

# Allergy-Like Asthma and Rhinitis

## A Cross-Sectional Survey of a Respiratory Cohort and a Diagnostic Approach Using the Autologous Serum Skin Test

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### Key Words

Allergy · Asthma · Autologous serum skin test ·  
Epidemiology · Rhinitis · Urticaria

### Abstract

**Background:** Chronic idiopathic urticaria is considered an allergy-like skin disorder, and in some cases an auto-reactivity caused by mast cell degranulating factors has been demonstrated. A positive reaction to the autologous serum skin test (ASST), reflecting the presence of factors capable to degranulate the mast cells, is regarded as a reliable in vivo diagnostic test in chronic urticaria patients. About one out of three patients complaining for rhinitis or asthma does not show sensitization to any allergenic source. No data are available on the application of ASST to respiratory patients. **Methods:** A cohort of respiratory patients, aged 1 to 80 years, complaining about suspected respiratory symptoms was screened using a panel of inhalant allergens by means of SPT and IgE. Current and past information on skin and respiratory symptoms were recorded for each patient. Patients were divided in 'allergy' and 'allergy-like' whether or not they react to at least one allergenic source. Age and gender distribution were analyzed. A control group fulfilling criteria for chronic idiopathic urticaria (CIU) was selected as well. The ASST was applied

to the control CIU group, to the allergy-like and allergy subsets, and to a group of 58 healthy adult subjects. **Results:** Allergy and allergy-like patients were differently distributed in relation to age and gender. Allergy-like children were prevalent before age six, whereas the prevalence of allergics steadily increased with age representing the large majority of patients in late childhood. The ratio of allergy/allergy-like subjects continues to increase in adult males, whereas adult females represent the large majority of the adult patients mainly within the allergy-like subset. Fifty-eight percent of CIU control patients reacted to the ASST. Among allergy-like patients ASST was positive in 47% of the adults (male 26%, female 54%), and in 84% of the children (no gender differences). The percentage of reactive subjects increased in the adult allergy-like subset if a CIU was associated (65%). Eighty-six percent of the pediatric patients and 61% of the adults having an allergy respiratory disease associated with an allergy-like condition had a positive ASST reactivity. Pure allergy patients reacted in 73% and 40% of the cases in the children and adults subgroups, respectively. Forty-five percent of healthy controls reacted to the ASST as well, and statistically significant gender differences were still recorded. **Conclusions:** An ASST reactivity is reported for the first time in allergy-like respiratory patients. A higher prevalence of reactive subjects has been recorded in all the pediatric sub-

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sets without gender differences, whereas female reactivity is prevalent among adults. The ASST reactivity seems to parallel the patient distribution within the entire respiratory cohort. Further studies are needed to demonstrate that the serum factors causing the ASST reactivity in respiratory patients are the same as for CIU affected subjects. In the light of recent *in vitro* findings, the ASST reactivity of healthy subjects could lead to a new interpretation of the autologous serum reactivity.

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## Introduction

Allergen exposure is considered the main trigger and maintenance factor of the bronchial asthma inflammation in sensitized individuals [1]. However, subjects with persistent bronchial symptoms mimicking an allergic disorder without allergic sensitization do exist [2]. This group of patients has been defined as having 'intrinsic asthma' in opposition to the allergen-induced IgE-mediated form (extrinsic asthma). Nevertheless, the two forms seem to lead to an almost identical immunopathological pattern [2]. The same dichotomy exists for the upper respiratory tract where allergic and non-allergic mechanisms have been claimed to cause perennial rhinitis whether or not associated with bronchial asthma [3–8]. Applying an extended panel of allergenic extracts for respiratory allergy screening, we recorded allergy-like symptoms in about 30–35% of the subjects with suspected respiratory allergy disease [9, 10].

The cutaneous counterpart of persistent allergy-like symptoms without evidence of an IgE-mediated sensitization is chronic idiopathic urticaria (CIU) [11, 12]. An autoimmune mechanism has been identified as the cause of 30–60% of CIU cases [13, 14]. The presence of autoimmune IgG antibodies directed toward the  $\alpha$ -chain of the high affinity IgE receptor (Fc $\epsilon$ RI $\alpha$ ), present on mast cell and basophil membranes [11–14], and, less frequently, of IgG directed toward the IgE molecule has been shown to cause CIU [13, 15–18]. Other histamine-releasing factors (HRF) have been claimed as a possible cause of mast cell degranulation in CIU patients [19]. In addition to the histamine release test, that allows the *in vitro* functional detection of anti-Fc $\epsilon$ RI $\alpha$ , anti-IgE, or other HRF [13, 14, 18, 19], the autologous serum skin test (ASST) is an *in vivo* validated test, performed injecting the patient's own serum into the skin in order to mimic the CIU autoreactivity [13, 18–22]. The ASST has been reported positive in 40–60% of CIU patients [13, 18, 20–22]. In a personal

experience, a 60% positive ASST results has been recorded in a selected CIU adult population [Mari, unpubl. data]. Little is known about prevalence and pathogenesis of chronic urticaria in childhood [23–26]. A positive assay for anti-Fc $\epsilon$ RI $\alpha$  has been reported in three out of seven adolescents [23].

None of the studies on CIU patients seems to report the presence of respiratory symptoms in such patients [11, 12, 22, 27], and no ASST reactivity studies in intrinsic asthma or in allergy-like rhinitis or in respiratory allergy have been published so far.

In the present study an epidemiological survey of a cohort of respiratory patients was first carried out, trying to define the age and gender distributions of subjects with an underlying IgE-mediated mechanism versus those having allergy-like non-IgE-mediated respiratory symptoms. A cohort including pediatric and adult subjects complaining about suspected respiratory allergy symptoms has been studied. In the second part of the study, the ASST was applied to several subsets of respiratory patients. This followed a preliminary observation confirming the existence of the respiratory-symptom-free CIU adult patients, but even recording the existence of a subgroup of CIU patients with associated perennial allergy-like rhinitis or with combined perennial rhinitis and intrinsic asthma. A positive ASST recorded in some subjects belonging to the latter group suggested the presence of a similar auto-reactivity in other subgroups of patients with intrinsic symptoms not included in previous CIU studies. A positive ASST has been recorded in either adult patients with respiratory allergy-like clinical condition or even in those associating a true allergy-related disease. Furthermore, ASST was applied to children having intrinsic cutaneous and respiratory symptoms. The average prevalence of positive ASST results in all the children subsets was higher than that in adults and no differences between genders were recorded. Positive ASST results in adult healthy controls pose the need of a new interpretation of the autologous serum skin reactivity.

