

Evaluation of autologous serum skin test results in patients with chronic idiopathic urticaria, allergic/non-allergic asthma or rhinitis and healthy people

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Summary

Background. Recent data indicate that the autologous serum skin test (ASST) shows a high rate of reactivity not only in chronic idiopathic urticaria (CIU) but also in cases with non-allergic asthma and rhinitis (NAAR), multiple drug allergy syndrome (MDAS) and even in some healthy people.

Aim. To evaluate ASST reactivity in patients with CIU, allergic/non-allergic asthma or rhinitis and in healthy controls.

Methods. We studied 80 patients with CIU, 40 non-atopic patients with NAAR, 57 patients with allergic rhinitis (AR) and allergic bronchial asthma (ABA), and 45 healthy controls. ASST was performed in all patients and controls, and it was considered positive when a serum-induced weal with a diameter 1.5 mm greater than the negative (saline) control, surrounded by erythema, was present.

Results. In total, 42 patients with CIU showed ASST reactivity (52.5%). ASST was found to be positive in 8 of 40 patients with NAAR (20%). The rate was similar (17.5%) in the AR/ABA patient group. However, 25 healthy controls (55.5%) also had positive ASST. The highest rate was in female controls and in individuals in the 18–30-year-old age group.

Conclusion. The data indicate that ASST positivity might be a nonspecific phenomenon, influenced by many factors. In the light of the results of this study, we suggest that the significance of ASST reactivity should be re-evaluated in CIU. In addition, the importance of ASST reactivity in patients with AR/ABA and in patients with NAAR remains unclear, and further controlled studies are needed.

Introduction

The autologous serum skin test (ASST) based on the intradermal injection of autologous serum is a simple screening diagnostic procedure used in chronic urticaria (CU). A positive ASST suggests that there are functional

autoantibodies (anti-FcεRI and anti-IgE) or histamine-releasing factors present in the serum.^{1,2} Studies have shown that ASST is positive in almost half of patients with chronic idiopathic urticaria (CIU).³

Bronchial asthma (BA) and rhinitis, which are chronic inflammatory diseases of the airways, may be caused by allergic or nonallergic factors. Allergy plays an important role; at least 80% of children with asthma and almost 50% of adults with asthma were found to be atopic.^{4–6} However, although they have no documented allergic sensitization, some patients show clinical features of respiratory allergic diseases [allergic BA (ABA) and allergic rhinitis (AR)]. These cases of

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intrinsic, nonallergic origin are described as 'nonallergic asthma and rhinitis' (NAAR). From an immunohistopathological point of view, NAAR and respiratory allergic diseases are similar. There are some preliminary studies in the literature revealing high rates of ASST reactivity in NAAR.^{7,8} These have been based on the hypothesis that NAAR, characterized by allergy-like symptoms without evidence of an IgE-mediated sensitization, can be regarded as the respiratory counterpart of CIU.⁷

It is well known that patients with allergic BA are characterized by predominant activation of T-helper (Th)2 lymphocytes, with increased production of interleukin (IL)-4, IL-5 and IL-13.^{4,9,10} Nonallergic asthma is also an inflammatory airway disease immunologically characterized by increased expression of IL-2 and IL-5 (not Th2 cytokines).^{11,12} Similarly, infiltrating T lymphocytes in CIU are a mixture of Th1/Th2 cell types or show a Th0 phenotype (IL-4, IL-5 and interferon- γ mRNA+cells).^{13,14}

In this study, we aimed to show and re-evaluate the significance of ASST reactivity in three patient groups (CIU, NAAR, AR/ABA) and healthy controls.

Methods

This study was carried out between 2004 and 2006 in the allergy and dermatology clinics of Gülhane Medical Academy. In total, 80 patients with CIU (46 men and 34 women; mean age 34.74 ± 14.94 , range 11–67), 40 nonatopic patients with NAAR (13 men and 27 women; mean age 35.53 ± 13.15 , range 12–67), 57 patients with AR/ABA (41 men and 16 women; mean age 26.89 ± 6.76 , range 16–48), and 45 healthy nonatopic controls (22 men and 23 women; mean age 30.04 ± 7.83 , range 18–44) were

studied (Table 1). The control group had no history of atopy (atopic respiratory disease, atopic dermatitis or food allergy), urticaria, nonatopic respiratory disease, or any drug allergy and/or intolerance. Antihistamines were withdrawn from anyone using them at least 1 week before skin testing was performed. The exclusion criteria for patients with CIU were: presence of multiple drug allergy syndrome (MDAS), pregnancy, pure physical urticaria, and urticaria lasting < 6 weeks. Exclusion criteria for patients with AR/ABA, patients with NAAR and healthy controls were: presence of MDAS, multiple intolerances to nonsteroidal anti-inflammatory drugs (NSAIDs), and pregnancy.

