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POSITION PAPER

EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria

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Abstract

An autoimmune subset of chronic spontaneous urticaria is increasingly being recognized internationally, based on laboratory and clinical evidence that has accrued over the last 20 years. This evidence has been reviewed by a taskforce of the Dermatology section of the European Academy of Allergy and Clinical Immunology. Functional autoantibodies in chronic urticaria (CU) patient sera have been demonstrated against IgE and FcεRIα by basophil and mast cell histamine release assays and by basophil activation assays. Antibody specificity has been confirmed by immunoassay, but there is a poor correlation between functionality and immunoreactivity. Approximately 25% of CU patients have a positive basophil histamine release assay and show autoreactivity (a positive autologous serum skin test), whereas 50% are negative regarding both. Functionality of CU sera appears to be complement dependent on mast cells but not exclusively on basophils. Basophil activation by CU sera is predominantly restricted to IgG1 and IgG3 subclasses. Circumstantial evidence for CU being an autoimmune disease comes from an observed association with other autoimmune diseases, a strong association between serum functionality and HLA-DR4 haplotype and the good response of CU patients to immunotherapies. It was proposed that a study should be undertaken to prospectively validate potentially relevant clinical criteria (from the history, examination and routinely available clinical investigations) against a new 'gold standard' for the diagnosis of ACU (positive autoreactivity, functional bioassay and immunoassay) to define preliminary criteria sets for the diagnosis of ACU based on clinical and laboratory features with highest individual sensitivity and specificity.

Abbreviations
ACU, autoimmune chronic spontaneous urticaria; ASST, autologous serum skin test; BHRA, basophil histamine release assay; cyclosporin A, CsA; CIU, chronic idiopathic urticaria; CU, chronic spontaneous urticaria; ELISA, enzyme-linked immunosorbent assay; FcεRI, high-affinity IgE receptor; MC, mast cell; MCHRA, mast cell histamine release assay; WB, Western blot.

The current nomenclature of urticaria endorses the use of a clinical rather than an aetiological classification to recognize that some patients with chronic spontaneous urticaria (syn. chronic ordinary urticaria: CU) may have an autoimmune rather than idiopathic aetiology (1).

An EAACI taskforce panel was established in 2010 to review the laboratory and clinical evidence for CU being an autoimmune disease in a definable subset of patients by examining whether there is sufficient direct, indirect and

circumstantial evidence currently available to fulfil Witebsky's postulates (2). A systematic review of the English language medical literature up to December 2011 was performed (for more details, see online repository). Critical evaluations included the definition of autoimmunity according to existing consensus reports and evaluation of common features of CU. Consensus was reached on information provided by each member of the panel at the meeting, followed by an in-depth review of the published data by all authors in serial revisions until the final document prior to submission.

Evidence for the existence of functional autoantibodies in chronic spontaneous urticaria

IgG and/or IgM antibodies against IgE were first identified by immunoassay in patients with CU, urticarial vasculitis and cold urticaria by Gruber and co-workers in 1988 (3). The serum of one patient with cold urticaria containing an IgM anti-IgE was shown to release histamine from healthy nonatopic donor basophils, but the possible relevance of this finding was not elaborated. IgG histamine–releasing autoantibodies with properties of anti-IgE were described 3 years later by Grattan et al. (4) in sera of CU patients. Most of the same sera were also shown to be vasoactive *in vivo* by eliciting a weal and flare response to autologous serum skin test (ASST), raising the possibility for the first time that some patients previously regarded as having idiopathic CU (CIU) might have an autoimmune disease. These initial findings were extended by the same group in 1993 (5), and importantly, novel functional autoantibodies with specificity against the high-affinity IgE receptor (FcεRI) were identified by neutralization of the histamine-releasing activity of IgG prepared from CU sera with the soluble alpha chain of FcεRI. These findings were confirmed by Fiebiger et al. (6) 2 years later and in other centres (7–11).

