Age, gender and reactivity to allergens independently influence skin reactivity to histamine

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Abstract. *Background.* The ability to mount an IgE response to allergens is a prerequisite for the development of positive allergen skin tests. Histamine is commonly used as a positive control in skin prick testing and provides a measure of nonspecific skin reactivity, similar to bronchial hyper-responsiveness.

Methods. To determine whether allergen responsiveness, age, gender and season of the year contribute to histamine sensitivity, 620 subjects (502 of them with at least one known sensitizing allergen and the remaining 118 non-allergic controls) were prick-tested with a panel of allergens common in the Northern Italy semi-rural area where the patients lived, and with 10 mg/ml histamine dihydrochloride.

Results. We found higher histamine reactivity in allergic versus control individuals (median value 23.7 versus 19.8 mm^2; p=0.0497). Likewise, we found in allergic subjects a correlation between allergen responsiveness in terms of number of positive allergens at skin prick test and sensitivity to histamine (mono- sensitized versus poly-sensitized subjects: p=0.0015). Moreover older age and male sex were associated with a higher response to histamine, also when separately considering allergic subjects (p<0.0001 in both cases: correlation coefficient for age versus histamine reactivity: r=0.3408). The correlation between allergen responsiveness and sensitivity to histamine was maintained also when statistically balanced for age and sex.

Conclusion. Allergen responsiveness, gender and age allow more accurate prediction of histamine sensitivity than either parameter alone.

Key words: skin reactivity, allergen, histamine, skin-prick test, age, gender.

Resumen. La capacidad de generar una respuesta IgE a alérgenos es un prerrequisito para el desarrollo de pruebas cutáneas positivas a los alérgenos. La histamina se utiliza comúnmente como control positivo en la prueba de punción cutánea y proporciona una medida de reactividad cutánea no específica, parecida a la hiperreactividad bronquial.

Métodos. Determinar si la reactividad a los alérgenos, la edad, el sexo y la época del año contribuyen a la sensibilidad a la histamina. Para ello se realizó prick test a 620 pacientes (502 con por lo menos un alérgeno sensibilizante conocido y 118 controles no alérgicos) con un panel de alérgenos común en la zona semirrural del norte de Italia, donde vivían los pacientes, y con 10 mg/ml de diclrhidrato de histamina.

Resultados: Se detectó mayor reactividad a la histamina en los pacientes alérgicos frente a los del grupo de control (media 23.7 frente a 19.8 mm^2; p=0.0497). Del mismo modo, en los pacientes alérgicos se observó una correlación entre la reactividad a los alérgenos, en términos de número de alérgenos positivos en el prick, y la sensibilidad a la histamina (pacientes monosensibilizados frente a polisensibilizados: p=0.0015). Además, la edad avanzada y el sexo masculino se asociaron con una mayor respuesta a la histamina, incluso cuando se consideraron por separado los pacientes alérgicos (p<0.0001 en ambos casos: coeficiente de correlación para edad frente a reactividad a la histamina, r=0.3408). La correlación entre la reactividad a los alérgenos y la sensibilidad a la histamina también se mantuvo en el caso de grupos estadísticamente equilibrados en cuanto a edad y sexo.

Conclusión: La reactividad a los alérgenos, el sexo y la edad permiten realizar una predicción más precisa de la sensibilidad a la histamina que cada uno de los parámetros por separado.

Palabras clave: reactividad cutánea, alérgeno, histamina, prick test, edad, género.

Introduction

Histamine is likely the most important mediator released during the immediate hypersensitivity reaction. Skin reactivity to histamine has long been used in *in vivo* diagnostic tests aimed at detecting sensitizations to specific allergens as a standard and/or as a positive control. In fact, before data were available on the potency of single allergenic extracts used for diagnostic purposes, results of skin prick tests were necessarily expressed as comparisons of the size of the allergen-induced wheal with that of histamine dihydrochloride [1]. Although at present standardization of allergens has greatly improved, the usage of histamine as a standard included in skin prick testing is still recommended [2-4], and its usefulness extends beyond the need of an indicator that the patient has not taken any antihistamines before the skin test. In particular, the recommendation by the EAACI subcommittee on Allergen Standardization and Skin Tests in 1993 [5] was to use a cut-off limit of wheal size of >3 mm diameter (or 7 square mm area), and to give the concentration of both histamine and allergen, since both parameters can be determined. Notably, it was reported that the higher the number of total positive skin prick tests, the higher the histamine reactivity in terms of wheal area [6]. Thus, there might be hyper-responsiveness to histamine in the skin, as is the case of the lower respiratory tract.

