occur after immigration in 83% of adult immigrants, who had otherwise been asymptomatic while living in their native country (89).

Going back to the rural versus urban gap, it is possible that urbanization associated with high levels of vehicle emissions may be responsible of increased pollen-induced respiratory allergy in urban compared to rural populations (90). Literature vastly describes the role of outdoor (but also indoor) air pollutants in causing adverse health effects. It was claimed that moving from a rural to an urban area leads to exposure to a mixture of natural (wildfires, volcanoes, biological decay, dust storms) and human-made pollutants (motor vehicles, biomass burning, power plants, industrial facilities, waste incinerators, pesticides). In addition to this, sulfur dioxide, nitrogen oxides, carbon monoxide, and particulate matter are typical outdoor air pollutants from fuel combustion or motor vehicle emissions (91). Indoor air pollution is becoming increasingly troublesome due to the habit of some societies to spend the vast majority of time indoors, where tobacco smoke, solid fuels, stoves, construction materials, ambient particulate matter and biological materials can be found (92). Some studies investigated the pollutants capacity to directly promote the development of allergic disease. Traffic-related air pollution (TRAP) and tobacco smoke in allergic disease and asthma are indeed able to cause asthma exacerbation in children (93). Few studies have shown how near-roadway exposure is associated with increased asthma prevalence, chronic lower respiratory symptoms, phlegm production, bronchitis, wheeze, and medication use (94), decreased lung function, lifetime diagnoses and symptoms of allergic rhinitis or allergic sensitization among school-aged children (95). In another study, an estimated 14% of incident childhood asthma and 15% of childhood asthma exacerbations were caused by exposure to pollutants from roads with high vehicle traffic (96). Finally, other studies have shown how a large portion (40-83%) of the increased risk of aeroallergen sensitization by age 4 and increased risk of food allergy by age 8 could be linked to TRAP exposure (97). Despite the number of studies, however, meta-analyses of American and European cohorts observed substantial heterogeneity across studies that limited the ability to draw conclusions about the relationship between TRAP exposure and allergic outcomes (98).

It has been claimed that air pollutants, such as CO2, O3 and NO2 levels, interact with airborne allergens enhancing the risk of allergic sensitization and exacerbation of symptoms in sensitized individuals. Climate change, especially the global warming phenomenon, is one of the most important factors acting on allergic disease risk because it affects air quality, plant distribution and production, pollen count and fungal growth, being responsible for modifications of both allergenicity and season onset of aeroallergens spreading (4). Dietary factors may be

important in modulating immune-responses. Migration is usually associated with a change in dietary habits, and therefore the impact of these changes on allergy development has been investigated. Few studies have confirmed that the consumption of fast-food and take-away foods combined with the low intake of fruit and vegetables in the diet correlate with the increase in asthma and other atopic disorders prevalence (99). On the other hand, Mediterranean diet, especially if started since the first years of life, would exert to be a protective factor for atopic diseases (100). Some authors have observed that the alteration of the intestinal microbiota may represent one of the mechanisms by which the consumption of food in industrialized societies results in harmful effects (101).

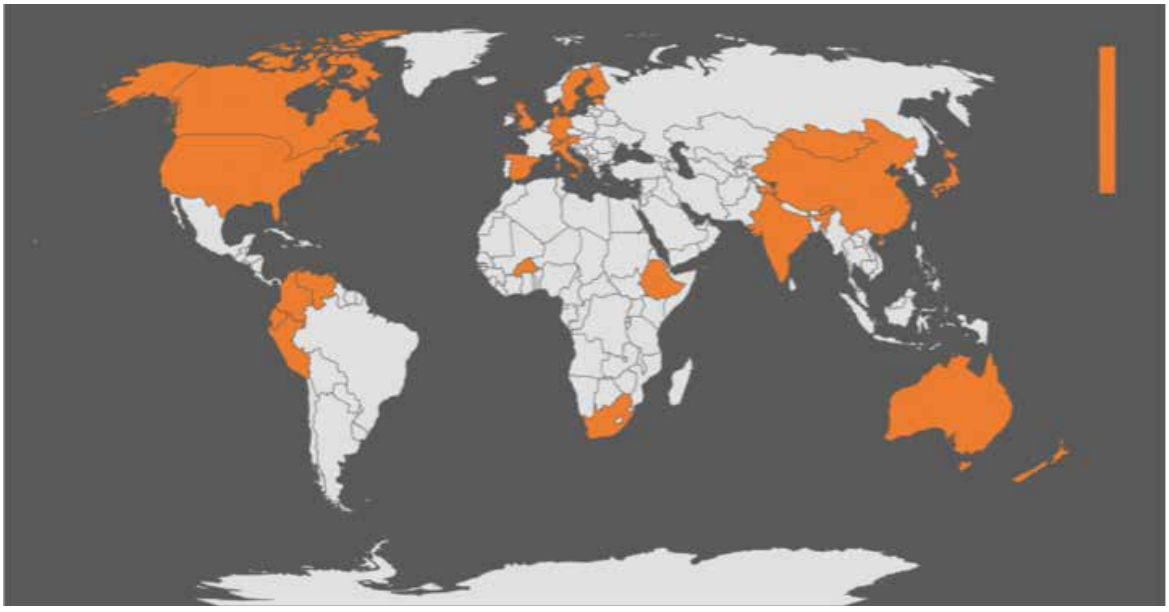
Migration status and allergic disease

Despite infection and parasitic pressure, asthma and allergic diseases in general are also increasing in low and middle-income countries, where the complexity and severity of atopic diseases especially affect the youth population who carry on the greatest part of this epidemiological tendency (102). Migration status could heavily contribute to this trend, due to the abrupt exposure of the migrant populations to a new set of pollutants and allergens and the dramatic changes in diet, housing conditions and accessibility to medical services after the arrival in a new country (103). This phenomenon has an enormous economic impact as nearly one-seventh of the world population is now living in a different location from the birthplace. With one billion people having moved in 2018, migration is a global reality and one of the greatest political issue in the contemporary world. The "disease load" among migrants is heterogeneous and dynamic because of a variety of interacting factors, such as genetic background as we have already touched on, pre-migration state of health, socio-economic and environmental conditions, local disease patterns, cultural norms and behaviours, access to medications before and during the migration process (81).

In the last two decades several papers on migrants have been published (**Figure 2**) with great heterogeneity regarding types of immigrant communities, comparison between local population and immigrants, which is often sporadic, possible lack of the country of origin. All these items may represent a big challenge for data synthesis (87). As a fact, the key variables affecting the external validity of migration studies and their results are represented by heterogeneity of immigrant population in terms of ethnicity and country of origin.

Overall, the findings of this great amount of published studies suggest that the burden of allergic diseases and asthma in immigrants from less affluent countries is lower than in the high-income host country. The ISAAC study involving 48 countries and 111 centres found that 6- to 7-year-old and 13- to 14-year-old individuals recently migrated to Western countries (high-income and high-allergy prevalence), had a lower prevalence of asthma and allergic disorders compared to local population (82). These results were not confirmed by the European Community Respiratory Health Survey (ECRHS), involving adults aged 20–45 years living in countries with high-asthma preva-

Figure 2 *- A representation of the Countries where the studies about immigrants have been performed.*



lence (Europe, USA, Australia, and New Zealand), founding no differences in asthma prevalence between resident migrants and non-migrants (104). However, these studies both present at least two limitations: sensitization instead of true allergy was taken into account in the ISAAC study, whereas in ECRHS asthma symptoms were considered, without any phenotyping into allergic and non-allergic one.

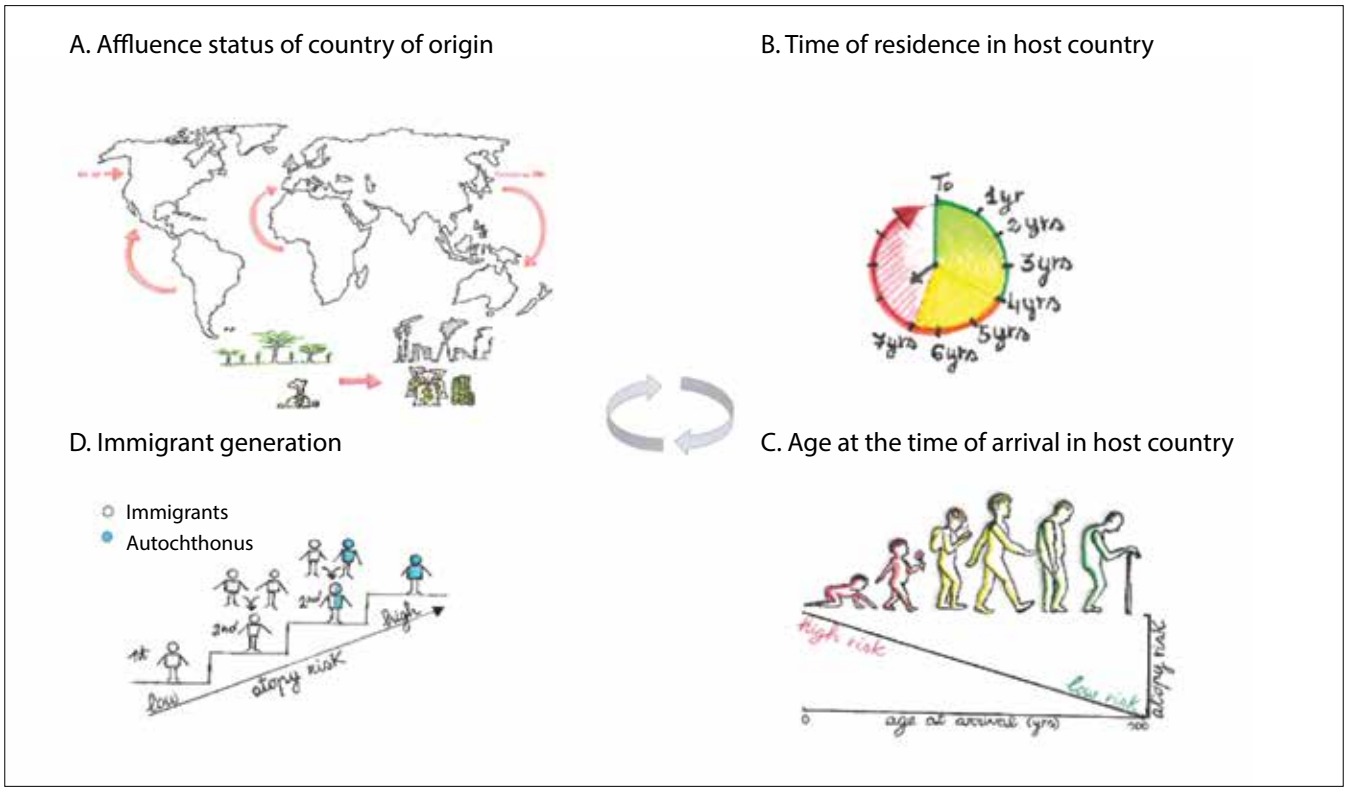
Most of the migration studies found that the prevalence of allergic disorders in immigrants tends to coincide with the general prevalence of the local population through a time-dependent process, confirming the strong role of the environment on the development of allergic sensitization and disease initiation. Analysing the ISAAC study, the protective effect of migration against allergy progressively declined with the increase of residence duration in the host country (82).

