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POLLEN COUNTS AS A TOOL FOR CLINICAL RESEARCH

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It was an English doctor, John Bostok, who, in 1819, was the first to describe the existence of a complaint similar to coryza which affected the nose and eyes, but on the contrary to the common cold it only appeared during the summer months. He suggested that the cause of this complaint were the efluvia from hay, which is why he called it "hay fever".[1]

Over half a century went by until another English doctor, Charles Harrison Blackley, published, in 1873, that the cause of this illness was produced by grass pollen and not by hay Blackley, who was really ahead of his time, designed a pollen sampler made up by a wind vane on which he attached a glass slide previously coated with glycerine. Every 24 hours he took down the slide and examined its contents using an optical microscope, identifying and measuring the different types of pollens that had remained stuck in the glycerin per cm3.

He found that during the months of June-July (the period of his patients' symptoms) there was grass pollen in the atmosphere in Manchester.

He also checked that the hay fever symptoms could be reproduced with his patients in his laboratory by placing small amounts of grass pollen into their noses.

These patients, contrary to the rest of the normal population, developed a cutaneous response with wheals and erythema within minutes of applying grass pollen grains to their previously scarified skin.

Latter it was corroborated that, in fact, Blackley had been right and that grasses are overall the greatest cause of pollinosis not just in England but in most parts of the world due to its great allergenicity and its wide distribution, since they represent 20% of the plant surface area on the planet.

Nevertheless, other pollens have been described as being responsible for pollinosis, such as Ambrosia which pollinates from August to October (the greatest cause of pollinosis in the U.S.A.). In the U.S.A., Ambrosia alone causes more hay fever patients than all the other pollens put together. *Betula*, together with grasses, are the two most allergenic pollens in Scandinavian countries or *Parietaria* spp, the most important cause of pollinosis in coastal areas in the Mediterranean.[2-5]

Nowadays we have much more sophisticated methods for diagnosis than those used at first by Blackley to be able to study this illness.

So by using Radio or Enzyme Immune Assays techniques, we can discover whether there are specific IgE antibodies in the patient's blood serum against the proteins extracted from the pollen grains. Using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS PAGE), we can see that one species of pollen contains not one but, on the contrary, numerous proteins with different molecular weights. Using immunoblotting techniques, we can see that only some of those proteins (not all of them) are capable of establishing the specific IgE. That is to say, only some of the many proteins that contain pollen grains turn out to be allergenic.

When these immunoblot techniques are carried out on separate blood serum samples from several patients suffering from pollinosis and allergic to that pollen, we can see that usually

only one or two of those multiple allergens are capable of establishing the IgE in most of the serums.

One might think that these immunoallergic techniques (both in vitro and in vivo) would be enough by themselves to be able to carry out a pollinosis study. Nothing further from the truth, these techniques simply tell us whether the patient is allergic or not to a given type of pollen and they are useful for research into pollen allergens, but they do not tell us whether natural exposure to that pollen will cause symptoms for him/her or not, though more and more through these techniques we are now able to detect allergies to pollens which do not even exist in the geographical area where the patient lives.

Below, we can see some examples of how absurd it would be to make a "pollinosis map" based solely on the cutaneous samples without an in-depth aerobiological study.

According to the cutaneous samples, *Amaranthus retroflexus* would be an important cause of pollinosis in Madrid, since 45% of the patients with pollinosis in that area are prick test positive to this pollen, however, according to the pollen counts Chenopo Amaranthaceae represent hardly 1.5% of the yearly pollen totals and what is more important is that most of these patients do not show any symptoms during its period of pollination which goes in August and September.[6]

A more dramatic example may be seen with *Ambrosia trifida*, since 15% of the patients with pollinosis in Madrid show to be prick test positive to this pollen, however this plant does not even exist in our country.

And the fact is that subclinical allergies (i.e., *Amaranthus*) or the presence of crossed reactivities (i.e., *Ambrosia-Artemisia*)[7] make it very difficult to be able to determine which pollens are clinically relevant in a given geographical area without a prior aerobiological study.

Therefore, it is still strange that, in spite of all the technology we have available at the present time, when we have to carry out a pollinosis study we have to use the same system that Blackley invented over 120 years ago.

That is to say, firstly we look at which pollens are the ones floating over the heads of our patients during the period in which they suffer these symptoms and secondly we confirm the allergy mainly by using cutaneous tests.

Though at present the collection mechanisms are more precise, in principle they are based on the one thought up by Blackley, that is to say, using wind vanes on which we place slides or tapes coated with vaseline, on which the pollens are trapped.

The skin tests, though at the present time they are carried out with a standardised methodology, are based on the same principle used by Blackley, observing a wheal an erythema response to allergenic pollens.

Thomen drew up some postulates which clearly show the conditions required to consider a given pollen to be the cause of pollinosis in a given area.

- Pollen must contain an excitant of hay fever.
- Pollen must be anemophilous.
- Pollen must be produced in large enough quantities.
- Pollen must be sufficiently buoyant to be carried long distances.
- Plants producing pollen must be widely and abundantly spread.

