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Autologous serum skin test vs autologous plasma skin test in patients with chronic urticaria: evaluation of reproducibility, sensitivity and specificity and relationship with disease activity, quality of life and anti-thyroid antibodies

Recent concerns have arisen about the specificity and interpretation of the autologous serum skin test (ASST), suggesting that ASST might produce false-positive results, and proposing the use of autologous plasma (APST) instead for intradermal testing in autoreactive urticaria. We investigated autoreactivity to autologous plasma and compared the results for reproducibility, sensitivity, specificity and accuracy and evaluated their association with quality of life and anti-TPO antibodies. 70 adults with chronic spontaneous urticaria (CU) and 62 controls underwent testing with ASST and APST and the tests were repeated two days after the first visit. Blood tests measured anti-TPO levels. Disease activity was assessed by urticaria activity score (UAS-7) and quality of life impairment was assessed by DLQI and CU-Q2oL. There were no statistically significant differences between ASST (+) and ASST (-) and also APST (+) and APST (-) patients with regard to disease duration, anti-TPO antibodies, urticaria activity scores, DLQI scores and CU-Q2oL scores. The results of first ASST and APST were well correlated with the results of second ASST and APST. The specificity of the two tests was similar, while ASST had a higher sensitivity and accuracy. Our results showed that there is no need to use autologous plasma instead of autologous serum for intradermal testing in CU.

Key words: autologous serum skin test, autologous plasma skin test, chronic urticaria, anti-thyroid antibodies, quality of life

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Chronic spontaneous urticaria (CU) describes a 6-week or longer history of widespread, transient, itchy cutaneous swellings with or without angioedema which affect 25% of the population at some time in their life [1, 2]. Pathogenic mechanisms such as chronic infections, food intolerance, non-infectious chronic inflammatory processes (gastritis, oesophagitis) have been elucidated to be responsible for the development of CU and there have been long discussions on the role of atopy during the past years [3-5]. Recently, an underlying autoimmune mechanism involving anti-high affinity IgE receptor (FcεRI) antibodies or anti-IgE antibodies that are capable of activating mast cell and basophil degranulation was proposed [6]. In a subset of CU patients, intradermal injection of autologous serum (autologous serum skin test, ASST) elicits a wheal-and-flare skin reaction which suggests the presence of a serum factor [7]. The ASST is an *in vivo* validated test performed by injecting the patient's own serum into the skin which results positive in 30-67% of patients [8, 9]. Even though ASST is widely used and well established, there are concerns about the interpretation and specificity of the test. Some authors suggested that ASST might produce false-positive results because of the

generation of large quantities of bradykinin during the clotting process and direct cleavage of C5 by tryptaselike plasma proteases secreted by neutrophils [10, 11]. Asero *et al.* proposed using autologous plasma (APST, autologous plasma skin test) instead of autologous serum which would be expected to result in a better specificity of testing [12].

The first aim of this study was to investigate whether CU patients are reacting to autologous plasma injections and then to compare the results of ASST and APST with regard to reproducibility, sensitivity, specificity and accuracy and evaluate their association with quality of life and anti-thyroid antibodies.

Subjects and methods

Subjects

This prospective study included 70 spontaneous chronic urticaria patients who reported daily or almost daily symptoms, lasting for at least 6 weeks. Patients with prominent

physical urticaria and/or dermographism and pregnancy were excluded. The control group consisted a total of 62 subjects who had no history of an autoimmune and/or systemic allergic disease: 11 healthy volunteers, 8 lichenoid dermatitis (localized lichen planus, lichen simplex chronicus, hypertrophic lichen), 24 eczema (localized irritant contact dermatitis, hand eczema, dyshidrotic dermatitis, eyelid eczema, neurodermatitis), 6 psoriasis (localized chronic plaque type or palmoplantar pustulosis), 4 localized pruritus (genital, scrotal, anal pruritus), 1 erythema annulare centrifugum, 2 acne rosacea, 1 postinflammatory hyperpigmentation, 2 folliculitis, 1 seborrheic dermatitis and 2 localized dermatophyte infection.

