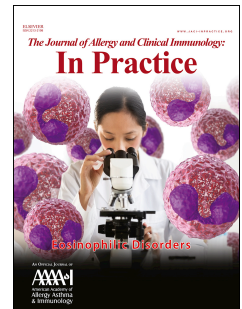


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Autoimmune chronic spontaneous urticaria detection with IgG anti-TPO and total IgE

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82 **Abbreviations.**

- 83 aiCSU: autoimmune chronic spontaneous urticaria
- 84 aTPO: IgG anti-TPO
- 85 aTPO $\uparrow$ IgE $\downarrow$: a group of CSU patients with high IgG anti-TPO and low total IgE levels
- 86 ASST: autologous serum skin test
- 87 BAT: basophil activation test
- 88 BHRA: basophil histamine release assay
- 89 CSU: chronic spontaneous urticaria
- 90 IgE: immunoglobulin E
- 91 sgAH(s): second generation H1-antihistamine(s)
- 92 TPO: thyroid peroxidase
- 93 UAS: urticaria activity score

Background: Autoimmune chronic spontaneous urticaria (aiCSU) comes with high disease activity and poor response to treatment. Recently, elevated levels of IgG anti-thyroid peroxidase (aTPO) and low levels of total IgE were reported to be common in aiCSU.

Objective: Here, we investigated how high aTPO and low IgE individually and combined are linked to features of aiCSU, including treatment responses.

Material and methods: We analyzed records of CSU patients from two independent cohorts (n=1,120) for demographic, clinical, laboratory parameters and treatment responses. Total IgE and aTPO were measured, and four markers of aiCSU were analyzed: autologous serum skin test (ASST), basophil activation test (BAT), eosinophil and basophil numbers. Cut-off values were ≥ 34 kU/L (high aTPO) and < 40 IU/mL (low total IgE).

Results: One of ten CSU patients had both high aTPO and low IgE (aTPO $\uparrow$ IgE $\downarrow$, 11%, n=123). aTPO $\uparrow$ IgE $\downarrow$ was linked to higher age at CSU onset, being female, angioedema and shorter CSU duration. aTPO $\uparrow$ IgE $\downarrow$ was associated with markers of aiCSU, i.e. BAT and ASST positivity, basopenia, and eosinopenia ($p < 0.01$ for all). Almost half of the aTPO $\uparrow$ IgE $\downarrow$ (44%, 19/43) patients had a positive BAT, the best single marker for aiCSU, versus 12% (43/344) of non-aTPO $\uparrow$ IgE $\downarrow$ patients ($p < 0.001$). Relative risk of showing BAT positivity for an aTPO $\uparrow$ IgE $\downarrow$ patient is 3.636 (95% CI, 2.382 to 5.551). aTPO $\uparrow$ IgE $\downarrow$ patients showed low response rates to antihistamine treatment as compared to non-aTPO $\uparrow$ IgE $\downarrow$ (30 vs 47%, $p = 0.01$).

Conclusion: Our findings suggest that aTPO $\uparrow$ IgE $\downarrow$ is a useful diagnostic marker for aiCSU in everyday clinical practice.

1. What is already known about this topic?

Tests for diagnosis of autoimmune chronic spontaneous urticaria (aiCSU) are not widely available and have limitations. Elevated levels of IgG anti-thyroid peroxidase and low levels of total IgE have been reported to be common in aiCSU.

2. What does this article add to our knowledge?

Combination of high IgG anti-thyroid peroxidase and low IgE levels are linked to known clinical and biological features of aiCSU including positive basophil activation test and poor treatment responses to second generation H1-antihistamines.

3. How does this study impact current management guidelines?

we developed a novel approach to diagnose aICSU in everyday clinical practice using a combination of easy-to-perform and cost-effective parameters, IgG anti-thyroid peroxidase and total IgE.