Data from these early studies suggested that almost 40% of the patients previously characterized as having CIU had circulating autoantibodies that might be implicated in urticaria pathogenesis (4, 5). Their functionality was defined by the release of histamine from healthy donor basophils or mast cells (MCs). An additional 5–10% have functional IgG anti-IgE autoantibodies. The ratio of functional anti-FcεRIα to anti-IgE ranges from 3 : 1 to 4 : 1 (8, 12). The specificity of these autoantibodies has been confirmed by either Western blot (WB) (6, 8, 9, 12–15), ELISA (10, 16) or immunoenzymetric (17) assays. However, no clear correlation between binding and functional assays has been found.

There is some evidence that other autoantibodies may be also involved in CU. Anti-FcεRII/CD23 autoantibodies have been identified in CU sera that elicit MC degranulation indirectly, via major basic protein release from eosinophils, resulting in noncytotoxic degranulation (18). Antiendothelial cell autoantibodies were present in some CU sera although their biological significance is unclear (19). IgE autoantibodies against thyroid peroxidase were detected by ELISA in CU sera in one study (20), but not in another (21). These autoantibodies might, in theory, elicit MC degranulation by binding soluble thyroid antigens or other cross-reacting self-antigens. It is possible, if not likely, that other autoantibody systems exist that may be directly or indirectly relevant to weal formation or persistence in CU patients.

A model of 'conditional autoimmunity' has been proposed on the premise that natural anti-FcεRIα autoantibodies can be found in healthy subjects and may become pathogenic locally following a dynamic shift between FcεRI occupancy by its natural ligand, IgE, and its unoccupied state. Removal of IgE from basophils resulted in their activation and MC mediator release by natural anti-FcεRIα autoantibodies, while resensitization with IgE prevented this autoantibody-dependent histamine release (22, 23). This finding may provide a model for cellular events in the skin that determine whether MC receptor activation, degranulation and weal formation occur as a result of exposure to anti-FcεRIα autoantibodies or not. There is evidence that IgE receptor occupancy is relevant in CU as most patients have anti-FcεRIα that will bind the α1 domain of the FcεRI despite IgE sensitization. However, in a minority, the autoantibody competes with IgE for the receptor, binding to its α2 domain, and will not trigger histamine release from basophils that are already presensitized with IgE (24). Local heat or pressure from clothing contact often appears to induce weals in spontaneous urticaria and might, perhaps, promote the local conditions necessary for changes in IgE receptor occupancy resulting in MC degranulation.

Although most of the studies have been conducted in adults, there is evidence that a similar percentage of children with CU have the same 'functional' autoantibodies (25, 26).

Basophil and mast cell assays for functional autoantibodies in CU

Histamine release assays

Both blood basophils (5, 6, 8–10, 14) and cutaneous MCs (7, 9) from healthy donors have been used in bioassays to evaluate CU sera containing anti-FcεRIα or anti-IgE autoantibodies. *Ex vivo* activation of these cells currently provides the gold standard for the characterization of CU sera as being functional (i.e. having an effect on a target cell) or nonfunctional.

After pooling available data from published studies (5, 7, 12, 13, 25–56), among patients with CU, 32.5% (median, 95% CI: 12–39.1%) have a positive whole serum–induced basophil histamine release assay (BHRA). Approximately 25% of CU patients [median: 26.5% (95% CI: 10.3–32.8%)] have a positive ASST and a positive BHRA, 49.5% (median, 95% CI: 23.9–66.2%) have a negative ASST and a negative BHRA, 15.8% (median, 95% CI: 1.6–64.1%) have a positive ASST and a negative BHRA, and 4.1% (median, 95% CI: 0–11.5%) have a negative ASST and a positive BHRA.

