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Skin prick-test reactivity to aeroallergens and allergic symptoms in an urban population of central Italy: a longitudinal study

M. L. Dottorini*, B. Bruni*, F. Peccini*, P. Bottini*, L. Pini†, F. Donato‡, G. Casucci* and C. Tantucci†

*Dipartimento di Medicina Interna e Scienze Endocrino Metaboliche (DiMISEM), Università di Perugia, Perugia, Italy, †Cattedra di Malattie dell' Apparato Respiratorio, Università di Brescia, Brescia, Italy and ‡Cattedra di Igiene, Università di Brescia, Brescia, Italy

Clinical and Experimental Allergy

Summary

Background Cross-sectional studies report an increasing prevalence of allergic diseases, such as rhinitis and asthma. Not thoroughly known, instead, is the natural history of allergic sensitization and the progress of the allergic disease-related symptoms.

Aim The purpose of this study was to evaluate longitudinally the skin reactivity for the most common aeroallergens and the allergic symptoms in an urban population living in Perugia, a town of central Italy with a low-level of air pollution exposure.

Methods In the 1998–1999 period 788 subjects were tested for skin reactivity to a panel of aeroallergens and underwent the administration of a questionnaire. These same subjects were part of a cohort of 1200 subjects who participated in a previous epidemiological study performed in 1984–1985 using the same tools. Subjects were aged between 14 and 64 years at the time of the first survey.

Results In the present survey 196 subjects (24.9%) had skin reactivity to at least one aeroallergen, while in the previous survey 143 subjects (18.1%) had skin prick-test reactivity. The increase of the skin reactivity between the two observations was highly significant ($P < 0.001$) and was mainly observed in subjects < 40 -years old. The greatest increment in skin reactivity was seen to *Dermatophagoides pteronyssinus* (house dust mite) allergen. Data obtained from questionnaires showed that subjects who declared allergic symptoms increased from 341 (43.3%) to 380 (48.2%). However, the increase was significant ($P < 0.01$) only in subjects who had a positive association between allergic symptoms and prick-test reactivity and was greater for rhino-conjunctivitis than for asthma-related symptoms.

Conclusions In a cohort of urban population of the centre of Italy, exposed to a low and stable level of air pollution, the sensitization to common aeroallergens increased with time, mostly in people < 40 -years of age. The greatest increment was found for indoor allergens such as *Dermatophagoides pteronyssinus*. A significant increase in allergic symptoms, mainly related to rhino-conjunctivitis, was observed only in the presence of positive prick test.

Keywords aeroallergens, atopy, epidemiology, skin prick test

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Correspondence:

M. L. Dottorini, Via del Favarone 8

06126, Perugia, Italy.

E-mail: marcodottorini@qubisoft.it

Introduction

Many studies have shown that the prevalence of allergic diseases, such as allergic rhinitis (AR) and asthma, has been increasing, particularly in developed countries [1–6]. However, the reported prevalence rates vary between different nations, and this has been attributed to differences in diagnostic methods. The increase of allergic diseases seems due to a real increase in incidence [7], but also to other factors such as an easier access to family

doctors or hospitals in the earlier stage of the disease, greater efficiency of diagnosis, increased knowledge of allergens. Alternatively, the higher prevalence may be due to an increased use of industrial chemical substances, exotic fruits and additives for food conservation.

Most epidemiological studies dealing with prevalence rates of allergic diseases are cross-sectional ones [1–6]. Indeed, few studies are longitudinal, containing large cohorts and spanning long periods of time. In addition, a standard definition of allergic sensitization, based on the

specific serum IgE dosage and/or the skin reactivity to aeroallergens (prick test), is lacking [7–13].

In 1984–1985, a cross-sectional survey of an urban area in the centre of Italy (city of Perugia) with a low level of atmospheric pollution was performed to determine the prevalence of sensitization to the most common aeroallergens and the prevalence of self-reported allergic symptoms in a sample representative of the general population [14, 15]. The present study was planned 14 years later (1998–1999) testing the same subjects to assess the longitudinal changes in skin reactivity to the same aeroallergens in the different age groups. Secondary aims of this study were to estimate progression of symptoms of allergic diseases (in particular conjunctivitis, rhinitis and asthma) and the association between skin reactivity and symptoms of allergic diseases.