Keywords: autoimmune chronic spontaneous urticaria; IgG anti-TPO; total IgE; marker; double positivity; thyroid peroxidase

Introduction

Chronic spontaneous urticaria (CSU) is a common and disabling mast cell-driven skin disease characterized by the development of wheals, angioedema, or both for more than 6 weeks.^{1, 2} Recent studies revealed major insights on the pathogenesis of CSU indicating the presence of two endotypes, type I (also called “autoallergic”) and type IIb autoimmune CSU.^{1, 3, 4} Type IIb autoimmune CSU (aiCSU) is associated with IgG, IgM and IgA autoantibodies against the high-affinity IgE receptor, FcεRIα, that activate skin mast cells.^{1, 3} aiCSU makes for at least 8% of CSU cases and comes with high disease burden, i.e. high disease activity, high rates of autoimmune comorbidity, and poor response to treatment.^{1, 5, 6} aiCSU can be difficult to diagnose, because the three defining tests, i.e. the autologous serum skin test (ASST), autoantibody immunoassays, and basophil testing, are not widely available and have limitations.

The ASST is used to assess CSU patient sera for skin mast cell-activating effects and is considered a screening test for aiCSU. Although it is a simple *in vivo* test, it is invasive, can be positive in patients with diseases other than CSU and healthy individuals, and only has a moderate sensitivity and a limited positive predictive value for the detection of serum autoreactivity.^{7, 8} Immunoassays for the quantitative measurement of mast cell-activating autoantibodies are of limited use because they lack a clear correlation with functional assays. Moreover, these assays are not readily available, not sufficiently validated and cost intensive.^{9, 10} Basophil tests, i.e. the basophil histamine release assay (BHRA) and basophil activation test (BAT), are the best single tests for identifying patients with aiCSU.^{9, 10} These functional tests evaluate *in vitro* basophil activation, as a surrogate for *in vivo* skin mast cell activation, by serum factors. Basophil testing is widely used in clinical CSU studies but rarely done in routine clinical practice, mostly because of limited availability and associated costs. Although it would be beneficial for all patients to be assessed for aiCSU by the combined use of these three tests, or at least the BAT or the BHRA, this is not possible in many cases and does not happen. The availability of simple, valid and reliable biomarkers for aiCSU would improve routine clinical care by guiding a personalized approach to CSU management including the prediction of its clinical course and response to treatment.

Recently, several markers have been reported to be linked to aiCSU including eosinopenia¹¹ and basopenia,⁵ elevated levels of antinuclear antibodies and IgG anti-TPO (aTPO)^{5, 6}, and low levels of total IgE.^{5, 12} The latter two are especially interesting. The prevalence of elevated aTPO in CSU is high, up to 25%, and linked to comorbid thyroid autoimmunity and non-response to omalizumab treatment, two important clinical features of aiCSU.⁶ Non-response to omalizumab treatment, in CSU patients, is also predicted by low total IgE levels, and two of every three aiCSU patients have low total IgE, i.e. levels of <40 IU/mL.^{5, 12, 13} In the recent PURIST (Profiling Urticaria for the Identification of Subtypes) study, a high ratio of aTPO to total IgE was significantly linked to aiCSU.⁵ However, the use of this ratio is

limited as it has to be calculated and depends on a cut-off value. The combination of elevated aTPO and low IgE, if equally predictive, may be less prone to error and easier to do in clinical practice for diagnosis of aiCSU.

Here, we investigated more than 1,000 patients with well characterized CSU from two independent cohorts. We determined the rates of patients with high aTPO, low IgE, and both. The specific questions we addressed with this information were whether the combination of high aTPO and low IgE, and both of them individually are linked to demographic, clinical or laboratory features of aiCSU, as well as treatment responses. Our overall aim was to determine if IgG anti-TPO and total IgE, two routine clinical markers, can help to identify patients with aiCSU.

Materials and Methods

Patients

We analyzed data from 1,120 patients from two large cohorts of CSU patients (the Russian cohort, n = 355, and the German cohort, n= 765). The Russian cohort included combined data from 3 urticaria centers located in Moscow and the German cohort consisted of data from the Charité Urticaria Center of Reference and Excellence (UCARE)¹⁴ in Berlin. Data were pseudonymized and analyzed retrospectively. The study followed Good Clinical Practice guidelines and the Declaration of Helsinki, ethics approval was obtained by each of the cooperating centers as required, and all patients provided signed informed consent. CSU was diagnosed as wheals and/or angioedema reoccurring for more than 6 weeks without definite triggers. Patients with CSU without wheals, i.e. isolated recurrent angioedema, urticarial vasculitis and isolated chronic inducible urticaria were excluded. The demographic characteristics of patients are presented in Table I. Some data from the German cohort have been previously reported.^{6, 11, 15}