In the present study, we investigated the influence of the number of specific allergen sensitizations, age, gender and season of the year on histamine reactivity in a large panel of allergic individuals and in control, non-allergic subjects. The aim of the present work was to study the importance and the problems of histamine hyper-responsiveness by simultaneously evaluating several factors, which are known to influence skin reactivity to this inflammatory mediator.

Materials and methods

Subject selection

Six-hundred and twenty consecutive Caucasian individuals who required allergological examination at our outpatient clinic, in a semi-rural area of Northern Italy, upon request of their general practitioner were included in this study. They were recruited over one year. Subject demographic characteristics are indicated in Table 1.

Table 1. Subject characteristics.

	N	Age, mean (SD)	Male	Female (%)
Total	620	23.89 (15.38)	307 (49.5)	313 (50.5)
Allergic	502	23.3 (14.4)	261 (52.0)	241 (48.0)
Non-allergic	118	26.3* (18.9)	46 (39.0†)	72 (61.0)

* p = 0.0489 allergic *versus* non-allergic.; † p = 0.011 allergic *versus* non-allergic male individuals.

Allergenic extracts

All individuals were skin prick-tested with each of the following standardized allergens (ALK-Abellò, Milan, Italy*): Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; grass mix (*Poa pratensis, Lolium perenne, Phleum pratense, Dactylis glomerata, Festuca prratensis*); *Olea europaea; Parietaria judaica, Artemisia vulgaris, Betula verrucosa* and *Corylus avellana*. The allergen was prepared in saline solution containing glycerol 50% volume/volume and phenol 0,4% weight/volume. According to the information given by the supplier, all diagnostics had a biological potency of 100 BU/ml, corresponding to an average skin reactivity of 75 square mm [7, 8]. For some of these tests, the supplier also indicated the major allergen concentration [9, 10] as follows:

- *Dermatophagoides pteronyssinus*: Der p 1 40 µg/ml; Der p 2 20 µg/ml
- *Dermatophagoides farinae*: Der f 1 40 µg/ml; Der f 2 20 µg/ml
- Grass mix: group 5 allergens 10 µg/ml
- *Olea europaea* Ole e 1 60 µg/ml
- *Parietaria judaica*: Par j 1 6 µg/ml
- *Artemisia vulgaris*: Art v 1 160 µg/ml

A negative control solution, containing the same concentration of glycerol and phenol was included. As a positive control, histamine dihydrochloride 10 mg/ml was used, which also represented the specific read-out for the purposes of the present work.

The prick test was performed on the volar area of the forearm by introducing the tip of a lancet with a 1-mm tip (Allergy pricker, Bayer DHS, Milan, Italy) into the skin through the allergenic or the control solution, with gentle pressure and without causing any bleeding [11].

Thirty minutes later the areas of the wheal and erythema were marked with a fine-tipped ballpoint pen and transferred onto paper with adhesive tape (Scotch Tape, 3M Italia, Italy) for subsequent planimetric determination of the wheal area. Wheals with an area of less than 7 square mm (i.e., less the 3 mm in diameter) were considered negative [5].

Statistical analysis

The distribution of histamine reactivity and of age were evaluated for normality with the Shapiro-Wilk test, and the symmetry of distribution with the Skewness/Kurtosis test for normality. This was applied both to non-transformed and to log-transformed data. On the basis of this preliminary analysis, comparisons of histamine reactivity in different groups were then performed with the Mann-Whitney two sample statistic for non-parametric data, whereas comparisons of age in different groups were performed with the two-sample t test with equal variances. In order to test whether the dichotomous outcome (belonging to the allergic or to the non-allergic group) was predicted by one or more of the considered independent variables (size of the reaction to histamine,

Table 2. Histamine reactivity (expressed in square mm) in allergic (= at least one positive allergen at skin prick test) versus non-allergic subjects.