Only a few studies relate anti-FcεRIα or anti-IgE autoantibodies in CU patients' sera detected by immunoassay to functional bioassay results (8, 9, 12–14). After pooling the available data from these studies, anti-FcεRIα autoantibodies are found in 49.2% (range, 41.6–72%) of CU patients; 26.4% (range, 20–52%) have both autoantibodies and a

positive BHRA, while 16.5% (range, 0–22.7%) have a positive BHRA with undetectable levels of autoantibodies. The study by Sabroe et al. (12) concluded that CU patients can be classified into five subsets: 26% with histamine-releasing anti-FcεRIα antibodies (group 1), 15% with immunoreactive nonfunctional anti-FcεRIα antibodies (group 2), 9% with MC-specific histamine-releasing factors, 9% with functional anti-IgE antibodies and 41% with no identifiable factors (group 5). Using a panel of basophils and MCs from healthy donors increased the number of strongly positive ASST CU sera evoking significant histamine release to 95% (43) from the expected level of around 50% for all positive ASST CU sera tested on single or paired basophil donors. Isolation, culture and use of MCs is much more laborious, difficult to perform and hard to standardize than the BHRA. On the other hand, the human basophil secretory response to FcεRI-dependent stimuli varies widely. Basophils from 10% to 20% of unselected healthy individuals are nonreleasers to FcεRI-dependent stimuli (57, 58). Consequently, the BHRA is dependent on the individual characteristics of the healthy donors from whom they originate. Unfortunately, there has been no international standardization of this test to date including a lack of agreement on the positive threshold for the BHRA. Further research including the characterization of more suitable assay populations such as human MC lines (16, 59) is needed to standardize and improve this method.

There seems to be a reasonably good concordance between basophil and MC histamine release in response to CU sera, suggesting a common pathway of cell activation (7, 9).

Basophil activation marker expression

Basophil activation tests have also been used to identify functional serum factors in CU patients. The induced basophil activation markers found to be most reliable for assessing functional activity in CU sera are CD63 (34, 35, 46, 60–63) and especially (64) CD203c (36, 62). Expression of CD63 has been closely related to basophil degranulation (65), while CD203c is upregulated after basophil activation independent of degranulation (66).

CD203c expression on allogeneic basophils from a healthy donor was found to correlate significantly with histamine release from the same donor after stimulation with CU sera (36). In the same study, IgG depletion of three CU sera significantly reduced CD203c expression, suggesting an IgG-mediated basophil activation mechanism.

Similarly, a CU serum positive for anti-FcεRIα autoantibodies by WB induced significantly higher CD63 expression on basophils from highly IgE-sensitized donors as opposed to basophils from nonatopic donors, while this was not observed in a CU patient without these autoantibodies (46). Neither the presence of anti-FcεRIα antibodies nor histamine-releasing activity of sera from CU patients was associated with consistent CD63 or CD203c expression on basophils from a healthy donor, although these data were based on a small sample size (62).

CD63 expression correlated linearly with healthy donor basophil histamine release after activation with CU sera in two studies (35, 67), whereas in another study, it was found that only 14 of 20 CU patients with positive ASST showed activity in CD63 expression of a healthy donor (60). CD63 increase and degranulation are correlated after anti-IgE or formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation (62). Both Frezzolini et al. (34) and Gyimesi et al. (46) concluded that sera from ASST-positive CU patients are able to significantly induce CD63 expression on basophils, providing a sensitivity up to 95.5%.

Use of different methodologies appears to influence the outcome. A tricolour flow cytometry method (based on identification of CD63, CD123 (interleukin-3 receptor) and absence of HLA-DR-expression) may improve diagnostic performance by avoiding IgE labelling as it is known that FcεRI expression may vary considerably between basophil donors (34). Elimination of *in vitro* activation (e.g. with IL-3) and use of washed leucocytes instead of whole blood may also explain sensitivity and specificity discrepancies (46).

