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ORIGINAL ARTICLE

Autologous serum skin test reactions in chronic spontaneous urticaria differ from heterologous cell reactions

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Abstract

Background Autoimmune chronic spontaneous urticaria (CSU) is due to mast cell (MC)-activating autoantibodies, which are screened for by the autologous serum skin test (ASST) and basophil tests (BTs). Many CSU patients are positive in only one of these tests. How often this occurs and why is currently unknown.

Objectives To characterize the prevalence of mismatched ASST and BTs in CSU patients, and to investigate possible reasons for these mismatches.

Methods We determined the rates of ASST+/BT− and ASST−/BT+ mismatches in published CSU studies. We assessed sera from 48 CSU patients by ASST, two BTs (basophil histamine release assay, BHRA; basophil activation test, BAT), a MC histamine release assay (MCHRA) and by *ex vivo* skin microdialysis (SMD).

Results The ASST/BT mismatch rate in published CSU studies was 31% (ASST+/BT−: 22%, ASST−/BT+: 9%). In our patients, the ASST/BHRA and ASST/BAT mismatch rate was 35.4% (ASST+/BHRA−: 18.8% and ASST−/BHRA+: 16.7%) and 31.3% (ASST+/BAT−: 6.3% and ASST−/BAT+: 25.0%), respectively, and the two BTs were significantly correlated ($P = 0.0002$). The use of heterologous MCs, *in vitro* and *in situ,* instead of basophils produced similar results (MCHRA mismatch: 47.9%, ASST+/MCHRA−: 18.8%, ASST−/MCHRA+: 29.2%; SMD mismatch: 40.0%, ASST+/SMD−: 10.0% and ASST−/SMD+: 30.0%), and the MCHRA was highly correlated with SMD results ($P = 0.0002$).

Conclusions The ASST and BTs show divergent results in a third of CSU patients. Mismatches cannot be explained by the choice of basophil assay, the type of heterologous cells exposed to CSU serum *in vitro* (basophils vs. mast cells), nor the experimental setting of heterologous skin mast cells (*in vitro* vs. *in situ*). Thus, serum-induced whealing, in CSU patients, seems to involve autologous skin signals modulating MC degranulation.

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Conflicts of interest

Dr. Baumann has nothing to disclose. Dr. Marcelino has nothing to disclose. Dr. Skov has nothing to disclose. Dr. Pereira Santos has nothing to disclose. Dr. Wyroslak has nothing to disclose. Dr. Scheffel has nothing to disclose. Dr. Altrichter reports grants and personal fees from AstraZeneca, non-financial support from Moxie, grants from Sanofi, grants from Novartis, grants and non-financial support from Allakos, outside the submitted work. Dr. Woetmann has nothing to disclose. Dr. Costa has nothing to disclose. Dr. Maurer reports grants and personal fees from Allakos, personal fees from Aralez, grants from AstraZeneca, grants and personal fees from FAES, grants and personal fees from Genentech, grants and personal fees from Menarini, grants from LEO Pharma, grants and personal fees from Moxie, grants and personal fees from MSD, grants and personal fees from Roche, grants and personal fees from Sanofi, grants and personal fees from UCB, grants and personal fees from Uriach, grants and personal fees from Novartis, outside the submitted work.

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Introduction

Chronic spontaneous urticaria (CSU) is a common and devastating disease that presents with itchy weals and/or angioedema.[1–6] In most patients, CSU is a putative autoimmune disease,[7] i.e. due to type I autoimmunity (also referred to as 'autoallergy') or type IIb autoimmunity, with mast cell-degranulating antibodies against the high-affinity IgE receptor, FcεRI, or receptor-bound IgE.[8–10]

By definition, type IIb Autoimmune CSU (aiCSU) is diagnosed in patients who develop a weal in response to intradermal injection of their own serum (i.e. a positive autologous serum skin test; ASST), show serum reactivity in basophil tests (BTs; the basophil histamine release assay, BHRA, or the basophil activation test, BAT) and have IgG-anti-FcεRI and/or IgG-anti-IgE autoantibodies present in their serum.[11] The ASST is used as a screening test for aiCSU,[12,13] and a positive result is linked to comorbid autoimmune thyroid disease, a prolonged disease course,[12] the response to autologous serum therapy[14,15] and antihistamine treatment,[12] and the time to response to omalizumab.[16,17] The BHRA and BAT are both commercially available and have a high predictive value of aiCSU; 88% and 68%, respectively.[18]