Clinical and laboratory parameters

Blood was drawn and analyzed for routine patient care. The patients' files were screened for information regarding the demographic characteristics of patients and some laboratory parameters where available: age, gender, duration of CSU, disease activity, presence of concomitant angioedema, comorbid inducible urticaria, NSAID hypersensitivity, complete blood cell count including eosinophils and basophils, antinuclear antibodies and C-reactive protein. BAT and detection of antinuclear antibodies were performed only in the German cohort of patients. Disease activity was determined using the urticaria activity score, which was calculated as the sum of the itch and the wheal scores of 7 consecutive days (UAS7) as reported before.²

The measurement methods and reference values of the measured indicators were the same in all Russian centers and, therefore, were pooled for analysis in this cohort of patients. Laboratory data from the German cohort were analyzed separately and also pooled with the data from the Russian cohort where available (Table 1, Table E1).

IgG anti-TPO and total IgE levels

Levels of aTPO and total IgE were measured in the local central laboratories of all centers. Reference values were defined as <34 kU/L for aTPO and <100 IU/mL for total IgE. A reference range for aTPO provided by the laboratories from two Russian centers was 0-60 kU/L and these aTPO values were adjusted to the reference values of other centers (<34 kU/L) using methodology according to the manufacturer's instructions. For the definition of low total IgE, we

used a cut-off of <40 IU/mL as reported previously. We defined elevated aTPO levels as ≥ 34 kU/L as provided by central laboratories.

Based on the cut-off values of aTPO and IgE levels, patients were divided into two main groups: 1) with high aTPO and low IgE (aTPO $\uparrow$ IgE $\downarrow$); and 2) all other patients (non-aTPO $\uparrow$ IgE $\downarrow$). Additionally, we compared aTPO $\uparrow$ IgE $\downarrow$ patients with single positive patients (high aTPO or low total IgE) where relevant (Table E1).

Markers of autoimmune CSU

Four markers of aiCSU were analyzed: ASST, BAT, eosinophil and basophil numbers. The ASST is considered a screening test for aiCSU that, if positive, indicates the presence of histamine-releasing serum factors of any type, including IgG autoantibodies. The positive ASST is regarded as positive when the serum wheal is 1.5 mm or more as compared with that of the negative control (saline).⁸

The BAT was performed using frozen patient serum as previously described.¹⁵ In short, the serum was thawed and incubated with fresh basophils from heparinized whole blood, taken from the same healthy donor for all samples. As a positive control, we used anti-human IgE, and phosphate-buffered saline served as a negative control. Following washing and centrifugation, the supernatant was aspirated and cells were stained with CD3, CD193 and the basophil activation markers CD63 and CD203c for measurement in flow cytometry after red blood cell lysis. The BAT was considered positive, if more than 7.77% of the basophils were both CD63 and CD203c positive, here the 95th percentile of double positive cells induced by control sera (n=51) was used.

Peripheral blood absolute eosinophil and basophil counts were measured by automated hematology analyzers in the local central laboratories. Reference values were the same and cut-off values for eosinopenia and basopenia were $<0.05 \times 10^9/L$ and $<0.01 \times 10^9/L$, respectively.¹¹

Additionally, aTPO $\uparrow$ IgE $\downarrow$ was compared to the ratio of IgG anti-TPO to IgE, another known biomarker of aiCSU, reported by PURIST study (results are in Online Repository Text files and Tables E1-3).⁵

Response to treatment

The response to treatment with a standard dose of second generation antihistamines (sgAHs) was evaluated in 349 patients with CSU in the Russian cohort. In most patients, efficacy was assessed by decrease in disease activity measured by UAS7, ie patients scored 16 or more were defined as non-responders. In 118 patients, the response to treatment with sgAHs was

evaluated as described before by asking the patients how much they suffered from CSU symptoms, how often the treatment for CSU was not enough to control symptoms, and how they considered the overall efficacy of treatment in the last 4 weeks.