Influence of IgG subclass on histamine-releasing activity of FcεRI autoantibodies

The role of autoantibodies as a stimulus for MC degranulation is not straightforward as they may be nonfunctional as well as functional (10, 14, 68). This functionality may be attributable to the complement-fixing IgG_1 and IgG_3 antibody subclasses as there is evidence suggesting that IgG_2 and IgG_4 (most commonly found in patients with other autoimmune cutaneous diseases) are less specific and more likely to be nonfunctional than IgG_1 and IgG_3, which are found predominantly in CU (10). Anti-FcεRIα autoantibodies have been detected by immunoassay in other autoimmune diseases (pemphigus vulgaris, dermatomyositis, systemic lupus erythematosus and bullous pemphigoid) (10), atopics (17) and even healthy individuals (17, 49). This may explain the lack of a significant correlation between the results of immunoassays and those of histamine release studies in CU. It may also account for the lack of correlation between the desensitization to anti-IgE-induced basophil histamine release observed in CU basophils and the detection of FcεRI-specific autoantibodies in CU (69).

The role of complement in mast cell and basophil degranulation

Complement dependence has been shown for histamine release from healthy donor cutaneous MCs in response to IgG from CU patients (11). However, complement does not appear to be obligatory for CU serum–induced basophil release (4, 10, 68). The observation that CU serum histamine-releasing activity of basophils is thermostable at 56 °C argues against complement involvement (5, 7, 38, 70, 71), although reduction in heating-dependent histamine release (11, 30) and complement-augmented histamine-releasing activity of IgG from CU sera (72) have also been reported. Under certain circumstances, monovalent rather than bivalent cross-linking of these receptors by anti-FcεRIα autoantibodies may occur (11), resulting in immune complex

formation and complement activation via the classical pathway (14, 72) with generation of C5a (11), followed by MC activation and histamine release (11, 14, 71) through binding of the C5a receptor (72). It has been proposed that histamine release due to C5a generation is primarily cutaneous rather than systemic because C5a receptors are expressed only by skin MCs (73), a hypothesis that may explain why CU patients do not commonly experience respiratory symptoms.

Evidence for intrinsic functional abnormalities of basophils and mast cells in CU

Basophils from CU patients have been shown to exhibit increased spontaneous basal histamine release *ex vivo* compared with basophils of normal individuals (4, 74). Incubation of basophils of CU patients with heterologous sera from either healthy or CU patients resulted in enhanced histamine release (4, 74). Analogous to basophils, cultured peripheral blood MCs from CU patients have also been shown to exhibit increased basal histamine release compared with MCs from normal individuals (75). In another study, histamine release from skin MCs of CU patients stimulated by compound 48/80 *in vivo* using a skin chamber technique was higher than in normal control subjects (76). Incubation of basophils of patients with CU with heterologous sera from CU patients resulted in increased histamine release (74). Stored autologous sera taken during CU activity resulted in positive ASST during CU remission, while the ASST was negative if autologous serum was taken and tested during remission (77).

However, in CU remission, direct (17, 78) or indirect (17) FcεRI-mediated histamine release with anti-IgE reportedly increases in basophils, while cutaneous skin MC releasability appears to decrease (76, 79), at least to a nonimmunological stimulus like compound 48/80.

Evidence for basophils and mast cells as targets of functional autoantibodies in lesional and uninvolved skin of CU

Basophils

Several studies have shown peripheral blood basopenia in patients with active CU (27, 80–82). Basophils are found in biopsies from CU lesions, ASST-induced weals and unaffected skin from CU patients (83, 84). In addition, blood basopenia is inversely related to the severity of urticaria (82). The last two observations suggest that basopenia may be attributed to basophil recruitment from the circulation into weals or to the destruction of basophils by circulating autoantibodies (27). CU patients with severe basopenia also have the highest level of autoantibodies by immunoenzymetric assay (17).