The recent PURIST study[18] indicates that many CSU patients exhibit a mismatch between serum reactivity (measured by BTs) and skin autoreactivity (assessed by ASST). In most of these mismatch patients, the ASST is positive and the BT is negative, but ASST−/BT+ patients are also described. The rate of these mismatches and what causes them is currently unknown.

Possible explanations for a positive ASST and a negative BT with the serum of the same patient include that serum signals degranulate (i) mast cells but not basophils, (ii) autologous (ASST) but not heterologous cells, i.e. donor basophils, (iii) skin mast cells indirectly, via effects on skin cells other than mast cells, or at a lower threshold. A patient with a negative ASST may have a positive BT if (i) serum signals degranulate basophils but not skin mast cells, (ii) the mast cells in the patient's skin are refractory when the ASST is performed, e.g. because the ASST is done at a skin site that was recently affected by spontaneous weals, or (iii) the patient is treated with antihistamines or other medications that inhibit the ASST.

To investigate why the serum of many ASST+ patients fails to degranulate basophils and why the serum of some ASST− patients degranulates basophils, we analysed ASST+ and ASST− sera of 'Component-resolved study of autoreactive CSU' (CORSA) patients by use of basophil activation testing; BHRA and BAT, a mast cell activation test, i.e. the mast cell histamine release assay (MCHRA), and *ex vivo* skin microdialysis (SMD). Specifically, this study aimed to explore if a mismatch between ASST and BTs is due to (i) the choice of the basophil activation assay used, i.e. BHRA vs. BAT, (ii) the cell type exposed to the patient's serum, i.e. heterologous basophils vs. heterologous skin mast cells, or (iii) the test settings, i.e. *in vitro* vs. *in situ* testing.

Materials and methods

Patients

Forty-eight patients, which were part of CORSA, a cross-sectional study conducted at the Immunoallergology Department, Hospital de Santa Maria, Lisbon, Portugal, were analysed. Patients were eligible if they: (i) had a medical diagnosis of active CSU by an immunoallergologist, (ii) were treated at the Immunoallergology out-patient clinic, (iii) required daily medication for urticaria control, and (iv) were not and had never been on immunomodulating drugs (specifically omalizumab and cyclosporine; corticosteroids were allowed). CORSA was approved by the corresponding ethics committee (Comissão de Ética do Centro Hospitalar Lisboa Norte e Centro Académico Médico de Lisboa; 129/17 and 339/19) and conducted according to the Declaration of Helsinki, Good Clinical Practice and local regulations. Informed consent was obtained from all patients.

Assessment of the rates of ASST/BT mismatch in published CSU studies

A literature search of PubMed was conducted to identify studies that assessed CSU patients by the use of the ASST and a BT. The search identified studies published before September 2020 using the terms 'chronic urticaria', 'autologous serum skin test', 'BHRA' and 'BAT'. The eligibility criteria for included reports were (i) study included 10 or more patients with CSU, (ii) information on the outcome of the ASST and at least one BT was provided for all patients included in the study. Twenty studies fulfilled the eligibility criteria and were included (Table 1). The following information was extracted from each study: (i) first author's name and the publication year; (ii) number of tested patients, (iii) rates of ASST+, ASST+/BT+, ASST+/BT−, ASST−/BT−, and ASST−/BT+ patients.

