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Research Article

Multiplex Methodologies in the Study of Occupational Allergies Due to Animal and Plant-derived Allergens Exposure

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Abstract

Background: The air quality guidelines published by WHO in 2021 underlines the need to study multipollutant exposures to investigate additive, synergistic or antagonistic effects of air pollutants including pollens. An important research area concerns the study of action mechanisms and the advanced methodologies for the evaluation of such exposure.

Multiplex methodologies are important tools that can improve the study of health responses followed exposure to agents from different sources and in particular in the research involving allergy. The use of multiplex methods can define the individual allergic profile, addressing the investigation of the action mechanisms and the identification of new biomarkers.

The aim of this paper is to promote the use of innovative methodologies applied to occupational allergies, showing the results obtained with two multiplex systems.

Methods: A total of 43 workers enrolled in an animal research facility and 105 patients were screened with two multiplex methods (ImmunoCap ISAC and ALEX) to evaluate allergic reactivity towards about 100-300 allergens.

Results: The results obtained with ImmunoCap ISAC evidenced a higher reactivity for plant allergens, while ALEX methodology showed that the pollens were the allergens with the higher IgE values.

These results highlight the role of plant allergens, in particular pollen, able to interact with chemical agents, as well as with several factors such as microclimate and meteorological variables. Pollen exposure is also affected by the ongoing climate change as well as by anthropic factors such as occupants in indoor settings.

Conclusion: The multiplex methodologies may be applied on biological and environmental matrices to improve the knowledge of the action mechanisms of allergies. The identification of novel biomarkers using multiplex methodologies is a further opportunity in the study of occupational allergies.

Keywords: Allergens; Occupational Allergy; Multiplex Methodologies; Occupational Health; Plant Allergens; Animal Allergens

Introduction

Allergic diseases represent a growing public health problem, being increasing worldwide, especially in children, although there are differences among countries. Allergy affects about 20-30% of the population worldwide putting together asthma, rhinitis, dermatitis, drug and food allergies. Epidemiological studies show the increasing prevalence of asthma (more than 300 million people in the world suffers from it) and rhinitis (globally, rhinitis affects approximately 400 million people) [1,2]. In this context, the issue of occupational allergic diseases needs a deeper understanding due to the high prevalence of these diseases and the consequent socio-economic impact. Occupational asthma represents approximately 25% of respiratory diseases attributed to occupational exposure and 15-17% of adult asthma is attributable to allergens found in the workplaces [1,3].

Approximately 300-400 substances (high-molecular-weight plant and animal (glyco) proteins as well as low-molecular-weight compounds) commonly encountered in occupational settings may be responsible for several allergic conditions, causing or exacerbating occupational asthma, occupational rhinitis and cutaneous reactions in sensitized workers [1-5].

In this regards, several studies showed that different categories of workers such as farmers, laboratory animal workers, workers in food industry, healthcare workers and cleaning service workers are exposed to biological allergens and may develop allergic diseases or exacerbate pre-existing ones, including asthma, rhinitis and dermatitis [6-15].

Allergies are the result of complex interactions between endogenous and exogenous risk factors. Essentially, indoor/outdoor exposure of susceptible individuals to several allergens or sensitizing agents in both living and working environments may occur [8,16-20].

In the last decades, the increase in the occurrence of allergic diseases is linked to environmental factors and climate change [21-26]. Meteoclimatic variables such as temperature, relative humidity, wind speed and direction as well as rainfall significantly affect pollen release and transport [27,28]. Climate change may increase production, release and dispersion of pollen, as reported in numerous papers. Moreover, it may modify pollen allergenic potential and, affecting the duration of pollen season, may prolong the development of clinical features associated to allergies [29-35]. Furthermore, climate change could amplify the effect of air pollution on pollen, being known the interactions among biological-derived allergens and chemical pollutants. NO_2 and O_3 represent the airborne chemical pollutants more studied in this regard. However, an increasing recognized role is played by particulate matter (PM), especially in urban settings. In fact, PM is able to increase the frequency of allergic respiratory diseases and to exacerbate the immune-physiological responses in sensitized individuals [29,36-39]. Higher concentrations of air pollutants worsen not only outdoor but also indoor air quality. As reported by World Health Organization (WHO), asthma, lung and other respiratory diseases are largely due to unhealthy air, breathed by over 90% of people [1,2,40].