Materials and methods

Study population

All the subjects included in the first epidemiological study (1984–1985), with the exception of those who died ($n = 49$), moved to other cities ($n = 95$), were sick ($n = 2$), refused to participate ($n = 236$) or were not traceable ($n = 30$), were contacted with an invitation letter or by phone. Thus, we were able to recruit 788 of the original 1200 subjects [14]. At the time of the first survey the subjects were aged between 14 and 64 years and were recruited, as representative of the urban population of the city of Perugia, through a randomized sampling, stratified for age, social–economical status and family group.

The sample ($n = 788$) observed in the 1998–1999 survey was homogeneous and similar for sex, age stratification and smoking habit to the sample ($n = 1200$) examined in the 1984–1985 survey.

Furthermore, the non-participants to follow-up ($n = 412$) were not different as compared with the participants to follow-up ($n = 788$) with regard to baseline parameters, in particular sex, skin reactivity and presence of allergic symptoms.

Subjects in the study gave written consent and then they underwent skin prick test (prick test) and administration of the same questionnaire used in the previous study (1984–1985). Lung function tests were also performed, but these results will be published in a subsequent paper. None of the 788 subjects used medications that would mask sensitization, such as antihistaminic drugs.

Allergy tests

The same allergens used in the first study were tested again *Graminaceae* (grass), olive, *Urticaceae* (parietaria), plants of the *Compositae* family, *Dermatophagoides pteronyssinus* (house dust mite), *Alternaria tenuis*, cat and

dog dander, horse dandruff, *Aspergillus fumigatus*. All the allergens, the diluents and the positive control (histamine 10 mg/mL) were prepared by Lofarma Allergeni (Milan, Italy), which supplied the same allergens for the previous study. Allergens were chosen according to the pollen status of the area where the subjects enrolled in the study were living. In this area *Graminaceae*, *Parietaria* and olive were the most prominent outdoor allergens and their levels did not change in the period between the two studies. [15, 16].

The prick test was performed as in the previous study [15]. Briefly, single drops were put on volar surface of the forearm, spaced 3.5 cm from each other, with the first and the last drop at the same distance from elbow and wrist. A needle was used to scarify the skin just below the single drops. Prick tests were read after 20 min. Positive prick-test reactivity was defined as the presence of a mean weal diameter of at least 5 mm.

Questionnaire

The questionnaire administered was prompted by the National Research Council (NRC) and it was the same as used previously [17]. This questionnaire was composed of questions about disease history, allergic and respiratory symptoms, smoking habits, occupational history and environmental and domestic conditions.

Questions about allergic symptoms included atopic eczema; rhinitis; respiratory distress with bronchial wheezing; itching; burning and redness of the eyes; and allergic symptoms secondary to food or drug ingestion, allergic symptoms secondary to contact with insecticides, flours, detergents, dusts, synthetic fibres, feathers and hair sprays or other.

The study was approved by the local Ethics Committee.

Air pollution

The Epidemiological and Public Health Laboratory of the Local Sanitary Unit of Perugia reported that the pollution levels (SO_2 , NO_2 and particulate) were lower than threshold values at the time of the 1984/1985 study ([18], see Appendix A). In 1992 and 1998 the pollution levels had not changed (see Appendix A). The air pollution was essentially due to domestic heating systems and motor traffic, taking into account that the productive activities of Perugia are mainly due to artisan and commercial settlements [19].

Allergen prevalence

During the second study and between the two studies, the environmental prevalence of allergens tested did not change. Main plant allergens were *Graminaceae*, *Urticaceae* and *Compositae*; the main tree allergen was olive [16, 20].

Table 1. Changes of skin test reactivity according to the age groups

Age (years)	P ⁻ /P ⁻	P ⁻ /P ⁺	P ⁺ /P ⁺	P ⁺ /P ⁻	Difference (%)	Total
< 19	41 (54%)	11 (14.5%)	23 (30.2%)	1 (1.3%)	13.2	76
20–29	86 (65.2%)	14 (10.6%)	28 (21.2%)	4 (3%)	7.6	132
30–39	110 (65.5%)	17 (10.1%)	38 (22.6%)	3 (1.8%)	8.3	168
40–49	141 (75.8%)	18 (9.7%)	19 (10.2%)	8 (4.3%)	5.6	186
50–59	(82.5%)	(7.2%)	(7.2%)	(3.1%)	4.2	166
> 60	55 (91.6%)	(5%)	1 (1.7%)	(1.7%)	3.3	60

P⁻/P⁻, negative prick test in the 1984/1985 and 1998/1999 surveys; P⁻/P⁺, negative prick test in the 1984/1985 survey but positive in the 1998/1999 survey; P⁺/P⁺, positive prick test (one or more) in the 1984/1985 and 1998/1999 surveys; P⁺/P⁻, positive prick test (one or more) in the 1984/1985 survey but negative in the 1998/1999 survey; difference (%), percent difference in the positive prick-test between the 1984/1985 and 1998/1999 surveys.