Autologous serum skin test

Patients were required to stop any antihistamine medication for 5 days and any systemic corticosteroids for at least 2 weeks prior to the test. Venous blood was collected and centrifuged, and

Table 1 Studies on CSU patients assessing ASST and basophil activation (BHRA or BAT)

Study	Patients assessed	Ratio of ASST+	Ratio of ASST+/BT+	Ratio of ASST+/BT−	Ratio of ASST−/BT−	Ratio of ASST−/BT+
Niimi *et al.* (1996)[25]	163	60% (98)	29% (47)†	31% (51)†	38% (62)†	2% (3)†
Grattan *et al.* (1997)[60]	24	42% (10)	25% (6)†	17% (4)†	54% (13)†	4% (1)†
Sabroe *et al.* (1999)[33]	155	45% (69)	25% (38)†	20% (31)†	45% (70)†	10% (16)†
O'Donnell *et al.* (1999)[61]	100	55% (55)	29% (29)†	26% (26)†	45% (45)†	0% (0)†
Wedi *et al.* (2000)[62]	40	50% (20)	23% (9)†	28% (11)†	43% (17)†	8% (3)†
			35% (14)‡	15% (6)‡	28% (11)‡	23% (9)‡
Asero *et al.* (2001)[63]	118	74% (87)	16% (19)†	58% (68)†	25% (30)†	1% (1)†
Caproni *et al.* (2004)[64]	68	34% (23)	27% (18)†	7% (5)†	66% (45)†	0% (0)†
Gyimesi *et al.* (2004)[65]	30	40% (12)	37% (11)‡	3% (1)‡	50% (15)‡	10% (3)‡
De Swerdt *et al.* (2005)[66]	61	36% (22)	25% (15)‡	11% (7)‡	38% (23)‡	26% (16)‡
O'Donnell *et al.* (2005)[67]	182	49% (90)	22% (40)†	27% (50)†	51% (92)†	0% (0)†
Platzer *et al.* (2005)[19]	28	57% (16)	43% (12)†	14% (4)†	29% (8)†	14% (4)†
Tedeschi *et al.* (2005)[68]	117	74% (87)	10% (12)†	64% (75)†	24% (28)†	2% (2)†
Frezzolini *et al.* (2006)[69]	64	34% (22)	33% (21)‡	2% (1)‡	59% (38)‡	6% (4)‡
Szegedi *et al.* (2006)[21]	72	56% (40)	47% (34)†	8% (6)†	40% (29)†	4% (3)†
			51% (37)‡	4% (3)‡	39% (28)‡	6% (4)‡
Tedeschi *et al.* (2007)[70]	34	50% (17)	26% (9)†	24% (8)†	44% (15)†	6% (2)†
Yasnowsky *et al.* (2006)[22]	28†	64% (18)†	50% (14)†	14% (4)†	32% (9)†	4% (1)†
	32‡	69% (22)‡	53% (17)‡	16% (5)‡	25% (8)‡	6% (2)‡
Altrich *et al.* (2009)[23]	31	77% (24)	42% (13)†	35% (11)†	19% (6)†	3% (1)†
			35% (11)‡	42% (13)‡	19% (6)‡	3% (1)‡
Gentinetta *et al.* (2011)[71]	110	21% (23)	18% (20)‡	3% (3)‡	49% (54)‡	30% (33)‡
Curto-Barredo *et al.* (2016)[31]	139	57% (79)	18% (25)‡	39% (54)‡	42% (59)‡	1% (1)‡
Endo *et al.* (2020)[72]	20	35% (7)	15% (3)‡	20% (4)‡	20% (4)‡	45% (9)‡
	1584 in total	**51% on average**	**31% on average**	**22% on average**	**39% on average**	**9% on average**

Ratios are listed as percentages of the total number of patients in each study, with the numbers in parentheses indicating the number of patients in each group. Only studies clearly denoting patient subsets with regards to positive/negative results in the ASST and BTs are included in the table. Studies encompassing ASST-positive patients only are not listed.
†Basophil histamine release assay (BHRA). ‡Basophil activation test (BAT), ASST: Autologous serum skin test, BT: Basophil test (BHRA or BAT).

intradermal testing was performed by injecting 50 μL serum with an insulin needle. The result was read after 30 min and considered positive if the serum weal diameter was ≥1.5 mm larger than that of the saline control.