The pollen counts, apart from being essential therefore to determine, in a given area, which pollens are the most important ones overall for pollinosis, may also be used for attempting to determine in highly allergic patients which pollens are the ones that are really causing the pollinosis symptoms. Pollen counts together with a "diary card of symptoms score" could be used by doctors as yet another tool to be able to differentiate, for a given patient, clinical allergies from subclinical ones, a fact which becomes especially interesting in those patients who are going to undergo specific immunotherapy.

Recently, thanks to the use of these correlations of "Pollen counts-Symptoms score", we have found patient with hay fever symptoms in Spring, skin tests positive to grasses and therefore initially diagnosed as pollinosis caused by grasses and treated with immunotherapy against grasses, in those people, on the contrary, whose real responsible agent was *Platanus* pollen.[8] We have discovered similar findings among patients allergic to grasses and *Olea* (prick tests) in whom the symptoms appeared to be almost exclusively induced by grass pollen alone (Fig.1-2).

Nevertheless, these studies raise two problems mainly, one is the need for the patient to live within a radius of at least 5 to 10 kilometres from the pollen sampler and the other one being that the patient should fill in his symptoms score card every day, which usually turns out to he quite difficult.

A bigger question for clinical researchers is that of finding out what is the threshold pollen count to elicit pollinosis symptoms. However, establishing a dose-response relationship between pollen exposure and symptoms is difficult. The severity range for different individuals is quite wide (what is low for one patient may be high for another) and symptoms often reflect concurrent exposure to several allergens. The response usually increases with ongoing short-term exposure (priming) and when exposure involves aerosol fractions as well as full pollen grains.[9]

In London, Davies and Smith found 50 grains/m3 of grass pollen elicited rhinitis in all of the clinically sensitized grass patients.[10]
In some patients who are ragweed sensitive and primed, 7 to 15 grains/m3 provoked symptoms. In Norman's paper, ragweed allergy patients varied considerably in their "threshold dose" and he reported much higher counts. Generally speaking, it is difficult to establish the mean pollen count required to elicit symptoms. We only have good data available for evaluating the thresholds for ragweed or grass allergy.[11-12]

Predictions of the star and intensity of the season may also prove to be of interest for doctors

and patients alike.

Previous studies demonstrated that by using an appropriate combination of meteorological variables, it is possible to predict the atmospheric pollen count. Estimates have been made with a certain degree of accuracy, e.g., in the case of Poaceace (grasses), *Ambrosia* spp (ragweed), *Betula* spp (birches) and *Quercus* spp (oak trees).[10, 13-16]

Davies and Smith,[10] by means of a linear regression formula and by using the average temperature in April and May and the accumulated daily averages (grass/m3 of air) recorded in May as independent variables, were able to predict the beginning and severity of the grass pollination season in London. Equally, Goldberg et al.[13] found strong dependency between the mean monthly temperature for May and the total amount of Poaceae pollen in the next season, on the basis of data from Copenhagen.

In Madrid, we have found a strong, statistically significant correlation, based on simple and multiple linear regression, between the seasonal grass count total and the preseasonal rainfall from October to March. Good correlation was found between the March estimates of wheat, rye and barley crops and the grass count total. A model was designed for predicting in April the annual variation in the atmospheric concentration of grass pollen, based on the aforementioned meteorological factors and the grain crop estimates.
This data may help clinical researchers to predict and get ready for the intensity of the grass pollen season and for explaining yearly variations in the severity of the symptoms.

Finally, those people who travel either for work or leisure have to have reliable information about the likelihood of seasonal allergies when they visit another country. That is why knowledge about atmospheric pollen concentrations to be found in different parts of the world is of great interest to clinical researchers and allergy patients in order to achieve greater control over their hay fever symptoms.

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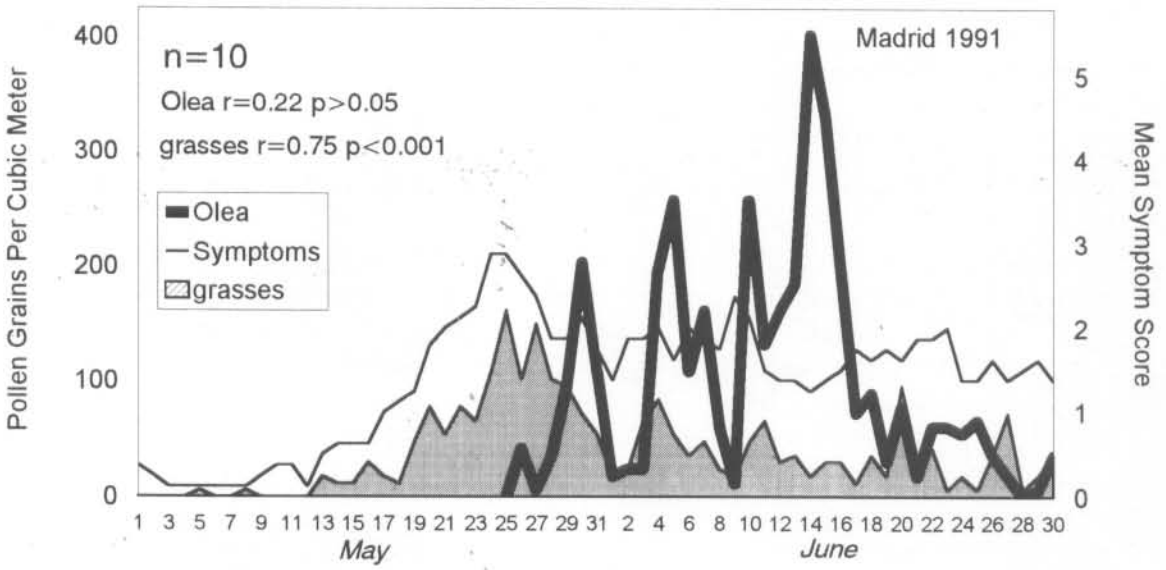