Statistical tests

Statistical analyses were done with R (R Foundation for Statistical Computing) and the Statistical Package for the Social Science (IBM SPSS version 25.0; IBM Corp, New York, NY). Demographic characteristics of patients with CSU were reported using descriptive statistics. As data were not normally distributed nonparametric tests were used. Results are expressed as median and interquartile range. Groups were compared using chi-square test of homogeneity, Fisher's exact test (categorical variables) and the Mann-Whitney U test as well as the Kruskal-Wallis test with multiple comparisons with a Bonferroni correction (continuous variables). Post hoc analysis involved pairwise comparisons using the z-test of two proportions or multiple Fisher's exact tests with a Bonferroni correction. Correlations were assessed by Spearman rank test. P values <0.05 were considered significant.

The combination of high IgG anti-TPO and low IgE is common in CSU patients and linked to higher age at disease onset and being female

The rate of CSU patients with high IgG anti-TPO (aTPO $\uparrow$) and low total IgE (IgE $\downarrow$) was 22% (n=245/1,120) and 32% (n=361/1,120), respectively. Of 1,120 patients, 11% (n=123) had both, high aTPO and low IgE (aTPO $\uparrow$ IgE $\downarrow$, Table 1). non-aTPO $\uparrow$ IgE $\downarrow$ patients (89%) included patients with low total IgE only (21%, n=238), patients with high aTPO only (11%, n=122) and patients with normal levels of aTPO and IgE (57%, n=637).

At the onset of their CSU, aTPO $\uparrow$ IgE $\downarrow$ patients were older than non-aTPO $\uparrow$ IgE $\downarrow$ patients (median: 41 vs 36 years, p=0.016, Table E1). Patients who developed CSU after their 50th birthday had higher rates of aTPO $\uparrow$ IgE $\downarrow$ (18%) than those who contracted CSU earlier in life (9%, p<0.05). aTPO $\uparrow$ IgE $\downarrow$ patients were more likely to be female as compared to non-aTPO $\uparrow$ IgE $\downarrow$ patients (93% vs 70%, p<0.001, Table E1). Female patients had higher rates of aTPO $\uparrow$ IgE $\downarrow$ (14%) than male patients (3%, p<0.05).

aTPO $\uparrow$ IgE $\downarrow$ is linked to clinical features of CSU, i.e. angioedema and shorter disease duration

aTPO $\uparrow$ IgE $\downarrow$ patients were more likely to have angioedema (76%, n=92/121) as compared to non-aTPO $\uparrow$ IgE $\downarrow$ patients (53%, n=512/962, p<0.001, Figure 1, Table E1). Patients with angioedema had higher rates of aTPO $\uparrow$ IgE $\downarrow$ (15%, n=92/604) than patients without (6%, n=29/479, p<0.001). CSU duration was shorter in patients who were aTPO $\uparrow$ IgE $\downarrow$ as compared to those who were not, and patients with a short disease duration, i.e. one year or less were more likely to be aTPO $\uparrow$ IgE $\downarrow$ (Table E1).

aTPO $\uparrow$ IgE $\downarrow$ is strongly associated with markers of autoimmune CSU

aTPO $\uparrow$ IgE $\downarrow$ patients had higher rates of BAT and ASST positivity, basopenia, and eosinopenia, all markers of aiCSU, as compared to non-aTPO $\uparrow$ IgE $\downarrow$ patients (Figure 1-3, Table E1). Almost half of the aTPO $\uparrow$ IgE $\downarrow$ patients (44%, 19/43) had a positive BAT, the best single marker for aiCSU, versus 12% (n=43/344), 21% (n=24/113), 17% (n=5/29) and 7% (n=14/202) of non-aTPO $\uparrow$ IgE $\downarrow$, low IgE, high aTPO, and double negative groups of patients, respectively (p<0.001, Figure 2). Relative risk of showing BAT positivity for an aTPO $\uparrow$ IgE $\downarrow$ patient is 3.636 (95% CI, 2.382 to 5.551). aTPO $\uparrow$ IgE $\downarrow$ patients showed higher rates of antinuclear antibodies positivity as compared to non-aTPO $\uparrow$ IgE $\downarrow$ patients (58%, n=22/38, vs 36%, n=163/450, p=0.008, Table E1).

aTPO $\uparrow$ IgE $\downarrow$ patients show low response rates to H1-antihistamine treatment

298 aTPO↑IgE↓ patients had lower rates of response to treatment with a non-sedating second
299 generation H₁-antihistamine (sgAH; 30%, n=20/66), as compared to non-aTPO↑IgE↓ patients
300 (47%, n=133/283, p=0.014, Figure 4, Table E1). Of sgAH non-responders, 24% (n=46/196)
301 were aTPO↑IgE↓ versus 13% of sgAH responders (n=20/153, p=0.014).