Basophil tests and histamine detection

The BAT was carried out by the Laboratory of Clinical Immunology, Faculdade de Medicina, Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal as described in Appendix S1 (Supporting Information). The BHRA was carried out by RefLab, Copenhagen, Denmark as described before.[19] The supernatants were extracted for histamine quantification in the histamine release (HR) assay as described in [20]. The total histamine content was determined by lysing the basophils using 7% perchloric acid, and the HR was expressed as % of total. A response with >16.5% of the spontaneous release was considered positive.

Skin specimens

Human skin was obtained from individuals undergoing cosmetic surgery with ethical permission from the Institutional Review Board at Charité – Universitätsmedizin Berlin (EA4/193/18) and with informed consent from the donors. Abdominal skin specimens were used for microdialysis studies and breast skin was used for mast cell isolation.

Purification of skin mast cells

Mast cells were isolated from human breast skin from four individual donors ($n = 4$) by mechanical and enzymatical digestion of the tissue as described in Appendix S1 (Supporting Information).

Mast cell histamine release assay

Histamine release was studied using purified human mast cells as described in Appendix S1 (Supporting Information).

Skin microdialysis and histamine detection

Histamine release from skin-resident mast cells was studied by microdialysis in excised human skin after injection of serum from 10 selected CSU patients as described in Appendix S1 (Supporting Information).

Statistical analysis

All statistical analyses were made using GraphPad Prism 8.2.1 (GraphPad Software, San Diego, CA, USA). Correlations between continuous variables were evaluated using the Spearman correlation, whereas dichotomized variables were compared using the Fisher's exact test. The area under the curve (AUC) was calculated using the trapezoid rule.

Results

In CSU patients, the results of the ASST and of BTs are often mismatched

Based on our review of studies that assessed CSU patients by the use of the ASST and a BT (20 studies with 1584 patients in total), 2%–64% of patients had a positive ASST and a negative BT, and 0–45% patients per study had a negative ASST and a positive BT (Table 1). The average rate of mismatch (both ASST+/BT− and ASST−/BT+) across the studies was 31%, with 22% of the patients being ASST+/BT− and 9% ASST−/BT+ (Table 1).

The frequency of ASST-mismatches is independent of which basophil test is used, and the BHRA and BAT are significantly correlated

To explain the high frequency of mismatches between ASST and BT results found in the literature, 48 patients were investigated in this study. Of these, 17 (35.4%) showed a mismatch between ASST and BHRA results; 18.8% were ASST+/BHRA− and 16.7% were ASST−/BHRA+ (Table S1, Supporting Information). To assess whether the choice of BT affects the rates of ASST/BT mismatches, we compared sera of CSU patients for their basophil activating effect by the use the BAT in addition to BHRA. BAT testing showed similar results as BHRA testing (Fig. 1), i.e. a mismatch between ASST and BAT was found in 15 (31.3%) of the 48 patients investigated; 6.3% were ASST+/BAT− and 25.0% were ASST−/BAT+ (Table S1, Supporting Information). Also, the two tests were significantly correlated ($\rho = 0.5184$, $P = 0.0002$, Spearman correlation).

Heterologous basophils and mast cells show similar *in vitro* responses to CSU sera, and ASST/MCHRA and ASST/BT mismatch rates are similar

To address the impact of the cell type used for testing, we assessed mast cells instead of basophils, *in vitro*, for their activation by CSU sera. The rate of ASST/MCHRA mismatch was 47.9%, similar to the mismatch rates of the ASST and the BTs, with 18.8% of the 48 patients being ASST+/MCHRA− and 29.2% ASST−/MCHRA+ (Table S2, Supporting Information). The amount of histamine released from purified skin mast cells and the levels of basophil activation measured by BTs were significantly correlated; BHRA: $\rho = 0.6189$, $P < 0.0001$ (Spearman correlation; Fig. 2a) and BAT: $\rho = 0.6301$, $P < 0.0001$ (Spearman correlation; Fig. 2b).

