

Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria (aiCSU): Results of the PURIST Study

Short Title: Autoimmune Chronic Spontaneous Urticaria

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53 47 **Acknowledgements:** We gratefully acknowledge the support of the urtikaria network e.V.
54
55 48 (UNEV, www.urtikaria.net) and of the GA²LEN UCARE network (www.ga2len-ucare.com).
56
57 49 We also thank Dr. Sabine Altrichter for helpful discussions.
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For Peer Review

Conflict of Interest: All authors declare that they have no conflict of interest that would have affected the design, presentation or conclusions of this study.

Abstract word count: 245 words

Text word count: 3601 words

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58 **Abstract**

59 **Background:** Autoimmune chronic spontaneous urticaria (aiCSU) is an important subtype
60 of chronic spontaneous urticaria (CSU) in which functional IgG autoantibodies to IgE or its
61 high affinity receptor (FcεRI) induce mast cell degranulation and subsequent symptom
62 development. However, it has not been tightly characterized. This study aimed to better
63 define the clinical and immunological features and to explore potential biomarkers of
64 aiCSU.

65 **Methods:** This was a multinational, multicenter study of 182 CSU patients. The clinical
66 features studied included: urticaria activity and impact (UAS7 and quality of life);
67 autologous serum skin test (ASST); IgG-anti-FcεRI and IgG-anti-IgE; IgG-anti-
68 thyroperoxidase (IgG-anti-TPO); total serum IgE; and basophil reactivity (BASO) using the
69 basophil activation test (BAT) and basophil histamine release assay (BHRA).

70 **Results:** Of the 182 patients, 107 (59%) were ASST+, 46 (25%) were BASO+ and 105
71 (58%) were IgG-anti-FcεRI+/IgE+. Fifteen patients (8%) fulfilled all three criteria of aiCSU.
72 aiCSU patients appeared more severe (UAS7 21 vs 9 P < 0.016) but showed no other
73 clinical or demographic differences from non-aiCSU patients. aiCSU patients also had
74 markedly lower total IgE levels (P<0.0001) and higher IgG-anti-TPO levels (P<0.001). Of
75 biomarkers, positive BAT and BHRA tests were 69% and 88% predictive of aiCSU,
76 respectively.

77 **Conclusions:** aiCSU is a relatively small but immunologically distinct subtype of CSU that
78 cannot be identified by routine clinical parameters. Inclusion of BHRA or BAT in the
79 diagnostic work up of CSU patients may aid identification of aiCSU patients, who may
80 have a different prognosis and benefit from specific management.

Keywords: Chronic spontaneous urticaria (CSU); autoimmune CSU (aiCSU); autologous serum skin test (ASST); IgG autoantibodies; basophil activation assays.

Abbreviations

aaCSU Autoallergic CSU

aiCSU Autoimmune CSU

ASST Autologous Serum Skin Test

BASO+ positive result in either the basophil activation test or basophil histamine release assay

BAT Basophil Activation Test

BHRA Basophil Histamine Release Assay

CSU Chronic Spontaneous Urticaria

FACS Fluorescence-activated cell sorting

FITC Fluorescein isothiocyanate

FSC Forward Scatter

FcεRI the high affinity receptor for IgE

IgE Immunoglobulin E

IgG Immunoglobulin G

PE Phycoerythrin

PBS Phosphate buffered saline

PURIST Profiling Urticaria for the Identification of Subtypes

RPMI Rosswell Park Memorial Institute (Buffer)

SSC Side Scatter

TPO Thyroperoxidase

UCARE Urticaria Center of Reference and Excellence

Introduction

Chronic spontaneous urticaria (CSU) is defined by the EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria (1) as the spontaneous appearance of wheals, angioedema, or both for > 6 weeks. However, the underlying basic pathomechanisms of CSU are still largely unclear.

The first suggestion that CSU may have an autoimmune basis came from Rorsman in 1962 (2), who suggested an antigen-antibody reaction as a potential cause of the observed peripheral blood basopenia. It has subsequently been proposed that this autoimmunity may have two possible forms, Type I and Type IIb (3-6).

A possible role of IgE-mediated Type I autoimmunity, also referred to as autoallergy, was postulated following observations of IgE autoantibodies to thyroperoxidase (7). More recently, CSU patients have also been shown to have IgE against double stranded DNA (8), interleukin-24 (9), tissue factor, and thyroglobulin (10). This is supported by the high rate of CSU patients with elevated IgE levels (11) and the fast onset of the beneficial effects of omalizumab (anti-IgE) in many CSU patients (12-16).

The hypothesis for Type IIb autoimmunity in CSU was initiated by reports that autologous sera injected intradermally produced a wheal at the site of injection (17). This is the autologous serum skin test (ASST) (1, 6). Subsequent studies showed that IgG antibodies to the patient's own IgE or its high affinity receptor (FcεRI) were instrumental in causing basophil and mast cell degranulation *in vitro* and are believed to be directly relevant to urticaria pathogenesis (3, 18-21). This response has been reviewed in detail recently (5, 22-24)

Autoimmune chronic spontaneous urticaria (aiCSU) is defined here by the presence of functional and relevant mast cell-degranulating IgG autoantibodies to IgE or its high affinity receptor Fc ϵ RI (6). The diagnostic criteria of aiCSU are 1) positive *in vivo* autoreactivity (a positive ASST) as evidence of serum factors capable of an inflammatory wheal response; 2) positive *in vitro* basophil reactivity (BASO+) (by basophil histamine release assay (BHRA) or basophil activation test (BAT)) as evidence of serum factors causing histamine release, basophil activation or both; and, 3) a positive immunoassay for specific identification of IgG autoantibodies against Fc ϵ RI and/or anti-IgE (western blot or ELISA) (6). As of now, these criteria have not been applied together to define a specific subgroup of autoimmune CSU. Consequently, the clinical features and potential biomarkers of aiCSU remain unknown.