Basophils from patients with active CU showed reduced histamine release when stimulated with anti-IgE or anti-FcεRI autoantibodies (81). This suppression of histamine release is not evident with FcεRI-independent stimuli, like compound 48/80, fMLP, monocyte chemoattractant protein-1, bradykinin or calcium ionophore, pointing to an FcεRI pathway–specific defect (15, 74, 78, 81). A desensitization process of the FcεRI pathway via anti-FcεRIα autoantibody activity has been proposed as a potential explanation of this observation (81). However, there was no clear relationship between histamine releasability of basophils from CU patients in response to anti-IgE stimulation and the presence of autoantibodies in their sera, as detected by immunoassay, suggesting that mechanisms other than FcεRI-dependent immunological desensitization may also be important (15, 17).

Mast cells

There is direct evidence from electron microscopy that MC degranulation occurs with an overall reduction in stainable MCs in positive ASST responses of CU patients (85). Similar changes have been shown after skin tests with the experimental MC degranulating agent, compound 48/80 (86). In addition, local tachyphylaxis has been demonstrated by reinjection of autologous serum at the same site (70). All these observations suggest that MC mediators are likely to be involved in the immediate weal and flare response to autologous serum. The cutaneous response provides an *in vivo* marker of serum-induced MC degranulation, which is supported by *in vitro* functional studies (4), although other influences, including hyper-responsiveness of MCs and the microvasculature, are likely. In fact, this hyper-responsiveness has been demonstrated with compound 48/80 and codeine sulphate in active CU, while the absolute number of MCs does not appear to increase (87). In CU remission, this hyper-releasability resolves for compound 48/80, but not for codeine (76, 79).

Association with other autoimmune diseases

Although there has been no systematic study of an association between CU and autoimmune diseases, there have been reports of CU patients with vitiligo, pernicious anaemia (88), rheumatoid arthritis (89), juvenile rheumatoid arthritis (90), type 1 diabetes mellitus (91, 92), Grave's disease (91, 93), coeliac disease (31, 94–96) and Raynaud's phenomenon with anticentromere autoantibodies (97). There have also been many studies describing a high prevalence of IgG antithyroid antibodies ranging from 15% to 30%, and in particular Hashimoto's thyroiditis, among patients with CU (50, 93, 98–104). Positive thyroid antibodies are more frequent in CU patients with a positive BHRA than in those without (67, 105). Positive thyroid antibodies are also found more often in ASST-positive CU patients than in ASST-negative CU patients (42, 67, 101, 106, 107), although this was not the case in all studies (31, 50, 53, 93). This increased incidence appears to reflect the involvement of common genetic factors in pathogenesis rather than shared epitope cross-specificity between IgG antithyroid and anti-FcεRIα antibodies (16).

Molecular mimicry of host structures by the saccharide portion of lipopolysaccharides (LPS) of *Helicobacter pylori* (HP) with the FcεRI has been proposed (108). An

association has been described in ASST-positive CU patients infected with HP and autoimmune thyroiditis (42). This might explain anti-FcεRIα autoantibody formation in genetically predisposed individuals and provide an indirect aetiological explanation of autoimmunity initiation in CU.

In addition, many CU patients reportedly have a non-organ-specific increase in autoimmune markers such as rheumatoid factor and antinuclear antibodies (32, 109).

The autoimmune basis of CU is further supported by the observation that p21Ras expression is increased, whereas the overall expression of the p21Ras stimulatory element hSOS1 (son of sevenless homolog 1) is downregulated in peripheral blood mononuclear cells of patients with CU. This aberrant signalling through the p21Ras pathway has been also described in other autoimmune diseases, such as systemic lupus erythematosus and type I diabetes mellitus. The decreased hSOS1 expression was present only in ASST-positive patients (41).

CD40L expression was higher on *in vitro* activated CD3 T lymphocytes from CU patients and the antiapoptosis marker Bcl-2 was expressed higher in severe than in moderate CU. This overexpression may be responsible for the polyclonal hyper-reactivity of B cells to produce anti-FcεRI antibodies, in keeping with the concept of an autoimmune disorder. Evidence of T- and B-cell activation in CU emphasizes that CU should be regarded as an immune disorder (110). Total IgE was shown to be elevated in nonatopic CU patients as a possible marker of polyclonal B-cell activation (111).