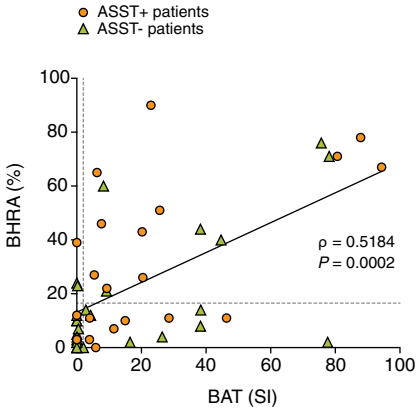

Figure 1 Correlation between the basophil activation test (BAT, expressed as stimulation index; SI) and the basophil histamine release assay (BHRA, expressed as per cent of total histamine content) with serum from 48 CSU patients. Solid line: best linear fit, dotted lines: assay cut-off for BAT and BHRA. ρ: Spearman correlation coefficient, *P*: *P*-value.

Heterologous skin mast cells, *in situ*, show similar responses to CSU sera as heterologous basophils and heterologous purified mast cells, *in vitro*, including similar rates of mismatch with the ASST

The potential impact of the skin environment on the effects of CSU sera in relation to heterologous skin mast cells *in situ* was investigated using SMD after injection of serum from 10 CSU patients into human skin *ex vivo*. The release of histamine from skin mast cells *in vitro* (assessed using MCHRA) was compared with the release *in situ* after exposure to CSU sera (assessed using SMD). The rate of ASST/SMD mismatch was very similar to the mismatch rates of the ASST with MCHRA, i.e. 40.0%, with 10.0% ASST+/SMD- patients and 30.0% ASST−/SMD+ patients (Table 2). The SMD and the MCHRA showed very similar (Fig. 3) and strongly correlated results ($\rho = 0.9394$, $P = 0.0002$, Spearman correlation).

Discussion

This study aimed at exploring the prevalence of mismatches between ASST and BT results as well as potential explanations for this phenomenon. Our review of published studies showed that ASST and BT mismatches are common, with up to 64% and an average of 31% CSU patients affected, more commonly by ASST+/BT- than by ASST−/BT+ mismatches. Are these mismatches between the ASST and BTs related to methodology? In line with other reports,[21–23] we found a significant correlation between BAT and BHRA, but the low correlation coefficient might point towards methodological variations between the two BTs (e.g. the use of a single basophil donor vs. different donors). Still, the similar frequencies of ASST/BHRA and ASST/BAT

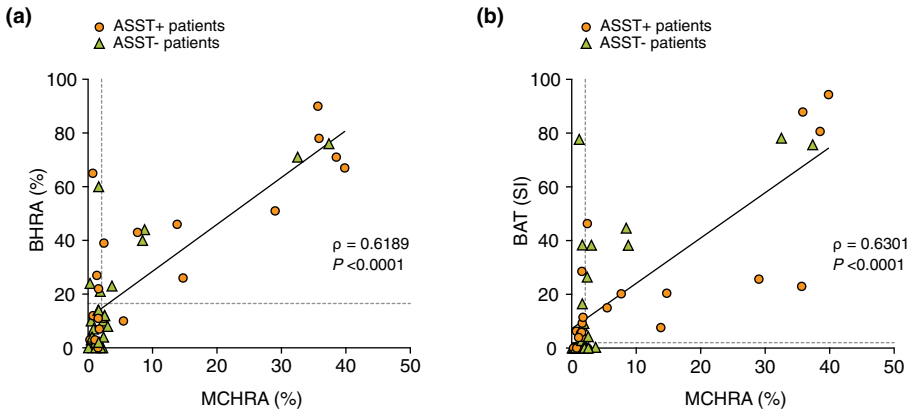

Figure 2 Correlation between basophil assays (a) basophil histamine release assay; BHRA, (b) basophil activation test; BAT) and the mast cell histamine release assay (MCHRA) with serum from 48 CSU patients. BHRA/MCHRA: per cent of total histamine content, BAT: stimulation index (SI). ρ: Spearman correlation coefficient, *P*: *P*-value. Solid line: best linear fit, dotted lines: assay cut-offs.