Patients with rhinitis and asthma were diagnosed as having AR/ABA or NAAR on the basis of clinical history, nasal smear, blood eosinophil count, serum total IgE concentration, pulmonary function tests and skin-prick and/or intradermal tests. The Global Initiative for Asthma (GINA) workshop report and the Allergic Rhinitis and its Impact on Asthma (ARIA) document were considered as diagnostic criteria^{15,16} for AR/ABA and NAAR. After obtaining written informed consent, all patients and controls underwent skin-prick testing (SPT) with a panel of 40 inhalant allergens including house dust mites, cockroach, animal epithelia and feathers, grass/tree/weed pollens, moulds and cockroach (Stallergenes, Antony, France).

We performed an intradermal test with fresh autologous serum on the 222 subjects (177 patients with CIU, NAAR, or AR/ABA, and 45 healthy controls). Venous blood samples were drawn into sterile tubes without clotting accelerator (Vacurette; Greiner Bio-One GmbH, Kremsmünster, Austria). After the blood samples were allowed to clot at room temperature for 30 min, they were centrifuged at 500 *g* for 15 min in order to separate the sera. All ASSTs were performed and evaluated by the same two investigators (OT and AK).

Autologous serum (0.05 mL) and 0.9% sterile saline (negative control) were injected into the volar aspect of the forearm. The result was evaluated after 30 min, and judged to be positive when a serum-induced weal with a diameter of 1.5 mm greater than the negative control, surrounded by an erythema, was present.

Statistical analysis

The χ^2 test, Mann–Whitney *U*-test and logistic regression analysis were used with SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). *P* < 0.05 was considered significant.

Table 1 Autologous serum skin test positivity in patients and controls.

Group	ASST	Male, <i>n</i> (%)	Female, <i>n</i> (%)	Total, <i>n</i> (%)
CIU (<i>n</i> = 80)	+	24 (52.17)	18 (52.94)	42 (52.50)
	–	22 (47.83)	16 (47.06)	38 (47.50)
NAAR (<i>n</i> = 40)	+	1 (7.69)	7 (25.92)	8 (20)
	–	12 (92.31)	20 (74.08)	32 (80)
AR/ABA (<i>n</i> = 57)	+	7 (17.07)	3 (18.75)	10 (17.54)
	–	34 (82.93)	13 (81.25)	47 (82.46)
Controls (<i>n</i> = 45)	+	9 (40.90)	16 (69.56)	25 (55.55)
	–	13 (59.10)	7 (30.44)	20 (44.45)

+, positive; –, negative; ABA, allergic bronchial asthma; AR, allergic rhinitis; ASST, autologous serum skin test; CIU, chronic idiopathic urticaria; NAAR, non-allergic asthma and rhinitis.

Results

Skin-prick test

The NAAR group comprised 40 SPT-negative patients: 29 with pure non-allergic rhinitis (NAR) and 11 with NAR/BAs. There were 27 patients with AR and 30 with AR associated with ABA patients in the AR/ABA group. There was no patient with pure BA in either group. SPT with inhalant allergens gave a positive result for at least one inhalant allergen in all patients with AR/ABA, and 35% of patients with CIU also had SPT reactivity. None of the healthy controls had a positive reaction to SPT with inhalant allergens.

Autologous serum skin test

In total, 42 patients with CIU (52.5%) showed ASST reactivity. However, 25 healthy controls (55.5%) also had a positive ASST (Table 1). The difference between patients with CIU and healthy controls was not significant ($P > 0.05$).

Autologous serum skin test was found to be positive in 8 of 40 patients with NAAR (20%) and 10 of 57 patients with AR/ABA (17.5%) (Table 1). There was no significant difference between NAAR and AR/ABA patients for ASST positivity ($P > 0.05$).

The highest rate of ASST positivity was found in women; almost 70% of healthy women had a positive ASST. Female gender was found to be more frequently associated with ASST positivity in all groups (Table 1). In addition, eight female controls still had ASST positivity 6 months after the completion of the study. The frequency of ASST positivity was found to be higher in patients and controls for the age group 18–30 years.

In all, 41 of 122 men (33.6%) and 44 of 100 women (44%) had positive ASST. The overall rate of ASST positivity in the patient groups (CIU, NAAR, AR/ABA) was 33.9% (60 of 177 patients), but 55.5% of controls (25 of 45 healthy people) had a positive ASST. As emphasized above, patients with NAAR had a low rate of ASST positivity (20%). This rate was comparable with that obtained in the AR/ABA group (17.54%).