Occupational exposures to animal allergens may occur in several occupational settings. For instance, workers involved in animal health care and veterinary practices may be affected by allergy due to laboratory animals (Laboratory Animal Allergy - LAA), recognized by NIOSH as an occupational risk since 1998 [41]. Several scientific papers are published on this topic [8,42-49], some of them reporting the occurrence of severe anaphylactic reactions [50,51].

The topic of biological allergens is very important and displays a lot of related issues. In this regard, the recent guidelines on air quality published by WHO in September 2021 recognized, among priorities: "… the study multipollutant exposures to determine the relative importance of specific air pollutants (such as nitrogen dioxide, carbon monoxide) and components of PM, with an examination of additive, synergistic or antagonistic effects, including in the presence of pollens or other airborne allergens. This is an area where mechanistic research will likely play an important role. Moreover, study the interaction with other environmental and behavioural factors such as traffic noise, green space and allergen exposure; physical activity and diet; and high and low temperatures and other climatic conditions. In addition, assess multiple sources of exposure in different locations (including home indoor, work indoor and transportation) and time–activity patterns" [52]. Thus, for instance, the monitoring of plant allergens such as pollens represents a priority to protect public and occupational health.

The research area on both exposure sources and action mechanisms of immunoallergic reactions of workers exposed to plant, animal and food allergens should be supported by multiplex methodologies. The last ones may also be applied to study the potential interactions of allergens with other occupational and environmental agents as well as to assess specific health effects linked to allergic responses.

Multiplex technologies are increasingly used and represent a potential powerful tool to detect novel biomarkers of exposure, effect and susceptibility, such as specific IgE associated to immunological response of sensitized individual and workers exposed to plant, animal and food allergens. The application of such methodologies could allow a better understanding of molecular mechanisms involved in allergic diseases [53,54].

The objective of this study is to report preliminary results obtained by applying multiplex methodologies and to promote the use and application of these innovative technologies in occupational settings in order to obtain the IgE specific reactivity patterns induced by exposure to biological-derived allergens.

Materials and Methods

This study was conducted on 43 workers (31 females and 12 males; mean age: 41 years) of a laboratory animal facility undergoing routine health surveillance and on 105 patients with allergy (52 females and 53 males; mean age: 40 years). All subjects were screened with multiplex methodologies. In particular, ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC) 112 microarray (ThermoFisher Scientific, Uppsala, Sweden) was used for workers and Allergy Explorer (ALEX/ALEX^2) (MacroArray Diagnostics, Vienna, Austria) was applied on patients. Both multiplex-specific serum IgE evaluation assays are able to identify the reactivity towards a high number of animal, plant and food allergens, namely 112 allergens and more than 300 allergens for ImmunoCAP ISAC 112 and ALEX respectively [55]. All enrolled subjects signed informed consent and received no other treatment. This study may be regarded as a non-interventional research, based on definitions of the European Clinical Trials Directive 2001/20/EC, for which the approval of an ethics committee was not requested [56]. It was conducted according to the Declaration of Helsinki and followed the International Code of Ethics for Occupational Health Professionals of the International Committee of Occupational Health [57]. Health data were acquired and treated anonymously, with no possibility of individual identification.