	N	Median	Mean	Min-Max	Quartiles
Total	620	21.65	23.75	7.1-54.75	17.75-28.45
Allergic	502	23.7	24.08	7.1-54.75	17.75-28.45
Non-allergic	118	19.8*	22,36	9.6-44.2	15.9-28.3

* p = 0.049 allergic *versus* non-allergic; p < 0.009 if sex and age were incorporated in a logistic regression analysis and the difference was recalculated.
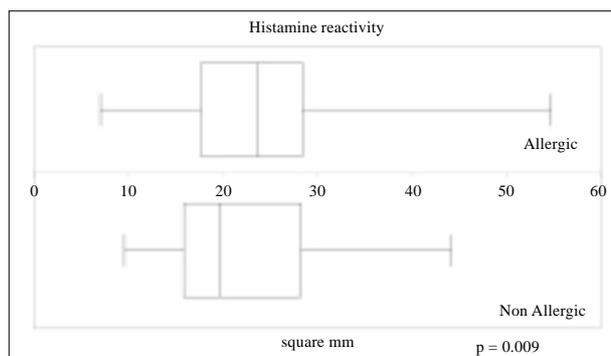

Figure 1. Box-and-whisker representation of values of histamine reactivity (in square mm) in allergic and non-allergic subjects. The box indicates the lower and upper quartile and the central line is the median. The points at the ends of the «whiskers» indicate upper extreme values. Allergic subjects are those with at least one positive allergen at skin prick test analysis. The «p» value refers to the result of the comparison of the two groups when age and sex were incorporated in a logistic regression analysis as independent variables.

sex, age), a logistic regression analysis was performed. This analysis generated a formula that predicted the probability of the occurrence as a function of the independent variables. The Spearman test was used to evaluate the nonparametric correlation between histamine reactivity and age. All statistical analyses were done with the BMPD statistical software package (BMPD Inc., Los Angeles, CA, USA). All statistical tests were two-sided, with a significance level of 0.05.

Results

The population that we studied included 502 and 118 allergic and non-allergic subjects, respectively. Patient characteristics are indicated in Table 1. Age for non-allergic individuals was higher than that of allergic patients (p=0.0489) and the proportion of male subjects prevailed over that of females among allergic individuals (p=0.011) (Table 1).

We found that histamine reactivity was higher in allergic versus non-allergic individuals, cumulatively considered (p=0.049) (Table 2 and Fig. 1). This held true at higher significance level when age and sex were

Table 3. Histamine reactivity (expressed in square mm) and sex.

A: ALL

	N	Median	Mean	Min-Max	Quartiles
Male	307	23.95	25.90	8.35-54.75	17.75-30.75
Female	313	19.80*	21.98	7.10-47.25	16.1-26.0

* p < 0.0001 male *versus* female.

B: ALLERGIC ONLY (N = 502)

	N	Median	Mean	Min-Max	Quartiles
Male	261	26.0*	25.98	8.4-54.8	18.5-33.2
Female	241	19.8	21.96	7.1-47.3	16.1-26.0

* p < 0.0001 male *versus* female.

C: NON-ALLERGIC ONLY (N = 118)

	N	Median	Mean	Min-Max	Quartiles
Male	46	20.7*	22.77	9.6-44.2	15.95-30.75
Female	72	19.8	22.04	9.6-41.8	15.90-26.0

* p < 0.77469 male *versus* female.

incorporated in a logistic regression analysis and difference were recalculated ($CHI^2_3 = 16.47$, p=0.009).