## Materials and Methods

### *Patients' Selection*

An unselected population referred to an allergy outpatient department of the National Health Service was examined. Almost all the patients were previously evaluated for their disease either by the general practitioner or family pediatrician, or by other non-allergy specialists (ENTs, dermatologists, pneumologists) before referred to the allergy unit. Consecutive subjects ( $n = 5,567$ ) complaining about respiratory symptoms of suspected allergic rhinitis whether or not associated with suspected allergic asthma were enrolled.

Criteria for the clinical diagnosis of suspected allergic rhinitis matched those reported in the ARIA document [28]. GINA diagnostic guidelines were used to define patients with asthma [29]. COPD patients were excluded on the basis of international guidelines [30]. Patients fulfilling the clinical criteria for a CIU diagnosis were selected as well [11, 12, 27].

Patients or parents were fully interviewed about current and past history of any symptoms suspected to be allergic in nature. All patients underwent standardized skin prick test (SPT) with a wide panel of respiratory allergenic sources (Allergopharma, Reinbeck, Germany; Stallergenes, Antony, France) [9, 10, 31]. Pediatric patients aged six or younger were also tested with a panel of food allergens including wheat, soy, tomato, milk, egg white, fish, and shrimp extracts (Allergopharma). In case of all negative SPT, IgE detection to a panel of inhalant and food allergens was carried out in all patients (milk, wheat, egg white, mites, cat, Alternaria, grass; ImmunoCAP system, Pharmacia, Uppsala, Sweden). All the adult patients with a current or past history of suspected allergic skin diseases (e.g. occasional or intermittent urticaria, atopic dermatitis) also underwent SPT with an extended panel of food extracts (Allergopharma). A positive food SPT having a causative role in the skin disease led to the exclusion of the patient. Patients with physical, cholinergic or vasculitic urticaria were excluded. Patients fulfilling all the clinical criteria but having all negative in vivo and in vitro allergy tests were classified as having an allergy-like respiratory symptoms or a CIU. The term 'allergy-like' has been adopted to easily summarize the clinical condition of the respiratory patients. The recently suggested EAACI nomenclature [32] of 'non-IgE-mediated allergic rhinitis or asthma or urticaria' has not been adopted because it includes several other immunopathogenetic mechanisms excluding the histamine release from mast cells. Respiratory subjects are defined throughout the present study as 'allergy' or 'allergy-like' depending on the above mentioned diagnostic criteria. As no differences were found in either rhinitis or rhinitis plus asthma distribution within the examined cohorts, patients in both conditions are herein reported as having 'respiratory' symptoms.

Family physicians were involved in the enrollment of adult healthy subjects. Seventy subjects were selected. From their personal histories and records none had evidence of a current or past presence of cutaneous and/or respiratory allergy or allergy-like symptoms, or autoimmune and any other relevant disease. All underwent SPT to foods and inhalants. Twelve of 70 (17.1%) had a positive SPT to at least one allergenic extract and, though asymptomatic, were excluded. A control group of 58 healthy adult subjects was established. Age and gender distribution matched those of the other adult groups. A pediatric healthy control group couldn't be established due to ethical committee limitations. The study has been carried out between years 1999–2003.

#### *Autologous Serum Skin Test*

The ASST was applied to patients with either chronic urticaria or with respiratory symptoms, both with active disease at the time of testing. Blood samples were drawn in fasting patients using 10-ml vacuum sterile tubes without clotting accelerator (Venoject, Terumo, Leuven, Belgium). Blood was allowed to clot at room temperature for 2 h. Serum was separated by centrifugation at 500 g for 15 min and recovered under sterile conditions soon before its use in the ASST. The intradermal injection was performed using a sterile single use 27G-needle syringe (Myjector, Terumo, Leuven, Belgium). The following methods and criteria were applied to the ASST: the test was

carried out in triplicate by the intradermal injection of about 50 µl of fresh autologous serum and of an equal amount of a saline solution as control test. Histamine (Stallergenes) was used as positive control to exclude any residual inhibitory effects of drugs. The test was applied on the volar surface of the forearm. In case of urticaria patients, areas involved in spontaneous whealing in the last two days were avoided. A gap of at least 3 cm was left between each injection site. Only three out of three positive concordant reactions allowed the subject to be included in the positive subset. A negative reaction to the saline control test was required as well. Furthermore, ASST reactions were scored positive when a serum-induced wheal with a diameter 1.5 mm greater than the saline control was present, and surrounded by an erythema [20, 33]. All the tests were applied and read at 30 min by the same operator. In all the subjects antihistamines were withdrawn at least fourteen days before the test. A 7-day suspension was allowed for patients with severe chronic urticaria when a relapse of the wheals occurred within two days following the drug withdrawal. None of the patient was under treatment with astemizole or ketotifen. Patients on oral steroids or immunosuppressants were enrolled if drugs could be stopped for at least two months. Patients with respiratory symptoms were allowed to use topical steroids and  $\beta_2$ -active drugs as needed.

Subsets of ASSTed patients were defined by clinical and diagnostic criteria as reported in the Results section. Age range of ASSTed children was 2–12 years. Some of the reported ASST results show selected data from a 20 to 50 years old subject subset. This has been done in order to keep this subgroup as homogeneous as possible, and to allow a clear-cut inter-generation comparison.

Subjects undergoing ASST were not randomized. The ASST was performed in consecutive subjects as soon as the informed consent for testing was obtained. The study was approved by the local ethics committee.

#### *Data and Statistics*

Data were stored by means of customized databases and retrieved by means of study-tailored query programs. Statistical analyses were carried out by means of the Prism package (Graphpad Software, version 3.0, San Diego, CA, USA). The Fisher's exact test was applied where appropriate. Statistical significance was accepted at  $p < 0.05$  and reported on graphs.

## **Results**

### *Epidemiological and Clinical Data*

A cohort of 5,567 subjects with a suspected allergic respiratory disease was examined. Patients' age ranged between 1 and 80 years, and male/female ratio was 0.84. Age and gender distribution of the entire examined population is reported in figure 1a. Among adults in the second, third, and fourth decades, female patients were largely more represented than males. The difference was reduced in the last two decades. About one of three patients of the whole respiratory cohort was in the pediatric age ( $n = 1,762$ , 31.6%). The overall gender ratio among children was in favor of male subjects ( $M/F = 1.33$ ), though differences started to be evident for patients older than four years (fig. 1b).