Statistical analysis

The categorical variables related to different positive prick tests for at least one allergen were analysed. Association analysis with the history of allergic diseases was carried out using the common techniques of analysis of data in proportions [21].

All the statistical tests used were two-tailed, using the 5% threshold ($P < 0.05$) to reject the null hypothesis. Data were analysed using STATA statistics program (statistical software: Release 8.0, State Corporation, College Station, TX, USA).

Results

Prevalence of skin reactivity

All the 788 subjects recruited for the second study, (424 males and 364 females) agreed to repeat prick tests. One hundred and ninety-six subjects had a positive prick test to one or more allergens (24.9%) and 592 (75.1%) had a negative test, while in the previous survey 143 (18.1%) of the same sample had a positive prick test for at least one allergen. Compared with the previous survey a significant increase of subjects with a positive prick test was found ($P = 0.001$).

Although the prevalence rates were increased for both genders in the 1998–1999 survey, no difference was seen in the prevalence for the prick-test reactivity between genders in the first as well as in the second survey. In fact, in the 1984–1985 survey 79 males (18.7%) and 63 females (17.3%) were positive to one or more allergens, while in the 1998–1999 survey 103 males (24.3%) and 91 females (25%) were positive to one or more allergens, respectively.

Seventy-five subjects with a negative prick test in the 1984–1985 survey become positive to one or more allergens in the 1998–1999 survey, while 22 subjects with a positive skin prick test to one or more allergens in the 1984–1985 survey become negative in the 1998–1999 survey. When samples were stratified by age, the percentage of the prick-test conversion (P⁻ to P⁺) was greater

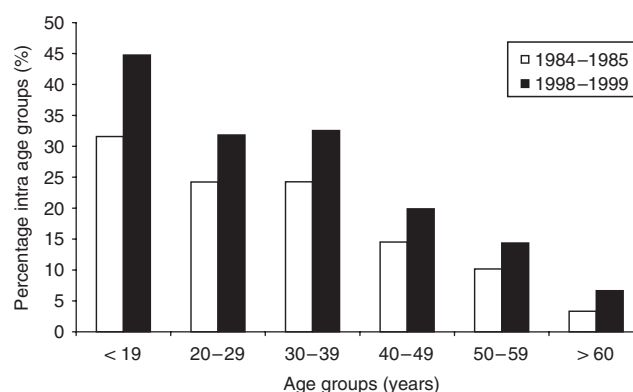


Fig. 1. Prevalence of the prick-test positivity for different age groups in the two surveys.

in the first age group. Reversion (P⁺ to P⁻) was distributed, without significant percent differences, in every age group (Table 1).

The distribution of the prevalence of skin reactivity for age groups was the same as reported in the first study, that is higher in the first age group and progressively lower with increasing age. The differences in prevalence of sensitization for age groups were not statistically different between the two surveys (Fig. 1).

Mono- and poly-sensitization

In the present study 12.6% of the overall sample had a positive prick test for only one allergen, while 8.4% of the same subjects was sensitized to one allergen in the 1984–1985 survey. In the 1998–1999 survey the poly-sensitized subjects were 12.3%, while in the 1984–1985 survey they were 9.8%.

Comparison between the 1984–1985 and 1998–1999 surveys showed that the prevalence of mono-sensitization increased in all age groups as well as the prevalence of poly-sensitization, with a higher percentage of poly-sensitized subjects in the younger age groups (< 40 years) as compared with the other groups (> 40 years). The differences in each age group between the two surveys

Table 2. Age-specific prevalence of prick test positivity after 14 years

Year of observation	Atopy for age-groups (years)			
	30–39	40–49	50–59	60–64
1984–1985	24.4% (<i>n</i> = 41/168)	14.5% (<i>n</i> = 27/186)	10.2% (<i>n</i> = 17/166)	3.3% (<i>n</i> = 2/59)
1998–1999	40.6% (<i>n</i> = 52/128)	28.9% (<i>n</i> = 44/152)	27.5% (<i>n</i> = 55/200)	13.9% (<i>n</i> = 11/79)
<i>P</i>	< 0.01	< 0.01	< 0.001	< 0.05

