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Review article

EAACI/GA^{2}LEN task force consensus report: the autologous serum skin test in urticaria

Injection of autologous serum collected during disease activity from some patients with chronic spontaneous urticaria (CU) into clinically normal skin elicits an immediate weal and flare response. This observation provides a convincing demonstration of a circulating factor or factors that may be relevant to the understanding of the pathogenesis and management of the disease. This test has become known as the autologous serum skin test (ASST) and is now widely practised despite incomplete agreement about its value and meaning, the methodology and the definition of a positive response. It should be regarded as a test for autoreactivity rather than a specific test for autoimmune urticaria. It has only moderate specificity as a marker for functional autoantibodies against IgE or the high affinity IgE receptor (FcϵRI), detected by the basophil histamine release assay, but high negative predictive value for CU patients without them. It is usually negative in other patterns of CU, including those that are physically induced. Positive ASSTs have been reported in some subjects without CU, including those with multiple drug intolerance, patients with respiratory allergy and healthy controls, although the clinical implications of this are uncertain. It is essential that failsafe precautions are taken to ensure that the patient's own serum is used for skin testing and aseptic procedures are followed for sample preparation and handling. CU patients with a positive ASST (ASST$^+$) are more likely to be associated with HLADR4, to have autoimmune thyroid disease, a more prolonged disease course and may be less responsive to H1-antihistamine treatment than those with a negative ASST (ASST$^-$) although more evidence is needed to confirm these observations conclusively.

G. N. Konstantinou[1], R. Asero[2], M. Maurer[3], R. A. Sabroe[4], P. Schmid-Grendelmeier[5], C. E. H. Grattan[6]

[1]Allergy and Clinical Immunology Department, 417 NIMTS, Army Hospital and Allergy Research Center National & Kapodistrian University of Athens, Greece; [2]Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugano, Italy; [3]Department of Dermatology and Allergy, Charité, Universitätsmedizin Berlin, Germany; [4]Department of Dermatology, Barnsley Hospital, Barnsley, UK; [5]Department of Dermatology, University Hospital, Zurich, Switzerland; [6]Norfolk and Norwich University Hospital, Norwich and St John's Institute of Dermatology, St Thomas' Hospital, London, UK

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Dr C. E. H. Grattan, MD, MA, FRCP
Dermatology Centre
Norfolk and Norwich University Hospital
Norwich NR4 7UY
UK

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The autologous serum skin test (ASST) has been widely adopted internationally as a clinical test to demonstrate circulating endogenous proinflammatory or weal-inducing factors in urticaria patients since it was first described in 1986 (1). It is often interpreted simplistically as a test of functional autoantibodies or autoimmune urticaria[1] with the implication of a good response to immunomodulatory therapies but the evidence for this is not convincing. This has resulted in some confusion about its interpretation and importance in clinical practice. A lack of standardization of the method has also hindered comparison of results from different centres. An expert panel, representing the Dermatology section of EAACI (chair, Dr C.E.H. Grattan), met in London on 20 December 2007 to review the evidence for best practice, where it exists, and to draw on the experience of its members where the evidence is incomplete or does not exist and to provide guidance on the use and interpretation of the ASST, with the intention of clarifying its place as a clinical tool in the investigation of urticaria.

Abbreviations: ASST$^+$, positive autologous serum skin test; ASST$^-$, negative autologous serum skin test; BHRA, basophil histamine release assay; CS, ciclosporin; CU, chronic spontaneous urticaria; ELISA, enzyme linked immunosorbent assay; NPV, negative predictive value; PPV, positive predictive value; RCF, relative centrifugal force; RPM, revolutions per minute.

[1]A diagnosis of autoimmune urticaria should include clinical, immunological and other laboratory criteria which, to date, have not been formally defined.

Definition

The ASST is an *in vivo* test which assesses autoreactivity. Autoreactivity is characterized by an itchy weal and flare

response to intradermally injected factors in autologous serum acting either indirectly through the release of mediators from cutaneous mast cells or other cells or directly on the microvasculature of skin. Autoreactivity does not define autoimmune urticaria but may be an indication of mast cell activating autoantibodies in ASST positive ($ASST^+$) CU patients. Functional autoantibodies need to be confirmed by the basophil histamine release assay (BHRA) and their specificity confirmed by immunoassay (Western blot or ELISA), where these tests are available.

Methodology of review

A systematic review of the English language medical literature through March 2008 was performed using web-based search engines provided by PubMed (for Medline database), Excerpta Medica Database (Embase) and Scopus with the key words 'autologous serum', 'skin test', 'chronic urticaria' or 'idiopathic urticaria'. Review articles, correspondence and most case reports were excluded.

Original data in the public domain were considered at the consensus meeting. Critical evaluations included the definition of autoreactivity, preparation of autologous sera and skin testing technique, criteria for positivity, association with autoimmunity and clinical implications of autoreactivity. Consensus was reached on information presented by each member of the panel at the meeting, followed by an in-depth analysis and meta-analysis of the published data by the lead author, serial e-mail revisions and subsequent peer review of the final document prior to publication.