Fig. 1 Fig. 1 Correlation between rhinitis symptoms observed by the patients on their diary cards and grasses and *Olea* pollen counts.
Note the close correlation of symptoms only with the grass pollen counts in spite of all of the 10 patients showing a positive skin prick test to both pollens (grass and *Olea)*.

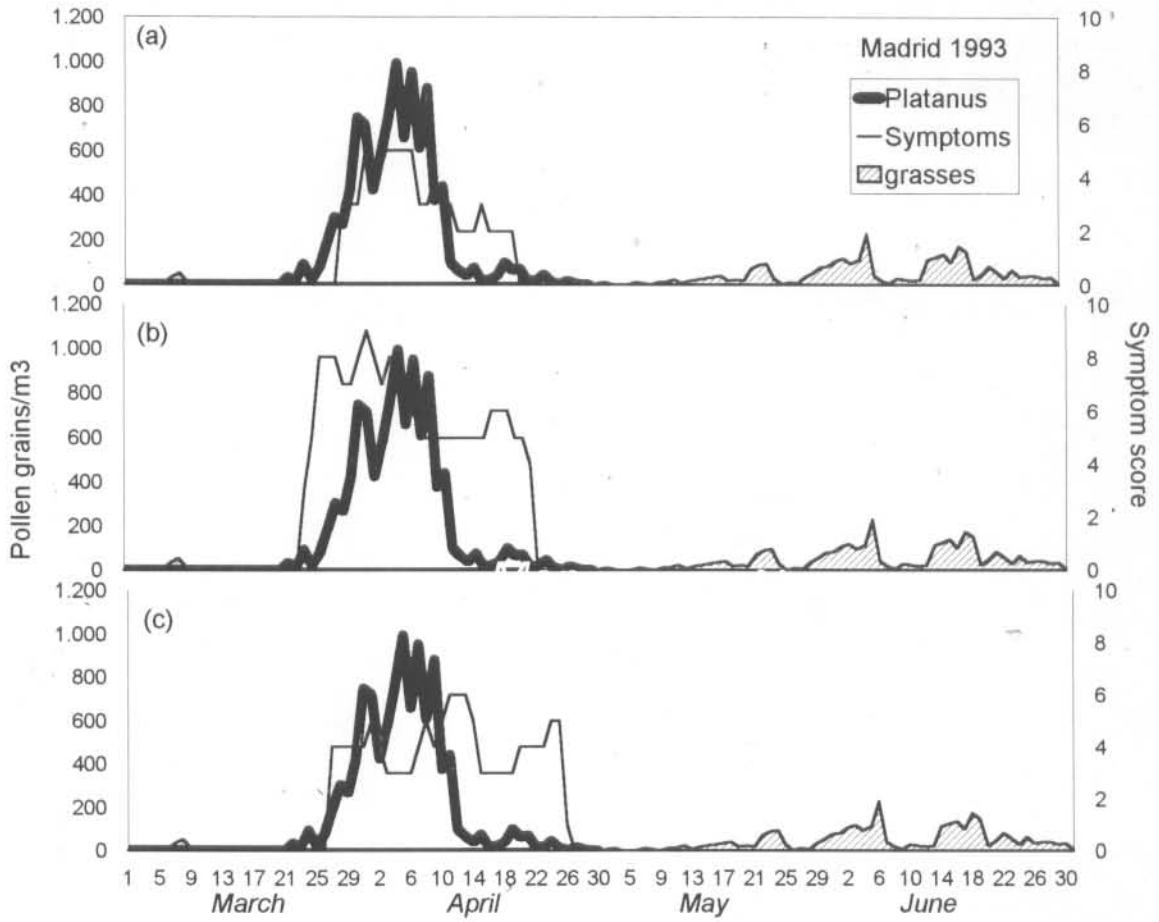

Fig 2. Correlation between asthma symptoms observed by three patients (a, b and c) on their diary cards and *Platanus* pollen counts obtained during the same period using a Burkard spore trap.
Note the close correlation of their symptoms only with the *Platanus* pollen counts in spite of all of patients showing a positive skin prick test to both pollens (grass and *Platanus*).