



From pollen count to pollen potency: the molecular era of aerobiology

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Molecular aerobiology may improve measurements of pollen to better represent allergen exposure
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In 1873, Charles Harrison Blackley recognised pollen as the cause of hay fever and “hay asthma” [1]. For the first time, he collected pollen grains with a kite that had a sticky tail and then he counted them. Being affected by seasonal asthma himself, he also performed the first skin prick test on his own arm. Since then, aerobiology (from Greek *αἴρ*, *aēr*, “air”; *βίος*, *bios*, “life”; and *-λογία*, *-logia*), a branch of biology that studies airborne organic particles, including pollen grains and fungal spores, has played a key role in the study of the relationship between allergic diseases and pollen. Although thousands of experimental studies have supported the role of pollen in the pathogenesis of allergic rhinitis, asthma and conjunctivitis, data from epidemiological studies were controversial in many cases, casting doubt on the pollen count (*i.e.* the number of pollen of the same type per cubic metre of air) as a reliable proxy of allergen exposure.

Several reasons can be claimed for the inconsistency of these results. One is that effects of pollen cannot be considered the same for all species. The mix of allergenic pollen to which the allergic population is exposed varies dramatically both qualitatively (types of pollen) and quantitatively (amount of pollen), according to the characteristics of the local flora. Diversity of exposure profile accounts for the impossibility of finding a “universal” concentration of pollen that is able to induce symptoms, *i.e.* the clinical threshold, as is possible in the case of urban pollutants, for which limits are established at a European level. In fact, the clinical threshold of pollen varies in different geographical areas and among different pollen taxa, as extensively reviewed by DE WEGER *et al.* [2]. As a consequence, for instance, threshold values ranged between 4 and 20 grains·m⁻³ air (in Israel and Poland, respectively) even if same criteria (“first symptoms”) and same pollen type (grass pollen) are taken into consideration. Although two large studies showed independently that a level of 30 grains·m⁻³ air for grass pollen was able to increase the risk of asthma exacerbations in two different areas [3, 4], common clinical thresholds of pollen levels for large areas, *e.g.* the whole of Europe, seem unthinkable, even for a specific pollen type. In addition to this problem, variations exist in individual [5] and disease-specific [6] susceptibility in the response to pollens and in the expression of symptoms.

The issue of the assessment of exposure to pollen is a further factor affecting the studies on the effects of pollen on respiratory allergic diseases. Pollen count has been used for over 50 years for the assessment of allergen exposure both in clinical practice and clinical and experimental studies and, more rarely, in epidemiological studies [7–9]. However, proof is lacking that pollen count is representative for allergen exposure. The method, proposed by HIRST [10] in 1952, is based on identifying and counting with a microscope pollen and spores collected with a volumetric trap, which is usually placed at 20–25 m above the street level (usually on the roof of a university/hospital building). Although this location reduces the effects of obstacles on the pollen collection, it does not reflect the exposure of an allergic individual. In addition, it is assumed that a single pollen trap is able to represent the pollen distribution over an area of about 20 km

Received: June 06 2013 | Accepted: June 14 2013

Conflict of interest: None declared.

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range [11]. It is clear that such coverage of pollen monitoring is insufficient in most of cities, at present. For example, in the area of London, which covers an area of 1570 km², there are only two pollen traps, while pollutants are monitored in more than 110 sites [12]. Thus, current levels of pollen monitoring cannot be considered the gold standard for assessment of the effect of pollen on the allergic population. Other than the enhancement of pollen networks, development and validation of new portable devices might be able to provide us with a more reliable measurement of pollen exposure. Despite these limitations, some recent studies were able to confirm the association between grass pollen count and exacerbations of asthma and rhinitis at the population level [7, 8, 13, 14], even after accounting for potential confounders including air pollution. Data on other pollen types are less consistent.