The CU patients underwent an extensive laboratory work-up including complete blood count, metabolic panel, anti-TPO antibodies, urine and stool analysis, complement and immunoglobulin levels, hepatitis markers and chest and sinus X-rays.

Skin tests

We performed intradermal tests with both autologous serum, autologous plasma, normal saline (negative control) and histamine (positive control) as previously described [12, 13]. After cessation of antihistaminic treatment at least for 3 days, venous blood samples were drawn into sterile tubes without clotting accelerator for ASST. Tubes containing Na citrate (0.125 mol/L of Na-citrate) were used to inhibit coagulation for APST. The samples were allowed to clot for 30 minutes at room temperature and centrifuged at 500 g for 15 minutes. We performed 0.05 mL of intradermal injections which were replaced 3 cm away from each other on the volar surface of the left arm and we waited for 30 minutes to evaluate the wheal and flare reactions. A wheal with a diameter of at least 1.5 mm greater than that of the negative control was considered as positive. To check the reproducibility of ASST and APST, we withdrew blood again and repeated the tests after two days on the same arm. The first and second readings were performed by the same investigator (EK) without checking the result of the first test. None of the patients was taking systemic corticosteroids or immunosuppressive drugs at time of testing. The patients and controls gave informed consent and the study was approved by the local Ethics committee.

Measurements

The disease activity was assessed by UAS7; a validated scoring system combining the intensity of itch and the number of wheals every day for one week [14]. To evaluate the quality of life impairment due to urticaria we used Dermatology Life Quality Index (DLQI) and Chronic Urticaria Quality of Life Questionnaire (CU-Q₂oL). The DLQI is a quality of life instrument for any kind of skin disease which has been validated for use in Turkey [15, 16]. The CU-Q₂oL is a quality of life questionnaire specifically designed for CU which was originally developed in Italy [17]. The author (EK) performed the validation study of the questionnaire which is in the process of being published. The patients scored their urticarial symptoms one week before testing on a daily basis and filled out the DLQI and CU-Q₂oL questionnaires the day before testing.

Statistical analysis

For interpretation of the results, descriptive statistical methods (mean, standard deviation) as well as unpaired T-test for comparison of groups, Chi-Square test and odds ratio for comparing quantitative data were used.

We used weighted kappa statistics to define the concordance between the first and second ASST and APST; and intra-class correlation coefficient to compare serum and plasma diameters. Sensitivity, specificity, positive and negative predictive values and likelihood ratios between ASST, APST and anti-TPO values were calculated. The results were evaluated within P values of <0.05 and a confidence interval of 95%. The NCSS 2007 statistical software was used for statistical analysis.

Results

Characteristics of the study sample

A total of 70 CU patients (77% female; age: 28-53 years; 40.6±12.7) participated in this study. The control group consisted of 62 subjects. There were no statistically significant differences with respect to age and gender between patients and the control group. The mean duration of urticaria was 32±64.7 (range 2-360) months. Forty-one (57%) had angioedema and 16 (23%) had positive anti-TPO antibodies while only three subjects (8%) had positive anti-TPO antibodies in the control group. Anti-TPO positivity was found three-fold increased in CU patients. Only three (4%) of the patients had NSAID intolerance and eight (11%) had accompanying autoimmune and/or allergic diseases: two had allergic rhinitis, two had vitiligo, one had asthma and four had an established autoimmune thyroid disease. One of the patients had both vitiligo and asthma. Mean UAS7 score was 20±11.4 (range 0-42), mean DLQI score was 9.7±7.6 (range 0-30) and mean CU-Q₂oL score was 35±19.4 (range 4.3-94.6).

The characteristics of the patients and controls are shown in *table 1*.

