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R. Asero[1], E. Pinter[2], A. Tedeschi[3]

35 years of autologous serum skin test in chronic spontaneous urticaria: what we know and what we do not know

[1]Allergology Clinic, Clinica San Carlo, Paderno Dugnano, Milan, Italy
[2]UOC Internal Medicine and Clinical Immunology, University of Rome La Sapienza, Policlinico Umberto I, Rome, Italy
[3]Department of Immunology Outpatient, UO General Medicine, Bolognini Hospital, ASST Bergamo Est, Seriate, Bergamo, Italy

Key words

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Corresponding author
Riccardo Asero
Allergology Clinic
Clinica San Carlo
via Ospedale 21
20037 Paderno Dugnano, Milan, Italy
ORCID: 0000-0002-8277-1700
E-mail: r.asero@libero.it

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Summary

The autologous serum skin test (ASST) has been used in patients with chronic spontaneous urticaria (CSU) as a means to detect an autoreactivity state for thirty-five years now. Nonetheless, several aspects of this old diagnostic test are still insufficiently defined. Particularly, the nature of the factor(s) responsible for the appearance of the wheal-and-flare skin reaction is still poorly characterized. This article will review our current knowledge about the clinical significance of the ASST and the factors possibly associated with the occurrence of the skin reaction following the intradermal administration of autologous serum that are known so far.

Impact statement

35 years after its introduction in the clinical practice, the autologous serum skin test still shows some unclear aspects that are addressed by the present review.

Introduction

Chronic spontaneous urticaria (CSU) is a quite common and frequently very disturbing disease characterized by the recurrent eruption of itchy wheals, often associated with angioedema for more than 6 weeks. The pathogenesis of CSU has remained obscure for decades until Grattan and co-workers, more than 30 years ago (1), found that a significant proportion of CSU patients develop a wheal-and-flare reaction in the site of the intradermal injection of a small amount of autologous serum (autologous serum skin test, ASST). Grattan's work actually resumed and developed the observations of Malmros, who evaluated the effects of intradermal injection of autologous serum in 956 patients with different disorders and found a wheal-and-flare reaction in 53 patients, 16 of whom with asthma and 6 with urticaria (2). The paper by Grattan and co-workers prompted the existence of circulating histamine-releasing factors in patients with CSU and has been the milestone of all subsequent studies that have led to our current understanding of the pathogenesis of this disease. We now know that the large majority of CSU patients have either an auto-allergic disease (type I, after Gell and Coombs), characterized by IgE directed against an array of auto-allergens (3), or an autoimmune disease (type IIb, after Gell and Coombs) characterized by functional IgG autoantibodies to IgE or to the high-affinity receptor (FcεRI) (4, 5). Recent studies found that the latter autoimmune process may be also IgM and/or IgA-mediated (6), and that the autoimmune and the auto-allergic pathogenic mechanisms co-exist in many patients (7) influencing to various extent their response to anti-IgE therapy. The ASST has been the silent witness in the background of all these advances throughout the decades, although its nature and clinical significance are far from being fully understood. The present article will review our knowledge about this "old" diagnostic method and will try to highlight the doubts that still surround its nature and clinical significance.

The ASST as a test for autoreactivity

About 10 years ago, two taskforces of the European Academy of Allergy and Clinical Immunology (EAACI), authored a couple of position papers, one dealing with the correct way to perform the ASST (8), and the other establishing criteria to define autoimmune urticaria (9). The task forces concluded that the ASST should be regarded as a test for autoreactivity rather than a specific test for autoimmune urticaria, as it shows only moderate specificity as a marker for functional autoantibodies against IgE or the high affinity IgE receptor (FcεRI) (8). Nonetheless its negative predictive value for autoimmune urticaria seemed excellent (8).
What does "autoreactivity" mean? A number of studies of ASST in CSU have been carried out in the past, showing large variability in positive skin testing, ranging between 4% and 76% (10). Although this may reflect differences in patients' selection and interpretation of positive results, the fact remains (and is generally accepted) that the ASST scores positive only in a variable proportion of chronic urticaria patients. In other terms, no "autoreactivity" can be detected in a variable proportion of patients with CSU.

The relationship between the ASST and serological tests for autoantibodies to IgE and/or FcεRI