Molecular aerobiology could shed a new light on the issues of both exposure and clinical thresholds. Allergy diagnosis has changed in the last 10–15 years, moving from the use of extracts for both *in vivo* and *in vitro* diagnosis to so-called “molecular diagnosis”, which characterises each patient’s immunoglobulin (Ig)E antibody profile to individual allergen components, thereby discriminating between genuine sensitisation to certain specific allergen sources and cross-reactivity. As expected, aerobiology is developing along the same line. Since the early observations by SCHÄPPI *et al.* [15] at the end of the 1990s, molecular aerobiology now represents the most intriguing achievement of research on allergenic pollen. Special devices, such as high-volume cascade impactors, and availability of purified and recombinant allergens, allow the collection of a high volume of ambient air and the measurement of allergens. The content of major allergens in the most important allergenic pollen has been measured in outdoor air, albeit with different methods, in Europe and Australia. Taken together, findings on Phl p 5 and Lol p 1 in grass pollen, Bet v 1 in birch pollen, Ole e 1 in olive tree pollen and Par j 1 and Par j 2 in wall pellitory pollen [16–19] showed that allergen content deviates from pollen count and varies with several environmental and botanical factors, as well as with the year and location of collection. In other words, allergenic potency of pollen is variable and therefore pollen count is not the best method to assess the allergenic load to which the allergic population is exposed. These preliminary results have been confirmed in a multicentric European project, HIALINE (Health Impacts of airborne ALlergen Information Network), funded by the Executive Agency for Health and Consumers of the European Union [20]. The major allergens from the top three airborne allergens in Europe (Phl p 5, Bet v 1 and Ole e 1) were sampled with a cascade impactor and analysed with a sandwich ELISA and data compared to pollen counts. First findings confirmed that the content of Ole e 1 [21] and Bet v 1 [22] deviates from pollen count and that it varies in different countries. Together with the molecular allergology, this new data is contributing to a new view on the relationship between pollen exposure and both allergic sensitisation and appearance of symptoms. Inconsistency of some studies on the association between pollen count and exacerbations of asthma, as well as the dramatic differences of levels of clinical thresholds of pollen in both population-based and experimental studies, might be due to differences in terms of pollen potency. Factors affecting allergen content are environmental, such as weather variables and climate, and plant related, such as the cultivar (*i.e.* type of plant belonging to the same species) [23]. Therefore, local meteo-climatic conditions and the specific flora determine the sensitisation profile of the population living in the area. In this regard, the case of grass pollen allergy is particularly suggestive. The prevalence of sensitisation to Phl p 1 and Phl p 5, two major and highly cross-reactive allergens of group 1 and 5 grass pollen allergens, respectively, varied significantly in the regions of Spain [24]. Conversely, the very same group 5 allergens are absent in subtropical grasses, as shown by DAVIES *et al.* [25]. Thus, the molecular view shows that allergy to grass pollen is not the same all over the world and that the concept of major allergen cannot be universal. In addition to the scientific importance of this approach, diagnostic and therapeutic implications are even more substantial. Commercial extracts for diagnostics and immunotherapy, which take into account molecular profile of exposure and sensitisation of local population, might improve their performance and efficacy, respectively. Finally, molecular allergology has introduced the concept of panallergen, which is a family of related and widely distributed proteins; some of them, *e.g.* lipid transfer protein (LTP), profilin and the PR-10 proteins, can be found in both pollen and food and are implicated in the so-called pollen-food allergy [26]. Other than the clinical and diagnostic importance, the concept of panallergen is shedding new light on the mechanisms of sensitisation to plant food and its geographical differences. LTP allergy is paradigmatic: while in southern European countries the source of sensitisation is fruit (mainly peach), in other countries, where peach is rarely eaten, the source of sensitisation to LTP is pollen, as shown in a recent Chinese study [27].

In the current issue of the *European Respiratory Journal*, CANOVA *et al.* [28] put the role of pollen in asthma exacerbation under the spotlight at last. This large epidemiological study on 2500 young adults with asthma from the European Community Respiratory Health Survey (ECRHS) confirms the seasonal pattern of pollen-associated asthma, which has been known for decades by clinicians. Other than supporting the use of seasonal therapeutic schemes in clinical practice, the article by CANOVA *et al.* [28] fosters multidisciplinary research on the role of pollen in respiratory allergic diseases. Although epidemiological evidence is

accumulating [7, 8, 13, 14], the molecular approach could further improve the tools for the assessment of pollen exposure. Future research should include the implementation of standard pollen count with allergen measurement as a proxy of exposure in epidemiological studies and clinical trials. This is most important due to climate change [29–31], which affects plants, pollen season and pollen allergenicity. With this in view, research both on allergenic pollen and national pollen networks should be continuously supported.

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