Frequency of the ASST response in patients with and without CU

Frequency of positive ASST responses in adults with CU

The frequency of $ASST^+$ responses for adult CU patients in published studies ranges from 4.1% to 76.5% using different criteria for positivity (Tables 1 and 2). These differences could be attributable to patient selection, disease severity, methodology and response interpretation or even to the true prevalence of autoimmune urticaria in the populations tested, but this needs further study. This variation underlines the need for standardization of the ASST. The frequency of ASST positivity after pooling available data from Tables 1 and 2 using a difference in diameter between serum and saline induced weals of 1.5 mm as a positive criterion is 45.5% (95% CI, 24.7–74.4%) and when using 2 mm the frequency is 43.5% (95% CI, 34.8–62.1%).

ASST in children with CU

CU has been well documented in adults but there is much less published data about its prevalence and aetiopathogenesis in childhood. In one study, all four children with CU included had a positive ASST (2). In a second study, Brunetti et al., demonstrated that 22/49 (44.9%) had a positive ASST (3) and in a third study, 18/44 (40.9%) had a positive response (4). Positivity criteria for these studies are shown in Table 2.

ASST in patients without CU and healthy controls

General experience, including that of the panel, is that healthy controls and patients without CU do not have positive ASST responses (Table 3). In contrast to most previously published studies, some have demonstrated a relatively high prevalence of positive ASST reactivity in 30–50% of adult patients with allergic or nonallergic respiratory symptoms, reaching up to 80% in childhood populations (2, 5–8). In two of these studies 40–45% of healthy individuals also had a positive ASST (2, 5). The meaning of these discrepancies is unclear. However, it would be interesting to investigate whether or not a positive ASST in healthy controls is a risk factor for the future appearance of CU and whether these individuals also show histamine releasing activity on basophils *in vitro*.

Methodology: recommendations of the panel

Although performing the ASST would appear to be straightforward, variations in methodology have been reported ranging from the conditions of clotting and centrifugation, volume of injection, choice of negative and positive controls, measurement of the response and, most importantly, the criterion for positivity (Tables 1, 2 and 3). The recommended methodology is summarized in Fig. 1.

Blood collection and serum preparation for skin testing

Venous blood should be collected into sterile glass tubes without accelerator or anticoagulant. Red-topped 7 ml BD Vacutainer® blood collection tubes (no additive) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) are recommended because most of the published literature relates to blood clotted in glass tubes. However, with the advent of plastic blood collection tubes across Europe it is appropriate to consider using them (after ensuring that they do not include any clot-activator additives) when glass tubes are not available with the caveat that comparative information on optimal clotting and centrifugation conditions and the incidence of false positive and negative results is not yet available. Blood should be allowed to clot at room temperature for 30 min before separation. This is usually done with a bench centrifuge at relative centrifugal force (RCF) of 450–

Table 1. Prevalence of positive ASST and performance characteristics (sensitivity, specificity, positive and negative predictive values) of different ASST positivity criteria compared with serum-induced histamine release assays (basophil or mast cell based) in patients with CU (most of them calculated from the relevant published original data)

Reference	Patients tested	ASST positivity criterion (mm)	Positive ASST	Time measured (min)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)
(72)	163	≥2	60.1%	30	94[1]	54.9[1]	48[1]	95.4[1]	66.9[1]
(73)	24	≥2	43.5%	30	85.7[1]	75[1]	60[1]	92.3[1]	78.3[1]
		≥1	47.8%		100[1]	75[1]	63.6[1]	100[1]	82.6[1]
(34)	155	≥0.5	66.5%	30	92.6[1]	47.5[1]	48.5[1]	92.3[1]	63.2[1]
		≥1	54.2%		81.5[1]	60.4[1]	52.4[1]	85.9%[1]	67.7[1]
		≥1.5	44.5%		70.4[1]	69.3[1]	55.1[1]	81.4[1]	69.7[1]
		≥2	34.8%		55.6[1]	76.2[1]	55.6[1]	76.2[1]	69[1]
		≥2.5	24.5%		44.4[1]	86.1[1]	63.2[1]	74.4[1]	71.6[1]
	84	≥1.5	63.1%	60	92.3[1]	48.3[1]	45.3[1]	93.3[1]	61.9[1]
(50)*	100	≥2	55%	30	100[1]	63.4[1]	52.7[1]	100[1]	74[1]
(15)	118	Weal >50% of histamine ID with negative NS ID	73.7%	15	95[2]	30.6[2]	21.8[2]	96.8[2]	41.5[2]
(65)	117	Unequivocal ASST weal response	69.2%	15 & 40	100[2]	38.3[2]	28.4[2]	100[2]	50.4[2]
(74)	78	≥1.5	34.6%	30	66.7[3]	82.4[3]	66.7[3]	82.4[3]	76.9[3]
					70.4[4]	84.3[4]	70.4[4]	84.3[4]	79.5[4]
(47)	68	≥1.5	33.8%	30	100[2]	90[2]	78.3[2]	100[2]	92.7[2]
(75)	43	≥1.5	41.9%	30	92.9[5]	82.8[5]	72.2[5]	96[5]	86.1[5]
(44)	61	≥2.5	36.1%	20 & 60	48.4[6]	76.7[6]	68.2[6]	59[6]	62.3[6]
(76)	117	≥1.5	74.4%	30	85.7[2]	27.2	13.8[2]	93.3[2]	34.2[2]
(39)	182	≥2	49.5%	30	100[1]	64.8[1]	44.4[1]	100[1]	72.5[1]
(77)	64	≥1.5	34.4%	30	84[6]	97.4[6]	95.5[6]	90.5[6]	92.2[6]
(78)	72	≥1.5	55.6%	30	91.9[7a]	82.9[7a]	85[7a]	90.6[7a]	87.5[7a]
					95.7[7b]	63.3[7b]	55[7b]	96.9[7b]	73.6[7b]
79)	28	≥2	64.3%	30	93.3[8]	69.2[8]	77.8[8]	90[8]	82.1[8]
(80)	34	≥1.5	50%	30	81.8[2]	62.5[2]	52.9[2]	88.2[2]	70.6[2]