The main factors influencing atopic risk in migrant populations are resumed in **box 1** and **Figure 3**.

Box 1 *Main factors influencing atopic risk in migrant populations*

- The protective effect of migration is confined for migrants moving from a low-income country to a country with a high prevalence of allergic diseases
- The risk of developing asthma and allergic disease increases with the duration of residence in the host country (4-7 years)
- The risk of developing atopic disease is increased in people with younger age at the time of migration
- First-generation immigrants have a lower allergy prevalence compared to the local population and to second-generations. Regarding the second generation, people with two foreign-born parents have a lower allergy prevalence compared to those with only one foreign-born parent

Figure 3 *- Main factors influencing atopic risk in immigrant people: A. The protective effect of migration is confined to migrants moving from a low-income country to a country with a high prevalence of allergic disease; B. The risk of developing asthma and allergic disease increases with the duration of residence in the host country; C. 1st generation immigrants have a lower allergy prevalence compared to 2nd generation. Regarding the 2nd generation, people with two foreign-born parents have a lower allergy prevalence compared to those with only one foreign-born parent.*



Asthma and Allergic Respiratory Diseases in migration studies

We summarize herein the principal findings of the most significant studies about asthma and allergic respiratory diseases in immigrants, grouping by the continent of the host country where the study was conducted and, when possible, dividing by ethnicity/country-of-origin of the immigrated study group.

North America

In 2007-2008 the National Survey of Children's Health studied 91,642 children with a cross-sectional questionnaire about atopic diseases, founding that children born outside the United States had significantly lower odds of any atopic disorders than their peers born in the USA (logistic regression OR, 0.48; 95% CI, 0.38-0.61), including ever-asthma (OR 0.53; CI 0.39-0.72), current-asthma (OR 0.34; CI 0.23-0.51) and hay fever (OR 0.39; CI 0.27-0.55) (105).

The National Health Interview Survey conducted early on between 1997 and 2011 demonstrated that foreign-born American adults from all regions of birth had lower odds of ever-asthma (adj OR 0.52, 95% CI 0.49–0.55) or current-asthma (adj OR 0.50, 95% CI 0.46–0.54) than US-born adults and that this risk increases after prolonged US residency. In fact, adult immigrants with prolonged residency in the USA (beyond 10 years) had greater odds of developing ever-asthma (OR 1.28, 95% CI 1.18–1.38) and current-asthma (OR 1.70, 95% CI 1.31–2.19) compared to those who had lived in the USA for less than 4 years (106).

Data analysis from the Canadian Community Health Survey demonstrated a lower prevalence of self-reported and physician-diagnosed non-food allergies among immigrants compared with non-immigrants, with diminishing difference along with the longer duration of residence (107).

A recent population-based retrospective cohort study in Ontario found that asthma incidence was lower among immigrants compared with long-term residents (IRR 0.30; 95% CI 0.30-0.30). On the other hand, Ontario-born children of immigrants from different world regions had significantly higher asthma incidence when compared to children of long-term residents (IRR 1.44; 95% CI 1.43-1.45).

Mexican Immigrants in USA

In the Chicago Asthma School Study, living in the USA instead of Mexico in the first year of life was associated with higher prevalence of physician-diagnosed asthma (OR 1.79, 95% CI 1.09–2.94). Along with this, long-term Mexican immigrants living in the USA for more than 10 years, had an increased risk of asthma compared with short-term immigrants, independently of country of residence in the first year of life (OR 1.93; 95% CI 1.00–3.73) (108).

Chinese Immigrants in USA

Adult Chinese immigrants living in the USA participating in the cross-sectional Community Assessment of Freeway Exposure and Health (CAFEH) study were less likely to have asthma (OR 0.20, 95% CI 0.09–0.48) compared to US-born whites thus showing that first generation immigrant status may be protective with a long-lived effect, as long as two decades (109).

Chinese Immigrants in Canada

Elaboration of data from the International Study of Asthma and Allergies in Childhood (ISAAC) phase III in 2008, comparing the prevalence of asthma and asthma-related symptoms (current wheezing, ever-wheezing, ever-asthma, wheezing attacks) in Chinese adolescents born in Canada, Chinese adolescents immigrated to Canada and Chinese adolescents living in China, demonstrated that asthma symptoms were lowest among mainland China residents, greater for Canada-immigrated and highest among Canada-born individuals. In detail, the prevalence of asthma in Chinese adolescent immigrants was 7.7% when residing in Canada for less than 7 years, 11.2% when living in Canada since more than 7 years and 15.9% when born in Canada (trend p=0.006) (110).

Australia

Early studies in the 1980s and 1990s observed that children born outside Australia had a lower incidence of asthma than natives. Moreover, foreign children tend to manifest a severe phenotype following a time-dependent pattern (111,112).

Asian Immigrants in Australia

It has been showed that in patients under 20 years of age the prevalence of asthma is distributed according to a gradient, from the more prevalent to the less prevalent, non-Asian Australians – Asian Australians – Asian migrants. However, this distribution was different when considering the prevalence of allergic rhinitis, which resulted more prevalent in Asian migrants and that was directly correlated with the levels of serum IgE (113,114).

Asia

The prevalence of allergic rhinitis in a cohort of new migrants from mainland China to Singapore was 9% compared to greater than 40% in Singapore-born subjects. The prevalence of allergic rhinitis increased up to 22% in the immigrated group after 8 years residence in Singapore. Moreover, less than 30% of China-born new immigrants were sensitized to house dust mites in comparison with 80% of Singapore-born subjects. However, after 3–8 years of residence, house dust mites in China-born migrants climbed to 50%, further increasing to 60% after more than 8 years. This study masterfully showed the

time-depending influence of the environment on the allergic sensitization process (115).
The prevalence of asthma at age 17 on Israeli adolescents of the Israel defence forces was higher in native born Israelis compared with Ethiopians (4.7% vs 2.6% respectively, p<0.0005) or immigrants from the former Soviet Union (FSU). The younger age of immigrants from Ethiopia and FSU when arriving to Israel, the higher was the prevalence of asthma at the age of 17 (20).

Europe
A different approach was used in a 2006 study, analysing the number of prescribed inhaled corticosteroids (ICS) as indicator of asthma in Swedish residents of different origin. A 3- to 4-fold higher rates of asthma medication was found in International adoptees and Sweden-born children from foreign-born parents when compared with foreign-born children. The odds ratios of asthma medication use declined persistently with age at immigration (116). More recently, in a selected disadvantaged immigrant population with highly precarious housing and potentially harmful environmental exposures (indoor moisture or mould, smoking), the atopic burden was indeed very high, also underlining the importance of unmet medical needs of certain immigrated communities (117).
One of the first Italian study conducted in Milan observed that a very high percentage of immigrants from outside Europe (84.5%) reported allergic/asthmatic symptoms developed after an average period of 4 years and 7 months from their arrival in Italy, while being asymptomatic in their country of origin. Aeroallergen sensitization patterns were similar to the local population (118). In 2011 it was found that new immigrants to Northern Italy compared to the resident population displayed a time-dependent increase in the number of sensitizing aeroallergens, which correlated with the duration of residence (30). The Viadana study, on the contrary, enrolled children aged 3–14 years living in Northern Italy who were compared to children born in Italy from Italian parents, demonstrating that immigrant children had a lower incidence rate of wheezing (7.9 vs. 36.6 per 1,000 persons/year) (119). In a wider cross-sectional study involving 21 Italian allergy units prevalently sited in the North and a very few in the Centre, taking into account immigrants referred to allergy services for respiratory symptoms, the onset of allergic respiratory disease occurred in 83% of previously asymptomatic adult immigrants. A higher rate of monosensitization was observed without any other relevant difference into the sensitization pattern, even though asthma and rhinitis were more severe in immigrants than in Italians (89). In a further and more recent cross-sectional multicentre study on rhinitis/asthma involving children born in Italy from Italian parents in comparison with children born either in Italy or abroad but from immigrants, the latter group showed a lower prevalence of rhinitis compared to Italians (68.3% vs. 87.6%, p=0.003) without any difference in terms of severity. However, significant differences in the pattern of sensitization was observed, inasmuch as immigrant children were more frequently sensitized to HDM than grass pollen (73.3% vs. 51%; p=0.002) (120).

Albanian Immigrants in Italy

In a pioneering Italian study on the Albanian immigrant community living in Southern Italy, a lower prevalence of reported asthma and sensitization compared to the local Italian population was found (83). Over the time, the Albanian population developed allergic symptoms due to an increased prevalence of sensitization to local aeroallergens and acquired a pattern of sensitization typical of the host country (olive tree pollen).

Other Atopic Diseases in migration studies

The evidences about the prevalence, development and burden of atopic disorders other than respiratory diseases in migrant populations are definitely lower in numbers and more fragmented. We reported some interesting findings below.

Anaphylaxis
A Danish register-based study using nationwide data revealed that hospitalizations for anaphylaxis were less frequent in non-Western immigrants compared to Danish-born people (121).
In a recent study held in Australia about allergy and anaphylaxis, Asia-born children migrated to Australia exhibited a lower risk of food allergy (OR 0.33, 95%CI 0.20-0.55), eczema (OR 0.37, 95%CI 0.24-0.57) and asthma (OR 0.29, 95% CI 0.21-0.40) than non-Asian children. However, they were more likely at risk of anaphylaxis induced by both food and other triggers (122). The triggers of anaphylaxis differ between Asia- compared to non-Asian children, as lower for milk, peanuts and tree nuts but higher for soy, wheat and non-food related.

Food allergy
The population-based Health Nuts study in Melbourne (Australia) showed that peanut allergy was more frequent in infants with at least one parent born in East Asia than infants with both two Australia-born parents (OR 3.4, 95% CI 2.2–5.1) and comparable to children with at least one UK/Europe-born parent (OR 0.8, 95% CI 0.4–1.5) (123).
In the Canadian 2005-2006 National Health and Examination Survey investigating parental nativity, US-born children and adolescents had higher odds of sensitization to food, compared to outside US-born children, (OR 2.05, 95% CI 1.49-2.83, p<0.001). In this case, levels of specific IgE to either milk, egg or peanuts of 0.35 kU/L or greater were considered. In foreign-born children, those arrived at 2 years of age or less had

higher odds of food sensitization than the older ones (OR 2.68, 95% CI 1.19-6.08, p=0.02), even if the US-born children with immigrant parents continued to be at the highest risk (OR 1.53, 95% CI 1.05-2.24, p=0.02) (86).