Table 2 Serum characteristics of the 10 CSU patients tested in SMD

Patient ID	ASST	BHRA	BAT	MCHRA	SMD
P11	+	+	–	+	–
P25	+	+	+	+	+
P27	–	+	+	+	+
P34	+	+	+	+	+
P35	+	+	+	+	+
P37	+	+	+	+	+
P43	–	+	+	+	+
P47	–	+	+	+	+
P53	+	–	+	–	+
P69	+	+	+	+	+

ASST, Autologous serum skin test; BHRA, basophil histamine release assay; BAT, basophil activation test; MCHRA, mast cell histamine release assay (n = 3 skin donors); SMD, skin microdialysis (n = 3 skin donors).

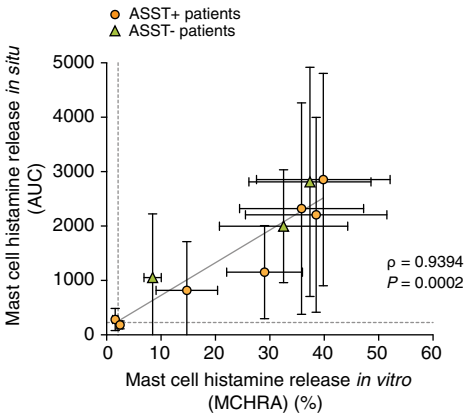

Figure 3 Correlation between histamine release from skin mast cells *in vitro* (mast cell histamine release assay; MCHRA) and *in situ* (measured by microdialysis, expressed as AUC) after incubation with serum from 10 patients. Both assays: n = 3 skin donors. Depicted: mean values ± SD, Solid line: best linear fit, dotted lines: assay cut-offs.

mismatches observed in this study indicated that the discrepancy between the results of the ASST and basophil testing is not due to the choice of basophil assay.

Can the mismatch between skin reactivity to autologous serum and the serum effects on heterologous cells *in vitro* be explained by the fact that the latter uses basophils rather than mast cells? To answer this question, we purified mast cells from donor skin to obtain effector cells with a skin phenotype, as opposed to the basophils used in the BTs. The application of purified heterologous skin mast cells (in the MCHRA), as compared to the use heterologous basophils (in BTs) for the *in vitro* assessment of serum reactivity, did not reduce the rate of ASST mismatch. In fact, the MCHRA results were significantly correlated with both BTs. This confirms the findings previously reported by several groups,[24–26] which, together with our results, point to a common activation mechanism in the two cell types.

Are mismatches between the ASST and *in vitro* tests due to the fact that cells, in the latter, are exposed to serum in isolation rather than in their cellular environment, i.e. the skin? We found a strong and highly significant correlation of the HR by skin mast cells *in vitro* and *in situ* after stimulation with CSU sera, and their ASST mismatch rates were very similar. This suggests that the cellular setting does not impact the release of histamine from heterologous mast cells; instead, it points to a difference between the response of mast cells in healthy skin vs. the skin of CSU patients.

How could this hypothesis explain the different mismatches? An ASST+/BT- mismatch might be due to the presence of signals in the skin of CSU patients that facilitate the degranulation of mast cells or the response to it. It is also possible that mast cells

residing in the skin of CSU patients are more susceptible to degranulation compared to mast cells in the skin of healthy people. Furthermore, previous reports of positive ASST results in healthy controls and patients with allergic rhinitis suggest that there is a risk of false positives when using the ASST to screen for aiCSU.[27–29]

How can ASST−/BT+ mismatches be explained? Our study did not specifically investigate this, however, possible explanations include that a negative ASST in BT-positive patients may be caused by exhausted mast cells in the tested skin area or by the downregulation of histamine receptors. In general, BT-positive patients are known to often exhibit high disease activity.[30–32] Despite performing the ASST on seemingly unaffected skin, a previous weal at the same location may have rendered skin mast cells refractory to degranulation, thus causing a negative ASST result.[12,33] In line with this, Grattan *et al.* showed that ASST results are not constant and dependent on disease activity.[34]