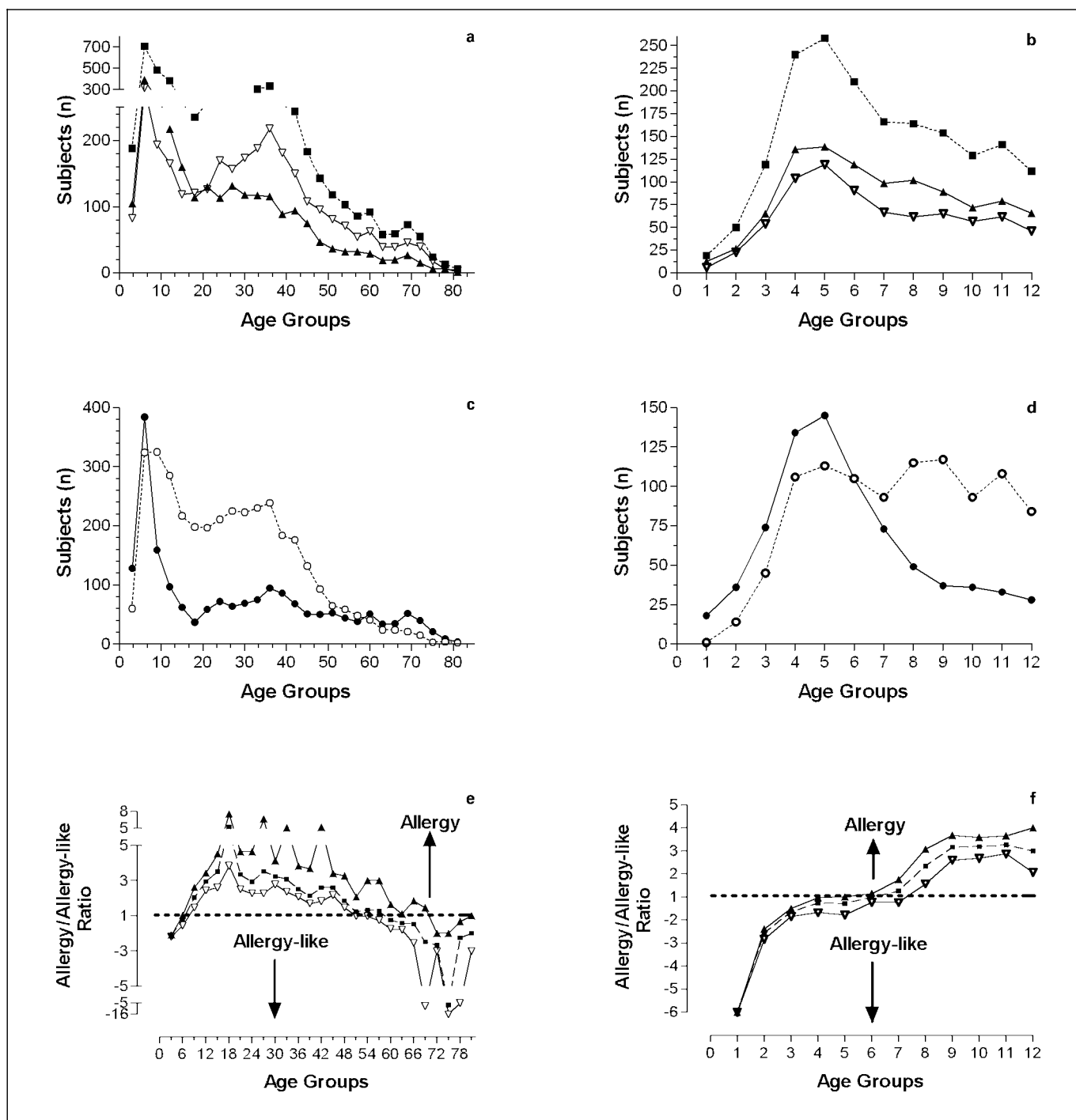


**Fig. 1.** **a** Age and gender distribution of 5,567 respiratory patients with either allergy or allergy-like symptoms at the first allergy consult. Male/female ratio = 0.84. Total = Filled square; male = filled triangle; female = empty triangle. **b** Age and gender distribution of 1,762 pediatric respiratory patients with either allergy or allergy-like symptoms at the first allergy consult. Male/female ratio = 1.33. Total = Filled square; male = filled triangle; female = empty triangle. **c** Age distribution of 5567 allergy and allergy-like respiratory patients. Allergy = Empty circle; allergy-like = filled circle. **d** Age distribution of 1,762 allergy and allergy-like pediatric respiratory patients. Allergy = Empty circle; allergy-like = filled circle. **e** Allergy/allergy-like ratio of 5,567 respiratory patients. Total = Filled square; male = filled triangle; female = empty triangle. **f** Allergy/allergy-like ratio of 1,762 pediatric respiratory patients. Total = Filled square; male = filled triangle; female = empty triangle. Subject distribution on left side graphs follows a three-year grouping. Subject distribution on right side graphs follows a year by year grouping.

buton of 1,762 allergy and allergy-like pediatric respiratory patients. Allergy = Empty circle; Allergy-like = filled circle. **e** Allergy/allergy-like ratio of 5,567 respiratory patients. Total = Filled square; male = filled triangle; female = empty triangle. **f** Allergy/allergy-like ratio of 1,762 pediatric respiratory patients. Total = Filled square; male = filled triangle; female = empty triangle. Subject distribution on left side graphs follows a three-year grouping. Subject distribution on right side graphs follows a year by year grouping.

Among the entire respiratory cohort, a positive SPT and related respiratory symptoms was recorded in 3,625 subjects (65.1%) whereas 1,942 (34.9%) did not react to any of the allergenic sources included in the screening tests. Distribution of patients with an allergy or an allergy-like condition varied with age and gender (fig. 1c). The absolute number of older patients (60–80 years) with an allergy-like disease was slightly greater than the allergic ones (fig. 1c). The number of allergic patients was greater than the non-allergic ones starting from age 7 to the end of the fourth decade (fig. 1c). A progressive rise of the non-allergic subjects was recorded from the third decade onward, and the absolute number of patients in either the allergy group or in the non-allergy group was almost the same in the fifth decade. In very young children (1–6 years of age) the absolute number of subjects in both conditions increased in a parallel way up to age six (fig. 1d), being the allergy-like condition always the most recorded one. At six years of age a crossover in the number of visited subjects was recorded (fig. 1d). The allergy-like patients rapidly declined from age 7 onward, whereas allergic patients largely exceeded the non-allergic ones until the end of the second decade of life (fig. 1c, d).