Eczema

Data from the German Interview and Examination Survey for Children and Adolescents (KIGGS) suggest that migration status has a significant inverse association with eczema (OR 0.63, 95% CI 0.49–0.80) (124).

This finding has been replicated in the multicentre ISAAC study, where children migrated at 10 years of age or older had lower odds of eczema (OR 0.69, 95% CI 0.56–0.86) compared to children migrated at the age of 2 years or younger. Nevertheless, eczema in children migrated at the age of 2 or older was more likely to be severe than earlier migration (82).

In USA the already mentioned National Survey of Children's Health found that outside US-born compared to US-born children had lower odds of eczema and food allergies in addition to lower respiratory allergic disorders (105).

In Italy, two already mentioned papers also accounted for allergic manifestations other than respiratory diseases. The first one is the cross-sectional multicentre study on adult immigrants with the finding of a lower prevalence of atopic eczema, food and drug reactions (89). The second one, the Viadana study, carried on children living in Northern Italy, confirmed that immigrant children had a lower incidence of eczema compared to Italy-born children from Italian parents (5.5 vs. 28.4 per 1,000 persons/year) (119).

Conclusion and future perspectives

Understanding the development of asthma and the allergic sensitization in the context of migration is a unique opportunity to reveal the complexity of gene-environment interactions, to identify risk factors and therefore to possibly find prevention strategies. In general, however, high-quality studies are still lacking, especially studies describing the longitudinal trajectory of illness in allergy (87). This should be advanced in future studies dealing more with basic immunological research, as unique findings might emerge following exposures and health status of immigrants before, during and after migration process and disclosing variation in their immune responses more in depth. Nevertheless, migration needs to be treated as a determinant of health and addressed as a global health priority, because the way the world will face human mobility in the near future will determine public health for the next decades (81).

A participatory approach in which migrants and local communities are included in the research process must be encouraged in order to best take care of the ever-rising number of sensitized patients, to predict who will become allergic and, among these, who will develop a more severe phenotype of atopy.

Conflict of Interest

The authors declare that they have no conflict of interest

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The growing importance of real-life studies in allergen immunotherapy

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Summary
Real-life studies offer the opportunity of obtaining outcomes suitable in clinical practice, as controlled trials do not mirror the real patients' population observed in clinical practice. This concept is particularly appropriate for allergen immunotherapy (AIT). Therefore, the current review will present and discuss the most recent and relevant studies published on this topic. Globally, 15 real-life studies on AIT efficacy are available until now, the total of patients amounts to 9090, with an average number of 699 patients per study. This high number significantly decreases the possibility that the observations from real-life study are casual, and confers to such studies a key role in the next years to assess issues other than efficacy and safety, especially those scantly investigated thus far.

Background

The concept of real-life studies was introduced in the 1970s as an optional approach to laboratory studies (1), but in the following years the actual reference to be used was the randomized controlled trial (RCT), which was pioneered in the 1940s (2) and became the gold standard to establish the efficacy of a medical treatment, such as the "evidence". The basis of an RCT is the random allocation of patients participating to the trial to receive either the treatment under investigation or placebo (a treatment already demonstrated as effective may be also used). The double-blind fashion results in clear advantages in terms of minimization of causality and bias commonly affecting open studies. In 1998, a level II evidence was attributed to a single RCT and a level I (the highest) evidence was attributed to a systematic review of RCTs (3). However, the advantage of the rigid control and patients' selectivity in RCTs is counterbalanced by the unlikely applicability to patients managed in routine clinical practice, because the "highly selected population of RCTs only partially represents the real-life population" (4). This issue plainly concerns also allergen immunotherapy (AIT) for respiratory allergy, which has clear evidence of efficacy and safety as assessed by meta-analyses, but the validity and applicability of the observations resulting from RCTs data, especially in the context of real-life settings, is debatable (5). To confirm the applicability to common practice of AIT products demonstrated as effective in RCTs, real-life (also defined real-world) studies are needed. This model was increasingly used in recent years and, especially when based on large populations of patients, provides very useful data to optimize the prescription and the performance of AIT in clinical practice. Here, we will discuss the significance of the outcomes that were achieved in such studies.

Real-life studies on AIT

The first paper mentioning the term real-life in its title was published in 2004. This study assessed the treatment outcome in 192 patients with allergic rhinitis (AR) with or without asthma treated only with drugs, and in 319 patients treated with sublingual immunotherapy (SLIT) (6). The results showed that SLIT approximately halved the symptom-medication scores compared to the score registered in drug-treated patients. Since then, several real-life studies were performed, including 11 studies on SCIT (7-17), 15 on SLIT (6,18-29) and 5 on both (30-

34). **Tables I** to **III** show the main characteristics of these studies. We analysed the issues highlighted in real-life studies, which are represented by efficacy, safety and tolerability, quality of life, patient adherence and compliance to treatment, economic aspects, and physicians' prescription attitude.

Efficacy and safety of SCIT and SLIT

The major measures to assess efficacy of immunotherapy in RCTs are symptom and medication scores. Actually, in most real-life studies the major aim was efficacy assessment. In 11 studies (3 on SCIT, 7 on SLIT and 1 on both) only efficacy was evaluated, while in 3 studies (1 SCIT and 2 SLIT) also safety was evaluated. In other 2 studies, safety was the only object of assessment. Thus, a global number of 14 real-life studies on efficacy (4 on SCIT, 9 on SLIT and 1 on both) are available. In particular, two studies (1 on SLIT and 1 on SCIT and SLIT) included very large number of patients.
Zielen et al. performed a retrospective multiple regression analysis of data from a German prescription database consisting of 2851 patients treated with grass pollen SLIT tablets, and 71,275 control patients (25) in a time horizon of 7 years. As indicators, changes over time in symptomatic drug consumption after SLIT stopping, use of medications for asthma, and time of asthma onset in patients with AR were used. The results showed a significant difference in favour of SLIT 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. The authors overall conclusions highlighted that the treatment with grass pollen SLIT tablets results in better disease control and less frequent onset of asthma in patients with AR, as well as in slower disease progression in patients with asthma. The other large real-life study included, using the same German prescription database and a time horizon of 2-6 years, a retrospective cohort of 9001 patients treated with SLIT or SCIT for birch pollen-associated

Table I *- Real-life studies on subcutaneous immunotherapy.*

Author, year (ref)	Study population	Issue addressed	Results
Zeldin et al. 2008 (7)	133 pts of all ages	efficacy and safety	significant decrease of symptoms and medication scores, mild to moderate reactions in 8%
Petersen et al. 2010 (8)		willingness to pay	patients with allergy select themselves appropriately according to need
Petersen et al. 2013 (9)	248 pts of all ages	quality of life (QoL)	improvement of QoL and decrease of sick days
Pfaar et al. 2015 (10)	2927 children and adolescents	safety	local reactions in 16.3%, systemic reactions in 1.6%
El-Qutob et al. 2016 (11)	409 pts of all ages	efficacy by physician-completed visual analogue scale (VAS)	58.1% clinical improvement
Droessart et al. 2016 (12)	800 pts of all ages	SCIT efficacy compared to drug treatment after a 3- year course	persistent symptoms in 18% of SCIT vs. 51% of drug treated, drug use in 30% of SCIT vs. 61% of drug treated
Li et al. 2016 (13)	272 pts of all ages	efficacy by symptom severity scores and VAS after 12 months	significant improvement in symptom scores and VAS
Gelincik et al. 2017 (14)	204 adult pts	adherence to SCIT	87.3% of pts were considered adherent
Reiber et al., 2017 (15)	307 adult pts	tolerability	adverse events in 23.3% of pts (mild-moderate in 14.8%, severe in 8.5%)
Allen-Ramey et al. 2017 (16)	6710 pts of all ages	healthcare costs	continued SCIT use associated to lower costs (decreased emergency room visits, inpatient stays, decreased oral corticosteroid use) compared with early discontinuation
Yang et al. 2018 (17)	311 pts of all ages	adherence to a 3-year SCIT course	global adherence rate at year 3 64.6%; 19% of pts dropped out in year 1, 10% in year 2, and 6.4% in year 3; higher adherence in children

AR and asthma and 45,005 matched patients treated only with symptomatic drugs as controls. Six different birch or tree (hazel, alder) pollen extracts were prescribed, including SLIT drops, natural pollen SCIT and 4 SCIT allergoid preparations (36). The multiple-regression analysis showed that at completion of the 6 years follow-up 65.4% of AIT treated patients used no more symptomatic drugs for AR compared with 47.4% of controls ($p < 0.001$), and 49.1% of AIT treated patients used no more drugs for asthma compared with 35.1% of controls ($p < 0.001$). Also, the risk of new-onset asthma was significantly lesser in AIT treated vs. controls (odds ratio 0.83, $p = 0.001$). The very large number of patients analysed in these two studies ensures the reliability of the efficacy data supporting a major role of AIT in the treatment of patients with respiratory allergy. Concerning the safety, though the number of studies was low (2 studies with safety as the only object of assessment, 4 studies