The aim of the international, prospective Profiling Urticaria for the Identification of Subtypes (PURIST) study was to better define and characterize features of “autoimmune” CSU that could be used in routine clinical practice to define the illness. PURIST was designed, funded, implemented and performed by a global consortium of urticaria centers of excellence, most of which are now UCARE centers (25). Here, we present the first results of the PURIST study with a focus on the demographic, clinical, and immunological profile of aiCSU and possible predictors.

Methods

Patients

A total of 182 chronic spontaneous urticaria (CSU) patients (134 female, median age 44 years, range 18 to 76 years) from 12 collaborating centres were recruited into the study. Because we wanted a predominantly ASST+ population, the number of ASST- patients

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3 161 who were recruited was no more than 75 patients, determined by a power calculation of
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5 162 the likely number of CSU patients with evidence of autoimmune urticaria based on
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8 163 literature available at the time. Ethics approval was obtained in each of the cooperating
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10 164 centres and the study followed Good Clinical Practice guidelines and the Declaration of
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12 165 Helsinki. All volunteers gave signed informed consent to be included in the study.
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15 166 Exclusion criteria included individuals less than 18 years of age, pregnant or lactating
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17 167 females or women planning pregnancy during the time of the study. Mentally incapacitated
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19 168 persons or persons patients protected by the law were also excluded from the study. Also
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22 169 excluded were patients with urticarial vasculitis and CSU patients who had undergone or
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24 170 were undergoing treatment with anti-IgE (omalizumab). Further exclusion criteria included
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26 171 the use of immunosuppressive drugs for 3 months or corticosteroids for 1 month before
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28 172 the screening visit or during the course of the study. The use of tricyclic antidepressants,
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31 173 doxepin, leukotriene receptor antagonists (LTRAs), H₂-antihistamines, sulphasalazine,
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33 174 dapsone, tranexamic acid, warfarin or heparin during the 4 weeks before the screening
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35 175 visit and during the course of the study was prohibited. The use of H₁-antihistamines was
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38 176 allowed in case of high urticaria activity (UAS \geq 6) or in case of an emergency during the
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40 177 baseline scoring for UAS7 over 2 weeks (see section on UAS7 and correction or
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42 178 antihistamines). However, no H₁-antihistamines were allowed during the 3 days prior to the
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45 179 diagnostic visit.

50 181 **Autologous Serum Skin Test (ASST)**

52 182 The autologous serum skin test was performed as recommended by the 2009
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55 183 EAACI/GA²LEN task force consensus report on the autologous serum skin test in urticaria
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57 184 (26) and as previously described (27). Wheal responses were measured at 30 min, and
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the ASST response was taken to be positive, when the red serum-induced wheal had a diameter at least 1.5 mm greater than the negative control (28).

Basophil Histamine Release Assay (BHRA)

The serum-induced BHRA was to detect autoantibodies in patients' sera that are directed against IgE or high affinity IgE receptors (FcεRI). The assay was performed by a single centre (RefLab, Copenhagen) as previously reported (14). Briefly, a buffy coat containing 1-2 % basophils was prepared from fresh blood from four individual donors. The buffy coats were mixed an equal volume of RPMI and stored overnight at 2-8 °C. with IL 3 in a final concentration of 1 ng/ml. The following day the buffy coats were centrifuged to remove plasma and RPMI, washed with physiological saline and subsequently exposed to low pH using a stripping buffer, pH 3.6 from RefLab, to partially remove IgE from the basophils. Finally, the IgE-stripped samples were resuspended in Pipes buffer from RefLab before incubation with patient sera. Patient sera, at dilutions of 20%, and stripped buffy coat cells were incubated in a total volume of 100 µl for 60 minutes at 37 °C. After centrifugation 25 µL supernatants were transferred to glass fibre coated microliter plates and histamine was measured according to RefLab instructions. Total histamine content was determined after lysis of cells with 7 % perchloric acid. The assay variation was < 7 % and its sensitivity 5 ng histamine /ml. A positive BHRA result was when histamine release was above 16.5% (29).

Basophil activation test (BAT)

The BAT was performed using sera as previously described (30, 31). Briefly, blood from healthy donors was centrifuged and the buffy coat collected and resuspended in stimulation buffer containing 2 ng/ml IL-3. The cells were then stimulated with 50 µl of

patient sera. After 30 min at 37°C, the stimulation was stopped and the serum was eliminated by washing the samples with cold washing buffer (PBS, 2 mM EDTA). Samples were labeled with anti-IgE FITC and anti-CD63 PE for 30 min at 4°C. The erythrocytes were lysed for 10 min at room temperature, and the samples were washed twice prior to analyzing them in a FACSCanto II flow cytometer (FACScan). Data were analyzed with FlowJo Tree Star software (Ashland, OR, USA). In all cases, dead cells were eliminated based on their forward (FSC) and side scattering (SSC) profiles (30). The test, which was performed in a single center, was considered positive when more than 5% of the total basophils were CD63-positive, the value of the 95th percentile of CD63+ cells induced by control sera as previously described (31).