Discussion

This is the first study to assess the combined use of IgG anti-TPO and total IgE levels in a large cohort of CSU patients. We found that aTPO↑IgE↓ is strongly linked to known clinical and biological features of autoimmune CSU (aiCSU) including treatment responses to second generation H1-antihistamines (sgAHs), the first line treatment of CSU.

How common are elevated aTPO levels and low levels of IgE in patients with CSU, and how often do patients have both? In our study, 22% and 32% of patients had elevated aTPO and low IgE, respectively. This is similar to previous reports, where the rates of aTPO and IgE were 21-26 and 27-34%, respectively.^{5, 12} Interestingly, 11% of patients had both elevated aTPO and low IgE, i.e. aTPO↑IgE↓. These rates are similar to those of aiCSU defined by a positive ASST, basophil test and autoantibody immunoassay in the recent PURIST study (8%)⁵. More importantly, aTPO↑IgE↓ patients showed many features of aiCSU including higher rates of female patients, higher age at disease onset, presence of angioedema, shorter disease duration, basopenia, eosinopenia, poor response to antihistamine treatment and, most importantly, BAT and ASST positivity, which define aiCSU.^{5, 16}

Female gender is known to be a risk factor for autoimmune diseases. In a large population study, female patients with chronic urticaria had a significantly higher incidence of rheumatoid arthritis, celiac disease, and other autoimmune diseases mainly diagnosed during the 10 years after the diagnosis of CSU.¹⁷ We and others have recently reported an association of comorbid autoimmune diseases, mostly autoimmune thyroiditis and vitiligo with female gender in patients with aiCSU.^{5, 6} Likewise, female gender was also associated with markers of aiCSU. For example, a recent study described a higher rate of females in BAT-positive patients¹⁸, and Asero reported that 76% of females with CSU as compared to 35% of male patients have a positive ASST.¹⁹ In line with this, female CSU patients, in our study, had markedly higher rates of aTPO↑IgE↓.

Autoimmunity, e.g. autoimmune thyroiditis and IgG anti-TPO, commonly develops in the second half of life^{20, 21}, and the average age-of-onset for most autoimmune diseases is 40-50 years.²² CSU patients who are 40 years old or older have autoimmune comorbidities more often than younger patients.⁶ This is in line with our finding that patients who develop CSU in their 50ies or later have higher rates of aTPO↑IgE↓. Thus, our data support that the later in life one develops CSU, male or female, the more likely it is aiCSU. Some studies^{23, 24} but not all²⁵ suggest that IgE levels may decrease with age. For instance, in a cohort of 3,206 adults from 25 European centers, aging was associated with lower levels of sensitization and lower total IgE levels after the age of 20 years, similarly in men and women.²³ Therefore, the hypothesis that the increase of rates of aTPO↑IgE↓ is linked to aging, rather than to aiCSU has to be tested. Importantly, IgG anti-TPO does not activate mast cells and is not held to be a driver of the pathogenesis of CSU.²⁶ However, we believe that elevated IgG anti-TPO and low total IgE are

related and associated with an autoimmune pathogenesis and signature in CSU patients. For example, there is evidence in the literature that patients with autoimmune diseases have lower IgA and IgE levels.^{27, 28} For example, 89% patients with Hashimoto's thyroiditis had mean levels of total IgE of 28 U/mL.²⁹ Recently, we reported an association between low IgA and low IgE in CSU patients.¹⁵ The underlying mechanisms are not clear and might include inherited IgE deficiency, B-cell defect and/or impaired protective function of IgE.

Our findings support the observation that aiCSU patients tend to have higher rates of angioedema.^{5, 30} In a recent meta-analysis, a positive ASST result was associated with a higher risk of angioedema.³⁰ Shorter disease duration may not be an independent feature of aiCSU. Patients with severe disease and shorter disease duration may be more likely to seek treatment at a center of reference, and all of the patients in this study are from such centers.