Table II *- Real-life studies on sublingual immunotherapy.*

Author, year (ref)	Study population	Issue addressed	Results
Marogna et al. 2004 (6)	511 pts of all ages	SLIT efficacy compared with drug treatment	significant improvement of clinical scores in the SLIT group
Marogna et al. 2007 (18)	65 adult pts	duration of SLIT efficacy 7-8 years after its stopping	significant difference in symptom-medication scores compared with untreated pts
Sieber et al. 2010 (19)	1052 adult pts	efficacy and safety of high dose SLIT	consistent improvement in symptom and medication score, better results with ultra-rush schedule. Adverse events in 24% of patients during titration with no difference between schedules
Trebuchon et al. 2012 (20)	1289 pts of all ages	SLIT effectiveness on rhinitis and asthma, compliance	symptoms of rhinitis and/or asthma improved in 66% and 63% of pts, respectively, concomitant reduction in medication intake. Compliance 84%
Wessel et al. 2012 (21)	628 pts of all ages	SLIT safety during 3-year treatment with 1-grass tablet	reactions requiring SLIT discontinuation or symptomatic medication: 15 (14 at initiation and 1 at reintroduction); mild- moderate reactions in 46.2%, 14.4% and 1.8% of pts, during the 1st, 2nd and 3rd year of SLIT, respectively
Pastorello et al. 2013 (22)	47 pts of all ages	SLIT efficacy in pts unresponsive to drugs	significant decrease of mean medication score (from 4.2 ± 1.3 before to 2.4 ± 2.0 after SLIT); significant increase in patient satisfaction after SLIT
Shah-Hosseini et al. 2015 (23)	1408 pts of all ages	SLIT effectiveness and safety	significant reduction of symptoms (49.9%) compared with the preceding pollen; mild-moderate reactions in 15.3% of pts
Zielen et al. 2018 (24)	2851 SLIT treated and 71275 untreated pts	SLIT long-term efficacy, asthma onset in pts with rhinitis	medication for rhinitis 18.8% lower and medication for asthma 16.6% lower in SLIT treated at cessation; asthma onset significantly less frequent ($p = 0.002$) in SLIT treated
Janson et al. 2018 (25)	207 adult pts	three-year completion of SLIT	55% of pts completed the SLIT course, 24% were still on treatment, 22% discontinued prematurely; asthma improvement twice as common among pts who completed
Schafer et al. 2017 (26)	253 adult pts	SLIT efficacy and patient's satisfaction	significant improvement of symptoms from baseline, reduced need for medications, good satisfaction
Novakova et al. 2017 (27)	191 adult pts	QoL after a 3-year course of SLIT	significant improvement of QoL compared with baseline
Nadir Bahceciler et al. 2017 (28)	90 children	steroid sparing effects in children with asthma	inhaled corticosteroids avoided in 70% of children; significantly higher avoidance in longer SLIT duration
Kiotseridis et al. 2018 (29)	399 pts of all ages	adherence and QoL in a 3-year course of SLIT	55% of pts completed the SLIT course; improvement of QoL at study end

Table III *- Real-life studies on subcutaneous and sublingual immunotherapy.*

Author, year (ref)	Study population	Issue addressed	Results
Schmidt et al. 2015 (30)	118,754 pts of all ages with allergic rhinitis but without asthma	risk of developing asthma	newly diagnosed asthma in 1.4% of pts treated with AIT for 3 or more years
McDonell et al. 2015 (31)	18,805 pts of all ages	AIT prescription for grass pollen allergy	SCIT is the preferred AIT in Germany but there was a marked increase in prescription of SLIT tablets
Schwanke et al. 2017 (32)	105 adult pts	QoL at initiation of AIT and after 6 d 12 months	improvement of QoL with both SCIT and SLIT but statistical significance only with SCIT
Wahn et al. 2017 (33)	1029 pts of all ages	AIT prescription in polysensitized pts	98% of physicians prescribed SCIT or SLIT, 58% with single-allergen and 42% with multiple-allergens; 74% of them were aware of latest AIT guidelines
Musa et al. 2017 (34)	236 adult pts	compliance at 3-year course of AIT	compliance of 58.7% with SCIT, 11.6% with SLIT
Wahn et al. 2018 (35)	9001 SLIT treated and 45,005 matched untreated pts	SLIT long-term efficacy, asthma onset in pts with rhinitis	significantly reduced AR and significantly decreased risk of new-onset asthma

analysing both efficacy and safety) the overall population investigated included 6148 patients. Such figure guarantees the reliability of the observations that indicate a very good profile of safety in real-life conditions. In fact, most adverse reactions, which ranged from 16.3% to 49.9% in the different studies, concerned local reactions in the oral mucosa, while systemic reactions were rare (7,10,19,21-23). Of interest, no fatal anaphylactic reactions to SCIT were reported. Such reactions have been a critical issue in the past, but the identification of the major risk factors, the highest being associated to the presence of uncontrolled asthma at the moment of the allergen extract injection, made the occurrence of anaphylactic reaction very rare (37). Based on the data from the available studies, it is likely that precautions to prevent anaphylaxis are adopted also in real life.

Other issues investigated

A single study evaluated as a measure of efficacy the steroid sparing effect of SLIT. This issue was previously explored concerning anti-asthmatic drugs, such as montelukast (38) also in controlled trials of allergen immunotherapy (39). The study by Nadir Bahceciler et al. evaluated 90 monosensitised or polysensitised children with asthma treated with single or 2-simultaneous and multiple-pollen-mix allergen SLIT, which resulted in 70% avoidance of inhaled corticosteroids. No significant difference was detected between mono- and poly-sensitized children. The rates of avoidance in mono-allergen, pollen-mixture and 2-simultaneous-allergen SLIT were 93.6, 83.3 and 73.7%, respectively. A significantly higher avoidance ($p = 0.0001$) was observed in children with longer-duration SLIT (28).

Another aspect evaluated in a single study was the ability to prevent the development of asthma in subjects treated for AR. The data were obtained from German National Health Insurance based on a cohort of 118,754 patients with rhinitis but without asthma, who were stratified to received AIT (SCIT or SLIT) or only drugs. In the 2431 AIT treated patients, a new asthma diagnosis was done in 1.4% of subjects, with a risk of asthma was significantly lower in AIT treated (risk ratio 0.60; 95% CI, 0.42-0.84) compared with patients treated only with drugs (31).

The other topics were addressed in multiple studies. The most investigated was adherence and compliance, which was the subject of 5 studies (15,18,26,30). In a short-term study on SCIT, 87.2% of patients were considered adherent (15), while in a 3-year study the adherence at the last year was 64.66% (18). In the two 3-years studies on adherence to SLIT, the same outcome was reported, 55% of patients completing the entire treatment (26,30). The only study comparing SCIT to SLIT reported a compliance rate of 58.7% in SCIT treated and 11.6% in SLIT treated patients (34). Except the first SCIT study, the rate of adherence in real-life is apparently lower than reported in controlled trials, but this is not surprising, based on the much more stringent criteria used to monitor the patients recruited in trials (40). The effects on quality of life (QoL) were analysed in 3 real-life studies. The first was a

prospective assessment of 248 patients with AR on the changes in QoL measured by the disease specific Rhino-conjunctivitis Quality of Life Questionnaire (RQLQ) after a SCIT course. The mean RQLQ-score significantly reduced from 3.02 at baseline to 2.00 at follow-up (9). Novakova et al. prospectively evaluated by RQLQ 191 adult patients with moderate to severe mite-induced or grass pollen-induced undergoing SLIT for 3 years. The mean RQLQ score decreased significantly from baseline to end of treatment for both mite (from 2.95 to 0.76) and grass pollen (from 2.83 to 1.22) SLIT (28). The study by Schwanke et al. used the same questionnaire to compare the variations of QoL in 29 SCIT treated and 11 SLIT treated patients with respiratory allergy from initiation to 6- and 12-months immunotherapy. In both groups of patients there was an improvement in QOL, but the change in the RQLQ score from both baseline to 6 months and baseline to 1 year was significant only in the SCIT group ($p = 0.002$). After 1 year of treatment, both SCIT and SLIT achieved the minimally important difference from baseline in the overall RQLQ score (33).

Economic aspects have increasing importance in any medical treatment. The first real-life study was limited to assessing the willingness to pay for SCIT in patients with respiratory allergy, concluding that subjects with allergy select themselves appropriately according to need and not to other characteristics, such as income or education (8). The more recent study by Allen-Ramey et al. evaluated medical and pharmacy claims from a US Database from January 2009 through February 2014 for adults and paediatric patients with more than 7 or less than 7 injection visits for SCIT within 60 days from starting (17). Each cohort included 6710 patients. Patients receiving more than 7 injections (continuers) used significantly less oral corticosteroids than patients receiving less than 7 injections (27.7% vs. 29.6%, $p = 0.018$). Other significant differences in favour of continuers included less respiratory-related emergency room visit, less outpatient visits in front of higher mean total rhinitis-related costs compared with discontinuers ($ 1918 vs. $ 646, $p < 0.001$). However, when adjusted with a generalized linear model, these costs were significantly lower among continuers ($p < 0.001$).

Lastly, two studies addressed in large populations the physicians prescribing attitudes. The first study, including 18,805 patients, reported that SCIT is the preferred AIT for grass pollen allergy in Germany, though a marked increase in prescription of SLIT occurred when sublingual tablets were made available (31). The other study estimated the AIT prescription in 1029 polysensitized patients. SCIT or SLIT were prescribed by 98% of physicians, using single allergens in 58% and multiple allergens in 42% of cases. The awareness of the updated AIT guidelines was ascertained in 74% of physicians (34).

Conclusions

AIT has received full evidence of efficacy and safety by a number of meta-analyses of placebo-controlled trials. Limiting the examination to the more recent meta-analyses, the evidence concerned both SCIT and SLIT. The analysis by Calderon et al., comprising 51 trials on SCIT in patients with AR, resulted in a highly significant reduction of symptoms and medication scores in active treatment ($p < 0.00001$ in both parameters), while severe systemic reactions requiring adrenaline occurred in 0.13% of patients (41). Radulovic et al. included 49 trials on SLIT in patients with AR: the same level of statistical significance ($p < 0.00001$) was detected for symptoms and medication need, along with the absence of severe systemic reactions requiring adrenaline administration (42). Dhami et al. performed a systematic review and meta-analysis including 89 trials (54 SCIT, 34 SLIT and 1 both treatments) on the efficacy of AIT in allergic asthma. Short-term symptom scores and medication scores were reduced, as shown by a standardized mean difference (SMD) of -1.11 (95% CI -1.66, -0.56) and -1.21 (95% CI -1.87, -0.54), respectively, though with potential publication bias. AIT resulted in a "of adverse events, systemic reactions being more frequent with SCIT but with no fatalities (43). This suggests that AIT in its two routes of administration is clearly indicated as an effective treatment in patients with AR or asthma. However, as hinted above, the efficacy and safety assessed by meta-analyses of rigidly controlled RCTs is unlikely applicable to common clinical practice, the average patient easily lacking the characteristics to be included in a trial. Thus, real-life studies are essential to favour the appropriate choices in daily practice in patients with respiratory allergy. A central aspect is represented by the number of patients: in the meta-analysis of 89 trials on the efficacy of SCIT and SLIT in asthma, a total of 7413 patients were enrolled, resulting in an average number of 83 patients per trial. Instead, in the 13 real-life studies on AIT efficacy available until now, the total of patients amounts to 9090, with an average number of 699 patients per study. This significantly decreases the possibility that the observations from real-life study are casual, and confers to such studies a key role in the next years to assess issues other than efficacy and safety, especially those scantly investigated thus far. Still, the real-life model has its pitfalls. For example, the lack of inclusion and exclusion criteria may result in marked differences in the proportion of patients in the groups to be compared (in the study by Zielen et al. the rate of patients in pediatric age was 48.6% in the SLIT group and 7.5% in the "non-AIT" group) (25).