All patients were tested both with ASST (intradermal injection of 50 μl of autologous serum) and histamine release assays. NS, normal saline.

*In this study patients with CIU and delayed pressure urticaria were included.

[1]The basophil histamine release assay (BHRA) was considered positive if histamine release was >5% (after correction for spontaneous histamine release) from the basophil leukocytes of (a) a poorly 'sensitized' donor (serum IgE < 1 IU/ml) and/or (b) a 'sensitized' donor (with serum IgE 145 IU/ml) (72, 81).

[2]The BHRA was considered positive if histamine release was >5% (after correction for spontaneous histamine release) from properly prepared mixture from three normal nonatopic donors whose basophils were previously shown to release 30% of total histamine content on challenge with an optimal dose of anti-IgE (15, 76).

[3]The BHRA was considered positive if histamine release was >5% (after correction for spontaneous histamine release) from basophil leukocytes of (a) a poorly 'sensitized' donor (serum IgE < 1 IU/ml) treated with lactic acid to strip residual receptor-bound IgE and/or (b) a 'sensitized' donor with serum IgE 145 IU/ml (74).

[4]Previous data (footnote number 3) and/or positive mast cells histamine release assay from skin slices from healthy donors (72) with >5% histamine release after correction for spontaneous histamine release.

[5]The BHRA was considered positive if histamine release was >16% (after correction for spontaneous histamine release) from basophil leukocytes of a donor (75).

[6]As a positive basophil histamine release assay was considered as CD63 expression either based on Basotest and above the 99 percentile of the controls expression (44) or above a predefined baseline.

[7]The BHRA was considered positive if histamine release was >5% (after correction for spontaneous histamine release) from basophil leukocytes of (a) a highly sensitized atopic donor (total serum IgE = 1468 IU/ml) and/or (b) an nonatopic healthy person with serum IgE = 1 IU/ml (78).

[8]The BHRA was considered positive if histamine release was >15% (after correction for spontaneous histamine release) from basophil leukocytes of a healthy donor (79).

500 *g* (expressed in units of gravity) for 10 min.[2] Centrifugation at higher speeds (up to 5000 *g*) for shorter periods does not appear to influence autoreactivity but cooling the sample during separation may be required to prevent overheating.[3] Fresh serum should be used whenever possible for immediate skin testing to minimize any risk of sample contamination (9) or labelling errors (10, 11). Deterioration of the serum does not appear to occur at room temperature over short periods but it is recommended that sera that cannot be used immediately should be frozen without delay between −20 and −70°C, at which it appears to be stable for months for *in vitro* assays.

[2]Many bench centrifuges only have settings for speed (revolutions per minute, RPM), not RCF. Consequently, a formula for conversion is required to ensure that the appropriate setting is used. The relationship between RPM and RCF is as follows:

$$g = (1.118 \times 10^{-5}) \times R \times S^2$$

where g is the relative centrifugal force, R is the radius of the rotor in centimetres, and S is the speed of the centrifuge in RPM.

[3]The manufacturer of BD Vacutainer® blood collection tubes recommends centrifugation for up to 10 min and not exceeding 1300 *g*.

Table 2. Prevalence of positive ASST in CU where information on serum basophil histamine releasing activity is not available