IgG-anti-FcεRI

IgG antibodies to FcεRIα in CSU and control sera were detected using ELISA. FcεRIα (50 ng/ml, Mybiosource, San Diego, CA) was used to coat ELISA plates (Corning 3690, Kennebunk, ME). Following blocking with 10% FBS-PBST, 1:100 diluted CSU and healthy control sera, pre-incubated for one hour with and without 100 ng/ml soluble FcεRIα, were added to the plate in parallel for a 2-hour incubation, AP-labeled goat-anti-human IgG (KPL, Gaithersburg, MD) was used as the secondary detecting reagent and a reduction of signal by more than 50% was considered to indicate the presence of IgG antibodies to FcεRIα.

IgG-anti-IgE

Anti-IgE antibodies were assessed by ELISA as described (27). Extinctions of >0.2 at 490 nm were considered positive.

IgG-anti-thyroperoxidase (IgG-anti-TPO) and total serum IgE

IgG-anti-TPO was measured by immunoassay (Elecsys, Roche Diagnostics) and total serum IgE by immunoassay (Immuno-CAP-Fluorescence-Assay, Thermo Scientific) or nephelometric analysis in each study centre using their own standard procedures. The cut off value that was used to determine a low IgE level was 40 IU/ml.

UAS7 and correction for intake of H₁-antihistamines

The seven-day once daily urticaria activity score (UAS7) including wheal number and itch severity scores were assessed as described by the 2017 revision and update of the EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis, and management of urticaria (1). The UAS7 was calculated as the sum score of 7 days (minimum: 0, maximum: 42), using the following modification to minimize the influence of on demand antihistamine medication. Antihistamine on demand medication was restricted to days on which patients had high disease activity, as reflected by a maximum UAS value of 6 for that day. The UAS score for the following day was not counted towards the UAS7, i.e. the day was censored, unless high disease activity was documented for this day, i.e. UAS = 6. The UAS7 was calculated by adding the UAS of the 7 non-censored days closest to the time point of evaluation.

Quality of Life assessment

Patients' quality of life (QoL) was assessed using the Chronic Urticaria Quality of Life questionnaire (CU-Q2oL) (32) validated in the local language as recommended by the 2017 EAACI/GA²LEN/EDF/WAO Guideline for the Definition, Classification, Diagnosis and

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3 258 Management of Urticaria (1). CU-Q2oL measures various aspects of QoL on a 0–100
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5 259 scale with low scores indicating better QoL.
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11 261 **Statistical Analyses**
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14 262 Binominal variables were analyzed using Chi-square test. The numbers of patients within
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16 263 groups were compared using Fisher’s Exact test. Continuous variables were compared
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18 264 using Mann-Whitney test. Correlations were calculated using Spearman's rank correlation
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21 265 coefficient and tested using asymptotic t approximation. $P < 0.05$ was considered to
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23 266 indicate statistical significance.
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31 269 **Results**
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35 271 **Many CSU patients exhibit one or two, but not all three diagnostic criteria of aiCSU**
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37 272 Of 182 CSU patients analyzed, 160 had at least one of the three criteria of aiCSU, 107
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40 273 had a positive ASST (ASST+, 59%), 46 had a positive result in either the basophil
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42 274 histamine release assay, the basophil activation test or both) (BASO+, 25%), and 105 had
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44 275 positive serum levels of IgG-anti-Fc γ RI, IgG-anti-IgE or both) (IgG-anti-Fc γ RI+/IgE+,
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47 276 58%). In total, 15 of 182 patients (8%) in the predefined PURIST population fulfilled all
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49 277 three criteria of aiCSU (Figure 1).
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54 279 **The demographic and clinical features of aiCSU are not unique**
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56 280 Patients with aiCSU were demographically and clinically similar to CSU patients who had
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59 281 no criteria of aiCSU (non-aiCSU) and to CSU patients with one or two, but not all three,
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criteria of aiCSU (partial-aiCSU, Table 1). There were large ranges in median patient age and duration of disease in all groups of patients with only small differences between them. The majority of patients in all three groups were female. There was no significant difference between the percentage of patients with angioedema, 56% - 80%, in the different groups. Urticaria activity and impact as assessed by UAS7 and CU-Q2oL, respectively, were compared. Except for higher disease activity (UAS7) in aiCSU patients vs non-aiCSU patients ($P = 0.016$), scoring was similar in all groups (Table 1). Additional demographic and clinical parameters were assessed, but were also not specific for aiCSU (Supplemental Table 1).

Patients with aiCSU exhibit a unique immunological profile

Patients with aiCSU had markedly lower total IgE levels, i.e. 22 vs 102 and 108 IU/ml ($P < 0.001$ and $P < 0.0001$) and higher rates of low total IgE (< 40 IU/ml, $P < 0.01$ and $P < 0.0001$) compared with non-aiCSU and partial-aiCSU patients respectively (Table 1). Also, IgG-anti-TPO levels were markedly higher in aiCSU ($P < 0.01$ and $P < 0.0001$), and the rates of elevated IgG-anti-TPO were greater ($P < 0.05$ and $P < 0.01$) than in either non-aiCSU or partial-aiCSU patients. The proportions of patients, who had low IgE levels and elevated IgG-anti-TPO levels, were higher in aiCSU patients (41%) than in partial-aiCSU patients (9%) and non-aiCSU patients (0%). Furthermore, the mean ratio of IgG-anti-TPO levels to IgE levels in aiCSU patients was some 15 times greater than in partial-aiCSU patients ($P < 0.0001$) and some 35 times greater than in non-aiCSU patients ($P = 0.025$) (Figure 2). Patients with aiCSU also showed higher rates of basopenia in comparison to partial-aiCSU patients ($P = 0.035$). The overall occurrence of autoimmune diseases, including vitiligo were found to be similar between groups (Table 1).