Conflict of interest

The authors declare that they have no conflict of interest

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Venom Immunotherapy: a 20-year experience with an ultra-rush protocol (210-min)

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Summary
Background. *Ultra-rush (UR) are induction protocols used in venom immunotherapy (VIT).* **Objectives.** *To evaluate the adverse reactions during a 210-minutes UR and determine possible risk factors.* **Methods.** *Retrospective study of 129 patients submitted to UR with VIT in the last 20 years.* **Results.** *In 114 (88.4%) patients, the 101.1 µg maintenance dose was reached in 210 minutes. Systemic reactions (SR) occurred in 22% of patients (71% mild). There were no severe SR, late reactions or fatalities. Adrenaline was administered in 10% of all UR. The SR were more frequent with honey bee VIT and had greater severity in the patients with a previous severe systemic sting reaction. No significant difference in the risk of SR was found with other demographic, clinical or laboratory factors. There were 5% of large local reactions (LLR), these being more frequent in females.* **Conclusion.** *Most SR during UR were mild with no need for adrenaline treatment. The honey bee venom and the severity of the anaphylaxis during the field sting were the only SR's risk factors for systemic adverse reactions during the UR.*

Introduction

It is estimated that between 56.6% to 94.5% of the general population has been stung, at least once in life, by a Hymenoptera (1,2). In Portugal, the most common Hymenoptera are the honey bee (Apis mellifera), the wasp (Vespula species) and the paper wasp (Polistes dominula).

Hymenoptera stings can be associated with local or systemic reactions (2,3). Local reactions that have a diameter greater than 10 cm and are maintained for a period of more than 24 hours, are called large local reactions (LLR) (1,2). The systemic reactions include anaphylactic, toxic and uncommon reactions (1,2). The most common reactions after Hymenoptera stings are LLR and systemic anaphylactic reactions (1,4,5).

Venom immunotherapy (VIT) is well recognized as the most efficient treatment to prevent further Hymenoptera systemic allergic reactions (1), being associated with a long-term protection in 85-90% of cases (1,6,7).

VIT is usually administered by subcutaneous injections with aqueous extracts and comprises an induction phase and a maintenance phase necessary to ensure a sustained effect over time (1). Since its development, several induction protocols have been proposed for VIT. These protocols differ from one another in the time required to reach the maintenance dose and in the interval between the injections (8,9,10). The risk of a new systemic sting reaction implies the need for the patient to reach the protection dose as quickly as possible. Thus, slow, conventional protocols with an induction period of 4 to 6 months and intervals between doses of 3 to 7 days have been progressively replaced by faster protocols (11,12). The latest include cluster or *rush*-modified protocols in which induction lasts generally 6 weeks (administration of 2 injections separated for 30 minutes every 3 to 7 days), *rush* protocols in which the induction lasts less than a week and the *ultra-rush* (UR) protocols in which the induction may last from 120 minutes to 2 days with doses being administered at intervals ranging from 20 minutes to 2 hours (11,12,13).

UR protocols allow a complete and rapid desensitization with a smaller number of injections and hospital visits. In these protocols the protective dose of 100 µg venom is reached in a quicker way, decreasing the potential risk of an anaphylactic sting reaction (1,8,14). Over the years, several studies have shown that this induction protocol is safe and effective (15,16,17,18). Nevertheless, there are still some concerns related to the occurrence of severe systemic reactions during the UR protocols.
The objective of the present study is to evaluate the frequency of local and systemic reactions associated with a 210-minute UR protocol with VIT and to identify possible risk factors for these reactions.

Materials and methods

Population

Retrospective study of 129 patients submitted to VIT using a 210-minutes UR protocol, from June 1998 to June 2018, in an Immunoallergology Department. Demographic, clinical and laboratory data were collected from the patients' file. VIT was prescribed to patients with a previous history of immediate systemic reaction after a Hymenoptera sting and sensitization to at least one of these venoms demonstrated by skin tests and/or specific IgE measurement, according to the criteria established by EAACI (1). The severity of the sting reactions was classified according to Mueller (19).
A written informed consent was obtained from all patients and/or their legal representatives before their diagnostic and therapeutic evaluation. Data were anonymized, and their confidentiality guaranteed, and this study protocol was approved by the Ethical Board of Centro Hospitalar Universitário de Lisboa Norte.

Skin tests

The skin tests with Apis mellifera, Vespula spp. and Polistes spp. venoms were performed with Stallergenes® or Bial-Aristegui / Roxall® extracts, at least three weeks after the last sting reaction (1). The skin prick tests were performed using a 100 µg/mL concentration and with 0.9% NaCl as negative control and 10 mg/ml histamine as a positive control. The intradermal tests were performed with increasing concentrations from 0.001 to 1 µg/ml and with a negative control (20).

Lab results

Specific IgE for Apis mellifera, Vespula spp. and Polistes spp. were determined in the sera of the patients by ImmunoCAP, Thermo Fisher Scientific (Uppsala, Sweden). All results > 0.35 kUL were considered positive. Basal serum tryptase was also determined and a tryptase value of < 11.4 ng/mL was considered normal.

Venom immunotherapy ultra-rush protocol

The induction protocol used was the 210-minute UR proposed by Birnbaum (21). In this, a cumulative dose of 101.1 µg, divided by 6 injections, is given as follows: an initial dose of 0.1 µg, followed by 1, 10 and 20 µg at 30-minute intervals. Then 30 and 40 µg were given every 60 minutes. The maintenance dose of 100 µg was repeated 15 days after the UR and administered at 4-6-week intervals over a period of 3 to 5 years, as established in the EAACI guidelines (1).
All injections were given by trained medical personnel in an Immunoallergology Day Hospital, equipped with material for the treatment of anaphylactic reactions. All patients had a venous access with saline during the procedure. Heart rate, blood pressure and peripheral oxygen saturation were continuously monitored. Patients received pretreatment with oral H1 antihistamine (cetirizine 10 mg, ebastine 10 mg or other equivalent 2nd generation H1 antihistamine) in the 2 days prior to UR and in the morning of the UR.
Therapy with ACE inhibitors or with cardio-selective beta blockers in patients with stable cardiovascular disease is continued during UR and VIT.

Classification of adverse reactions

Systemic reactions in UR were stratified according to the Mueller classification (19) and treated with intravenous corticosteroids, antihistamines and, if necessary, with intramuscular adrenaline. The UR was not finished in patients with systemic grade III or IV adverse reactions and in patients with grade I or II systemic reactions that had an unsuccessful response to its treatment. Regarding local adverse reactions, only LLR (mean diameter > 10 cm) were considered. All local reactions were treated with ice, topical corticosteroids and oral antihistamine.

Statistical analysis

Statistical analysis was performed using version 24 of SPSS® software for Windows (SPSS Inc., Chicago, Ill). The median value and the first and third quartiles [Q1, Q3] are presented for the results. The Chi-square test or the Mann-Whitney U test were used to calculate differences between variables and p values < 0.05 were considered statistically significant.

Results

Characteristics of the population

In the last 20 years, 129 patients (73% men, median age 42 years, minimum age 10 years, maximum age 74 years) were submitted to VIT with a 210-minutes UR protocol in our Immu-

noallergology Department. Nine of these were under 18 years of age (90% boys, minimum age 10, median age 16 years).
All patients had a previous history of anaphylaxis after insect sting (29% grade III and 34% grade IV). Demographic and clinical data and the results of in vivo and in vitro diagnostic tests are summarized in **table I**. None of the patients from our population had mastocytosis or any mast cell disorder. A total of 96 patients (74.4%) received VIT with honey bee, 19 (14.7%) with wasp and 14 (10.9%) with paper wasp. UR was performed with aqueous extracts purified from Hymenoptera venom (97% produced by Stallergenes® or Bial-Aristegui / Roxall®).

Adverse reactions during the ultra-rush protocol

From a total of 129 patients who underwent UR, 114 (88.4%) achieved a dose of 101.1 µg and 94 (72.9%) did not present any systemic reaction or large local reaction during the protocol (**table II**). There were no fatalities or late reactions.

Table I *– Demographic and clinical characterization of the studied population*

	Honey bee	**Wasp**	**Paper wasp**	**Total**
Patients - n (%)	96 (74.4)	19 (14.7)	14 (10.9)	129 (100)
Age - $\tilde{x}$ [Q1;Q3]	41 [30.3; 55.5]	52 [35; 57]	43.5 [31.8; 57.3]	42 [31; 56.5]
Age group (≤45/>45) – n (%)	56 (58.3)/ 40 (41.7)	7 (35.8)/ 12 (63.2)	7 (50)/ 7 (50)	70 (54.3)/ 59 (45.4)
Gender				
Male - n (%)	72 (75)	10 (52.6)	12 (85.7)	94 (72.9)
Female - n (%)	24 (25)	9(47.4)	2 (14.3)	35 (27.1)
Atopy - n (%)	37 (38.5)	6 (31.6)	5 (35.7)	48 (37.2)
Asthma - n (%)	11 (11.5)	1 (5.3)	1 (7.1)	13 (10.1)
Cardiovascular disease- n (%)	14 (14.6)	4 (21.1)	3 (21.4)	21 (16.3)
Beekeeper - n (%)	66 (68.8)	-	-	66 (68.8)
Beekeeper direct family member - n (%)	16 (16.7)	-	-	16 (16.7)
Severity of anaphylactic reaction after Hymenoptera sting – n (%)				
Grade I	9 (9.4)	3 (15.8)	1 (7.1)	13 (10.1)
Grade II	32 (33.3)	1 (5.3)	2 (14.3)	35 (27.1)
Grade III	27 (28.1)	5 (26.3)	5 (35.7)	37 (28,7)
Grade IV	28 (29.2)	10 (52.6)	6 (42.9)	44 (34.1)
Use of adrenaline after Hymenoptera sting				
Yes - n (%)	31 (39.7)	6 (46.2)	1 (10)	38 (37.6)
Basal tryptase (ng/mL) - $\tilde{x}$ [Q1;Q3]	3,6 [2.3; 5.7]	5.4 [2; 9.5]	3.3 [1.8; 5.6]	3.6 [2; 5.8]
Apis mellifera sIgE (kU/L) - $\tilde{x}$ [Q1;Q3]	11.5 [3.9; 31.2]	-	-	-
V*espula spp*. sIgE (kU/L) - $\tilde{x}$ [Q1;Q3]	-	8.1 [1.2; 21.4]	-	-
Polistes spp. sIgE (kU/L) - $\tilde{x}$ [Q1;Q3]	-	-	8.7 [1.7; 21.7]	-
Positivity of Hymenoptera venom skin tests - n (%)				
Skin prick tests	12 (9.3)	0	1 (0,8)	13 (10)
ID 0.001 µg/mL	12 (9.3)	2 (1.6)	0	14 (10.9)
ID 0.01 µg/mL	35 (27.1)	9 (7)	8 (6.2)	52 (40.3)
ID 0.1 µg/mL	25 (19.4)	5 (3.9)	3 (2.3)	33 (25.5)
ID 1 µg/mL	10 (7.9)	2 (1.6)	1 (0.8)	13 (10)