Skin tests

ASST and APST scored positive in 44 (63%) vs. 27 (39%) of the patients, respectively. In the control group we found 9 (14.5%) subjects with a positive ASST and 10 (16%) subjects with a positive APST. ASST was positive in 2 healthy controls, one psoriasis, four eczema, one localized pruritus and one lichen planus patient. APST was positive in 3 healthy controls, 3 eczema, one dermatophyte infection and two psoriasis patients. One patient with eyelid eczema and one healthy control was positive on both ASST and APST. Twenty-five (36%) patients scored positive both on ASST and APST, while 19 (27%) patients scored positive on ASST and negative on APST. There were only two patients who scored positive on APST and negative on ASST. Twenty-four patients scored negative on both ASST and APST (*table 2*).

There was a statistically significant difference between patients and controls with respect to ASST and APST positivity ($p=0.0001$, $p=0.0001$); CU patients had a 10-fold and 3.2-fold increased risk for ASST and APST positivity compared to the control group, respectively.

Table 1. Clinical characteristics of the patients and controls.

		Control group		Study group		OR 95% CI	
Age		39.31±14.12		40.66±12.68		t:-0.5 p=0.564	
Gender	Female	52	83.90%	54	77.10%	χ^2 :0.94	0.65
	Male	10	16.10%	16	22.90%	p=0.332	0.27-1.56
ASST	Positive (+)	9	14.50%	44	62.90%	χ^2 :31.97	9.97
	Negative (-)	53	85.50%	26	37.10%	p=0.0001	4.23-23.48
APST	Positive (+)	10	16.10%	27	38.60%	χ^2 :8.21	3.26
	Negative(-)	52	83.90%	43	61.40%	p=0.004	1.42-7.49
Anti-TPO	Positive (+)	3	8.30%	16	22.90%	χ^2 :3.41	3.26
	Negative (-)	33	91.70%	54	77.10%	p=0.065	0.88-12.05

Table 2. Results of ASST and APST in CU patients.

	ASST (+)		ASST(-)		p
APST (+)	25	56.80%	2	7.70%	χ^2 :14.6
APST (-)	19	43.20%	24	92.30%	0.0001

There were no statistically significant differences between ASST (+) and ASST (-) patients with regard to gender, age, disease duration, presence of angioedema, NSAID intolerance and allergic or autoimmune diseases, urticaria activity scores, DLQI scores and CU-Q2oL scores ($p>0.05$). There were no statistically significant differences between APST (+) and APST (-) patients with regard to gender, age, disease duration, presence of angioedema, NSAID intolerance and allergic or autoimmune diseases, urticaria activity scores, DLQI scores and CU-Q2oL scores ($p>0.05$).

Of the 16 (23%) CU patients with anti-TPO antibodies, 9 (56%) scored positive on ASST and 6 (37.5%) scored positive on APST. The ones with positive APST were all positive on ASST. There were no significant differences between ASST (+) and ASST (-) groups and also between APST (+) and APST (-) groups with respect to anti-TPO positivity. In the control group, only one subject with a positive anti-TPO antibody reacted both to ASST and APST. Anti-TPO assay showed a specificity of 23% and a sensitivity of 92% between CU patients and healthy controls. The positive predictive value for CU patients was 84% and negative predictive value was 38%. The accuracy was low; 46% and the likelihood ratio was 2.74 (table 3).

The sensitivity and specificity of ASST when comparing CU patients to healthy controls were 63% and 85%, respectively. The positive and negative predictive values were 83%

and 67%, respectively. The test showed an accuracy of 73% and the likelihood ratio was 4.33. This meant that the risk of a positive ASST in CU patients was 4.33 fold increased compared to healthy controls.

The sensitivity and specificity of APST when comparing CU patients to healthy controls were 39% and 84%, respectively. The positive and negative predictive values were 73% and 55%, respectively. The test showed an accuracy of 60%, which was lower than the accepted levels. The likelihood ratio for APST was 2.39.