Histamine reactivity was then considered within allergic individuals by comparing subjects with one versus more than one, with two versus more than two, and with three versus more than three positive allergens at skin prick test, respectively (Fig. 2, panels A, B and C,
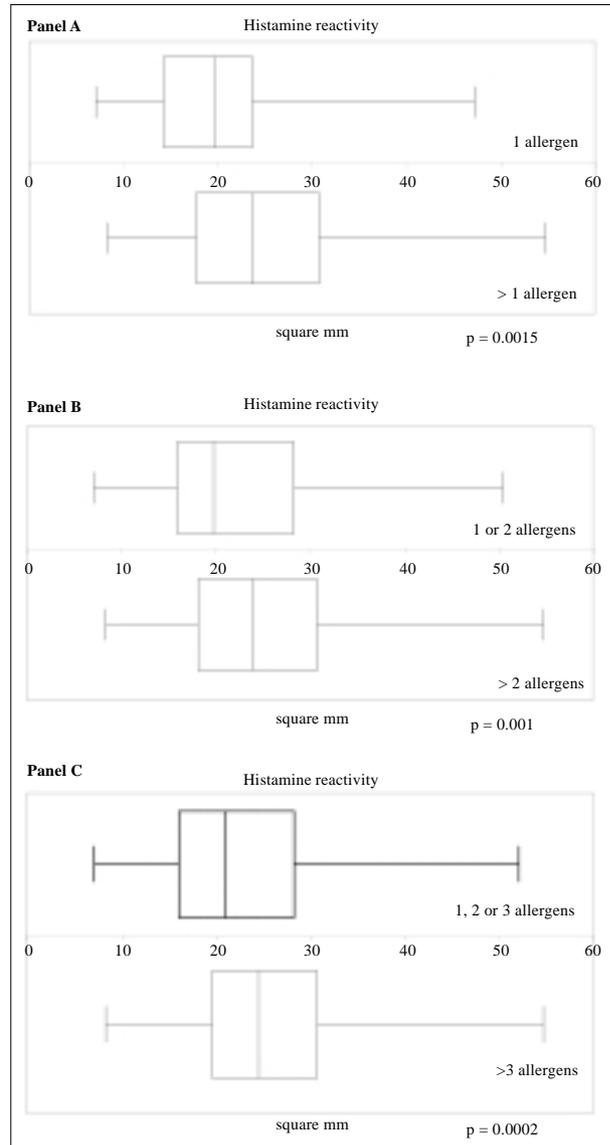

Figure 2. Box-and-whisker representation of values of histamine reactivity (in square mm) in allergic subjects with one, two or three positive allergens at skin prick test analysis versus subjects with more than one (panel A), more than two (panel B) or more than three (panel C) positive allergens, respectively. The box indicates the lower and upper quartile and the central line is the median. Points at the ends of the «whiskers» indicate upper extreme values. The «p» value refers to the result of the comparison of the two groups when age and sex were incorporated in a logistic regression analysis as independent, variables.

respectively). In each case we found significantly higher histamine reactivity in the group with higher number of skin test positivity, respectively, when age and sex were incorporated as independent variables (CHI^2_3 =19.60, p=0.0015; CHI^2_3 = 20.44; p=0.001 and CHI^2_3 = 26.21, p=0.0002, respectively).

The effect of gender on histamine reactivity was then considered. We found a higher skin prick test response to histamine in male versus female subjects, when considering the whole population (p<0.0001). This held true when separately analyzing allergic subjects (p<0.0001), but did not apply to non-allergic subjects (Table 3).

Age and histamine reactivity were significantly correlated in -the study subjects collectively considered (Spearman's rho = 0.3408, p<0.0001) (Fig. 3). This applied also to allergic subjects when they were separately analyzed (Spearman's rho = 0.3163, p<0.0001).

Patients were then grouped according to the season when the test was performed. Results are reported in Table 4. Although no significant differences were observed among considered groups when comparing the more divergent values, the histamine wheal tended to be larger (p=0.0623) in summer, as compared to autumn, in allergic individuals. This tendency was not observed when all studied individuals were cumulatively considered, or in non-allergic subjects only (not shown).