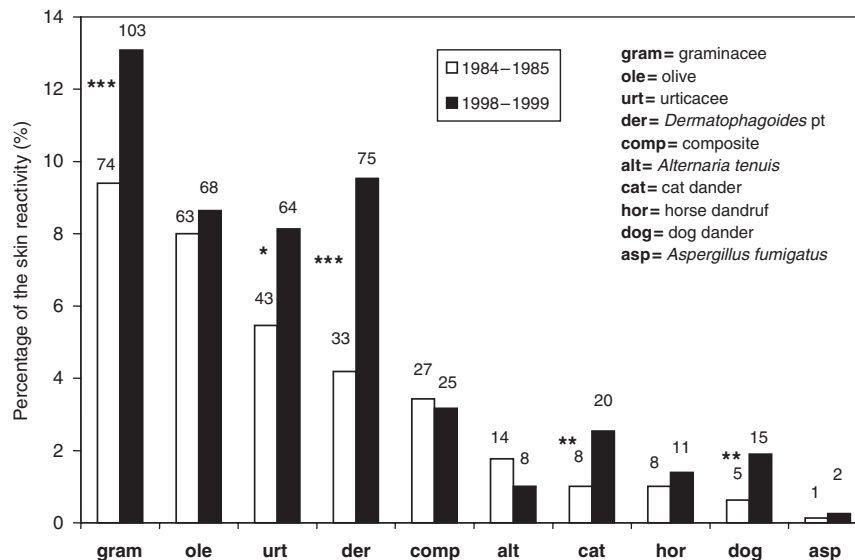


Fig. 2. Prevalence of the prick-test positivity to the different aeroallergens in the overall population (*n* = 788) in the two surveys. **P* = 0.05; ***P* < 0.05; ****P* < 0.001 vs. 1984–1985 survey.

were not statistically significant for both mono-sensitization and poly-sensitization.

Age specific prevalence of prick-test positivity

Although we were interested to the longitudinal changes in allergic sensitization, we also performed a sub-analysis to compare the positivity to prick test in those subjects with similar age examined in the two different surveys to assess the changes in the age-specific prevalence of allergic sensitization after 14 years. The results are shown in the Table 2 and clearly demonstrate a significant increase in prevalence of allergic sensitization, according to the skin test reactivity, in each age group considered.

Prevalence of reactivity to tested allergens

In the 1984–1985 survey subjects had the greatest reactivity to *Graminaceae*, followed by olive and *Urticaceae*.

The prevalence rates were similar between the two studies, except for *Dermatophagoides* pt., which was the fourth most prevalent allergen (after *Graminaceae*, olive and *Urticaceae*) in the 1984–1985 survey, becoming the second most common in 1998–1999 survey.

In the total sample the increase in positive prick tests, expressed as percentage unit, was 5.3% for *Dermatophagoides* pt. (*P* < 0.001), 3.7% for *Graminaceae* (*P* < 0.05), 2.7% for *Urticaceae* (*P* < 0.05), 1.5% and 1.3% for cat (*P* < 0.05) and dog dander (*P* < 0.05), respectively. It is worthy to note that increments in the prick-test reactivity for cat and dog dander and *Dermatophagoides* pt. reflect an increased sensitization to indoor allergens (Fig. 2).

In the 1984–1985 survey the stratification for age groups of the reactivity to most relevant allergens showed the prominence of *Graminaceae*, olive and *Parietaria* with the highest values observed in the younger age groups (Fig. 3). In the 1998–1999 survey a small not significant increase in the prick-test reactivity was found in all age groups for the above-mentioned aeroallergens with the same trend of distribution (Fig. 3). In contrast, the reactivity to *Dermatophagoides* pt. was markedly increased in all age groups, showing a significant (*P* < 0.005) increment in the 30–39 years and 40–49 years classes of age (Fig. 3).