Reference	ASST Positivity criterion	ASST volume (μl)	Frequency of positive ASST	Time measured (min)	Negative control
(82)	≥5 mm	50	72/132 (54.6)	30	NS or NS + Sepharose-protein G
(40)	≥0.5 mm	50	9/19 (47.4%)	30	NS
(35)	≥1.5 mm	50	42/102 (41.2%)	30	NS
(83)	≥5 mm	50	17/24 (70.8%)	45	NS
(46)	≥1.5 mm	50	167/257 (65%)	30	NS
(38)	≥1.5 mm	100	26/48 (54.2%)	30	Autologous serum + NS (1 : 1)
(42)	≥1.5 mm	50	38/50 (76%)	30	NS
(84)	≥1.5 mm	50	234/487 (48.1%)	30	NS
(85)	≥3 mm	20	22/95 (23.2%)	30	NS
(64)	≥1.5 mm	50	28/47 (59.6%)	30	NS
(3)	≥1.5 mm	50	22/49 (44.9%)	30	NS
(17)	≥5 mm	50	42/110 (38.2%)	10 min intervals up to 3 h	NS
(86)	≥1.5 mm	50	12/45 (26.7%)	30	NS
(87)	≥2 mm	ND	12/30 (40%)	30	NS
(2)	≥1.5 mm	50	≈58%	30	NS
(88)	ND	ND	6/18 (33.3%)	15	ND
(41)	≥1.5 mm	100	36/126 (28.6%)	30	NS
(89)	>9 mm^2	50	13/17 (76.5%)	60	Phosphate-buffered saline
(90)	≥1.5 mm	50	16/28 (57.1%)	30	NS
(62)	≥1.5 mm	50	34/73 (46.6%)	30	NS
(91)	≥3 mm	50	51/96 (53.1%)	30	NS
(4)	≥1.5 mm	50	18/44 (40.9%)	30	NS
(43)	≥2 mm	50	18/29 (62.1%)	30	NS
(36)	≥1.5 mm	50	21/85 (24.7%)	30	NS
(48)	≥1.5 mm	100	53/135 (39.3%)	30	Human serum albumin
(49)	≥1.5 mm	50	19/100 (19%)	30	NS
(92)	≥1.5 mm	50	53/95 (55.8%)	30	NS
(5)	≥1.5 mm	50	17/32 (53.1%)	30	NS
(93)	ND	ND	5/121 (4.1%)	ND	ND
(60)	≥1.5 mm	50	36/78 (46.2%)	30	NS
(94)	≥1.5 mm	50	8/21 (38.1%)	30	NS
(45)	≥1.5 mm	50	34/100 (34%)	30	NS

Different methodologies and positivity criteria used are included.
NS, normal saline; ND, not described.

Serum skin testing technique and control skin tests

Serum skin testing technique. The ASST should be performed with a 0.5–1 ml sterile syringe and needle 27G × 12.7 mm or finer (nominal outer diameter equivalent of less than 0.4 mm) (12) without dead space using 0.05 ml (50 μl) of fresh undiluted serum. Smaller and larger volumes have been used (Tables 2 and 3) without an appreciable influence on the response (after subtraction of the noninflammatory oedema of an adjacent negative control of the same volume) (13). Autoreactivity was diluted out by three serial twofold dilutions in saline (13). A superficial intradermal injection should be made by introducing the bevel of the needle uppermost and aiming to raise a palpable 'bleb' of fluid within the papillary dermis. Impalpable skin tests are usually too deep and should be repeated. A typical papule resulting from introducing 50 μl of fluid will measure 6–7 mm across but may not be easy to produce in aged or atrophic skin.

Control skin tests. The ASST response should be validated by performing a positive histamine control, either by skin prick testing (10 mg/ml) or by intradermal injection of 0.5–1 μg histamine (50 μl of 10–20 μg/ml histamine solution). As a negative control skin test, 50 μl sterile physiological (normal) saline is injected intradermally using the same method as for serum. Phosphate buffered saline may be used instead.[4]

Skin test site and timing

The volar forearm skin should be used after cleansing with antiseptic, avoiding the wrist and skin known to have had spontaneous weals in the previous 48 h [to avoid sites where mast cells may be refractory to further

[4]It should be appreciated that the most appropriate negative control skin test would be with normal sera which, however, cannot be performed in clinical practice for ethical reasons.

Table 3. ASST methodology and outcome in chronic urticaria and other groups of patients

Reference	Methodology				ASST reproducibility	Positive reactions in control subject	Other outcomes
	Controls	Positivity Criterion	Vol (μl)	Reading time			
(1)	NS	mean diameter ≥ 5 mm	100	30 min-2 h	Performed at different sites: $1^1/_2$–19 h after reproducible	• Healthy individuals: 0	• Predominantly neutrophilic infiltrate
(14)	Plasma & NS	mean diameter ≥ 5 mm	100	1–2 h	Performed at the same site: tachyphylaxis	• Healthy individuals: 0	• Mediator(s) 1000–15000 daltons molecular weight probable
(72)	NS	mean diameter ≥ 2 mm	50	30 min	ND	• Healthy individuals: 0	• Prevalence of basophil HRA positivity in CIU • Comparison of HRA from both basophils and mast cells • Predominantly neutrophilic infiltrate
(30)	NS + heparin NS + EDTA NS	mean diameter ≥ 0.5 mm	50	30 min	Performed at different sites: reproducible	• Healthy individuals: 0 • Atopic individuals: 0	• Heparin is able to inhibit the cutaneous response to histamine releasing factors
(34)	NS	mean diameter ≥ 0.5/1/1.5/2/2.5 mm	50	30 and 60 min	Performed at two different sites two separate days: reproducible	• Healthy individuals: 1/40 • Cholinergic Urticaria: 1/9 • Demographic: 0 • With atopic eczema: 0	• Role of ASST in identification of autoimmune urticaria
(15)	Heparinized plasma, NS	ASST/histamine (ID) weal ratio > 0.5	50	15 and 40 min	ND	• Atopic patients: 0	• Role of ASST in identification of autoimmune urticaria • Heparin inhibits histamine release from basophils and mast cells
(16)	NS	ASST/histamine (ID) weal ratio if > 0.5 & < 1:+ + if = 1:+ + + if > 1:+ + + +	50	15 min	ND	• Atopic individuals: 0 • Multiple NSAIDs intolerance: 20/22 • Single NSAIDs intolerance: 5/14	-
(83)	NS	mean diameter ≥5 mm	50	45 min	ND	• Healthy individuals: 0	• Aberrant signalling through the p21Ras pathway in lymphocytes of patients with CIU
(95)	NS	ND	50	20 min	ND	• Healthy individuals: 0 • Multiple antibacterial intolerance: 17/18 • Single antibacterial drug intolerance: 8/20	-
(41)	NS	mean diameter ≥1.5 mm	100	30 and 60 min	ND	• Healthy individuals: 0	• ASST may be a prognostic risk factor for urticaria duration and efficient treatments. • Coexistence of anti-thyroid antibodies and angio-oedema increases this probability.
(2)	NS	mean diameter ≥1.5 mm	50	30 min	Performed in triplicates Results not mentioned	• Healthy individuals: 26/58 • Nonatopics with respiratory symptoms: 58% • Allergic Children: 80% • Allergic Adults: 45%	• Positive ASST reactivity in non-CIU individuals