Discussion

Histamine is widely used as a standard in *in vivo* diagnostic assays for allergic diseases. Several reports indicated that different factors affect the extent of the reaction to histamine in single individuals, in a fashion that is reminiscent of aspecific bronchial hyper-responsiveness. These include age [12-14], gender, ethnic origin [15, 16], environmental exposure [17] or specific patterns of sensitization to allergens [6]. Here we report data on a comprehensive study which is simultaneously evaluating age, sex, number of sensitizations and season of the year in predicting the modification of histamine reactivity in allergic and control Caucasian subjects from a semi-rural area of Northern Italy over a one-year study period. We found that allergen responsiveness as measured by skin prick test, sex and age independently contribute to histamine sensitivity. In fact, we observed a positive correlation between the number of positive allergens at skin prick test and the wheal reaction to histamine. Also older age and male sex were associated with a higher response to histamine, both when studied subjects were cumulatively considered and when the evaluation was limited to allergic patients only. When the correlation between allergen responsiveness and sensitivity to histamine was statistically balanced for age and sex in a cumulative regression analysis, it was not only maintained, but even increased. Although a formal sample size calculation was not performed at the initial phase of our study, given the number of individuals

Table 4. Allergic only (N = 502).

	N	Median	Mean	Min-Max	Quartiles
Spring	169	22.10	24.12	7.10-54.75	16.10-30.75
Summer	28	26.60	27.90	12.75-47.25	19.14-35.95
Autumn	182	21.65	23.21	8.35-28.30	17.75-28.30
Winter	123	23.95	24.45	9.60-52.10	17.75-28.45

p = 0.06231 Summer versus Autumn. Histamine reactivity is expressed in square mm.
Spring: March, April, May; Summer: June, July, August; Autumn: September, October, November; Winter: December, January, February.

included in the study, we performed a power analysis (at the 5% significance level) and assessed that it was equal to 53% in the comparison of the whole allergic versus non-allergic population. Thus, our finding on histamine reactivity has enough power and its validity appears warranted.

We previously reported that in a cohort of allergic patients both reactivity to allergen and to histamine increased with age, whereas no correlation was found between age and the ratio allergen wheal/histamine wheal estimated through the skin-prick endpoint [13]. Along this line, Ronchetti et al. reported [18] that in two distinct cohorts of 9-year old children recruited 16 years apart in a given geographical setting, the prevalence of subjects with at least one positive (i.e., ≥ 3 mm) allergen-induced wheal reaction increased. In contrast, if the prevalence of positive skin-prick tests was expressed as the allergen/histamine wheal ratio, it remained virtually unchanged. This observation can be explained by the increased air pollution, since a direct effect of exhaust particles on histamine released by mastocytes has been reported [19, 20]. In this perspective, the allergen wheal/histamine wheal ratio, instead of the absolute allergen wheal, appears more appropriate in the evaluation of possible variations

over time of skin reactivity to allergens. On the basis of these observations, caution should be recommended when considering such relevant issues as the prevalence of positive skin-prick tests to airborne allergens in Western versus Eastern European countries, the age-dependent tendency in asthmatic children to become sensitized to new aeroallergens [21], or the seasonal variation of allergen sensitivity [22]. The relevant question here is: does skin hyper-sensitivity exist, similarly to bronchial hyper-responsiveness, and how should it be considered when evaluating results of skin prick testing? Indeed, it was reported that the skin wheal elicited by histamine 10 mg/ml is a variable that differs in children from different European and African [16] countries and that increases over time in the same place (Italy) [14]. These results, as well as our personal experience, make it unlikely that differences in the strength of pricking children as compared to adults may have a role in determining the data we report. Notably, Ronchetti et al. recently reported [16] in a multi-national survey that a given wheal area corresponds to a serum specific IgE concentration two to three times higher in children with low versus high histamine skin reactivity, suggesting that complex dynamic environmental interactions probably affect skin responsiveness which is measured by histamine and by another mediator (e.g., codeine, a marker of histamine releasability from mast cells) [15]. Among factors that could increase difficulty in the interpretation of skin reactivity, emotional changes may have a further confounding role. However, although it was reported that mood changes can indeed affect the time-dependent increase in the flare reaction elicited by histamine, no effect of emotion was observed for the wheal reactions [23] that are used as end-points in most studies, including this one.