Genetic predisposition

Immunogenetic studies have found a highly significant association between certain HLA class II molecules that are associated with autoimmune illnesses in general and CU in particular (29, 112, 113). The prevalence of DRB1*04 (DR4) and its genetically associated allele DQB1*0302 (DQ8) is markedly increased in CU patients as a whole and ASST-positive CU patients alone compared with the healthy population. In particular, HLA-DR4 is strongly associated with increased BHRA activity and positive ASST responses (29, 112), while HLA-DR4 and DQB1*0301/4 frequencies were significantly higher in ASST-positive CU patients than in ASST-negative CU patients. CU patients are more commonly HLA-Bw4 and HLA-DQ1 positive (113).

There was a higher frequency of CU in first-degree relatives of patients with CU than expected, and most of them had a positive ASST (114).

Response of CU to immunotherapies

Plasmapheresis (115) and intravenous immunoglobulin (IVIG) infusions (116) appeared to benefit severe CU patients with positive ASST and serum histamine-releasing activity in uncontrolled studies. In one patient who responded to plasmapheresis, the urticarial activity score mirrored the level of functional autoantibodies, whereas the peripheral blood basophil numbers showed an inverse relationship, thereby providing good anecdotal evidence for a pathogenetic relationship between these clinical and laboratory parameters. Six weeks later, reaccumulation of the autoantibodies and recurrence of basopenia coincided with clinical relapse (115). However, the most studied immunosuppressive agent for CU to date is ciclosporin A (CsA) (117–121) giving an average dose- and duration-dependent response rate of 83.2% (range, 51.7–100%). It is the only drug with a strong evidence for efficacy in CU. Patients with a positive serum BHRA responded better to CsA than those with a negative BHRA (117), while functional activity in serum was inhibited by treatment (117). This action can be explained by inhibition of either T-cell activation or T-cell-dependent antibody formation by B lymphocytes (122), while direct inhibition of basophil and MC histamine release has been also suggested as an explanation for the partial and temporary clinical improvement seen in some patients (123). Other immunosuppressive drugs used successfully in severe CU include mycophenolate mofetil (124), methotrexate (125) and cyclophosphamide (126, 127). It should not be forgotten that oral corticosteroids are the most widely used immunosuppressive and anti-inflammatory drug for CU worldwide (128), although an evidence base for their undoubted effectiveness is lacking.

Reports of patients with spontaneous and inducible CU responding to treatment with anti-IgE (omalizumab) raise the question as to how it might work in CU. Following an initial rapid reduction in free IgE levels after treatment, there appears to be a decrease in FcεRI function (129) and probably FcεRI numbers on basophils (130) and MCs (131). In most cases, this results in a rapid clinical improvement accompanied by reversal of laboratory abnormalities (i.e. increased blood basophils and enhanced FcεRI-dependent basophil histamine release) (132) similar to that observed in naturally recovered CU patients (17).

Autoimmune urticaria: is it a real entity?

According to the revisited Witebsky's postulates (2) evidence in support of an autoimmune origin of a human disease is grouped in three categories: level 1: direct evidence from transfer of pathogenic antibodies or T cells, level 2: indirect evidence based on reproduction of the disease under question in experimental animal models and level 3: circumstantial evidence from clinical practice.

Direct: The only direct *in vivo* proof of pathogenicity of humoral factors in CU comes from a report of intradermal injection of plasma from a CU patient with functional autoantibodies into a healthy volunteer producing a weal and flare reaction similar to the patient's ASST response, while an ASST in the same healthy volunteer was negative (133). However, cross-species passive transfer of human CU patients with positive ASST responses to macaque monkeys (134) and guinea pigs (135) was not demonstrated.