Discussion

Autologous serum skin test positivity, first described by Grattan *et al.*, has been suggested to indicate the presence of functional histamine-releasing autoantibodies or histamine-releasing factors in some patients with CIU.¹ It has been suggested that ASST positivity in patients with CIU might indicate the presence of functional circulating

autoantibodies to FcεRI and/or to IgE, with a sensitivity of 65–71% and a specificity of 78–81%.^{2,17} Although its sensitivity and specificity for basophil histamine release were found by Grattan *et al.* to be high, the test is not considered specific for autoimmune urticaria. In addition, recent data indicate that the ASST shows a high rate of reactivity not only in CIU, but also in patients with NAAR,⁷ those with multiple intolerances to NSAIDs,¹⁸ and those with MDAS.¹⁹

Asero *et al.*²⁰ showed that the autologous plasma skin test yielded more positive results than ASST in CIU. Moreover, the extrinsic pathway of the coagulation cascade, which possibly leads to thrombin generation, was found by the same group to be activated in plasma samples from patients with CIU. This strong expression of tissue factor by upper dermal inflammatory cells, providing evidence of coagulation pathway involvement in CIU, presented new perspectives.²¹

The ASST shows the highest rate of positivity (>90%) in MDAS, a condition characterized by a marked tendency to react to several chemically unrelated drugs. Almost 90% positivity has also been found in patients with multiple NSAID sensitivity.¹⁸ These findings suggest that MDAS and multiple NSAID sensitivity are characterized by the presence of histamine-releasing factors.

To our knowledge, our study is one of the most detailed undertaken on ASST in different patient groups. We performed an intradermal test with fresh autologous serum on 222 people (177 patients of CIU, NAAR or AR/ABA, and 45 healthy controls). In contrast to previous reports, we found that patients with NAAR had a low rate of ASST positivity (20%). A large number of controls (55.5%) also had a positive ASST, which was higher even than that found in the CIU patient group (52.5%).

Although it is generally regarded as a useful clinical procedure, there have been some reports that false-positive results might be seen in ASST. Bradykinin production and C5a formation during the clotting process have been put forward as causes for false positivity.¹⁴ However, as there are no clear data regarding false-positive reactions in ASST, the significance and the rate of false positivity remain unknown. There are also no data available on whether the serum-collecting procedure [fasting, activity of the disease at the time of blood drawing, rate (*g* value) and duration of spinning in the centrifuge] influences the results. In the literature, there are large variations, with rates of positivity ranging from 0% to 45% among healthy controls. Table 2 shows the positive ASST results for healthy people reported in the literature.^{7,8,17,19,22,23}

Table 2 Overall results of the studies in the literature: ASST positivity (%) in CIU and/or AR/ABA, NAAR and healthy controls.

Reference	CIU (%)	Controls (%)	NAAR (%)	AR/ABA (%)
7	58	45	47	40
8	—	0 (3 cases)	58	0 (27 cases)
17	44.51	2.5	—	—
19	—	0 (20 cases)	—	—
22	34.61	2.56	—	—
23	53.1	40.5	—	29.8 (SAR)
Present study	52.5	55.55	20	17.54

ABA, allergic bronchial asthma; AR, allergic rhinitis; CIU, chronic idiopathic urticaria; NAAR, non-allergic asthma and rhinitis; SAR, seasonal allergic rhinitis.

It is difficult to interpret zero,^{8,19} low^{17,22} and high^{7,23} rates of ASST reactivities in healthy populations. As shown in Table 2, Mari⁷ and Guttman-Yassky *et al.*²³ have reported 45% and 40.5% positivity, respectively, in healthy controls. Our results represent the highest rate (55.55%) of ASST reactivity in a healthy adult population; almost 70% in healthy women. In general, ASST was more often found to be positive in women than in men.^{7,8} The fact that the rate of positivity was highest in the young (female) population, age range 18–30 years, in our study suggests the role of immune status and female sexuality (e.g. hormonal factors, menstrual cycle) in ASST reactivity. The fact that eight female controls had still ASST positivity at the end of the sixth month after completion of the study supports this view.

Although high rates of ASST reactivity almost similar to those detected in CIU have been reported in some NAAR patient groups,^{7,8} the data obtained from our study are not consistent with the data of previous studies (Table 2). Our results (high rate of ASST positivity in healthy controls, low rate of positivity in AR/ABA and NAAR) cast doubt on the suggestion that ASST might reflect minimal persistent inflammation.^{7,24,25} In addition, the hypotheses that circulating histamine-releasing factors that might contribute to the inflammatory process in NAAR by activating pulmonary mast cells, and that a common autoreactivity may be operative in both CIU and AR/ABA and NAAR via chronic activation of mast cells^{8,23} seem highly controversial on the basis of the results of present study. Recent data in patient and control groups indicate that ASST positivity might be a nonspecific phenomenon, influenced by many factors.

In the light of the results of this study, we suggest that the significance of ASST should be re-evaluated in CIU. In addition, the importance of ASST in patients with AR/ABA and patients with NAAR remains unclear, and further controlled studies are needed.

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