Most, but not all, studies on mast cell numbers in the skin of CSU patients show that they are increased.[10,35–40] If higher numbers of mast cells are indeed found in the skin of CSU patients, the release of small amounts of histamine, by many cells, might lead to weal formation, whereas the release of such amounts *in vitro* or *in situ* would not be considered a positive response. It is also possible that other residing or infiltrating cells in the skin of CSU patients play a role,[39,41–44] e.g. by effects on signalling pathways,[40,45] by reducing the function or number of regulatory cells,[10,46–48] or by providing priming factors that increase the responsiveness of mast cells. Relevant priming factors may include neuropeptides (e.g. stress related),[49–51] agonists of the mas-related G-protein coupled receptor X2 (food/drug related)[40,52] or complement components (e.g. infection related)[53] (recently reviewed by Bansal and Bansal[54]). Data by Ferrer *et al*. indicate that HR from human skin mast cells after incubation with CSU serum is, in some cases, complement-dependent.[55] Complement involvement was not assessed in this study, but it is possible that the lack of mast cell-activating capacity of some sera is due to an absence of complement (as a result of storage and handling) in the serum samples tested in the mast cell assays (MCHRA and SMD).

How should we identify aiCSU patients in the future? Whether a CSU patient has aiCSU is, in clinical practice, often assessed by either the ASST or basophil testing alone, although the current diagnostic criteria proposed by the European Academy of Allergy and Clinical Immunology (EAACI) require triple positivity of a BT, the ASST, and IgG autoantibody immunoassay.[11] The ASST is a good screening test but comes with several limitations, e.g. refractory mast cells that may cause a false-negative ASST result. Anti-FcεRI assays are not widely available due to the lack of commercial anti-FcεRI autoantibody immunoassays, and the results obtained from anti-FcεRI assays are often mismatched with the ASST, BTs and levels of total IgE.[18] Basophil testing is the single best test for identifying patients with aiCSU and should, in our opinion, be performed in CSU patients treated by specialists, especially in patients with long-standing and treatment-resistant disease. Our results demonstrate that diagnosing true aiCSU requires more than single positivity in the ASST or a commercially available BT. Thus, a combination of autologous skin testing and *in vitro* testing is, at present, most likely to reveal autoreactivity in CSU patients. Where these tests are not available, elevated levels of IgG-anti-TPO and low levels of IgE are biomarkers that point to aiCSU[18,56].

Diagnosing aiCSU does not immediately impact the decision on how to treat the patient, as there are currently no endotype-specific treatments. The current step-up treatment algorithm, i.e. administration of an antihistamine, updosing of an antihistamine, add-on omalizumab, and add-on cyclosporine, therefore applies to all patients, independent of their CSU endotype. Nevertheless, the diagnosis aiCSU can help physicians to manage patient expectations with respect to antihistamine and omalizumab treatment effects. Also, earlier add-on treatment with cyclosporine or switching to cyclosporine monotherapy earlier may be considered in omalizumab-resistant patients with aiCSU. Taken together, it is advisable to screen patients for aiCSU and to assess them for markers of this endotype, especially in a specialist care setting.

It would be interesting to see in future clinical studies whether the combined ASST/BT signature of individual patients is indicative of the patient's response to anti-IgE treatment (omalizumab[57] or ligelizumab[58]). Perhaps the differential response from ASST−/BT+ and ASST+/BT− CSU patients indicates the existence of new patient sub-groups of diagnostic and clinical importance, and the additional skin factors, which seem to be present in ASST+/BT- patients, may prove to be future therapeutic targets.

A limitation of the present study is the small sample size, especially in relation to the low capacity of the SMD setup to screen serum samples. Our limited access to excised skin specimens necessitated the selection of 10 sera from the group of 48 patient sera, however, a statistically significant correlation was obtained even with a low number of patients and skin donors.

Conclusion

Our study demonstrates that one in three patients with CSU shows a discrepancy between the results of the ASST and BTs, which cannot be explained by the cell type used for the *in vitro* assays (basophils vs. mast cells), as the two cell types showed similar responses when exposed to CSU serum. The fact that skin mast cells responded similar to basophils, regardless of their setting (*in vitro* vs. *in situ*), suggests that the skin of CSU patients contains additional, disease specific mast cell and/or skin factors that modulate mast cells degranulation when autologous serum is injected.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Material S1. Materials & methods.
Table S1. Results of the ASST and the BTs for the 48 CSU patients assessed in this study.
Table S2. Results of the ASST and the MCHRA for the 48 CSU patients assessed in this study.