To describe the relationship between the data reported above and to exclude any bias in the patients' selection, the allergy/allergy-like ratio was used (fig. 1e, f). The ratio was largely in favor of the allergy-like forms in children younger than four (fig. 1f), with a decreasing trend as they become older. Allergy and allergy-like conditions paired at age seven, but female ratio raised slower than the male one (fig. 1f). From age eight onward allergy condition was largely more represented than the allergy-like one, but the ratio raised up to a value of eight for males whereas exceptionally was greater than two for females (fig. 1e, f). Male versus female differences were recorded for all the remaining adulthood decades. The female allergy/allergy-like ratio progressively declined from the third decade on, reaching the lowest values in the sixth and seventh decades (fig. 1e).

Current rhinitis or rhinitis plus asthma symptoms were recorded for each patient. The age and gender comparative distribution were evaluated. No differences were recorded in the distribution when rhinitis patients were compared to rhinitis plus asthma ones. Variations followed the gender and age factors reported above for both the clinical conditions (data not shown).

## *Autologous Serum Skin Test Data*

### *ASST in CIU*

ASST results of adult CIU patients enrolled for control purposes are reported in figure 2a (left part). About 58% reacted positive to autologous serum, but statistically significant differences were recorded between female patients (65.9%) versus males (34.4%) (fig. 2a, left part). Pure CIU pediatric patients were rarely found, being their cutaneous symptoms almost all the times accompanied by respiratory allergy-like ones. Only in 4 patients rhinitis or asthma were not associated to urticaria. All four reacted positive to ASST. Thus, the pediatric CIU control group could not be established and used for comparative evaluation neither with adult CIU nor with ASST results in allergy-like pediatric subjects.

### *ASST in Allergy-Like Respiratory Symptoms*

ASST applied to subjects with an allergy-like condition (fig. 2a, right part) raised about the same CIU percentage of positive reactions (57.9%), without gender differences, when the entire subset of respiratory subject was considered. No differences were found between rhinitis and rhinitis plus asthma subsets, either considering the whole population or in all the subsets reported below. ASST results in allergy-like tested subjects were further stratified for age and gender (fig. 2b, c, d). A higher number of positive ASST reactors (84%) were recorded in the pediatric age (fig. 2b, left part), even after further subgrouping for age (fig. 2c, d left). No differences were recorded between genders within the pediatric subset. About 48% of allergy-like adult subjects reacted positive to the ASST, significantly differing from pediatric results (fig. 2b), but not from the CIU adult control group (fig. 2b right, and 2a left). Statistically significant gender differences were recorded as for adult CIU control group (fig. 2b right, and 2a left). ASST reactivity was comparatively evaluated in pediatric and adult subjects whether or not they associate CIU symptoms to the allergy-like ones (fig. 3a, b). No differences were recorded in the two pediatric subsets (fig. 3a left and right), whereas higher statistically significant difference in ASST reactive subjects were recorded among adults when cutaneous and respiratory symptoms were associated (fig. 3b left and right). A statistically significant higher number of allergy-like adult male subjects with CIU were recorded positive to the ASST (75%). Among ASSTed allergy-like patients, but lacking the criteria for a CIU diagnosis, 31% in the pediatric subset and 19% in the adult one reported occasional or intermittent episodes of urticaria symptoms. Ninety-seven percent of these chil-