Results

The reactivity of workers against allergens grouped by source of exposure (Food allergens, Plant allergens and Animal allergens); was expressed as values of IgE, reported in ISU (ISAC Standardized Units), classified in: Low IgE level, Moderate-High IgE level and Very High IgE level, according to ImmunoCAP ISAC methodology instructions (Table 1). In the same way the reactivity of patients was evaluated according to ALEX methodology instructions (Figure 1), grouping allergens by source of exposure (Pollen, Moulds/Yeasts, Plant foods, Animal foods, Insect venoms, Dander/Epithelia, Mites/Cockroaches) and classifying the values of IgE, reported as kUA/L (kilo Units of Allergen/Liter), in: Low IgE level, Moderate IgE level, High IgE level and very high IgE level. The results obtained with the two multiplex methods show a greater reactivity towards plant allergens and particularly towards pollens, as evidenced by ImmunoCap ISAC (Table 1) and ALEX respectively (Figure 1).

Discussion

Aerobiological pollutants such as pollen are acquiring an increasing importance in the context of air quality [52]. Plants allergens and/or pollens have a crucial role in aerobiology and

	0,3-1 ISU*	1-15 ISU	>15 ISU
	1 – Low IgE level	2 – Moderate-High IgE level	3 – Very High IgE level
Food allergens	2		
Plant allergens	11	12	2
Animal allergens	1	4	2

Table 1: Allergens grouped by source of exposure and IgE levels of workers evaluated with ImmunoCap ISAC.

*Value of IgE were reported as ISU (ISAC Standardized Units).

Figure 1: Allergens grouped by source of exposure and IgE levels of patients evaluated with ALEX.

*Values of IgE were reported as kUA/L (kilo Units of Allergen/ Liter).

can interact with chemical pollutants, the last ones being able to increase the allergenic potential [31,36,37]. Climate change may modify the seasonality and pollen concentrations, so affecting frequency, severity and duration of respiratory allergic diseases [58]. Multiplex methodologies represent a useful and powerful tool if associated with a proper health surveillance, to improve the study and the management of risks due to allergen exposures in occupational and living settings.

The advancement of multi-omics technologies is improving the understanding of molecular mechanisms in allergic diseases such as asthma, extending the opportunity to detect specific biomarkers for different allergic reactivities, co-exposures, and biological profiles of susceptibility. The identification of novel biomarkers could represent a powerful tool in the prevention and treatment of allergic disorders, being included into the approaches characterizing the precision medicine [59]. Moreover, omics could represent a great support in the detection of cross-reactivity between pollen and foods, for instance in workers employed in food handling and processing [10,11]. In this regard, ALEX methodology is able to identify the reactivity towards approximately 300 animal, plant and food allergens (100 animal-derived and 150 food-derived), including 50 pollen types, so obtaining the individual immunoallergic profile based on allergen types and levels. Many studies using innovative methodologies to identify allergic reactivity towards animal and plant allergens in occupational settings were conducted [8,54]. Incidentally, the present study was intended to investigate any differences, attributable to occupational or living environments, in reactivity of subjects differentialy exposed to several categories of allergens using two different tests (ISAC and ALEX) based on multiplex methodologies.

As shown in other papers [8,53,54] the relevant presence of allergens of different origin (plant, food and animal allergens) in indoor occupational settings encourages the application of these innovative technologies to evaluate both the reactivity and susceptibility of subjects to allergens, in order to define the individual immunoallergic profile based on allergen types and levels.

The preliminary results of this paper show the predominance of a specific reactivity towards plant allergens, especially pollen. Furthermore, the greater reactivity to plant allergens of subjects

detected in this study supports the validity of such methodologies when applied in occupational setting to better investigate the susceptibility of workers and patients to pollen and to improve a targeted diagnosis of plant allergy.

Conclusion

Our findings are an initial contribution to a better investigation of the increasing global health problems due to allergic diseases. In the context of occupational health, the identification of workers affected by allergic diseases as well as of occupational settings at risk should be extended and improved. Moreover, the research on work-related allergies showed that the cut-off between chemical- and biological-derived allergens is not so evident. The assessment of occupational allergies including the use of new methodologies is also a great opportunity to improve workers' health surveillance and workers' information and training programs, encouraging the creation of multi- and interdisciplinary teams in order to achieve a better integration between clinics, public health and occupational health.

Conflict of Interest

None of the authors declare any interest or potential conflict of interest related to this publication.

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