NS, normal saline; ND, not described; ID, intradermal, HRA, histamine release assay.

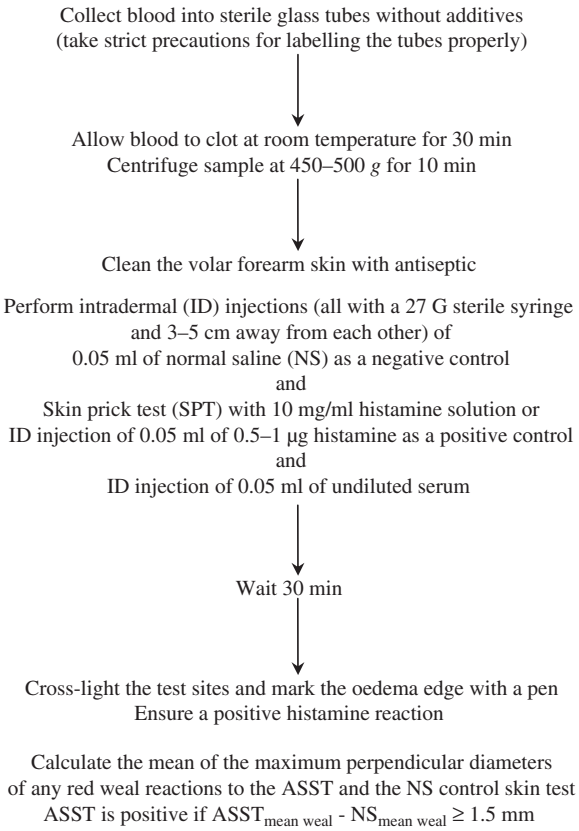

Figure 1. Protocol flow-chart for ASST procedure.

activation (local tachyphylaxis) (14)], leaving 3–5 cm gaps between each of the three injections (serum, positive and negative control) and from wrists and elbows. Equivocal ASSTs may be repeated but routine duplication of skin tests is not standard practice since the ASST has been shown to be reproducible (Table 3). The timing of venesection and skin testing in relation to daily activity of continuous urticaria does not appear to influence the test result (13). However, if the urticaria is episodic and is quiescent at the time of testing or has gone into remission, skin testing with fresh current serum is more likely to produce a negative test which contrasts with the observed positivity when stored serum taken during disease activity was used (1).

Skin test reading

The speed of onset and initial appearance of a positive ASST response is similar to the response to intradermally injected histamine but the reaction persists for longer. Even though a 30-min reading for the ASST has become standard practice a weal and flare response is usually apparent within 10 min. An additional 15-min reading has been performed in some centres. A positive ASST will often continue to enlarge with redness and a surrounding flare up to and beyond 30 min whereas the saline control injection site will usually become flatter and remain pale (Fig. 2A). Some residual skin coloured oedema is nearly always detectable at 30 min in both ASST$^-$ and negative control skin tests. Thirty-minute readings result in the best efficiency[5] when compared with the positive predictive value (PPV) for 15- and 60-min readings (Table 1). Because of the difficulty in assessing small differences in weal diameter between the serum-induced and the saline-induced reactions for weaker responses, some authors have compared the ASST response to a positive histamine control test (15–17) using a grading between 1+ to 4+, depending on the size of the difference. A clean transparent diascope or ruler may be pressed lightly onto the skin surface of a positive ASST to demonstrate the oedema within a skin test site more easily by blanching the erythema (Fig. 2B). Cross-lighting the skin tests and marking the oedema edge with a ballpoint pen before measuring between the points with a ruler marker in mm is probably the most accurate way of estimating diameter in clinical practice. The size of the red weal response should be documented and any atypical or late onset reactions. Taking the mean of the two longest perpendicular red weal diameters is recommended. The diameter of the surrounding flare is not informative. Weal redness is easy to assess and does not require special equipment, but is subjective. In addition, redness of both weal and flare reactions is difficult to see in pigmented skin.