Correlation between prick-test and history

In the 1984–1985 survey, 341 (43.3%) subjects of 788 reported symptoms (*S*⁺) related to allergic diseases in the questionnaire. Among these 341 subjects, 107 (31.2%) had

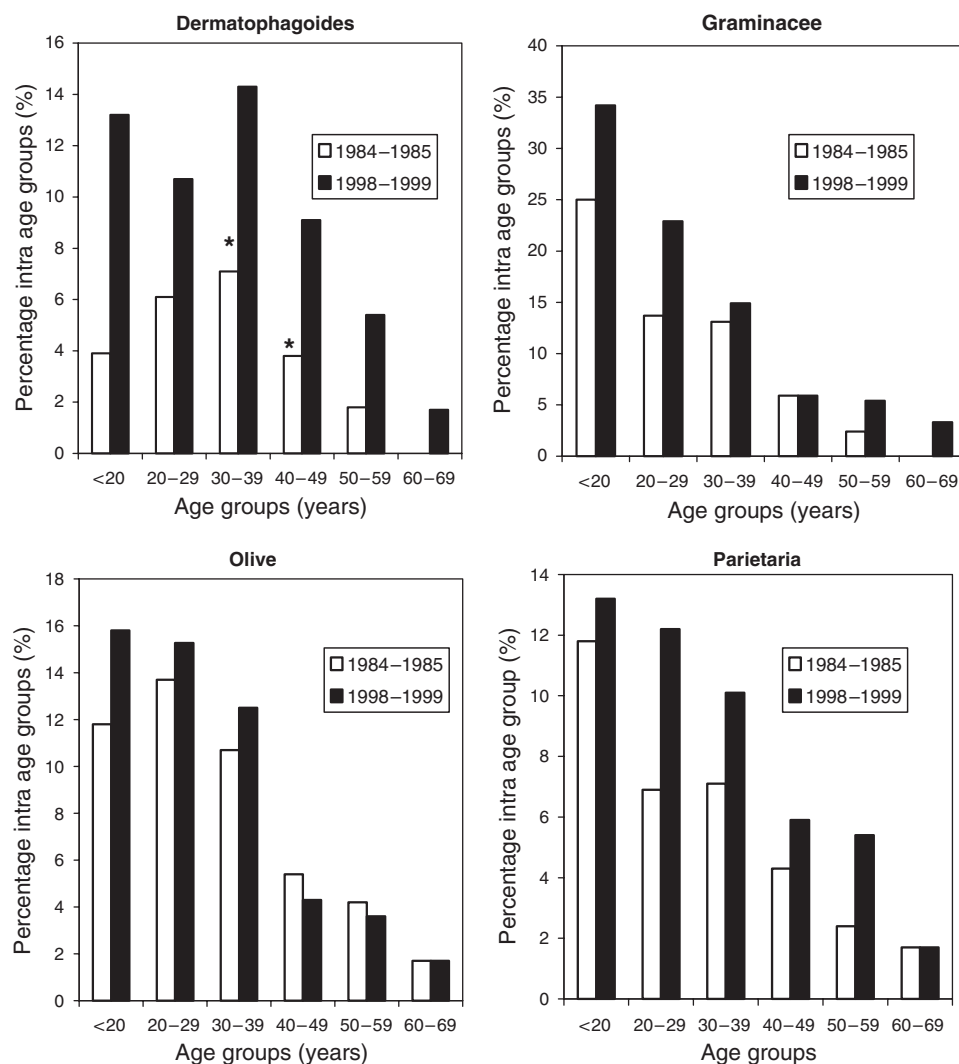


Fig. 3. Percentage of the positive prick tests to the main aeroallergens, according to age groups, in the two surveys. * $P=0.05$.

Table 3. Association between prick test sensitivity and history of allergic symptoms as referred in the two surveys (1984-1985 and 1998-1999)

	1984-1985 study			1998-1999 study		
	Positive history	Negative history	Total (%)	Positive history	Negative history	Total (%)
Positive prick	107 (74.8)	36 (25.2)	143 (18.1)	147 (75.0)	49 (25.0)	196 (24.9)
Negative prick	234 (36.5)	411 (63.5)	645 (81.9)	233 (39.4)	359 (60.6)	592 (75.1)
Total	341 (68.8)	447 (92.0)	788	380 (61.3)	408 (88.0)	788

Between brackets percentage values of prick test (positive or negative) relative to the history results (first row) and of history (positive or negative) relative to the prick test results (second row).

a positive prick test (P^+). Of 447 (56.7%) subjects with a negative clinical history (S^-), 36 (8.0%) had a positive prick test (P^+) (Table 3).