The relationships between the ASST and functional autoantibodies against IgE or the high affinity IgE receptor were investigated in a study carried out 20 years ago on > 300 CSU patients (11). In that study, the ASST scored positive in 67% of patients whereas sera from only 16.5% of patients were able to induce histamine release from basophils of normal donors (BHRA, basophil histamine release assay), suggesting the presence of functionally active histamine releasing autoantibodies. Interestingly, all BHRA+ patients showed a positive ASST, but only 22% of ASST+ patients were also BHRA+. In BHRA+ patients, ultrafiltered serum fractions > 100 kDa fully retained their *in vitro* histamine-releasing ability, whereas serum fractions < 100 kDa were unable to induce any histamine release from donors' basophils. Notably, the proportion of patients whose sera were able to induce histamine release from basophils of normal donors is very close to that of patients that were identified as having an autoimmune chronic spontaneous urticaria (aiCSU) in a recent international study (12).
One very important (but rather overlooked) study about the lack of relationship between the ASST and functionally active, histamine-releasing autoantibodies was published by an Italian group in 2000 (13). In that study, heat-decomplemented/IgG-depleted sera elicited wheal-and-flare reactions on intradermal testing that were comparable with those observed with untreated sera. The authors concluded that skin reactivity to autologous serum was caused by unidentified non-Ig reactants. This observation was in line with earlier findings by Grattan and co-workers who observed that heat-decomplemented serum retained its ability to induce a wheal-and-flare reaction and that a low molecular weight histamine-releasing factor (HRF) could be detected in sera from CSU patients (14).
In summary, these studies show that all BHRA+ patients score positive on ASST, whereas most ASST+ patients do not show functionally active circulating histamine-releasing autoantibodies, suggesting that autoreactivity on autologous skin testing can be caused by serum factors other than autoantibodies.
The basophil activation test (BAT) and the ASST did not show significant correlation in some older studies (15, 16), but in a more recent, international study (12) ASST-positive patients included virtually all patients whose sera scored BAT-positive. In contrast, a much larger discrepancy between positive ASST and sera positive for IgG anti-FcεRl or IgG anti-IgE detected by ELISA was observed (12).

Effect of anticoagulants on autologous skin test

The first observations about the inhibitory effect of heparin on the autologous serum skin test were made by Fagiolo and coworkers in 1999 (17). They found that a positive skin test with autologous serum turned into negative if heparinized autologous plasma was injected intradermally, and if heparin was added to autologous serum. Further, adsorption of CSU sera with solid-phase heparin abrogated or strongly reduced the ability to induce cutaneous reactions. In contrast, interestingly, the intradermal injection of EDTA (ethylene diamine tetra acetic acid) - anticoagulated plasma did not modify the results of the ASST, and no change in the cutaneous response to allergens was associated with locally administered heparin in five atopic patients with no history of CSU. The authors concluded that heparin inhibits the cutaneous response to HRFs present in the sera of patients with CSU, possibly by a direct interference (17). These findings were confirmed two years later by another Italian group (11). In that study 205/306 (67%) CSU patients scored positive on ASST, whereas only 8/57 (14%) responded to intradermal injection of autologous heparinized plasma. Notably, all those 8 patients were very strong ASST reactors. As reported in Fagiolo's study (17), the addition of heparin to a commercial grass pollen extract did not change the wheal-and-flare response induced by skin prick test (SPT) in grass pollen allergic subjects. Further, *in vitro*, heparin dose-dependently inhibited histamine release induced by sera and plasma, and by basophil agonists such as anti-IgE, formyl-methionyl-leucyl-phenylalanine, and interleukin (IL)-3. The study concluded that heparin inhibits histamine release from both basophils (*in vitro*) and mast cells (*in vivo*), possibly acting directly at a cellular level (11).
A study published in 2006 (18) reported for the first time that the intradermal injection of autologous plasma anticoagulated with sodium citrate produced much more frequently a wheal and flare reaction than autologous serum in CSU patients (86%

vs 53%, respectively). This observation, along with the detection of increased levels of plasma prothrombin fragment 1+2 paralleling urticaria severity, led to conclude that CSU is associated with the generation of thrombin, and that APST (autologous plasma skin test) and ASST only partially depend on the presence of circulating antibodies to FcεRI or to IgE. This work represented the starting point of a number of subsequent studies dealing with the activation of the coagulation cascade in CSU and comparing APST and ASST that will not be considered here. In the same study (18), K_2EDTA was also tried as anticoagulant to test plasma skin reactivity, but it was found it caused nonspecific skin reactions that were directly related to its concentration both in patients and in controls.

Interestingly, it has been recently demonstrated that activated coagulation factors, such as factor Xa, factor IIa, and plasmin, can induce human skin mast-cell and basophil degranulation via the production of complement C5a which in turn binds to the C5a receptor and causes histamine release (19). This represents an additional IgE-independent mechanism of mast-cell and basophil activation which may be operating in CSU. Further, activated coagulation factors may activate mast cells via the so-called protease-activated receptors 1 and 2 (PAR-1 and PAR-2). Thrombin is able to activate PAR-1, while tissue factor+factor VIIa (FVIIa) and factor Va (FVa)+factor Xa (FXa) complexes act via PAR-2. This may well represent another IgE-independent pathomechanism playing a relevant role in CSU (20, 21).