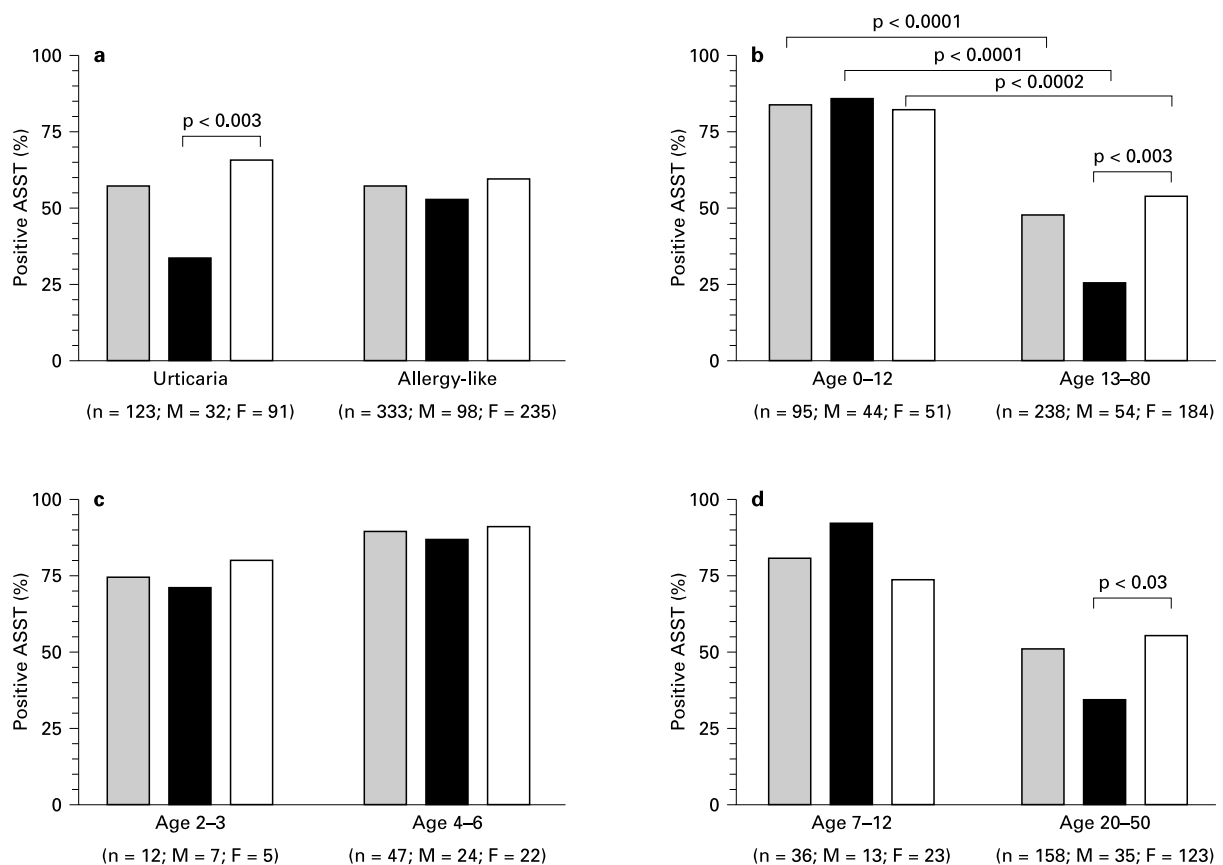


**Fig. 2.** **a** Autologous serum skin test in chronic idiopathic urticaria (CIU) adult patient (left part) and in allergy-like respiratory patients (right part). **b** Autologous serum skin test in allergy-like pediatric (left part) and adult respiratory patients (right part). **c** Autologous serum skin test in allergy-like respiratory pediatric patients: age subsets. Left: Children aged 2–3; right: children aged 4–6. **d** Autologous serum skin test in allergy-like respiratory pediatric and adult patients: age subsets. Left: Children aged 7–12; right: adults aged 20–50. Total = Grey bar; male = black bar; female = white bar. ASST = Autologous serum skin test. Statistics: Fisher's exact test. Statistically significant differences were also found comparing pediatric age subsets 4–6 and 7–12 with the adult subset (data not shown on graphs).

dren and 42% of the adults had a positive ASST. The onset age of respiratory symptoms was retrospectively defined in children belonging to the allergy-like subset. Eighty-four percent of these children started suffering of rhinitis or asthma as early as the first year of life. Eighty-seven percent of these children had a positive ASST. No differences in ASST reactivity were recorded when isolated allergy-like rhinitis and rhinitis *plus* asthma subsets were compared.

#### *ASST in Allergy plus Allergy-Like Respiratory Symptoms*

ASST was also performed in patients with clear-cut allergy symptoms (e.g. spring pollinosis) and thus classified as allergic, but associating long-lasting allergy-like respiratory symptoms not related with the diagnosed sensitization. Results reported in figure 3c show an ASST percent reactivity very close to those reported in previous conditions, in both pediatric and adult subsets. Non CIU but occasional or intermittent urticaria episodes were present in 59% of the children, 88% with a positive ASST,

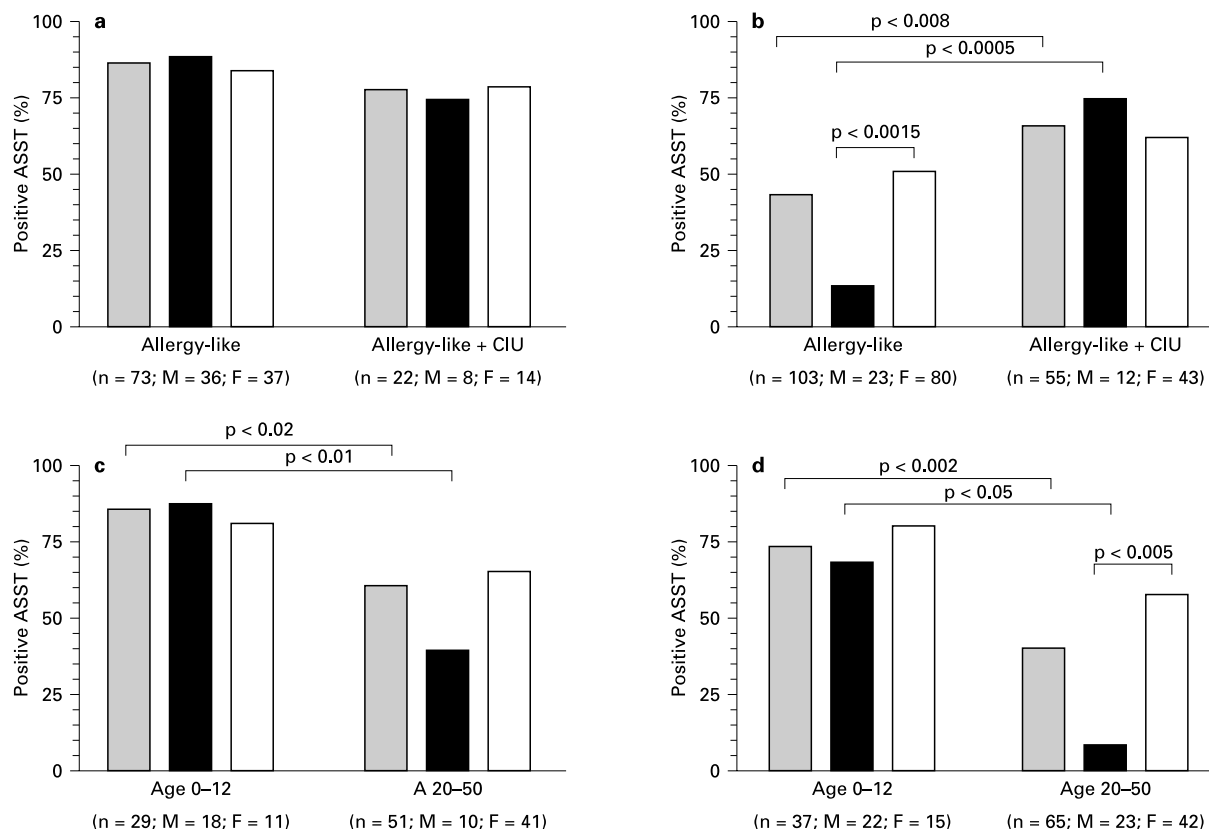


**Fig. 3.** **a** Autologous serum skin test in pediatric patients (aged 2–12) with allergy-like respiratory symptoms associating or not chronic idiopathic urticaria (CIU). Left: Allergy-like children; right: allergy-like plus CIU children. **b** Autologous serum skin test in adult patient (aged 20–50) with allergy-like respiratory symptoms associating or not CIU. Left part: Allergy-like adults; Right part: allergy-like plus CIU adults. **c** Autologous serum skin test in allergy plus allergy-like respiratory symptoms in pediatric and adult patient subsets. Left: Children aged 2–12; right: adults aged 20–50. **d** Autologous serum skin test in allergy pediatric and adult patients. Left: Children aged 2–12; right: adults aged 20–50. Total = Grey bar; male = black bar; female = white bar. ASST = Autologous serum skin test. Statistics: Fisher's exact test.

and in 59% of the adults, 73% with a positive ASST. The onset age of respiratory symptoms was retrospectively defined in children belonging to this combined allergy plus allergy-like respiratory symptoms subset. Eighty-six percent of these patients started suffering of rhinitis or asthma as early as the first year of life. Eighty-eight percent of these children had a positive ASST.