Mean wheal diameter for serum and plasma was 5.6 ± 4.7 mm (range 0-13) and 3.4 ± 3.9 mm (range 0-11), respectively. There were no statistically significant correlations between the mean wheal diameter for serum and age, gender, presence of angioedema, duration of disease, urticaria activity scores, DLQI and CU-Q2oL scores ($p>0.05$). There were no statistically significant correlations between the mean wheal diameter for plasma and age, gender, presence of angioedema, duration of disease and urticaria activity scores ($p>0.05$). The mean wheal diameter for plasma was positively correlated with DLQI and CU-Q2oL scores ($p=0.044$, $p=0.017$).

In patients with a positive ASST, the mean wheal diameter for serum and plasma was greater than that of patients with a negative ASST ($p=0.0001$). In patients with a positive APST, the mean wheal diameter for serum and plasma was greater than that of patients with a negative APST ($p=0.0001$, $p=0.0001$ and $p=0.004$, respectively). In 7 (16%) patients with a positive ASST, the mean wheal diameter for serum and plasma were equivalent. Plasma induced mean wheal diameter exceeded serum induced diameter in 4 of ASST (+) patients and in 5 of APST (+) patients. In 15 (55%) of APST (+) patients and 34 (77%) of ASST (+) patients, serum induced mean wheal diameter exceeded that induced by plasma.

The results of first ASST and APST were well correlated with the results of second ASST and APST ($\kappa_W=0.414$, $p=0.001$ and $\kappa_W=0.256$, $p=0.01$).

Table 3. The association between anti-TPO antibodies and the results of ASST and APST.

	Anti-TPO (+)		Anti-TPO (-)	
ASST(+)	9	56.30%	35	64.80%
ASST (-)	7	43.80%	19	35.20%
APST(+)	6	37.50%	21	38.90%
APST(-)	10	62.50%	33	61.10%

Discussion

Asero *et al.* suggested that the proportion of patients showing a wheal and flare reaction increases up to 86% when autologous plasma is injected intradermally instead of

autologous serum in patients with CU. They also found that APST test was positive in 70% of patients with a negative ASST and 98% of patients with a positive ASST. Only one patient with a positive ASST scored negative on APST [12]. Asero *et al.* hypothesized that coagulation cascade was involved in the wheal and flare reaction, induced by autologous plasma. He based this idea on the knowledge that thrombin has been shown to induce oedema through an increase in vascular permeability and to trigger mast cell degranulation [18-20]. Following this study, Godse KV from India reported 46% vs 46% positive results on both ASST and APST and the wheal and flare area induced by autologous serum was equivalent to that of autologous plasma in 13 of 14 cases [21]. Metz *et al.*, reported results from three centers; Spain, England and Germany. He reported that ASST was positive in 37.5% while APST was positive in 43% of chronic urticaria patients. In this study, patients with a positive ASST were found to have a more impaired quality of life and an increased disease activity when compared to patients with a positive APST [22]. In our study we found a positive APST in 39%, and a positive ASST in 63% of the patients, while 16% and 14.5% were positive in the control group, respectively. Twenty-five (36%) of the patients were positive on both ASST and APST. Of the ASST (+) patients, 56% scored positive on APST, while 92% of the APST (+) patients scored positive on ASST. There were only two patients who scored positive on APST and negative on ASST. This finding showed that ASST is a more sensitive tool than APST and has the ability to detect more patients with autoreactive urticaria.

It has been shown that there is no association between the ASST response and gender, age, personal or familial history of atopy and age at onset [13, 23-27]. Likewise, in our study we did not find any associations between ASST (+) and ASST (-), APST (+) and APST (-) groups with respect to age, gender, disease duration and personal atopy history.