Concomitant treatment

Skin testing should be done off antihistamines for at least 2–3 days, allowing up to 2 weeks for doxepin. A maximum of 72 h was thought to be an acceptable and tolerable time without antihistamines before assessment in the context of a clinical study (18). Longer periods of time have been advocated as the pharmacodynamic effects of antihistamines in skin may last longer than indicated by their plasma half-lives (e.g. loratadine, Table 4) (19–24) but are often unrealistic for patients with more severe disease that would be poorly controlled without treatment.

No published information exists on the potential inhibitory effects of leukotriene receptor antagonists which probably do not need to be discontinued before testing since the early phase of the ASST response is thought to be histamine-mediated. However, this needs to be clarified. Similarly, there have been no specific studies looking at the effect of corticosteroids on the ASST response. In the experience of the panel, patients on modest doses of systemic steroids (15 mg daily or less of prednisolone) can still be tested but prior use of high dose systemic steroids or potent topical steroids should be avoided. However, even when on modest doses negative

[5]The efficiency of a test is the percentage of the correctly classified patients (both true positive and true negative ones) by this test whereas PPV presents only the true positive percentage.

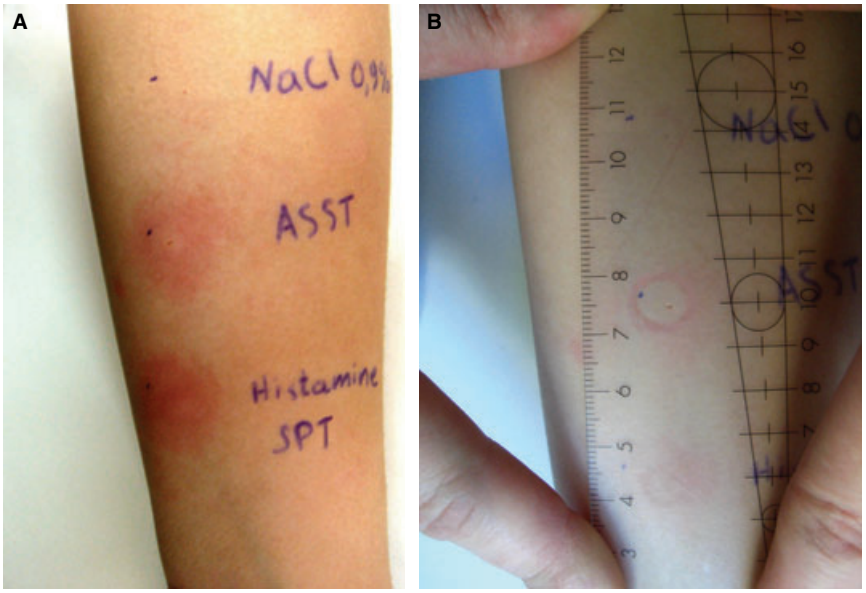

Figure 2. (A) A positive autologous serum skin test (ASST) at 30 min. The figure shows a negative control (NaCI 0.9%, normal saline), a positive weal response to autologous serum with a mean diameter of 7.5 mm and a fading positive histamine skin prick test response (positive control). (B) After pressure with a transparent ruler, the erythema blanches and the oedema within the skin test site (weal reaction) separates clearly and becomes much easier to define.

results need cautious interpretation. The same applies if antihistamines cannot be discontinued prior to testing because of disease activity. Skin testing with autologous serum on the day of finishing ciclosporin (CS) did not inhibit the autoreactive response to stored serum taken when disease was active implying that the primary effect of CS on CU activity is on reducing levels of histamine releasing autoantibodies rather than blocking cutaneous mast cell degranulation (25). Heparin has been shown to inhibit histamine release from mast cells and basophils by anti-IgE and Compound 48/80 in animals (26–28) and humans (15, 29–33). Any clinical relevance of concurrent and systemic administration of heparin for unrelated cardiovascular reasons on the results of skin testing is unknown but is likely to be negligible.

Table 4 Elimination plasma pharmacokinetics and duration of action, assessed by suppression of skin weal and flare reaction after histamine SPT, for various antihistamines (19–24)

	Elimination	Duration of skin weal and flare suppression	
Drug name/daily dose	$t_{1/2}$ (h)	Single dose (h)	Regular administration (days)
Acrivastine 8 mg	1.4–3.1	8	NA
Azelastine 4 mg	22	12	7
Cetirizine 10 mg	7–11	≥24	3
Cyproheptadine 8 mg	NA	NA	11
Dexchlorpheniramine 4 mg	NA	NA	4
Diphenhydramine	9.2 ± 2.5	NA	NA
Ebastine10 mg	10.3 ± 19.3	≥24	3
Fexofenadine 60 mg	14.4	24	2*
Hydroxyzine 0.7 mg/kg	20 ± 4.1	36	NA
Loratadine 10 mg	7.8 ± 4.2	24	7
Mizolastine 10 mg	12.9	24	NA
Levocetirizine 5 mg	7 ± 1.5	NA	4
Desloratadine	27	NA	NA
Doxepin 25 mg	17	4–6 (days)	NA

NA, not available.
*Duration of suppression is similar with 120 mg and 180 mg administration.