#### ASST in Allergy Symptoms

The last group of patients undergoing ASST was pure allergy patients. Though values of ASST reactive subjects

were slightly lower in both the pediatric and the adult subsets (fig. 3d), they did not reach statistical significance when compared with previous subsets. Reporting of occasional or intermittent urticaria symptoms was recorded in this subset as well. Thirty-three percent of the children and 19% of the adults reported occasional urticaria. Fifty-eight percent of the former and 52% of the latter had a positive ASST, respectively. The onset age of respiratory symptoms was retrospectively defined in children belonging to the pure respiratory allergy subset. Seventy-six percent of these patients started suffering of rhinitis or

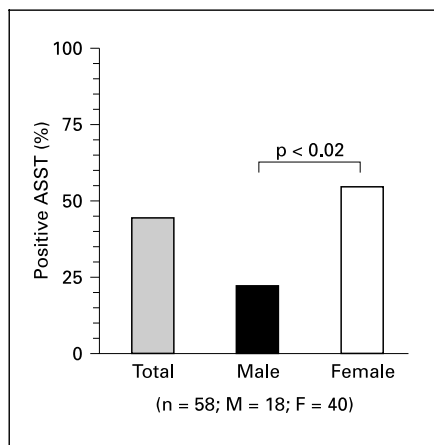


**Fig. 4.** Autologous serum skin test in healthy subjects (aged 20–50). Total = Grey bar; male = black bar; female = white bar. ASST = Autologous serum skin test. Statistics: Fisher's exact test.

asthma in the first year of life. Seventy-five percent of these children had a positive ASST.

#### *ASST in Healthy Subjects*

Twenty-six of 58 adult healthy subjects (44.8%) reacted positive to the ASST. Figure 4 reports statistically significant differences between genders.

## Discussion

The positive ASST reactivity in non-CIU subsets of patients is herein reported for the first time. The 58% prevalence of positive ASST reactivity found within our pure CIU adult population is close to those reported by other authors [13, 18, 20, 22, 34, 35]. The application of the ASST to adult patients with allergy-like respiratory diseases leads to a slightly lower positive result (47%). When a CIU was associated to the allergy-like condition, ASST results were equal to those obtained in pure CIU patients. Kinet preliminary reported the finding of anti-Fc $\epsilon$ RI $\alpha$  in four out of ten asthmatic subjects, and two of them were capable of inducing histamine release from basophils [36], whereas functional anti-IgE have been demonstrated in asthmatic patients by Nawata and co-workers [37]. It may thus be considered the hypothesis that a common autoreactive mechanism could underlie the activation of mast cell in different tissues and organs, causing one or more associated clinical symptoms resem-

bling an allergy disease [21]. The heterogeneity of symptom patterns observed among patients could be due to the patient variance in the contribution of several serum factors [38].

When the ASST was applied to children with the same clinical picture of allergy-like respiratory symptoms, it resulted in a higher number of reactive subjects than in adults. The presence of a CIU did not cause a different prevalence of the ASST reactivity in the pediatric subset. Noticeably, patients with pure CIU within the pediatric population were very rare because cutaneous symptoms, though sometime prominent, were always associated to respiratory ones. As preliminary reported by Greaves [23], an autoimmune mechanism based on the presence of autoantibodies anti-Fc $\epsilon$ RI $\alpha$  could be taken into account even for children. A unifying mechanism of mast cell activation may thus be hypothesized for children as well as for adults. This mechanism could be of pathogenic relevance starting very early during the life.

Marked differences were recorded comparing the pediatric and the adult subsets in either the allergy-like respiratory disease prevalence or in the ASST reactivity. No gender differences were recorded for ASST results among children in any of the studied conditions, whereas ASST reactivity in adults was always higher among females than males. This has been recently reported for adult CIU patients by Asero [35]. In the present study, the distribution of patients affected by respiratory symptoms suspected to be allergic in nature showed similar differences. This has been already reported by authors of several epidemiological studies [28, 39–41]. Noticeably, a higher prevalence of respiratory symptoms in adult females [41], and their very early onset age in children have been focused in previous cross-sectional and longitudinal studies [41–43]. In the present study the gender prevalence variations of rhinitis, whether or not associated to asthma, seem to be related to the potential underlying pathogenic mechanism highlighted by means of the positive ASST reactivity. In fact, this reactivity seems to vary according to the gender prevalence variations of the allergy and allergy-like respiratory diseases. Although the studied population may not be representative of the general population and may suffer of sampling bias, the parallel variation of the ASST reactivity between genders suggests such a potential relationship. Differences in gender prevalence and allergy/allergy-like ratios could also be a phenomenon reflecting changes between generations [44, 45]. All these hypotheses and observations certainly deserve further studies.

The overlapping presence of an IgE-mediated mechanism causing allergy symptoms and an allergy-like disease has already been reported [46–49]. The clinical picture of the two forms cannot be easily discriminated by means of their clinical signs [7]. In the present study, ASSTing of a patient subset reporting allergy plus allergy-like symptoms lead to positive results in both children and adults. Prevalence of positive ASST paired those recorded for other subsets. Interestingly, similar results were obtained even in patients with a pure allergy respiratory disease. In the latter subset the positive ASST could be explained and may be interpreted in the light of the minimal persistent inflammation [50, 51]. In all the respiratory subsets, urticaria, whether occasional or intermittent, was a common finding. Along with the early onset of respiratory symptoms in children, the presence of urticaria symptoms suggest the presence of a unique mechanism of mast cell activation, whose expression may involve several organs at different time points.

It may be also hypothesized, that the high number of ASST positive results could be biased by the lack of randomization of patients undergoing the test. More severe or multiple current or past symptoms could certainly lead patients or parents to consent the application of the ASST in order to achieve an explanation of features, duration and severity of the respiratory/cutaneous disease [52]. Further studies are needed to correctly define the ASST reactivity prevalence.