In the 1998-1999 survey, 380 subjects (48.2%) had a positive allergy history (S^+). Among these, 147 (38.7%)

had a positive prick test (P^+). Of 408 (51.8%) subjects with a negative clinical history (S^-), 49 (12.0%) had a positive prick test (P^+).

A significant increase ($P < 0.001$), from 13.6% (107/788) to 18.6% (147/788), was observed in the subjects

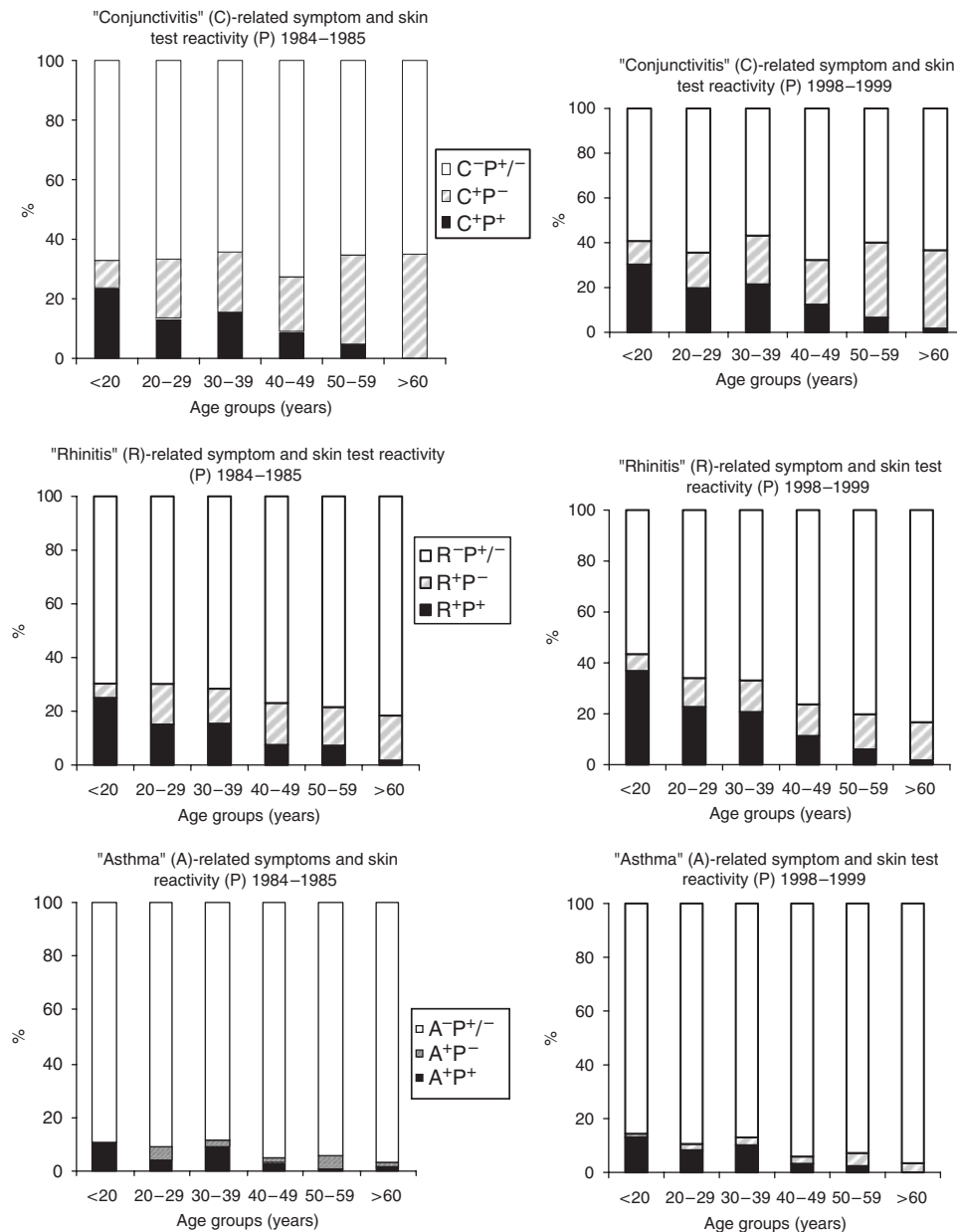


Fig. 4. Prevalence of the association between symptoms referable to allergic diseases and skin reactivity in the two surveys, stratified by age groups.

with a positive prick test and clinical history (S⁺ and P⁺) when comparing the two surveys. Instead, the difference in subjects with a negative clinic history but with a positive prick test (S⁻ and P⁺), from 4.6% (36/788) in the 1984-1985 survey to 6.2% (49/788) in the 1998-1999 survey, was not significant. The increase found in the (S⁺ and P⁺) prevalence between the two surveys, when stratified by age groups, was not significant.