Safety and side-effects

The blood collection procedure should ensure that the identity of the donor is verified and the labelling is correct to avoid any risk of skin testing with the wrong serum. Standard precautions are required for handling blood products to protect both the patient and the operator in accordance with European Council Directive 2002/98/EC (10, 11). Any risk of microbial infection appears to be negligible provided that careful attention is given to withdrawing and re-injecting serum using an aseptic technique in line with good clinical practice. Serum infection risk seemed to increase after 24 h when autologous serum drops were used for the management of ocular surface disorders (9) so it is recommended that fresh sera should be used for skin testing where this is practical and that it should be prepared by the same clinician who does the skin testing. No clinically important side-effects from autol-

ogous serum skin testing have been reported since the ASST was described 22 year ago and none of the panel members were aware of any adverse events in their personal practice.

The autologous serum skin test as a marker for functional autoantibodies

Most studies have recognized that the ASST has limited PPV for a positive BHRA, ranging from 13.8–85% (Table 1)[6]. The most widely quoted study (34) had a PPV of 55.1% for a positive BHRA using a 1.5-mm difference in mean weal diameter between the ASST and a normal saline control at 30 min. Despite this limitation, its low cost and simplicity make the ASST a practical clinical screening tool, although time consuming, mainly due to its high negative predictive value (NPV), ranging from 59% to 100% for different positivity criteria (Table 1). However, if a positive test is defined as one with a minimum difference in weal diameter of 1.5 mm then the average NPV is 92.8% (range 81.4–100%). This means that a negative ASST is a useful surrogate marker of the absence of circulating functional autoantibodies. Therefore, it is recommended as a reliable clinical tool to exclude the presence of functional circulating autoantibodies detectable by the BHRA.

Recommended criterion of the panel for a positive ASST

For routine clinical purposes, the ASST can be interpreted as positive if firstly, a red serum-induced weal response is present at 30 min (usually with a surrounding flare) in the absence of a reaction to the saline negative control skin test. Secondly, based on the experience of the panel and evidence from the published literature for the best efficiency and PPVs for basophil histamine release (Table 1), a minimum difference of 1.5 mm in mean perpendicular weal diameter between the autologous serum-induced response and the saline-induced response should be used to define a positive response. The ASST should be considered un-interpretable if a red weal reaction develops at the saline skin test site or the histamine test site is negative. The latter is likely to be due to prior inadvertent antihistamine use. It is recommended that the histamine response should not be used to score the ASST. Care should be taken not to extrapolate the outcome of testing for autoreactivity to predict the presence of functional autoantibodies by the basophil histamine release assay based on previous literature, without internal validation.

[6]The term sensitivity describes the probability of a test (e.g. ASST) being positive in a disease (e.g. CU). Specificity describes the probability of a negative test in the absence of disease. PPV corresponds to the probability of having the disease given that the test is positive and NPV is the probability of not having a disease given that the test is negative.

Clinical characteristics in ASST$^+$ and ASST$^-$ CU patients

There is no association between the ASST response and gender, age, personal or familial history of atopy and age at onset (3, 4, 35–45). However, one study showed that the prevalence of a positive ASST was significantly higher in women than in men (76% *vs* 35%) (46). There is controversy about the relationship between ASST positivity and disease severity. One study showed that patients with positive serum tests had more weals, in a wider distribution, higher itch scores and more systemic symptoms, than those with negative tests (18). Similarly, Caproni et al. (47) showed that the overall frequency of urticarial episodes during the week before skin testing and number of spontaneous weals, was greater in the ASST$^+$ group. Staubach et al. (48) demonstrated that ASST$^+$ patients required significantly more antihistamines than ASST$^-$ patients and experienced a marginally (but nonsignificantly) longer duration of CU. However, in another study, the serum-induced weal size of a positive ASST did not correlate with the severity or duration of CU (36). Similarly, Staubach et al. showed no difference between ASST$^+$ and ASST$^-$ patients in quality of life scores (49). One group demonstrated that CU patients with angio-oedema seemed to be ASST$^+$ more frequently than those without angio-oedema (35) but this has not been a consistent finding in all studies (18, 36, 43, 44, 48). The ASST remained positive even after disease remission in one study (36) but became negative during remission in another (1).

Association between autoreactivity and autoimmunity in CU patients

There is evidence to support CU having a genetic predisposition [e.g. strong HLA associations (50–52), familial inheritance (53)] and/or an autoimmune aetiology in some patients, especially those with a positive ASST response. There are also a few reports suggesting an association specifically between ASST$^+$ CU patients and autoimmune diseases such as autoimmune thyroiditis, coeliac disease (47), rheumatoid arthritis (54), Graves' disease and type 1 diabetes mellitus (55) and an increased frequency of autoimmune markers such as rheumatoid factor, anti-thyroid and antinuclear antibodies (44, 56).