Chronic dermatologic diseases other than CIU or healthy subjects have been used in previous studies for ASST control purposes. Negative or rarely positive results have been recorded [13, 18, 20]. In the attempt to establish reliable criteria to evaluate the ASST, Sabroe and coworkers recorded 4 out of 40 healthy controls reactive in the ASST [20]. Three tests were regarded as false positive by the authors. More recently they found only one out of 39 healthy control subjects reactive to the ASST [18]. Forty-five percent of 58 healthy controls enrolled in the present study were recorded positive to the ASST. This average reactivity of control subjects did not statistically differ from the symptomatic subsets. A relevant finding is that gender distribution of the ASST reactivity in adult healthy subjects parallels that of diseased patients. The striking difference with previously reported ASST results may be explained by different healthy control enrollment criteria. On the other hand, the reported findings of the presence of anti-Fc $\epsilon$ RI $\alpha$  in both CIU patients and in normal donors, both inducing histamine release [53] could lead to a different interpretation of the positive ASST in the healthy subjects. The *in vitro* data [53], along with the

herein reported positive ASST test in healthy subjects, could suggest the presence of autoantibodies as a pre-clinical sign of potential mast cell activation. Similarly, the finding of a positive skin test to allergens in asymptomatic healthy subjects, as also recorded during the enrollment phase of the present study, is frequently reported in clinical and epidemiological studies, and is generally considered a pre- or sub-clinical aspect of an IgE sensitization [50, 54–56]. This hypothesis further reinforces the need for a better understanding of the possible role of autoreactive mechanisms behind allergy or allergy-like respiratory symptoms.

In conclusion, if the mast cell activation by autoreactive mechanisms will be confirmed in ASST-positive respiratory subjects, the lack of immunopathological differences between the ‘intrinsic’ and the ‘extrinsic’ forms could thus be explained [2, 8, 21, 57]. Similarly to what has been shown in the HIV infection [58], the chronic activation of mast cell could lead to an increasing expression of the allergy phenotype either as direct expression of symptoms (release of mediators) or indirectly by enhancing the risk of developing an IgE-mediated disease (release of Th2 cytokines). Until a convenient and reliable laboratory method will be available [59], the ASST could represent a valid diagnostic approach to all the allergy-like conditions. Cutaneous and upper/lower respiratory symptoms could be probably seen within a unique framework of the mast cell activation in different tissues. Patient’s symptom phenotype further depends on gender, age and other pathophysiologic conditions certainly deserving further studies. Follow up studies are needed to define the positive ASST as a risk factor for reactive healthy subjects.

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## References

- Holt PG, Macaubas C, Stumbles PA, Sly PD: The role of allergy in the development of asthma. *Nature* 1999;402:B12-B17.
- Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, Kay AB: The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: More similarities than differences. *Immunol Today* 1999;20:528-533.
- Powe DG, Hiskisson RS, Carney AS, Jenkins D, Jones NS: Idiopathic and allergic rhinitis show a similar inflammatory response. *Clin Otolaryngol* 2000;25:570-576.
- Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS: Evidence for an inflammatory pathophysiology in idiopathic rhinitis. *Clin Exp Allergy* 2001;31:864-872.
- Carney S, Powe DG, Huskisson RS, Jones NS: Atypical nasal challenges in patients with idiopathic rhinitis: More evidence for the existence of allergy in the absence of atopy? *Clin Exp Allergy* 2002;32:1436-1440.
- Simons FE: Allergic rhinobronchitis: The asthma-allergic rhinitis link. *J Allergy Clin Immunol* 1999;104:534-540.
- Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D: Allergic vs nonallergic asthma: What makes the difference? *Allergy* 2002;57:607-613.
- Riccio AM, Tosca MA, Cosentino C, Pallesstrini E, Ameli F, Canonica GW, Ciprandi G: Cytokine pattern in allergic and non-allergic chronic rhinosinusitis in asthmatic children. *Clin Exp Allergy* 2002;32:422-426.
- Mari A: Multiple pollen sensitization: A molecular approach to the diagnosis. *Int Arch Allergy Immunol* 2001;125:57-65.
- Mari A: IgE to cross-reactive carbohydrate determinants: Analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002;129:286-295.
- Greaves MW: Pathophysiology of chronic urticaria. *Int Arch Allergy Immunol* 2002;127:3-9.
- Kaplan AP: Chronic urticaria and angioedema. *N Engl J Med* 2002;346:175-179.
- Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW: Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-1604.
- Kikuchi Y, Kaplan AP: Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;107:1056-1062.
- Grattan CE, Francis DM, Hide M, Greaves MW: Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;21:695-704.
- Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP: Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 1997;99:461-465.
- Ferrer M, Kinet JP, Kaplan AP: Comparative studies of functional and binding assays for IgG anti-Fc $\epsilon$ RI ( $\alpha$ -subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;101:672-676.
- Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, Black AK, Stingl G, Greaves MW, Barr RM: Classification of anti-Fc $\epsilon$ RI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002;110:492-499.
- Fagiolo U, Kricek F, Ruf C, Peserico A, Amadori A, Cancian M: Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 2000;106:567-572.
- Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza BA, Greaves MW: The autologous serum skin test: A screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol* 1999;140:446-452.
- Marone G, Spadaro G, Palumbo C, Condorelli G: The anti-IgE/anti-Fc $\epsilon$ RI $\alpha$  autoantibody network in allergic and autoimmune diseases. *Clin Exp Allergy* 1999;29:17-27.
- Asero R, Tedeschi A, Lorini M, Salimbeni R, Zanoletti T, Miadonna A: Chronic urticaria: Novel clinical and serological aspects. *Clin Exp Allergy* 2001;31:1105-1110.
- Greaves MW: Chronic urticaria in childhood. *Allergy* 2000;55:309-320.
- Dalal I, Levine A, Somekh E, Mizrahi A, Hanukoglu A: Chronic urticaria in children: Expanding the 'Autoimmune kaleidoscope'. *Pediatrics* 2000;106:1139-1141.
- Plumb J, Norlin C, Young PC: Exposures and outcomes of children with urticaria seen in a pediatric practice-based research network: A case-control study. *Arch Pediatr Adolesc Med* 2001;155:1017-1021.
- Simons FE: Prevention of acute urticaria in young children with atopic dermatitis. *J Allergy Clin Immunol* 2001;107:703-706.
- Greaves M: Chronic urticaria. *J Allergy Clin Immunol* 2000;105:664-672.
- Bousquet J, Van Cauwenberge P, Khaltaev N: Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108:S147-S334.
- National Institute of Health NHLBI: Global Initiative for Asthma: global strategy for asthma management and prevention. NHLBI/WHO workshop report 1993, Publ 95-3659, Jan 1995. 2001. Ref Type: Unpublished Work.
- Fabbri L, Caramori G, Beghe B, Papi A, Ciaccia A: Chronic obstructive pulmonary disease international guidelines. *Curr Opin Pulm Med* 1998;4:76-84.
- Mari A: Skin Test with a Timothy Grass (*Phleum pratense*) Pollen Extract v.s. IgE to a Timothy Extract v.s. IgE to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12: Epidemiological and Diagnostic Data. *Clin Exp Allergy* 2003;33:43-51.
- Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski ML, Mygind N, Ring J, Van Cauwenberge P, Hage-Hamsten M, Wuthrich B: A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813-824.
- Grattan CE, Dawn G, Gibbs S, Francis DM: Blood basophil numbers in chronic ordinary urticaria and healthy controls: Diurnal variation, influence of loratadine and prednisolone and relationship to disease activity. *Clin Exp Allergy* 2003;33:337-341.
- Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW: Chronic idiopathic urticaria: Comparison of the clinical features of patients with and without anti-Fc $\epsilon$ RI or anti-IgE autoantibodies. *J Am Acad Dermatol* 1999;40:443-450.
- Asero R: Sex differences in the pathogenesis of chronic urticaria. *J Allergy Clin Immunol* 2003;111:425-426.
- Kinet JP: The high-affinity IgE receptor (Fc $\epsilon$ RI): From physiology to pathology. *Annu Rev Immunol* 1999;17:931-972.
- Nawata Y, Koike T, Yanagisawa T, Iwamoto I, Itaya T, Yoshida S, Tomioka H: Anti-IgE autoantibody in patients with bronchial asthma. *Clin Exp Immunol* 1984;58:348-356.
- Kikuchi Y, Kaplan AP: A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002;109:114-118.
- Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM: Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988;319:1112-1117.
- Court CS, Cook DG, Strachan DP: Comparative epidemiology of atopic and non-atopic wheeze and diagnosed asthma in a national sample of English adults. *Thorax* 2002;57:951-957.
- De Marco R, Locatelli F, Cerveri I, Bugiani M, Marinoni A, Giammanco G: Incidence and remission of asthma: A retrospective study on the natural history of asthma in Italy. *J Allergy Clin Immunol* 2002;110:228-235.
- Wahn U, von Mutius E: Childhood risk factors for atopy and the importance of early intervention. *J Allergy Clin Immunol* 2001;107:567-574.
- Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD: Tucson children's respiratory study: 1980 to present. *J Allergy Clin Immunol* 2003;111:661-675.
- Upton MN, McConnachie A, McSharry C, Hart CL, Smith GD, Gillis CR, Watt GC: Intergenerational 20 year trends in the prevalence of asthma and hay fever in adults: The Midspan family study surveys of parents and offspring. *BMJ* 2000;321:88-92.
- Horak E, Lanigan A, Roberts M, Welsh L, Wilson J, Carlin JB, Olinsky A, Robertson CF: Longitudinal study of childhood wheezy bronchitis and asthma: Outcome at age 42. *BMJ* 2003;326:422-423.