Another way to demonstrate the pathogenicity of an autoantibody is to demonstrate its action on the target organ or cell *ex vivo* or *in vitro*. This is the case with the release of histamine from healthy donor basophils (4) and MC (7) by IgG purified from CU sera and inhibition assays using the soluble FcεRIα subunit (5). Positive BHRA activity data

provide direct evidence of a stimulatory effect of anti-FcεRIα antibodies on the FcεRI mimicking a receptor agonist.

Indirect: Unfortunately, there are currently no experimental animal models of chronic urticaria. However, a mouse model expressing human FcεRI (136) may be suitable for study. In another model, rats immunized with rat myeloma IgE, produced anti-IgE, which induced immediate skin test responses with the evidence of MC degranulation but only on intradermal injection (137).

Circumstantial: There is a growing body of circumstantial evidence for CU being an autoimmune disease, as detailed in the previous sections.

- Association with other autoimmune diseases.
- *Lymphocytic infiltration of target organ*: T lymphocytes are found in the upper and mid-dermis with a perivascular distribution in spontaneous CU weals (84) and the ASST response (85).
- Statistical association with a particular MHC haplotype.
- Favourable response to immunosuppression.

Consensus from the existing evidence

The panel agreed that the evidence for autoimmune chronic spontaneous urticaria (ACU) being a distinct disease entity was persuasive although more evidence is required to meet the full Witebsky's criteria, particularly the need for an animal model. It should be noted that a number of human diseases are frequently designated autoimmune even though they do not meet the previously described criteria (e.g. vitiligo, primary biliary cirrhosis, primary adrenal insufficiency, Addison's disease).

The main difficulties with identifying patients with ACU in clinical practice are as follows:

- The lack of a characteristic clinical phenotype which would allow patients with ACU to be distinguished confidently from those with other aetiological patterns of CU (infection-related, pseudoallergic or idiopathic) on clinical grounds for clinicians without access to functional bioassays.
- No single test is available that unequivocally demonstrates specific functional autoantibodies against FcεRIα or receptor-bound IgE on skin MCs or basophils. Functionality itself is considered to be the key criterion for ACU definition. The panel considered whether the BHRA or the ASST could be adopted as the 'gold-standard' tests for functional autoantibodies to define ACU. Although clinical and laboratory characteristics of patients with positive BHRA and ASST have been reported, nearly all the publications relate to the use of whole sera rather than purified IgG. The panel recognized that neither test alone could be regarded as sufficiently robust to offer a secure platform for the validation of diagnostic criteria for ACU. The limitations of the individual tests used in the assessment of ACU are summarized in Box E1 (see online repository).

Recommendations from the panel

To overcome these limitations, the panel proposed that the 'gold standard' for ACU diagnosis should be a combination of a positive bioassay, positive autoreactivity and a positive immunoassay. The details and referenced methodologies are summarized in Box 1.

Protocols and methodologies currently recommended as best practice are presented in the online repository.

In addition, it was agreed that the taskforce should prospectively validate potentially relevant clinical and laboratory criteria (from the history, examination and routinely available clinical investigations) to explore and identify potential surrogate parameters that can substitute for the combined major criteria of the newly adopted 'gold standard' (Box E2 in online repository) before reconvening to define preliminary criteria sets for a diagnosis of ACU based on individual criteria with highest individual sensitivity and specificity.

Summary Box 1 proposed gold standard for diagnosis of ACU

A A positive bioassay (BHRA or basophil activation marker expression) to demonstrate functionality *in vitro* AND.
B Positive autoreactivity (by means of a positive ASST) to demonstrate relevance *in vivo* to MC degranulation and vasopermeability AND.
C A positive immunoassay for specific IgG autoantibodies against FcεRIα and/or anti-IgE (WB or ELISA) to demonstrate antibody specificity.

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Conflict of interest

PSS is Consultant for RefLab, Denmark, that provides a commercial BHRA (HR-Urtikaria® test) but has no commercial interest in it. All the other authors do not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article found at: www.wileyonlinelibrary.com:

Data S1. Online repository.

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