Abbreviations: ID – Intradermal tests, $\tilde{x}$ – Median; sIgE – specific IgE

Table II shows that during the UR, 28 patients (27 with honey bee venom and 1 with paper wasp venom) had a systemic reaction. Considering the percentage of systemic reactions according the type of venom that was administered, 28% of patients submitted to honey bee venom UR protocol had a systemic reaction, 7% of those who received an UR protocol with paper wasp had a systemic reaction and there were no systemic reactions among the patients with wasp venom UR protocol.
Although most of the 28 systemic reactions were mild (71% grade I or II), 13 patients received adrenaline and one patient was admitted for surveillance. Systemic reactions were more frequent with 20 µg or higher venom doses ($p < 0.05$) (**table II**). Systemic reactions were more frequent in patients submitted to VIT with honey bee venom ($p = 0.003$).
There were no significant differences between the occurrence of systemic reactions and the patient's gender ($p = 0.85$), personal history of atopy ($p = 0.8$), asthma ($p = 0.10$) or cardiovascular disease ($p = 0.7$). Regarding age, we stratified patients in two age groups (≤ 45 years or > 45 years) and we did not find any difference between the age group and the development of systemic reactions during VIT ($p = 0.44$). Being a beekeeper ($p = 0.15$) or a direct family member of a beekeeper ($p = 0.8$) did not increase the frequency of systemic reactions. Different vaccine manufacturer also did not influence the frequency of systemic reactions ($p = 0.6$).
However, we found that severity of the reactions during the UR protocol was worse in patients who had a previous history of a severe anaphylactic reaction after insect sting ($p < 0.05$) (**table III**).
No statistically significant relationship was found between the existence of systemic reaction during UR and patients' baseline tryptase values ($p = 0.8$) or the venom concentration that elicited a positive response in the skin tests ($p = 0.6$).
Fifteen patients did not reach the cumulative dose of 101.1 µg on the UR protocol day (8 with grade III systemic reactions and 7 with grade II systemic reaction and unsuccessful response to the treatment). In all, UR was repeated 15 days after a reinforcement in premedication (30 minutes before starting the UR: clemastine 2 mg i.v. and hydrocortisone 100 mg i.v.). In the second UR, 10 of the 15 patients successfully completed the protocol without systemic reaction. The remaining 5 patients were included in other induction protocols.

Table II *- Systemic and local adverse reactions.*

	Honey bee	**Wasp**	**Paper wasp**	**Total**	**p-value**
Patients - n	96	19	14	129	-
Adverse reactions (locals and systemic) - n (%)	31 (32.3)	2 (10.5)	2 (14.2)	35 (27.1)	-
Systemic reactions - n (%)	27 (28.1)	0 (0)	1 (7.1)	28 (21.7)	0.003
Grade I	4 (14.8)	0 (0)	0 (0)	4 (14.2)	
Grade II	15 (55.6)	0 (0)	1 (100)	16 (57.1)	
Grade III	8 (29.6)	0 (0)	0 (0)	8 (28.6)	
Grade IV	0 (0)	0 (0)	0 (0)	0 (0)	
Reactions that required adrenaline - n (%)	12 (12.5)	0 (0)	1 (7.1)	13 (10)	-
Rate of systemic reactions per injection - %	6	0	1.2	5.4	-
Dose administered when the systemic reaction occurred - n (%)					
0.1 µg	0 (0)	0 (0)	0 (0)	0 (0)	-
1 µg	0 (0)	0 (0)	0 (0)	0 (0)	
10 µg	4 (14.8)	0 (0)	0 (0)	4 (14.2) **[4 GI]**	
20 µg	14 (51.9)	0 (0)	0 (0)	14 (50) **[7 GII; 7 GIII]**	
30 µg	4 (14.8)	0 (0)	0 (0)	4 (14.2) **[4 GII]**	
40 µg	5 (18.5)	0 (0)	1 (100)	6 (21.4) **[5 GII; 1 GIII]**	
Large local reactions (LLR) - n (%)	4 (4.2)	2 (10.5)	1 (7.1)	7 (5.4)	0.4

Abbreviations: GI-Grade I systemic reaction, GII – Grade II systemic reaction, GIII- Grade III systemic reaction – according to Muller et al.19

There were 7 LLR (**table II**): 4 with honey bee venom, 2 with wasp and one with paper wasp. From these, one reaction was identified after the administration of the 30-µg and the remaining after the 40-µg injection. All patients with LLR finished the 210-minutes UR in the first attempt. These reactions were more frequent in females (p = 0.02). We did not find any other factor that had a significant association with the occurrence of LLR.

Discussion

This study is a retrospective survey of the last 20 years of all patients undergoing VIT using a 210-minutes UR protocol. From a total of 129 patients included, 114 (88%) achieved the dose of 101.1 µg in the planned period of time, while 15 (12%) patients did not complete the UR in the first attempt. Of these, two thirds (n = 10) reached the cumulative dose of 101.1 µg in a further UR, 15 days later, with a premedication reinforcement and using the same UR protocol.

In contrast to other studies published to date (16,17,21,22) our study has much more patients submitted honey bee VIT, and also 13 patients that underwent an UR protocol with paper wasp venom. These are particular aspects related to the environmental exposure to insects' stings in our country.

The UR protocols allow a rapid desensitization and are associated with fewer injections and hospital visits. Additionally, they are associated with a reduced risk of anaphylaxis in case the patient is re-stung before reaching the protective dose (1,10,14). Despite being safe, these fast protocols are not totally risk free, as they can be associated with systemic and/or local adverse reactions.

Regarding the systemic reactions, we documented a frequency of systemic reactions of 22%, most of them being mild. We also found that only 10% of all our patients were treated with adrenaline during the protocol, and no fatalities were reported. The frequency of systemic reactions during VIT UR protocols (≤ 210 minutes) reported in previously published studies ranges from 0% to 30% (14,21,22,23).

Our results are similar to the ones reported by Birnbaum et al (21), who documented a frequency of systemic reactions of 30% in a subset of patients treated with honey bee venom and of 6.1% in patients treated with wasp venom, using a 210-minutes UR protocol. Roll et al (22), on the other hand, reported a lower frequency of systemic reactions. These authors analyzed 67 patients (total of 80 UR procedures) that received VIT with honey bee or wasp venoms and found a total of 12.5% of systemic adverse reactions (bee 5% and wasp 7.5%). Although, in this study (22), the overall percentage of adverse reactions is inferior to ours, the number of reactions in patients treated with wasp VIT is higher than the number of reactions that occurred in patients treated with honey bee VIT. This data is not in agreement to what is published in most studies where bee venom alone is a risk factor for systemic reactions (1,24,25,26). An explanation for this difference in systemic reactions between honey bee and wasp VIT could be the degree of purity of the Hymenoptera venom vaccines. It has been demonstrated through laboratory studies that honey bee vaccines have a lower concentration of non-allergenic proteins, whereas in the wasp vaccines the allergenic proteins are diluted with non-allergenic proteins from the venom bag (11). Several studies have been developed in order to identify possible clinical or laboratory risk factors for the occurrence of systemic adverse reactions (24,26).

In our study, in addition to the venom administered, we verified that there is a relationship between the severity of anaphylaxis after honey bee sting and the severity of the systemic reaction during UR with honey bee venom (p = 0.04). Birnbaum et al. (24) also demonstrated that patients who had grade III or IV anaphylaxis after Hymenoptera sting more frequently devel-

Table III - *Relation between the severity of the reaction after Hymenoptera sting and the severity of anaphylactic reaction during VIT-UR.*

Severity of the reaction@ after insect sting/ Severity of the reaction @during VIT-UR*		**Severity of the reaction after insect sting**			
		Grade I (n=13)	Grade II (n=35)	Grade III (n=37)	Grade IV (n=44)
Severity of the reaction during the VIT-UR	**Without reaction (n=101)**	13/11	35/25	37/30	44/35
	Grade I	13/1	35/3	37/0	44/0
	Grade II	13/1	35/7	37/2	44/6
	Grade III	13/0	35/0	37/5	44/3
	Grade IV	13/0	35/0	37/0	44/0

*p-value <0.05

oped a grade III or IV systemic reaction during the UR. This data may point out to the need of a reinforcement in premedication before UR in the subset of patients with a previous history of more severe systemic reaction after insect sting.

We did not find any relationship between the occurrence of systemic reactions and the patients' age (p = 0.44), gender (p = 0.85), personal history of atopy (p = 0.8), asthma (p = 0.1) or of cardiovascular disease (p = 0.7). Besides this, no statistically significant relationship was found between the existence of systemic reactions during the UR protocol and the results of the skin tests (p = 0.6) or the level of the baseline tryptase (p = 0.8). These data are in line with previous reports (5,24,25,27,28).

Regarding LLR, we also did not find any significant association between their occurrence and the patients' age, previous history of atopy, asthma or cardiovascular disease or, also, with the type of venom administered to the patient. However, as previously reported (20,29), we found that LLR were more frequent in females (p = 0.02). However, there were only 7 LLR reactions which limits interpretation of these results.

Another aspect that deserves mention is the fact that 10 of the 15 patients who did not complete the UR in a first attempt, achieved the 101.1 µg dose in a second UR session, which increased our success rate to 96%. It was not possible to identify any risk factors that led to the failure of the first UR in these patients.

Conclusion

In conclusion, over the past 20 years, in our Immunoallergology Department, 129 patients underwent a 210-minutes VIT UR protocol, with an overall completion rate of 96%, considering the 114 patients that completed VIT UR at a first attempt and the 10 patients that completed it at a second attempt with premedication reinforcement. This protocol was carried out in the Day Hospital and was performed by trained medical staff with quick access to the necessary equipment for the treatment of an anaphylactic reaction. UR protocols allow a quicker achievement of the protective dose; however, it is not a risk-free procedure. In our study, we documented a frequency of 22% of systemic reactions, most of them being mild and without need for adrenaline. The only predictive factors for a systemic reaction that we found in our study were the use of honey bee venom and the severity of systemic insect sting reaction.