Effect of antihistamines on autologous skin test

More recently, a study investigated the effect of H1-antihistamine treatment on the autologous plasma skin test in CSU patients, and found that 87% of them showed a large flare on APST while taking H1-antihistamines while the skin reaction to histamine 10 mg/ml was abolished or negligible (22). Little difference in the autologous plasma-induced flare was seen before and after the start of cetirizine therapy in 6 cases, whereas the drug exerted a marked effect on the histamine SPT as well as on the autologous plasma-induced wheal. The APST-induced flare was not associated with patients' response to H1-antihistamine. The study concluded that factors other than histamine are probably involved in the flare induced by APST in CU; such factors might play a pathogenic role particularly in patients not responding to standard H1-antihistamine treatments.

Are there other soluble histamine-releasing factors in sera from CSU patients?

In an *in vitro* study carried out using a mast cell line (HMC-1) missing the high affinity IgE receptor, most sera from CSU patients were able to promote the degranulation of mast cells, irrespective of a positive or negative autologous serum skin test (23). The study concluded that the combined degranulation and leakage assays used proved to be more sensitive than the ASST as a means to detect HRFs in patients with CSU. Subsequently, we found that both whole serum from CSU patients and serum fractionated at 100, 50, and 30 kDa, including fractions < 30 kDa, were able to activate LAD2 mast cells (carrying FcεRI receptors) significantly more than the corresponding fractions from normal control sera (24). These findings suggest that HRFs other than immunoglobulins may be involved in mast cell and basophil activation in CSU. In favor of this hypothesis also stands the observation that Mas-related G-protein coupled receptor-X2 (MRGPRX2), a protein that mediates IgE-independent activation of mast cells, basophils and eosinophils (25), is markedly upregulated in the skin of CSU patients (26). Some neuropeptides, such as substance P, and eosinophil-derived proteins, such as major basic protein and eosinophil peroxidase, can induce histamine release from human skin mast cells through MRGPRX2. It is conceivable that substance P as well as eosinophil-derived proteins may play a role in mast cell activation and contribute to CSU pathogenesis.

Other CSU-related conditions characterized by autoreactivity detectable by ASST

Patients showing a propensity to react (probably in a non-specific way) to several, chemically unrelated antibacterial drugs and termed as having a MDAS (multiple drug allergy syndrome) were found to show an extremely frequent skin reactivity (94%) upon intradermal injection of autologous serum (27). Interestingly, in the same study about one third of control patients with a history of hypersensitivity to a single antibacterial drug scored positive on ASST, whereas no normal control did. The study concluded that circulating histamine-releasing factors might play a role in drug-induced adverse reactions observed in these patients (27).

A similarly very high rate of positive ASST (91%) was detected in another study carried out in otherwise normal subjects with a history of hypersensitivity to multiple nonsteroidal anti-inflammatory drugs (NSAID) (28). These patients are currently defined as having a NIUA (NSAID-induced urticaria angioedema) (29). Interestingly, also in this case, ASST was positive in a significant proportion (36%) of patients with a history of single NSAID hypersensitivity (defined as having a SNIUA), whereas no normal control scored ASST-positive. One of these conditions, namely the NIUA seems quite correlated to CSU (30). The main features of ASST are summarized in **table I**.

Conclusions

There has been a rather limited interest in the ASST during the last few years, probably because attention was focused on the recent finding of autoreactive IgE supporting an "auto-allergic"

Table I *- Summary of the main features of the autologous serum and plasma skin test in CSU.*

Prevalence of positive ASST	Variable, generally about 50%
Addition of heparin to serum	Skin reactivity abolished
Skin test with heparinized plasma	Skin reactivity abolished
Skin test with K2EDTA anticoagulated plasma	Causes nonspecific positive reactions
Skin test with Na citrate anticoagulated plasma	Increases positive skin reactions in most studies
ASST with heat-decomplemented serum	No effect
ASST with IgG-depleted serum	No effect
Other CSU-related conditions characterized by ASST positivity	MDAS, NIUA

pathogenesis of the disease in a consistent proportion of CSU patients (2). Nonetheless, the nature and the clinical significance of this older diagnostic test remain an unsolved and fascinating problem. The evidence available so far suggests that skin reactivity to the intradermal injection autologous serum, albeit frequently associated with the presence of IgG autoantibodies directed against the high affinity IgE receptor or IgE, may also be due to HRFs other than antibodies. To our best knowledge, heparin is not able to bind immunoglobulins and inactivate them. In contrast, this might be the case with positively charged, low molecular weight histamine-releasing substances. In fact, heparin is a heavily negatively charged compound that might strongly bind and sequester similar substances. The demonstration of low molecular weight circulating HRFs in CSU patients and the immediate *in vivo* effect of heparin on ASST seems to support this view. In the light of the earlier findings of Grattan *et al*. on the occurrence of a low molecular weight serological mediator in CSU patients (14), and of our studies showing the presence of low molecular weight HRFs in sera from CSU patients (24) as well as their ability to activate human mast cells bypassing the high affinity IgE receptor (23), we believe reasonable to suppose their involvement both in the skin reaction to autologous serum and in CSU pathogenesis.

Fundings

None.

Conflict of interests

The authors declare that they have no conflict of interests.

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