The key finding of our study is that aTPO↑IgE↓ is strongly linked to true diagnostic markers of aiCSU. In particular, aTPO↑IgE↓ was tied to BAT and ASST positivity as well as eosinopenia and basopenia, more so than elevated aTPO and low IgE alone. In the PURIST study, aiCSU patients had significantly higher rates of low IgE and high IgG anti-TPO levels as compared to partial-aiCSU patients and non-aiCSU patients (41 vs 9 vs 0%). aiCSU patients had a 15 and 35 times greater mean ratio of IgG anti-TPO to IgE as partial-aiCSU patients and non-aiCSU patients, respectively.⁵

Since 2013 the diagnosis of aiCSU is made when three specific tests are positive, namely basophil tests, ASST and IgG anti-FcεRI/IgE detected by immunoassay.¹⁰ Of these three, basophil tests, i.e. BAT and BHRA, are considered to be best suited for diagnosing aiCSU. For example, BAT/BHRA positive (BAT/BHRA+) CSU patients had markedly lower levels of total IgE and higher levels of IgG anti-TPO than ASST positive (ASST+) or IgG anti-FcεRI/IgE positive (IgG anti-FcεRI/IgE+) patients.⁵ Importantly, total IgE and IgG anti-TPO inversely correlated in BAT/BHRA+ patients but not in ASST+ or IgG anti-FcεRI/IgE+ patients.⁵ Furthermore, the rates of aTPO↑IgE↓ patients were similar in "triple positive" aiCSU patients (41%) and BAT/BHRA+ CSU patients (39%) and higher than in ASST+ patients (18%) or IgG anti-FcεRI/IgE+ patients (19%).⁵ Finally, positive basophil tests allow for a 100% prediction of aiCSU against non-aiCSU as compared to 27% and 28% for ASST+ and IgG anti-FcεRI/IgE+, respectively.⁵

In our study, we saw a strong association between BAT+ and aTPO↑IgE↓. The rates of BAT+ were two to three times higher in aTPO↑IgE↓ patients as compared to single positive, double negative and non-aTPO↑IgE↓ patients. The ASST was positive in 24% of patients with normal anti-TPO and total IgE, whereas the BAT was positive in only 7% of double negative patients. In this context, aTPO↑IgE↓ may better predict aiCSU than ASST, low IgE or high IgG anti-TPO alone and should be regarded as a marker of aiCSU, in addition to basophil tests.

aiCSU patients are known to have a poor and slow response to omalizumab and a good response to cyclosporine^{32, 33} and fenebrutinib, a Bruton's tyrosine kinase inhibitor (Metz et al., personal communication). In addition, non-response to omalizumab was also associated with other markers of aiCSU such as low total IgE¹², elevated aTPO⁶ and elevated ratios of aTPO to IgE.³⁴ For example, Ertas and colleagues observed that non-response to omalizumab was predicted by baseline low total IgE levels and ratio of week 4 IgE to baseline IgE.¹² Recently, we described that the presence of antinuclear antibodies and aTPO was linked to non-response to omalizumab treatment.^{6, 35} Furthermore, Chen et al. recently reported higher median ratio of aTPO to IgE in omalizumab non-responders as compared to responders (1.22 vs 0.09, $p < 0.001$).³⁴ Finally, low IgE levels were associated with good response to cyclosporine treatment in CSU patients.^{36, 37} In the present study, we did not assess how aTPO↑IgE↓ is linked to treatment responses to omalizumab. We predict that aTPO↑IgE↓ patients show poor and slow responses to omalizumab and recommend to test this hypothesis prospectively.

Less is known about the response of aiCSU patients to the first line of CSU treatment, sgAHs. Here, we report for the first time that aTPO↑IgE↓ patients show lower response rates to sgAHs than single positive, double negative, and non-aTPO↑IgE↓ patients. This is in line with the notion that aiCSU patients have worse response to sgAHs. For example, antihistamine therapy was less effective in BAT+ patients with CSU as compared to BAT-negative patients³⁸, and basophil activation levels were significantly higher in CSU patients whose symptoms were not completely controlled with antihistamines.³⁹