In principle, a more accurate information on the quantitative amount of major allergens in extracts used for diagnosis (and therapy) should limit in the future the need of histamine as an internal reference for prick tests. Nevertheless, the standardization of skin-prick testing with an internal control for aspecific reactivity will certainly remain a useful tool in clinical practice in the next years. Studies on histamine reactivity expressed as histamine concentration eliciting a given wheal size [2-4, 24], using different concentration of histamine [15, 17, 18] and possibly employing strictly quantitative tools to measure the wheal areas [25-27] are promising approaches to finely dissect the intricacies of this issue [28].
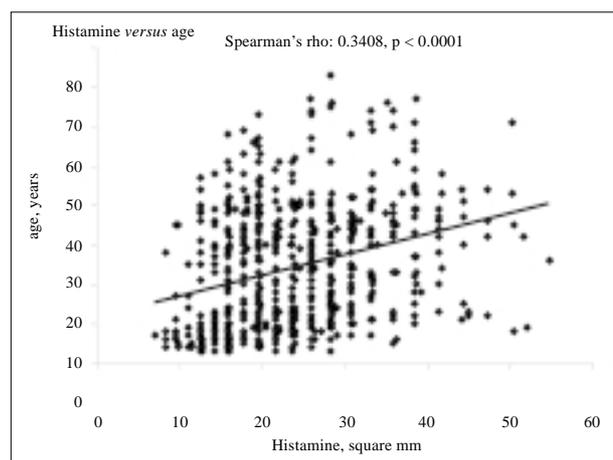

Figure 3. Scatter analysis of histamine reactivity (in square mm, on the x-axis) versus age (in years, on the y-axis) in study subjects collectively considered. Non-parametric Spearman's correlation yielded a rho coefficient equal to 0.3408 and a value of p < 0.0001. For representative purposes, the linear regression trend line is pictured.

Specific IgE are known to increase under environmental allergenic pressure both in blood and in target organs [29-31]. Thus, it could be speculated that the increase in skin reactivity simply reflects the increase in reaginic antibodies. However, this hypothesis is rightfully not accepted [4, 6, 32, 33]. In fact, circulating IgE are not equivalent, in terms of the locally elicited reaction, to cell-bound histamine-releasing active mediators. Accordingly, the slope of the histamine-dose relationship was to be found less steep than the allergen-dose slope [2], suggesting that different mediators and/or mechanisms are involved in the process triggered by allergen, as compared to the mere activation of histamine-receptor dependent mechanisms. Certainly, histamine acts directly on skin tissue components, causing vasodilatation, increased blood flow and edema, and measures the reactivity of the skin. In contrast, skin tests for allergen-specific IgE measure both the effect of histamine and of other preformed mediators such as leukotrienes and prostaglandins released by mast-cells. Thus, histamine challenge is supposedly influenced by the individual sensitivity to histamine itself, whereas allergen-skin test is affected both by the level of membrane-bound IgE on the mast cell and by the individual reactivity to the different categories of mediators they release, including histamine itself. Along this line, Petersen et al., reported that after allergen challenge histamine is present only in the center of the wheal, but not in the periphery, and that a relevant role in the extent of the overall response is played by neurogenic peptides [34].

The analysis of seasonal variations of histamine sensitivity we report here did not show clear-cut variations. However, a tendency to higher reactivity in the summer was observed in allergic individuals, which may depend on specific environmental conditions (e.g., atmospheric pollution). Notably, histamine reactivity was relatively homogeneous in non-allergic individuals, suggesting a role of allergic inflammation in its modulation. This aspect deserves further studies on a larger sample size.

The difference we observed in skin reactivity to histamine is on average quite small and could be considered clinically non-relevant for interpreting skin prick tests in most cases. However, our results may be relevant in the crucial context of recruiting allergic subjects for the purpose of performing the *in vivo* allergen standardization assays which are presently used to determine the biological potency of commercially available allergen extracts [7, 8].

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