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3 307 **The immunological profile of aiCSU patients is shared with BASO+ CSU patients,**
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5 308 **but not ASST+ or IgG-anti-FcεRI+/IgE+ CSU patients**
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8 309 Patients with aiCSU and BASO+ CSU patients had comparable levels of total IgE, which
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10 310 were markedly lower than those of ASST+ or IgG-anti-FcεRI+/IgE+ patients (Table 2).
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12 311 aiCSU patients and BASO+ CSU patients also had comparable levels of serum IgG-anti-
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14 312 TPO, which were markedly higher than those of ASST+ and IgG anti-FcεRI+/IgE+
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17 313 patients. Similar to aiCSU patients, levels of total IgE and of IgG-anti-TPO were inversely
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19 314 correlated in BASO+ patients ($r = -0.354$, $P = 0.03$), which they were not in ASST+ or IgG-
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21 315 anti-FcεRI+/IgE+ patients. Also, the rates of patients who had both, low IgE and elevated
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24 316 IgG-anti-TPO levels were similar in aiCSU patients (41%) and BASO+ CSU patients (39%)
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26 317 and higher than in ASST+ patients (18 %) or IgG-anti-FcεRI+/IgE+ patients (19 %).
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30 319 **Can a positive basophil reactivity assay predict aiCSU?**

31 320 From the data in Table 2 it was calculated that BASO+ was 100% predictive for aiCSU
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33 321 against non-aiCSU. In contrast, ASST+ and IgG-anti-FcεRI+/IgE+ were only 27% and 28%
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35 322 predictive for aiCSU against non-aiCSU, respectively. To investigate which individual
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37 323 basophil test was more predictive for aiCSU against non-aiCSU, the immunological
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39 324 profiles of the basophil histamine release assay (BHRA) and basophil activation test (BAT)
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41 325 were determined separately (Table 3). Calculations made from these data showed that the
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43 326 predictive value of BHRA of 88% was higher than that of BAT of 69%. No tests were
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45 327 significantly predictive for aiCSU against partial-aiCSU.
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53 329 **Discussion**

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55 331 The results of the PURIST study, the first study to assess CSU patients with three criteria
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57 332 to define a subgroup of patients with aiCSU as tightly as possible, show that aiCSU is
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distinct from non-aiCSU in its immunological profile. Low IgE and elevated IgG-anti-TPO are key features of this immunological profile and inversely correlated in aiCSU patients. Basophil tests for serum autoreactivity are best at predicting aiCSU.

This study, by design, included more ASST+ than ASST- patients, which is why 61% of our patients were ASST+. This is higher than the rate of ASST+ patients in the general population of CSU patients. In CSU patients tested consecutively at specialized urticaria centers, the average rate of ASST+ was 38%.(33) The ASST+ rate is likely to be even lower in patients seen by general practitioners or specialists outside of urticaria centers. Despite the positive selection towards ASST+ in the PURIST patient population, only 8% of patients had true aiCSU, i.e. fulfilled all three criteria of autoimmune CSU, ASST+, BASO+, and IgG-anti-Fc ϵ RI+/IgE+.

aiCSU patients did have higher disease activity as illustrated by a significantly higher UAS7, but did not show differences in disease duration, angioedema frequency, quality of life scores compared to CSU patients who do not have aiCSU. We identified a trend towards later onset of disease, higher female preponderance, shorter duration of disease and higher rates of angioedema, but these were not statistically significant. Previous studies, in which one or two criteria of aiCSU were assessed in CSU patients, demonstrated higher urticaria activity and impact in these patients. For example, Sabroe and coworkers reported that ASST+ patients had more wheals, with a wider distribution, higher itch scores and more systemic symptoms, than ASST- patients.(34) Caproni and coworkers showed that the overall frequency of urticarial episodes during the week before skin testing and number of spontaneous wheals were greater in ASST+ patients.(35) In another study, ASST+ and BAT+ CSU patients also had significantly higher UAS7 values

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358 than ASST- and BAT- CSU patients.(36) At present, we cannot explain why this is.

359 Differences in how disease activity was assessed may be relevant. PURIST, unlike earlier

360 studies, used a unique UAS7 assessment that allowed for measuring disease activity

361 without regular antihistamines. Possibly the numbers of aiCSU patients in PURIST were

362 too low to detect statistically different clinical features. What is clear though is that

363 physicians cannot know from what they learn from their CSU patients' history, whether

364 they have aiCSU or not. This, however, may be important and helpful and could improve

365 management and treatment decisions, as we discuss below.

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367 Importantly, aiCSU patients have low total IgE, (median 30 IU), and 2 of 3 aiCSU patients

368 have total IgE of less than 40 IU/ml. Previous studies have shown that serum

369 autoreactivity assessed by BAT, a feature of aiCSU, is inversely correlated to IgE levels

370 (37) and that total IgE serum levels are significantly lower in BAT+ than BAT- CSU

371 patients as well as in ASST+ vs ASST- CSU patients.(38) IgE levels in partial-aiCSU and

372 non-aiCSU PURIST patients were more than three times higher, on average higher than

373 100 IU/ml (the upper limit of normal). Their IgE levels and rates of elevated IgE were

374 similar to those reported previously for CSU patients.(11, 39)

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376 Our study does not provide an explanation for why patients with aiCSU have low levels of

377 IgE, and this needs to be addressed by future studies. The non- and possibly also the

378 partial-aiCSU PURIST patients may have type I autoallergic CSU (aaCSU). Their total IgE

379 levels may be elevated, because they make IgE to autoallergens. The presence of IgE

380 against autoallergens in CSU patients has recently been shown to be linked to elevated

381 total IgE levels.(40) Is the fact that aiCSU patients have low IgE levels clinically relevant?