The strengths of our study include its sizeable cohorts of well characterized CSU patients from two countries showing the same trends. As for its limitations, our study design was retrospective and we did not perform IgG anti-FcεRI/IgE immunoassays, for lack of access to validated tools. Moreover, BAT was performed only in one cohort and only with one donor. In addition, all patients from the Russian cohort and most of patients from the German cohort were of Caucasian/white race/ethnicity. The low rates of non-Caucasian patients is a limitation of our study and further studies should explore more diverse populations of CSU patients for the prevalence and relevance of high levels of IgG anti-TPO and low levels of total IgE. Follow-up studies should also aim to establish the sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of aTPO↑IgE↓ as a biomarker for aiCSU. This can be a challenge, because the cut-off values provided by assay manufacturers differ for both aTPO and total IgE. Further studies should address this issue by analyzing the different cut-off values for their corresponding sensitivity, specificity, NPV and PPV using the different assays. The lack of this information does not decrease the relevance of our findings for helping clinicians with the identification of patients with aiCSU. First, the decision whether a patient has high or normal aTPO can be easily made even if the cut-off set by manufacturers for high aTPO can slightly differ among the assays. Second, the results of total IgE measurements may vary among

laboratories. However, use of the same WHO 11/234 International Reference Preparation (IRP) for human IgE minimizes significant systematic bias and facilitates comparisons of total serum IgE results obtained across the majority of quantitative assays.⁴⁰ A cut-off of 40 IU/mL for defining a low total IgE is in line with previous reports of low IgE as a marker of aiCSU⁵ and is below the cut-off of 100 IU/ml reported as normal for assays calibrated to IRP WHO 11/234.⁴⁰

In conclusion, aTPO↑IgE↓ is strongly linked to aiCSU and associated with resistance to antihistamine treatment. As of yet, the stratification of patients into aiCSU and non-aiCSU is only possible by using a combination of BAT/BHRA, ASST and IgG autoantibodies by immunoassays, which are difficult to determine, only partly commercially available, not sufficiently validated, cost intensive as well as invasive.^{1, 10} Both, aTPO and IgE are readily assessed in routine clinical practice at low cost, and their use for identifying aTPO↑IgE↓ patients is straightforward. As compared to ratio of IgG anti-TPO to total IgE, aTPO↑IgE↓ may be the more useful marker for aiCSU, simply because one does not have to calculate the ratio. We do not suggest that aTPO↑IgE↓ patients, i.e. patients with aTPO levels of ≥34 kU/L and low total IgE levels of <40 IU/mL, should be treated differently. Rather, this information can help treating physicians, especially specialists, to better guide and educate patients on what to expect in terms of the course of their disease and response to treatment and to be sure that attention is paid to the management of these patients. Our results also provide a good marker for identifying patients for participation in clinical trials with aiCSU-targeted treatment. Furthermore, our results and aTPO↑IgE↓ as a marker may be of use for the identification of patients at risk for autoimmune thyroiditis/hypothyroidism and other autoimmune diseases.⁶ Algorithms based on aTPO and IgE and other aiCSU-related parameters could improve decision-making in everyday clinical practice. The development of such algorithms, however, requires more information and data, which need to come from further studies. These studies should further characterize appropriate cut-off values for aTPO/IgE, identify why aiCSU patients have lower than normal IgE levels and higher than normal IgG anti-TPO, and better define the strength of the association between aTPO↑IgE↓ and aiCSU-defining tests, and the predictive values, sensitivity, and specificity of the former to predict the latter.

Table 1. Demographic characteristics of CSU patients

Features	Russian cohort,* n=355	German cohort,* n=765	All, n=1,120
Female patients, % (n)	74.4 (264)	71.1 (544)	72.1 (808)
Age in years, median (interquartile range)	40.0 (30-55)	43.0 (31-53)	42.0 (30-54)
Disease duration in years, median (interquartile range)	1.1 (0.5-4.2)	2.0 (1.0-6.5)	2.0 (0.83-6.0)
Age at disease onset in years, median (interquartile range)	36.2 (26.5-52.4)	36.5 (25-48)	36.4 (25.5-48.7)
Disease activity (UAS7), median (interquartile range)	8.0 (0-16)	16.5 (9-24)	15.0 (7-23)
Presence of angioedema, % (n)	71.8 (255)	47.9 (349 ¹)	55.8 (604 ²)
aTPO↑IgE↓, % (n)	18.9 (67)	7.3 (56)	11.0 (123)
non-aTPO↑IgE↓, % (n)	81.1 (288)	92.7 (709)	89.0 (997)

¹total n=728; ²total n=1,083; aTPO↑IgE↓: IgG anti-TPO high and total IgE low; UAS7: Urticaria Activity Score over 7 days.