- 46 Liccardi G, Kordash TR, Russo M, Noschese P, Califano C, D'Amato M, D'Amato G: Why are nasal and bronchial symptoms mostly perennial in patients with monosensitization to *Olea europaea* pollen allergens? *J Invest Allergol Clin Immunol* 1996;6:371–377.
- 47 Settiple RA: Demographics and epidemiology of allergic and nonallergic rhinitis. *Allergy Asthma Proc* 2001;22:185–189.
- 48 Settiple RA, Lieberman P: Update on nonallergic rhinitis. *Ann Allergy Asthma Immunol* 2001;86:494–508.
- 49 Mercer MJ, van der Linde GP, Joubert G: Rhinitis (allergic and nonallergic) in an atopic pediatric referral population in the grasslands of inland South Africa. *Ann Allergy Asthma Immunol* 2002;89:503–512.
- 50 Ciprandi G, Buscaglia S, Pesce G, Pronzato C, Ricca V, Parmiani S, Bagnasco M, Canonica GW: Minimal persistent inflammation is present at mucosal level in patients with asymptomatic rhinitis and mite allergy. *J Allergy Clin Immunol* 1995;96:971–979.
- 51 Ricca V, Landi M, Ferrero P, Bairo A, Tazzer C, Canonica GW, Ciprandi G: Minimal persistent inflammation is also present in patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 2000;105:54–57.
- 52 Rothmier JD, Lasley MV, Shapiro GG: Factors influencing parental consent in pediatric clinical research. *Pediatrics* 2003;111:1037–1041.
- 53 Miescher SM, Horn MP, Pachlopnik JM, Baldi L, Vogel M, Stadler BM: Natural anti-Fc $\epsilon$ RI $\alpha$  autoantibodies isolated from healthy donors and chronic idiopathic urticaria patients reveal a restricted repertoire and autoreactivity on human basophils. *Hum Antibodies* 2001;10:119–126.
- 54 Moody A, Fergusson W, Wells A, Bartley J, Kolbe J: Increased nitric oxide production in the respiratory tract in asymptomatic pacific islanders: An association with skin prick reactivity to house dust mite. *J Allergy Clin Immunol* 2000;105:895–899.
- 55 Witteman AM, Mulder M, Aalberse RC, Jansen HM, Van Der Zee JS: Bronchial allergen challenge in subjects with low levels of allergic sensitization to indoor allergens. *Allergy* 1999;54:366–374.
- 56 Bodtger U, Poulsen LK, Malling HJ: Asymptomatic skin sensitization to birch predicts later development of birch pollen allergy in adults: A 3-year follow-up study. *J Allergy Clin Immunol* 2003;111:149–154.
- 57 Magnan A, Fourre-Julian C, Jullian H, Badier M, Lanteaume A, Vervloet D, Charpin D: Rhinitis alone or rhinitis plus asthma: What makes the difference? *Eur Respir J* 1998;12:1073–1078.
- 58 Marone G, Florio G, Petraroli A, De Paulis A: Dysregulation of the IgE/Fc $\epsilon$ RI network in HIV-1 infection. *J Allergy Clin Immunol* 2001;107:22–30.
- 59 Greaves M: Autoimmune urticaria. *Clin Rev Allergy Immunol* 2002;23:171–183.