382 Low IgE levels have been linked by several studies to lower rates of response to anti-IgE

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3 383 treatment with omalizumab in CSU patients,(15, 41-43) suggesting that aiCSU patients
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5 384 respond differently to omalizumab. In line with this, a positive BAT and/or ASST is linked to
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8 385 slower responses to omalizumab.(14)
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12 387 The other immunological marker that we identified in aiCSU patients, viz their high IgG-
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14 388 anti-TPO levels and >50% rate of IgG-anti-TPO elevation, is just as interesting and
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17 389 important. In a previous study, 31% of BHRA+ CSU patients were positive for thyroid
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19 390 autoantibodies (AABs), compared with only 12% of BHRA- patients.(7) In a recent
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21 391 systematic review, the frequency of elevated antithyroid AABs varied from 4% to 37% in 24
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24 392 studies on CSU patients, and two-thirds of these studies reported rates of increased
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26 393 antithyroid AABs in $\geq 10\%$ of CSU patients.(22) Taken together, these findings suggest that
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28 394 the increased rates of anti-thyroid AAb elevation and comorbid autoimmune thyroid
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31 395 diseases observed in the total CSU population is largely driven by the aiCSU
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33 396 subpopulation of CSU exhibiting these immunological features. The ability to distinguish
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35 397 aiCSU patients from other CSU patients would facilitate targeted screening of this
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38 398 subpopulation for autoimmune comorbidities,(44) which this study found to be more
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40 399 common in aiCSU patients.
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44 401 Interestingly, the immunological profile of aiCSU patients is shared by BASO+ CSU
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46
47 402 patients but not ASST+ or IgG anti-Fc ϵ RI/IgE+ CSU patients, and a positive BAT or BHRA
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49 403 predicts aiCSU. This is helpful information for clinical practice as both, the BAT and the
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51 404 BHRA, are relatively easy to perform and commercially available. Compared with
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54 405 performing the ASST and measuring autoreactivity by basophil testing and assessing IgG-
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56 406 anti-Fc ϵ RI and IgG-anti-IgE to identify aiCSU patients, the BAT or the BHRA alone is
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58 407 almost as sensitive and specific. Although this was not the focus of this study and further
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3 408 analyses are needed, between the BAT and the BHRA, the BHRA showed the higher
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5 409 predictive value for aiCSU, almost 90%.
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10 411 The PURIST study and these first analyses of its results come with several strengths and
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12 412 limitations, and they raise some important questions that need further research. The
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14 413 strengths include the large size of the multicentric and international PURIST CSU patient
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16 414 population studied and that we assessed all of these patients for three diagnostic criteria
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18 415 predefined for the purpose of aiCSU as well as the use of the two different assays
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20 416 available to screen CSU patients for serum autoreactivity, the BAT and the BHRA. Among
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22 417 its limitations, some of the routine assays were not done by a central laboratory, but rather
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24 418 by the centers involved, which may have increased the heterogeneity of data. Another
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26 419 interesting question to answer now is to figure out the underlying cause or causes in the
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28 420 more than 90% of CSU patients who do not meet the combined criteria for aiCSU. Testing
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30 421 non-aiCSU and partial-aiCSU patients for aaCSU, i.e. CSU due to IgE to autoallergens,
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32 422 should be an aim of future studies. Other questions raised by our results include whether
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34 423 the BHRA is truly better than the BAT in identifying aiCSU (and if so why) and why aiCSU
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36 424 patients have lower than normal IgE levels. In terms of the clinical implications of our
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38 425 findings, larger studies are needed to better define the distinct clinical features of aiCSU
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40 426 patients and their responsiveness to anti-IgE treatment and to cyclosporine. Previous
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42 427 studies have shown that partial-aiCSU patients respond less well to omalizumab, slower
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44 428 and less often,(15, 45) and better to cyclosporine.(46)
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54 430 In conclusion, the findings of this study show that aiCSU is a relatively small but
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56 431 immunologically quite distinct subgroup of CSU. The immunological profile of aiCSU
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58 432 patients suggests that they could benefit from specific management and treatment.
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Inclusion of the BHRA or the BAT in the diagnostic work up of CSU patients may allow for the identification of aiCSU patients in clinical practice.

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Table 1. The demographic, clinical and immunological features of autoimmune chronic spontaneous urticaria (aiCSU), non-autoimmune chronic spontaneous urticaria (non-aiCSU) and partial-autoimmune chronic spontaneous urticaria (partial-aiCSU).

	aiCSU n=15	non-aiCSU n=22	partial-aiCSU n=145
Demographic features			
Age in years, median (range)	62 (19 - 74)	47 (27 - 76)	42 (18 - 74) *
Gender = female, % (n)	87% (13)	64% (14)	75% (107)
Clinical features			
CSU duration in months, median (range)	15 (3 - 482)	42 (7 - 360)	18 (1 - 360)
Angioedema, % (n)	80% (12)	76% (16)	56% (79)
UAS7, median (range)	21 (0 - 42)	9 (0 - 31) *	17 (0 - 42)
CU-Q2oL, median (range)	26 (0 - 54)	25 (8 - 60)	28 (0 - 75)
Immunological features			
Total IgE in IU/ml, median (range)	22 (0 - 132)	102 (23 - 1401) ***	108 (2 - 909) ****
Total IgE <40 IU/ml, % (n)	86% (12)	26% (5) **	23% (32) ****
IgG-anti-TPO in kU/l, median (range)	153 (6 - 868)	10 (0 - 211) **	9 (0 - 1121) ***
IgG-anti-TPO positive, % (n)	62% (8)	17% (3) *	23% (27) **
Basopenia, % (n)	30% (3)	7% (1)	6% (5) *
Elevated CRP, % (n)	29% (4)	33% (6)	22% (28)
History of AID, % (n)	14% (2)	15% (3)	14% (18)
Vitiligo, % (n)	7% (1)	0% (0)	3% (4)