It is interesting to note that asymptomatic subjects with poly-sensitization in the 1984-1985 survey become symptomatic in the 1998-1999 survey in a greater percentage (six subjects over ten, equal 60%) than asymptomatic subjects with mono-sensitization (eight subjects over 20,

equal 30.8%). Owing to the small numbers of subjects changing status, statistical evaluation was not performed.

Most common symptoms in the subjects with positive prick test were associated with rhinitis, conjunctivitis and, to a lesser extent, with bronchial asthma in both studies (Fig. 4). Compared with that found in the 1984-1985 survey, the prevalence of the combination of the symptoms related to allergic diseases and positive prick tests increased in all the age groups, except the oldest two, in the 1998-1999 survey, particularly for rhinitis and conjunctivitis (Fig. 4). The prevalence of this combination, however, was not significantly increased when stratified for age groups between the two surveys.

Subjects with negative prick test (P^-) but declaring symptoms were present in both surveys with similar percentages.

Discussion

This present study is based upon a previous survey conducted in 1984–1985 on a 1200 subject cohort recruited from the urban population of Perugia in the centre of Italy. After 14 years we re-examined 788 subjects from the same cohort with a similar distribution for age, gender and smoking habits as in the 1984–1985 survey. Experimental procedures and analysis were identical in the two surveys, allowing for longitudinal assessment of the same sample in terms of allergen reactivity and symptom scoring. Possible selection bias due to non-participants to follow-up regarding age, sex, atopy and allergic symptoms were checked and excluded.

Sensitization to one or more aeroallergens as documented by positive prick test significantly increased from 18% to 25% in the overall sample after 14 years. Noticeably, subjects in the youngest age group showed the higher percentage of conversion from negative to positive prick test to one or more allergens when comparing the two surveys.

Other studies have reported similar increases in sensitization to one or more allergens [8–11]. In a 5-year study conducted on German schoolchildren [10] the prevalence of positive prick test to at least one of the six allergens tested, was increased from 19.3% to 26.7%.

In another study performed in a cohort of English adults [11] the prevalence of positive prick test to at least one of the three allergens tested, was increased from 17% to 28% during an observation period of 14 years.

In a longitudinal study planned in Tucson (Arizona) 1333 subjects of every age were tested [9]. The increase of positive prick test was of 11.6% (from 39.1% to 50.7%) after an observation period of about 6–10 years. Moreover, the increase of sensitization has been noticed in all the age groups but it was higher in the younger age groups, while the loss of sensitization was most notable after the 35-years of age. In two Australian studies, the first conducted on schoolchildren [12] and the second on adult subjects [13], there was an increase, although not significant, in the prick-test reactivity.

In line with these studies and in agreement with our results, a longitudinal increase in the prevalence of allergic sensitization to common inhalant allergens, looking at the changes of serum specific IgE, was also reported after 8 years in 386 subjects from an initial cohort of 599 subjects, aged 15–69 years, randomly recruited in general population in Copenhagen. The increase in serum-specific IgE was significant among subjects who were < 30 years at baseline [22]. In contrast, a multi-centre study, aimed to assess longitudinal changes in IgE sensitization by measuring serum-specific IgE to common allergens on two

occasions about 9 years apart in 6371 young adults living mainly in Western Europe, failed to show overall a significant change in the prevalence of sensitization to at least one of house dust mite, grass or cat dander [23].

As already observed in the 1984–1985 survey, there was no significant difference in the skin reactivity between the genders also in the present survey, being 24.9% and 24.3% the percentage with positive prick test for males and females, respectively. The absence of correlation between gender and sensitization we found, confirms the results obtained by other authors [9, 24, 25]. Furthermore, a similar increase in the prevalence in positive prick test was found for both sexes, equal to 5.6% for males and 7.7% for females.