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Conflict of Interest

The authors declare that they have no conflict of interest

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A successful topical treatment for cutaneous inflammatory diseases: an additional or alternative therapy to topical steroids

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To the Editor

Topical steroids are commonly used in several cutaneous pathologies, such as psoriasis, atopic dermatitis, irritative and allergic contact dermatitis, particularly in acute phase and often for long periods. However, topical corticosteroids can be associated with adverse effects, such as acneiform lesions, erythema, teleangiectasias, hypertrichosis, perioral dermatitis, photosensitivity and burning sensation (1).

The goal of our study was to find an alternative non-steroidal anti-inflammatory treatment for the management of cutaneous diseases. We selected 50 patients to test our non-steroidal inflammatory topical product. The patients were affected by mild or moderate psoriasis, atopic dermatitis and irritative contact dermatitis.

The patients were not performing neither systemic nor topical treatment at the moment of our observation. The age of our patients (both male and female) was between 20 and 65.

Exclusion criterion was a proven allergy to one of the three components of the cream: hemp seed oil, macadamia oil, rosa mosqueta oil.

The patients applied 1 ml of the cream, twice a day, on the inflammated lesions. Patients came to control visit after 1, 3, 7 and 12 weeks. The evaluation of the treatment efficacy has been estimated with a three-levels score: complete healing, partial healing (using %) with description, absence of visible effect.

The emollient and hydrating cream of the study was composed by water, cetearyl alcohol, cannabis sativa seed oil, rosa moschata seed oil, macadamia ternifolia seed oil, panthenol, propylene glycol, hydrolyzed glycosaminoglycans, proline, magnesium aspartate, citric acid, disodium EDTA, cetrimonium chloride, 2-bromo-2-nitropropane-1,3 diol, phenoxyethanol.

After 12 weeks, we observed a complete healing with *restitutio ad integrum* in 30 patients (**figure 1** and **2**). Among this group, one patient was affected by radiodermitits and another one by ex-

Figure 1- *Bilateral, erythematous and squamous lesions of the palms.*

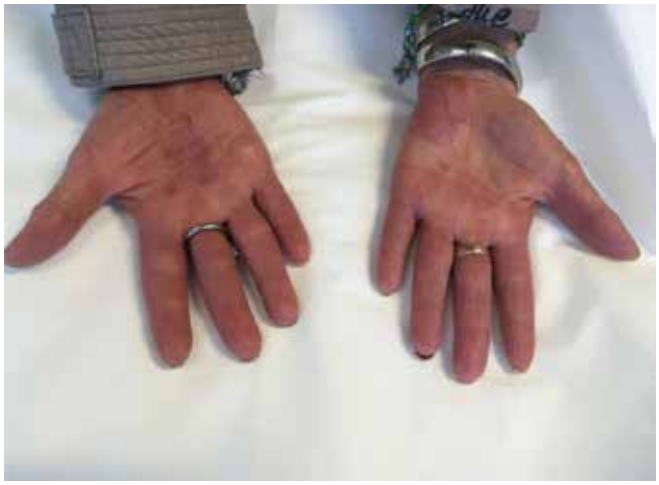

Figure 2 - *Cutaneous aspect after 12-week topical therapy.*

tramammary vulvar Paget. Both showed complete healing using our cream. In the other 20 patients, we revealed a partial healing. According to the guidelines, steroid creams should be used for limited periods to avoid the onset of side effects (1).

The cream of our study was composed by three different oils: cannabis sativa seed oil, rosa moschata seed oil and macadamia ternifolia seed oil. These oils are composed by omega-6 and -3 fatty acids, respectively linoleic acid and α-linolenic acid. Furthermore, it contained tocopherol, carotenoids and vitamin A, which have natural antioxidant action.

Cutaneous diseases, such as psoriasis and atopic dermatitis, are characterized by an alteration of the skin barrier, which is normally constituted by ceramides, free fatty acids and cholesterol. For this reason, the use of our product, containing essential fatty acids, has been helpful for the reconstitution of the cutaneous barrier.

Moreover, the components of our cream had also an anti-inflammatory effect. Indeed, essential omega-3 fatty acid has anti-inflammatory and immune-modulating actions and can be helpful in various conditions: atherosclerosis, blood pressure alterations, platelet function alterations, rheumatoid arthritis and cutaneous diseases (2).

These fatty acids are capable of partly inhibiting many aspects of inflammation, including leucocyte chemotaxis, adhesion molecule expression, and leucocyte-endothelial adhesive interactions and production of pro-inflammatory cytokines (3).

In conclusion, our cream showed anti-inflammatory actions and could reintegrate the cutaneous barrier. For this reason, it could be a valid substitute to topical steroids in case of *mild or moderate* cutaneous lesions. It could be also useful as a co-adjuvant approach in case of patients who are performing a topical or systemic steroid treatment. Lastly, this cream could represent an additional therapy in case of cutaneous lesions, such as radiodermitits or Paget's (4).

Conflict of interest

The authors declare that they have no conflict of interest

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Methylchloroisothiazolinone / methylisothiazolinone: epidemiological retrospective study

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To the Editor

Allergic contact dermatitis (ACD) is a common problem confronted in clinical practice and several authors have started to point to MCI/MI as an emerging allergen primary present in many daily cosmetic (1,2) since late 80s. The present study was carried out to described the epidemiological characteristic of patients with ACD to MCI/MI during a 10 years period. We want to elucidate factors associated and identify the clinical and epidemiological features of patients with ACD to MCI/MI.

During a period of ten years, every adult patient with signs or symptoms of eczema attending our Contact Dermatitis Department, located in the University Hospital Virgen del Rocío (Seville, Spain), were recruited. They underwent a clinical history, dermatological exploration and patch test with the standard series of the Spanish Contact Dermatitis and Skin Allergy Research Group (GEIDAC) (3). All patients were tested with certified allergen supplied by True Test® (TRUE test; Mekos Laboratories ApS, Denmark). Patches were applied to the upper back using Scanpore® and left on for 48 h; then read on day D2 (48 h), and D4 (96h). We considered positive responses those in which erythema, infiltrated papules or vesicles were detected. All patients' personal data were firstly anonymized and manually entered into a computerized database (Microsoft Excel 2011 for Mac, version 14.0.0). Data were analysed with the Statistical Package for Social Sciences (SPSS) program 25.0; several statistical analyses were performed when needed (chi-square test), and $p < 0.05$ was considered statistically significant. This study has been prepared respecting the fundamental principles established in the Declaration of Helsinki (1946). The research project has undergone evaluation by the Committee of Ethics and research clinic of our University Hospital and all patients have been informed about the project.

A total of 142 patients had a positive patch test reaction to MCI/MI. The allergic contact dermatitis to MCI/MI diagnosis was based on medical history and a relevant patch test reaction, which was found in 118 patients, representing 8% of the total.
The age range of patients was from 14 to 89 years (mean age 51 years); 58.5% of the patients were males (n = 69) and 41.5% (n = 49) were females. By age group, sensitization to MCI/MI was more frequent in adults over 40 years old (79.6%). The most frequent location was hands (62.9%, n = 73), followed by diffuse involvement of different body parts (16.4%, n = 21); 11.3% of patients had facial lesions (n = 13), 4.3% (n = 5) had axillar eczema, 4.3% (n = 5) had foot eczema, and only 0.9% (n = 1) had genitalia-perianal eczema. No statistically significant differences were found.
Furthermore, 12.7% had a pre-patch test diagnosis of atopic dermatitis (n = 15), 8.5% psoriasis (n = 10), and 0.9% seborrheic dermatitis (n = 1). We found that patients with atopic dermatitis had three times increased relative risk to and tested positive for MCI/MI with statistically significant differences (p = 0.016, OR 3, confidence interval of 95% [1.2/6.4]).
Of the patients, 70.7% (n = 83) were in careers with manual activities involving wet work, compared with 29.3% (n = 35) who did not use their hands in their professional activities. The most frequently registered profession was cleaners/janitors (40.2%, n = 37), followed by office workers (19.6%, n = 18). Only 5 patients were identified as having occupational dermatitis (4%), of which 2 were related to the use of paints and 3 were associated with cosmetic or household products used in their work. No statistically significant differences were found between locations or professions and a positive sensitization to MCI/MI.
The most common sources of exposure related to MCI/MI found in our group were bath products (46.6%, n = 55), baby wipes (26.27%, n = 31), household cleaning products (14.40%, n = 17), and beauty products (12.70%, n = 15).
Finally, we studied whether patients with positive patch test results for MCI/MI had concomitant sensitization to other substances, particularly other preservatives. These data are included in **table I**. We found that patients with positive patch test results for Quaternium 15 had an increased relative risk to or had sensitization to MCI/MI with statistically significant differences (p < 0.0001, OR 8, IC 95% [3.7/17.8]). Furthermore, patients with positive patch test results for formaldehyde also had an increased relative risk sensitization to MCI/MI with statistically significant differences (p < 0.0001, OR 6, IC 95% [2.5/13.2]).
Finally, in the MOAHLFA index (male, occupational dermatitis, atopic dermatitis, hand, leg, > 40 years old), 58.5% were men, 4.24% had occupational dermatitis, 57.7% had atopic dermatitis, 62.9% had hand eczema, 0% had leg eczema, 9.5% had facial eczema, and 79.6 % were over 40 years old.
A recent meta-analysis published in 2018 revealed that at least 20% of the general population have contact-allergic dermatitis due to common environmental allergens (4). Hypersensitivity reactions to metals are one of the most common (5), but a concerning increased sensitization to MCI/MI had been detected since the late '80s. Lundov et al. (6,7) revealed that MI ACD is estimated to have a prevalence of 1.5% in 2011, which has been increasing in the next years, mainly affecting middle-aged women with hand and facial dermatitis most often associated with cosmetics. In 2014, Aerts et al. showed an increase in sensitization rate to MCI/MI of 4.5% (7). Our group's sensitization rate to MCI/MI is 8%, significantly higher than in other papers (6,7,8). Most studies on ACD to MCI/MI revealed a predominance in women (7,9) whereas in our study group, we found little predominance of males affected (58.5%, n = 69). We think this can be a point of confusion because men were asked more frequently in our study sample. Atopic dermatitis was the most common dermatological disease registered in our series in contrast with dyshidrotic eczema in the de Unamuno series, which studied a population similar to ours (10). Furthermore, we described that patients with AD had three times higher risk to sensitization to MCI/MI probably associated with skin barrier alterations in this group of patients.
The most frequent concomitant allergen in the de Unamuno (10) group and other series was nickel sulfate whereas it was formaldehyde and Quaternium 15 in our study group, although we also detected a high number of patients with concomitant positive patch test to disclosure cobalt and nickel sulfate (**table I**). In fact, we want to emphasize that it is particularly interesting that formaldehyde and the formaldehyde releaser Quaternium 15 are not only allergens more often concomitant with MCI/MI sensitization, but we also found statistically significant differences between these allergens and MCI/MI. These substances are preservatives used to prevent contamination in cosmetics and household products. Warshaw et al. (5) reported in 2013 that formaldehyde, Quaternium 15, and MCI/MI were the preservatives that most frequently tested positive to the patch test in the North American Contact Dermatitis Group (NACDG) (5). A positive sensitization to MCI/MI was much more prevalent in janitors and cleaners (n = 37, 40.2%), followed by office workers (n = 18, 19.6%) and builders (n = 8, 8.7%).
Thus, we conclude that humidity affects the process of allergic contact dermatitis, but MCI/MI is so prevalent in daily cosmetic products that further studies are needed to elucidate whether this is associated with professions or daily contact with cosmetic and cleaning products. In addition, our result supports that the prevalence of MCI/MI is increasing in all developed countries. Many other studies are necessary to explain risk factors for ACD to preservatives.