Demographic, clinical, and immunological features of patients who fulfill all three criteria of chronic autoimmune urticaria (aiCSU), patients with no criteria of aiCSU (non-aiCSU) and patients with one or two criteria of aiCSU (partial-aiCSU). Statistical significance of differences from aiCSU patients are indicated as **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and no asterisk indicates no statistically significant difference.

Abbreviations: TPO thyroperoxidase; AID autoimmune disease; CRP C-reactive protein; CU-Q2oL chronic urticaria quality of life; UAS7 7 day urticaria activity score.

Table 2. Table showing that CSU patients who are positive in either the basophil histamine release assay or the basophil activation test (BASO+) have a similar immunological profile to those of autoimmune chronic spontaneous urticaria (aiCSU).

	aiCSU n=15	BASO+ n=46	ASST+ n=107	IgG anti- FcεRI+/IgE+ n=105
Total IgE in IU/ml, median (range)	22 (0 - 132)****	35 (0 - 655)****	85 (0 - 643)**	110 (0 - 909)
Total IgE <40 IU/ml, % (n)	86% (12)****	61% (25)****	36% (37)*	22% (21)*
IgG-anti-TPO in kU/l, median (range)	153 (6 - 868)***	70 (0 - 2444)****	12 (0 - 2444)	10 (0 - 868)
IgG-anti-TPO positive, % (n)	62% (8) **	55% (21)****	31% (27) *	25% (21)
Basopenia, % (n)	30% (3)*	21% (6)**	10% (7)	8% (5)
Elevated CRP, % (n)	29% (4)	41% (16)**	23% (21)	19% (17) *
History of AID, % (n)	14% (2)	33% (13)***	12% (11)	11% (10)
Vitiligo, % (n)	7% (1)	13% (5)***	4% (4)	1% (1)

Statistical significance of differences from patients who tested negative in each test used, i.e.

aiCSU vs aiCSU- (partial-aiCSU or non-aiCSU), BASO+ vs BASO-, ASST+ vs ASST- and IgG anti-FcεRI+/IgE+ vs IgG anti-FcεRI-/IgE- are indicated as **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. No asterisk indicates no statistically significant difference. Abbreviations: TPO thyroperoxidase; CRP C-reactive protein; AID autoimmune disease; aiCSU, autoimmune chronic spontaneous urticaria; BASO+ positive result in the basophil histamine release assay (BHRA) and/or the basophil histamine activation test (BAT); ASST+, positive result in the autologous serum skin test); IgG anti-FcεRI+/IgE+, positive for serum levels of IgG anti-FcεRI or IgG anti-IgE.

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Table 3. Comparison of the individual profiles of Serum autoreactivity shown by basophil testing predicts autoimmune chronic spontaneous urticaria (aiCSU).

	BASO+ n=46	BHRA+ n=20	BAT+ n=41
Immunological features			
Total IgE in IU/ml, median (range)	35 (0 – 655)****	22 (3 – 220)****	35 (0 – 655)****
Total IgE <40 IU/ml, % (n)	61% (25)****	84% (16)****	61% (22)****
IgG-anti-TPO in kU/l, median (range)	70 (0 – 2444)****	281 (0 – 2444)****	153 (0 – 2444)****
IgG-anti-TPO positive, % (n)	55% (21)****	65% (13)****	58% (19)****
Basopenia, % (n)	21% (6)**	31% (5)**	22% (5)*
Elevated CRP, % (n)	41% (16)**	28% (5)	34% (14)*
History of AID, % (n)	33% (13)***	35% (7)*	35% (12)***
Vitiligo, % (n)	13% (5)***	20% (4)***	15 % (5)***

Statistical significance of differences from patients who tested negative in each test used, i.e.

BASO+ vs BASO-, BHRA+ vs BHRA-, BAT+ vs BAT-, are indicated as **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, and no asterisk indicates no statistically significant difference.

Abbreviations: TPO thyroperoxidase; AID autoimmune disease; CRP C-reactive protein.

BASO+, positive in either BHRA or BAT; BHRA+, positive in the basophil histamine release assay; BAT+, positive in the basophil activation test.

Figure Legends

Figure 1. Venn diagram of the number of patients with constituent components of autoimmune chronic spontaneous urticaria (aiCSU). The diagram shows the numbers of 182 patients who were ASST positive (ASST+, n=107, 59%), positive in either the basophil histamine release assay or the basophil activation test (BASO+, n=46, 25%), positive for serum levels of IgG anti-FcεRI or IgG anti-IgE (IgG anti-FcεRI+/IgE+, n=105, 58%), positive for all three criteria of aiCSU (n=15, 8%), ASST+ and BASO+ (n=27, 15%), ASST+ and IgG anti-FcεRI+/IgE+ (n=40, 22%) and BASO+ and IgG anti-FcεRI+/IgE+ (n=4, 2%).