Thyroid autoimmunity

There is evidence of an increased prevalence of anti-thyroid autoantibodies among CU patients as a whole (57–59). Some studies have shown a statistically

significant association with a positive ASST (38, 39, 46, 60) whilst others have not (36, 47, 61, 62). Fusari et al. found no significant difference in the prevalence of antithyroid antibodies between CU patients with positive and negative ASST while the ASST was negative in the patients with thyroiditis but with no history of urticaria, who were used as negative controls (62).

HLA Class II association with ASST

A strongly positive association between certain HLA class II molecules and CU has been found, suggesting a possible genetic component in the pathogenesis of CU (50–52). CU patients as a whole and ASST$^+$ CU patients alone have a strikingly increased frequency of DRB1*04 and its associated allele DQB1*0302 compared with the healthy population. DRB1*04 and DQB1*0301/4 frequencies were also significantly higher in ASST$^+$ CU patients than in ASST$^-$ CU patients. No statistically significant difference was found between ASST$^-$ CU patients and healthy individuals (50). Of note, the above HLA genotypes are strongly associated with autoimmune disease, lending more support to a possible autoimmune basis to the disease.

Helicobacter pylori infection and ASST

A direct role for *Helicobacter pylori* (HP) infection in CU as a whole remains controversial. Similarly, limited clinical data suggest either a positive (38, 61, 63) or a negative (3, 64) relationship between HP infection and autoreactivity in CU patients. Bakos et al. (38) described not only a positive correlation between ASST positivity and infected patients but also noticed that most of the infected patients had autoimmune thyroiditis, raising the possibility of cross-reactivity between HP and target organs vulnerable to autoimmunity, such as the thyroid and skin.

NSAIDs intolerance, multiple drug allergy syndrome and ASST

The prevalence of NSAIDs intolerance seems to be similar in ASST$^+$ and ASST$^-$ patients with CU (65). However, a high proportion (91–93%) of patients presenting with new onset urticaria after the ingestion of several chemically unrelated drugs have a positive ASST (16, 17). This occurs not only in patients with NSAIDs intolerance but also in those with multiple antibacterial drug intolerance (16).

Prognosis and implication for management according to ASST response

There are limited data on the prognostic role the ASST response might have in predicting disease severity, duration and thus the best therapeutic approach in the management of CU. In one study, Staubach et al. (48) demonstrated that ASST$^+$ patients required significantly more antihistamines than ASST$^-$ patients and experienced a marginally (but nonsignificantly) longer duration of CU. Toubi et al. (41) also found that ASST$^+$ CU patients are more likely to have a prolonged disease course than ASST$^-$ patients, regardless of severity. In the same study, it was suggested that concurrence of angio-oedema and positive anti-thyroid antibodies in addition to a positive ASST substantially increases the probability of long standing disease. Positivity of the ASST correlates with disease activity in CU patients without anti-thyroid antibodies but positivity is more likely to persist into disease remission in ASST$^+$ CU patients with anti-thyroid antibodies (62) and thus the ASST loses its potential value as a marker of urticarial disease activity in this subgroup of patients.

Based on these findings early initiation of a third line treatment like ciclosporin (CS), in ASST$^+$ patients may be advantageous. Unfortunately, there is a lack of randomized, double blind, placebo-controlled clinical trials comparing the efficacy of CS or other immunosuppressive drugs in patients with positive and negative ASSTs. Although patients with a positive ASST often benefit from CS (25, 66, 67) a successful outcome does not appear to be dependent on the demonstration of autoreactivity (68). Studies of plasmapheresis or intravenous immunoglobulins showed that these treatments appeared to benefit ASST$^+$ CU patients (69–71) but none of them were placebo-controlled, randomized or compared ASST$^+$ with ASST$^-$ groups.

Areas requiring future research

Several aspects of the ASST deserve further investigation including:

1. The effect of variations in technique, the difficulty of defining a positive response accurately and interpretation of results.
2. Standardization of the ASST using objective measures of oedema, capillary blood flow and cellular inflammatory response.
3. Correlation of disease course and severity with the size of the serum-induced response.
4. Evaluation of the ASST in acute spontaneous urticaria.
5. The incidence and implications of positive ASSTs in subject groups without urticaria.

More information is needed to address the following questions:

1. What mediator or mediators result in autoreactivity?
2. What is the prognostic and management significance of autoreactivity?

Summary points

- The ASST is an *in vivo* test for assessing autoreactivity, rather than autoimmune urticaria.
- A minimum difference of 1.5 mm in mean maximum perpendicular weal diameter between the ASST and the negative control skin test, where the serum-induced weal is red, should be used to define a positive response
- Using this definition, a negative ASST can be used reliably as a clinical tool to exclude the presence of functional autoantibodies detectable in the BHRA.
- A positive ASST should ideally be performed in conjunction with the basophil histamine release assay to demonstrate functionality and immunoassay to demonstrate autoantibody specificity where facilities are available.
- Every centre using the ASST should ideally validate it against a standardized BHRA to ensure that the PPV and NPV are comparable with other centres.
- A positive ASST result may provide additional evidence for early initiation of a trial of immunomodulatory therapy (e.g. ciclosporin) but there is a lack of randomized, double blind, placebo controlled clinical trial data to support this approach.

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