Conflict of interest

The authors declare that they have no conflict of interest.

Table I *- Patients with positive patch test to MCI/MI and concomitant sensitisation to other substance in patch-test.*

Allergens		MCI/MI negative (N)	MCI/MI negative (%)	MCI/MI positive (N)	MCI/MI positive (%)
potassium dichromate	**negative**	1252	91.7%	110	93.2%
	positive	114	8.3%	8	6.8%
Caine mix	**negative**	1349	98.8%	113	95.8%
	positive	17	1.2 %	5	4.2%
fragrance mix	**negative**	1302	95.3%	110	93.2%
	positive	64	4.7%	8	6.7%
balsam of Peru	**negative**	1333	97.6%	113	95.8%
	positive	33	2.4%	5	4.2%
cobalt dichloride	**negative**	1260	92.2%	109	92.4%
	positive	108	7.8%	9	7.6%
paraben mix	**negative**	1357	99.3%	117	99.2%
	positive	9	0.7%	1	0.8%
carba mix	**negative**	1338	98.0%	112	94.9%
	positive	28	2.%	6	5.1%
Quaternium-15	**negative**	1349	98.8%	107	90.7%
	positive	17	1.2%	11	9.3%
p-phenylenediamine	**negative**	1317	96.4%	113	95.8%
	positive	49	3.6%	5	4.2%
formaldehyde	**negative**	1347	98.6%	109	92.4%
	positive	19	1.4%	9	7.6%
thiuram mix	**negative**	1345	98.5%	116	98.3%
	positive	21	1.5%	2	1.7%
diazolidinyl urea, Germall II	**negative**	1358	99.4%	117	99.2%
	positive	8	0.6%	1	0.8%
imidazolidinyl urea, Germall 115	**negative**	1361	99.6%	117	99.2%
	positive	5	0.4%	1	0.8%

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When to stop biologicals. Severe asthma exacerbation after mepolizumab discontinuation

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To the Editor

Severe asthma affects 5% to 10% of all asthmatic patients (1). To be defined as severe an asthmatic, patient must be treated with the best standard therapies at maximal doses, without achieving a full control, and therefore requiring also oral corticosteroids (OCS) (2). In Western Countries, severe asthma is responsible for at least 50% of the socio-economic burden, including both direct and indirect costs (3). The development of monoclonal antibodies (mAbs) allowed to expand the therapeutic approach in patients affected by this disease, especially in reducing the need for OCS and the exacerbation rate. Certainly, the most studied immunological aspect is that linked to the "type 2" inflammation, which involves eosinophils and other factors through the action of several cytokines, including interleukin (IL) 5 (4). Severe asthma also involves unrecognized or underestimated social costs, for instance due to the effects of systemic corticosteroids or work absenteeism (5). The novel biological drugs for severe asthma are able to reduce the direct and indirect costs, while maintaining a satisfactory control of disease. Mepolizumab (MEP), a humanized mAb against IL-5 was recently tested and commercialized for severe asthmatic patients (6,7).

We describe herein the clinical case of a patient with severe hypereosinophilic asthma, successfully treated with MEP, first during the MENSA (6) study, then with its open-label extension and commercial availability, who had a sudden episode of asthma exacerbation after MEP withdrawal after more than 3

years of therapy. Written informed consent was obtained from the patient for publication of this case report.

A never-smoker 64-year-old Caucasian male was followed-up by our clinic since about 10 years due to severe uncontrolled asthma. Asthma appeared around the age of 40, without any previous evidence of respiratory symptoms. Of note, between 40 and 60 years of age, the patient underwent 5 surgical interventions for nasal polyposis. Besides, an episode of isolated hypereosinophilia without symptoms was recorded. During clinical and historical examination, we found gastro-oesophageal reflux and a positive skin prick test to Parietaria. There was no evidence of occupational exposure to pulmonary pathogens. In the 2 years before enrolling him in MENSA study respiratory symptoms became increasingly severe, with a significant limitation to daily activity, despite the maximal therapy (fluticasone / salmeterol 50/500 mcg twice daily, theophylline 300 mg/die, proton-pump inhibitors and nasal steroids). To exclude that symptoms are related to a poor adherence to the therapy, we carefully checked it.

In the two years before enrolling him in MENSA, a mean of 4 asthma exacerbations per year were ascertained, with multiple hospital admissions. Finally, at the end of 2012 the patient was enrolled in the MEA 115588 trial (MENSA) (6), and treated with MEP. The baseline characteristic before MEP administration, was an increased number of blood eosinophils (770 cell/µl), a normal lung function, a mild hypertension and a normal saturation. At the beginning of 2013 the patient was randomized and included in the study. After the first month of therapy a clinical improvement could be observed, with an improvement in FEV_1 (from 2.45 L to 2.87 L). Afterwards the patient continued, with subjective and clinical benefit, with no more exacerbations or systemic steroids need. At the end of the study, he received regularly MEP for compassionate use then, after marketing, he continued for other 8 months without symptoms or drug related adverse events.

After 4 years and 11 months of therapy, at the end of November 2017, with a full control of asthma symptoms, no exacerbations, no OCS therapy and normal lung function, we attempted MEP discontinuation. After about 2 months of control we could assist to a progressive deterioration: worsening of respiratory symptoms with dyspnoea even at rest, wheezing, cough, and nocturnal awakenings due to shortness of breath and a normal haemoglobin saturation. His blood eosinophils level increased to 820 cell/µl and lung function test showed a progressive impairment. A home-based treatment with OCS (50 mg/day Prednisone), aerosolized steroids and bronchodilators was started, without a clinical improvement. The patient was hospitalized and treated with intravenous steroid and aminophylline. During the hospitalization, a bacterial superinfection occurred, for which an antibiotic therapy was set. A progressive objective and subjective improvement of symptoms was seen, with a reduction of blood eosinophils, and 500 mL FEV_1 improvement. After this, the MEP therapy was re-started during hospitalization and, in his clinical stability, patient was discharged. Currently, the patient is on regular MEP treatment, and remains well controlled, without exacerbations or OCS treatment needed (**Table I**).

All clinical trials with MEP, after appropriately selecting patients (serum eosinophils > 300/µl), confirmed the positive results in reducing exacerbations (6,7), in sparing OCS (8), in FEV1 improvement and in quality of life (6-8). In the current trials, a reassuring safety profile was also demonstrated with a small number of adverse events, similar to the placebo groups (9).

Table I *- Main data at baseline, after MEP therapy (MEA 115588, compassionate use and marketing), during and after exacerbation.*

	2013 baseline	**Nov 2017**		**Hospitalization (Feb 2018)**		**8 months later (Oct 2018)**
weight	65 kg	69 kg		69 kg		69 kg
height	173 cm	172 cm		172 cm		172 cm
serum eosinophils	770 cell/µl	150 cell/µl	**DISCONTINUATION**	800 cell/µl	**READMINISTRATION**	110 cell/µl
FEV1 (%)	79%	96%		74%		92%
FEV1 (L)	2.45 L	2.73 L		2.09 L		2.58 L
FVC (%)	85%	121%		104%		115%
FVC (L)	3.38	4.25 L		3.80 L		4.21 L
FEV1/FVC	72	61.5		55		61.3
ACT score	13	24		15		24
exacerbations / 12 monts	4	0		n.a.		n.a.
hospitalization	1	0		n.a.		n.a.

(n.a. = not available)

The available clinical trials with MEP clearly evidenced the efficacy of the biological drug in reducing exacerbations of severe asthma. One of the most important point to be clarified is how long to continue the administration of MEP (10). In vitro observations suggested a hypothetical risk of rebound of airways eosinophilic inflammation after discontinuation (11). Also, an increased stimulus of TH_2 cells to synthetize IL-5 was shown, associated to an upregulated expression of IL-5R by eosinophils and a persistence of preformed IL-5 in complex with the drug (12). In vivo, an observational study performed on 56 subject (27 on active arm) who suspend MEP after the trial, reported a slightly greater exacerbation rate at 12 months (non statistically significant) (3.1 vs. 3.9; rate ratio, 1.25; 95% CI, 0.71-1.91; p = 0.54). An increase of overall frequency of severe exacerbations, only in treated patients and not in placebo, from 0.56/patient at 0 to 3 months to 1.2/patient (p = 0.007) at 3 to 6 months (rate ratio, 2.11; 95% CI, 1.76-2.54; p < 0.001). As for exacerbations, also for blood eosinophils count, the increasing of value resulted higher in 0 to 3 months (p < 0.001) and 3 to 6 months (p = 0.004) in treated patients (11). As previously observed in the case herein described, the increase in blood eosinophils paralleled the clinical exacerbation. To better understand also how to continue with the administration of the therapy, the experience in real life will be very useful. Indeed, consistent data are available about the patients enrolled in clinical trials with biologicals, but few data in the real-life setting, especially about the duration of the therapy. In addition, the characteristics of real-life patients resulted to be different from those described in clinical trials (13,14).

The duration of the therapy is one of the main challenges in the management of these drugs. In fact, in the asthmatic patient, in which therapy is usually modulated according to the control of symptoms, the behaviour to be applied with biological drugs is not yet defined. Although it is common ground that a suspension of biologicals should be taken into account, at the moment the timing is not clear. With clinical practice it was possible to highlight that, after drug discontinuation, several patients responded by keeping control and others returning to have symptoms. A careful study of the biological parameters of patients treated with these drugs will be necessary in order to look for biomarkers that allow us to predict control of drug withdrawal. This clinical case is an interesting example of how cautiously MEP should be discontinued in clinical practice, being aware of the risk of exacerbations immediately after withdrawal.

Conflict of Interest

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