In our study we found that the increase of mono-sensitization (positive prick test to one allergen) was greater than the increase of poly-sensitization (positive prick test to more than one allergen) in all age groups with the exception of the class of 20–29 years. It is of interest to note that the poly-sensitization seems to increase more in the younger age groups until 40 years of age as compared with the older age groups. Other authors reported the same trend of the increase of sensitization, with a prevalence of poly-sensitization in younger subjects [26]. Such observations support the idea that the increment of sensitization and poly-sensitization diminishes in the older age groups, as reported in other studies [9]. Indeed, it is probable that in the general population, the sensitized subjects of advanced age have a progressive reduction in the IgE synthesis; moreover, the response of target organs (skin) to the mediators seems to be influenced by the ageing process, because of genetic and non-genetic factors such as different age-related skin reaction to the allergen exposure.

Sensitization to *Graminaceae* and *Urticaceae* is increased with time in the overall cohort, although it is more marked in the younger age groups (Figs 2 and 3). This is in agreement with the higher prevalence of these allergens in the aeroallergens in the geographic area where the two surveys were performed.

The most interesting observation, however, is the significant increment in the skin reactivity towards *Dermatophagoides* pt., a perennial indoor allergen, that was observed in all age groups (Figs 2 and 3). Hence, it is possible that the increase in allergic symptoms associated with positive prick test is due to an increased sensitization to *Dermatophagoides* pt., particularly in the intermediate age groups who tend to remain longer in indoor environments. Our findings support the results of previous cross-sectional studies where a higher prevalence of positive prick test to *Graminaceae*, *Urticaceae* and *Dermatophagoides* pt. was reported [27–29]. Our longitudinal data, however, indicate that with ageing in such populations a prevalent increase in sensitization would be expected essentially for indoor aeroallergens such as *Dermatophagoides* pt. and in middle age subjects.

Conversely, a loss of sensitization (from positive to negative prick test) was observed in 22 subjects (3% of the total sample); among these subjects 14 (66%) were over 40 years of age in the 1984–1985 survey, suggesting that other mechanisms, apart from the ageing process, may be involved. Indeed, Barbee et al. [9] have reported similar findings in their study, showing that disappearance of prick-test reactivity did not occur only in older population. The loss of sensitization cannot be explained by reduced allergen exposure, for instance a strict prevention from exposure to indoor allergens, as the subjects who reverted were almost all positive for outdoor allergens.

In the present study, the increase of symptoms related to allergic diseases associated to positive prick test was seen in all the age groups, but the tendency lessened from the younger age groups to the older ones. This fits with the results obtained in several cross-sectional studies showing that, at least in the western countries, the allergic diseases and especially the AR are increasing, particularly in younger population [6, 28–34].

A study conducted in the centre of Italy showed a prevalence of 8.1% for asthma and of 15.2% for rhinitis in a population aged between 0 and 69 years [32]; the association with positive prick test were present in 60–70% of the subjects with asthma, rhinitis or both. In a multi-centre study (ECRHS) where a sample of 13 558 subjects randomly selected from the general population of 16 European countries was recruited [35], it was reported that the association between atopy and asthma was present on average in about 30% of asthmatic subjects, with a variation between 4% and 61% among the different centres.

More interestingly, in a recent longitudinal study conducted in the North of Italy a questionnaire was sent by mail to subjects to determine the presence of symptoms related to allergic diseases. After 7 years a significant increase in the prevalence of AR-related symptoms was observed, in contrast to what was found for those related to asthma [31]. In agreement with this finding, in our study the increase of allergic sensitization, as shown by the prick tests, seems to be more related to conjunctivitis and rhinitis than to bronchial asthma, as documented by the corresponding symptoms.

In conclusion, the results obtained in the present study show that after 14 years in a sample representative of an urban population living in a region of low and stable air pollution: (i) allergic sensitization increased significantly and in all the age groups; (ii) the largest increment in the skin reactivity to aeroallergens was found for *Dermatophagoides pteronissimus* especially in the middle age subjects; (iii) increase in sensitization was associated with an increase of the symptoms related to allergic diseases, particularly to conjunctivitis and rhinitis rather than to asthma and mostly in subjects younger than 40 years.

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Appendix A

Table A1. Atmospheric pollution in the urban area of Perugia

Survey	1984	1992	1998	Safety threshold value
SO ₂	55	28	4	125
NO ₂	27	44	39	200
Particulate	109	56	73	150

Data are in mcg/m³ (annual mean value). The safety threshold values are those indicated by law in Italy (DPR no. 203 1988 and DM 1994).