Presence of angioedema was found not to be associated with ASST results [24, 28-31] except one study which suggested that presence of angioedema was more frequent in ASST (+) patients [23]. There was no association between presence of angioedema and skin test results in our series of patients.

Caproni *et al.* [32] showed that urticarial episodes and number of wheals was higher in the ASST (+) group. Another study showed that patients with positive ASST had more wheals and higher itch scores [28]. We found no differences between ASST (+) and ASST (-), APST (+) and APST (-) groups with respect to disease activity.

Staubach *et al.* [31] demonstrated that ASST (+) patients required significantly more antihistamines than ASST (-) patients and experienced a longer (but not significantly) duration of CU. We were not able to report this kind of information, because almost all of the patients stopped their antihistaminic drugs during the week they scored the UAS7. But duration of the disease was not affected by the ASST and APST test results in our study.

We found no differences between the ASST (+) and ASST (-) patients with respect to quality of life scores and disease activity, like Staubach *et al.* who could not show any differences between ASST (+) and ASST (-) patients in quality of life scores [31]. We also found no differences between the APST (+) and APST (-) patients with respect to quality of life and disease activity, which matched the results of

Metz *et al.* [22]. In our study, there were no correlations between the mean wheal diameter for serum and duration of disease, disease severity and quality of life scores, like Kulthanan *et al.*, who reported that serum-induced wheal size of a positive ASST did not correlate with the severity and duration of CU [24]. The mean wheal diameter for plasma was not correlated with duration of disease and disease activity, but was positively correlated with quality of life scores. The significance of this finding remained unexplained where the results of APST were not associated with quality of life.

In the study of Asero *et al.* in patients with a positive ASST, the mean diameter of the wheal and flare area induced by autologous plasma exceeded that induced by autologous serum in 51% and was equivalent in 46% of cases. In our study, the mean wheal diameter for serum and plasma were equivalent in 7 (16%) patients with a positive ASST. Plasma induced mean wheal diameter exceeded serum induced diameter in 4 of ASST (+) patients and in 5 of APST (+) patients. In 15 (55%) of APST (+) patients and 34 (77%) of ASST (+) patients, serum induced mean wheal diameter exceeded that induced by plasma. We saw that serum wheal was the prominent reaction in both ASST (+) and APST (+) patients in contrast to Asero *et al.* [12].

There is evidence of an increased prevalence of anti-thyroid autoantibodies among CU patients; Lezznoff reported that 14.4% of CU patients have anti-thyroid antibodies [33]. We found anti-TPO antibodies in 23% of our patient population, where only 8% of control subjects had these antibodies. Anti-TPO assay showed a high sensitivity albeit a low specificity between CU patients and healthy controls. In addition we found no association between anti-TPO antibodies and a positive ASST, while O'Donnell reported a significant association [27]. In their study, 82% of chronic urticaria patients with thyroid antibodies scored positive in ASST. In our study population, 56% of patients with positive anti-TPO antibodies scored positive on ASST and 37.5% of them scored positive on APST. And positive APST results were not associated with anti-TPO antibodies.

We compared ASST and APST with respect to their sensitivity, specificity and accuracy. The specificity of the two tests was similar, while ASST was found to be more sensitive than APST in discriminating between CU patients and controls. The ASST had a higher accuracy and likelihood ratio than APST. These findings showed that ASST was superior to APST with respect to sensitivity, accuracy and likelihood ratios.

The reproducibility of ASST has been evaluated earlier by Grattan and Sabroe in two separate studies [7, 8] and it was found reproducible. We aimed to compare the reproducibility of two tests and found that the results of both ASST and APST are reproducible.

Our results show that ASST is superior to APST in many aspects and has the ability to detect more patients with autoreactive urticaria than APST. Therefore there is no need to use autologous plasma instead of autologous serum for intradermal testing in autoreactive urticaria. The ASST is a reproducible and sensitive skin test that should be performed in all clinical settings where differentiation of autoreactive urticaria is demanded. ■

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