Figure 2. Ratios of serum IgG-anti-thyroperoxidase (TPO) to total serum IgE. The groups are autoimmune CSU (aiCSU, n=12), partial-aiCSU (n=12) and non-aiCSU (n=121).

Figure 1

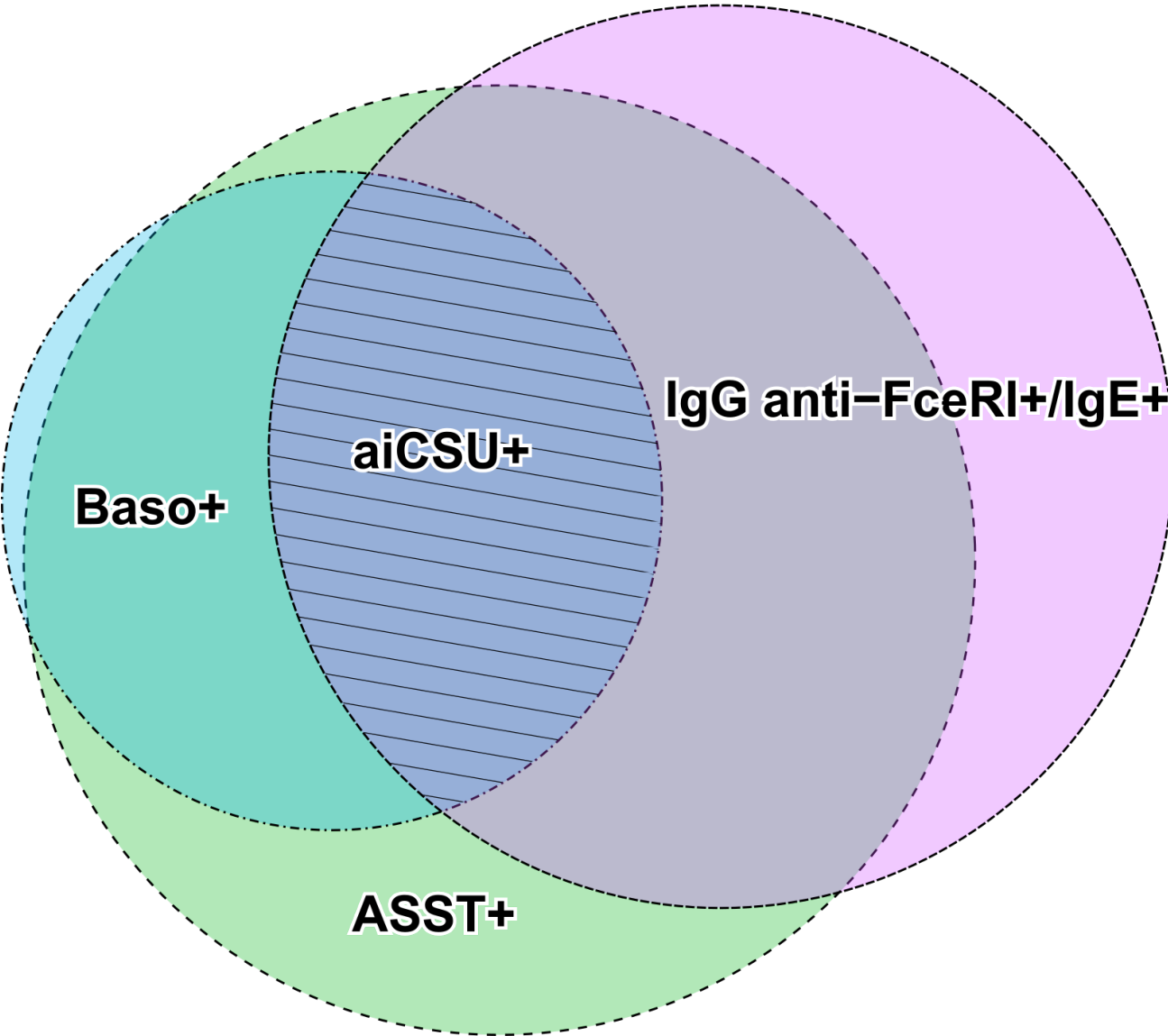
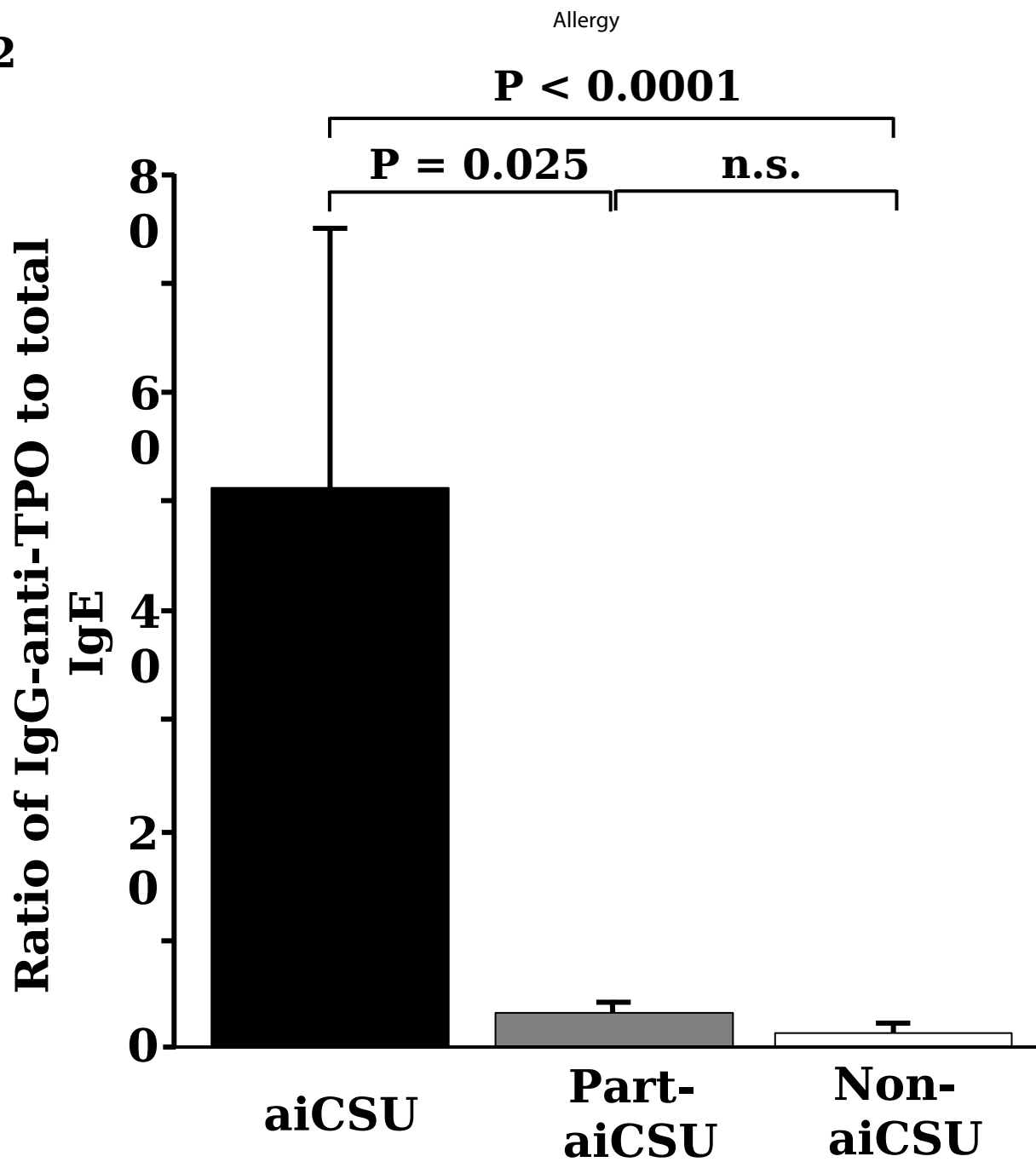
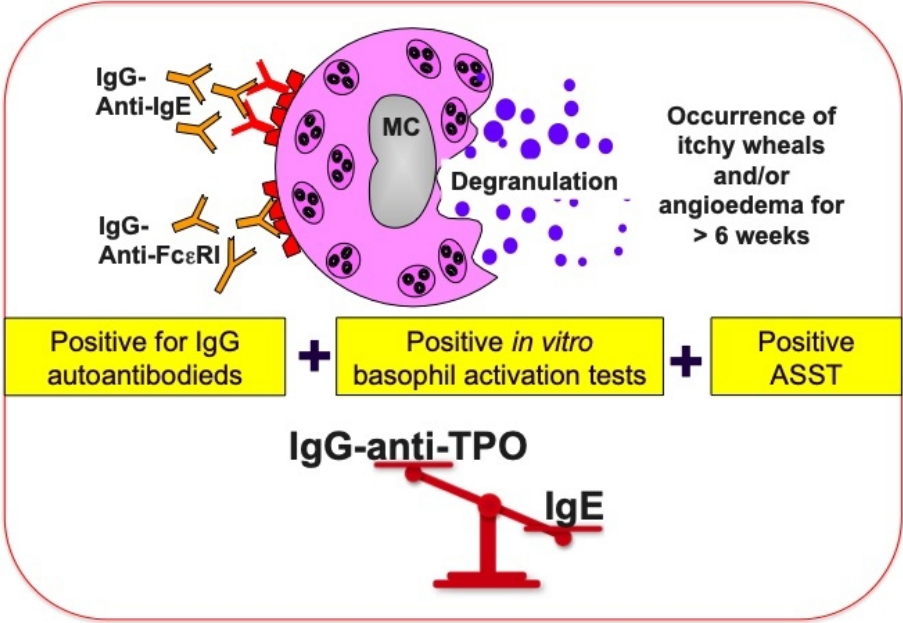


Figure 2

Autoimmune chronic spontaneous urticaria (aiCSU)



254x190mm (72 x 72 DPI)

Autoimmune Chronic Spontaneous Urticaria (aiCSU) is Immunologically Distinct: Results of the PURIST Study

Nicole Schoepke, Riccardo Asero, André Ellrich, Marta Ferrer, Ana Gimenez-Arnau, Clive EH Grattan, Thilo Jakob, George N. Konstantinou, Ulrike Raap, Per Stahl Skov, Petra Staubach, Arno Kromminga, Ke Zhang, Carsten Bindslev-Jensen, Alvaro Daschner, Tamar Kinaciyan, Edward F. Knol, Michael Makris, Nadine Marrouche, Peter Schmid-Grendelmeier, Gordon Sussman, Elias Toubi, Martin K. Church, and Marcus Maurer

Autoimmune chronic spontaneous urticaria (aiCSU) defined by IgG autoantibodies to IgE or FcεRI AND positive *in vitro* basophil activation test AND positive ASST.

14% of ASST+ patients have aiCSU. aiCSU patients have low IgE levels and elevated levels of anti-thyropoxidase (TPO).

Positive basophil activation tests (BAT) and basophil histamine release assays (BHRA) were 69% and 88% predictive of aiCSU.