*Statistically significant differences between Russian and German cohorts of CSU patients were seen for disease duration, disease activity, presence of angioedema, and rates of aTPO↑IgE↓ (p<0.05).

FIGURE LEGENDS

Figure 1. Associations between aTPO $\uparrow$ IgE $\downarrow$ and CSU patients' demographic, clinical and laboratory parameters. This figure represents the strength of association measured by Cramer's V between each pair of dichotomous variables, i.e. high values of Cramer's V and the big circles with intense color reflect a strong degree of association. The significance level was set to less than 0.05. Nonsignificant correlations were excluded and are not shown. Pooled data were analyzed (Table E1). aTPO $\uparrow$: high levels of IgG anti-TPO; IgE $\downarrow$: low levels of total IgE; BAT+: positive basophil activation test; AE: presence of angioedema; ANA: presence of antinuclear antibodies; ASST+: positive autologous serum skin test.

Figure 2. Box plot of percentage of CD63 and CD203c positive cells in the BAT after stimulation of healthy basophils with patient's serum. Boxes are displayed as median and interquartile range. The whiskers indicate the range. The observations displayed as dots. The data were analyzed using the Kruskal-Wallis test for comparison of four groups (aTPO $\uparrow$ IgE $\downarrow$, n=202, aTPO $\uparrow$ IgE $\downarrow$, n=29, aTPO $\uparrow$ IgE $\downarrow$, n=113, aTPO $\uparrow$ IgE $\downarrow$, n=43), and Mann-Whitney test for comparison of two groups (aTPO $\uparrow$ IgE $\downarrow$, n=43 and non-aTPO $\uparrow$ IgE $\downarrow$, n=344; Table E1). aTPO $\uparrow$ IgE $\downarrow$: CSU patients with low IgE and high IgG anti-TPO; aTPO $\uparrow$ IgE $\downarrow$: patients with low IgE only; aTPO $\uparrow$ IgE $\downarrow$: patients with high anti-TPO only; aTPO $\uparrow$ IgE $\downarrow$: patients with normal levels of both IgE and anti-TPO; non-aTPO $\uparrow$ IgE $\downarrow$: all patients apart from aTPO $\uparrow$ IgE $\downarrow$ patients.

Figure 3. Rates of positive markers for autoimmune urticaria among CSU patients grouped on the basis of anti-TPO and total IgE levels. Data were pooled from both cohorts (See the number of patients in Table E1). aTPO $\uparrow$ IgE $\downarrow$: CSU patients with high IgG anti-TPO and low IgE; aTPO $\uparrow$ IgE $\downarrow$: patients with low IgE only; aTPO $\uparrow$ IgE $\downarrow$: patients with high anti-TPO only; aTPO $\uparrow$ IgE $\downarrow$: patients with normal levels of both anti-TPO and IgE; non-aTPO $\uparrow$ IgE $\downarrow$: all patients apart from aTPO $\uparrow$ IgE $\downarrow$ CSU patients; ASST+: positive autologous serum skin test; BAT+: positive basophil activation test; Basopenia: % of patients with basopenia; Eosinopenia: % of patients with eosinopenia.

Figure 4. Rates of response to standard dose of second generation H1-antihistamines in CSU patients grouped on the basis of IgG anti-TPO and total IgE levels. Data were pooled from both cohorts. aTPO $\uparrow$ IgE $\downarrow$ (n=66): CSU patients with high IgG anti-TPO and low IgE; aTPO $\uparrow$ IgE $\downarrow$ (n=74): patients with low IgE only; aTPO $\uparrow$ IgE $\downarrow$ (n=55): patients with high anti-TPO only; aTPO $\uparrow$ IgE $\downarrow$ (n=154): patients with normal levels of both aTPO and IgE; non-aTPO $\uparrow$ IgE $\downarrow$ (n=283): all patients apart from aTPO $\uparrow$ IgE $\downarrow$ patients (Table E1).

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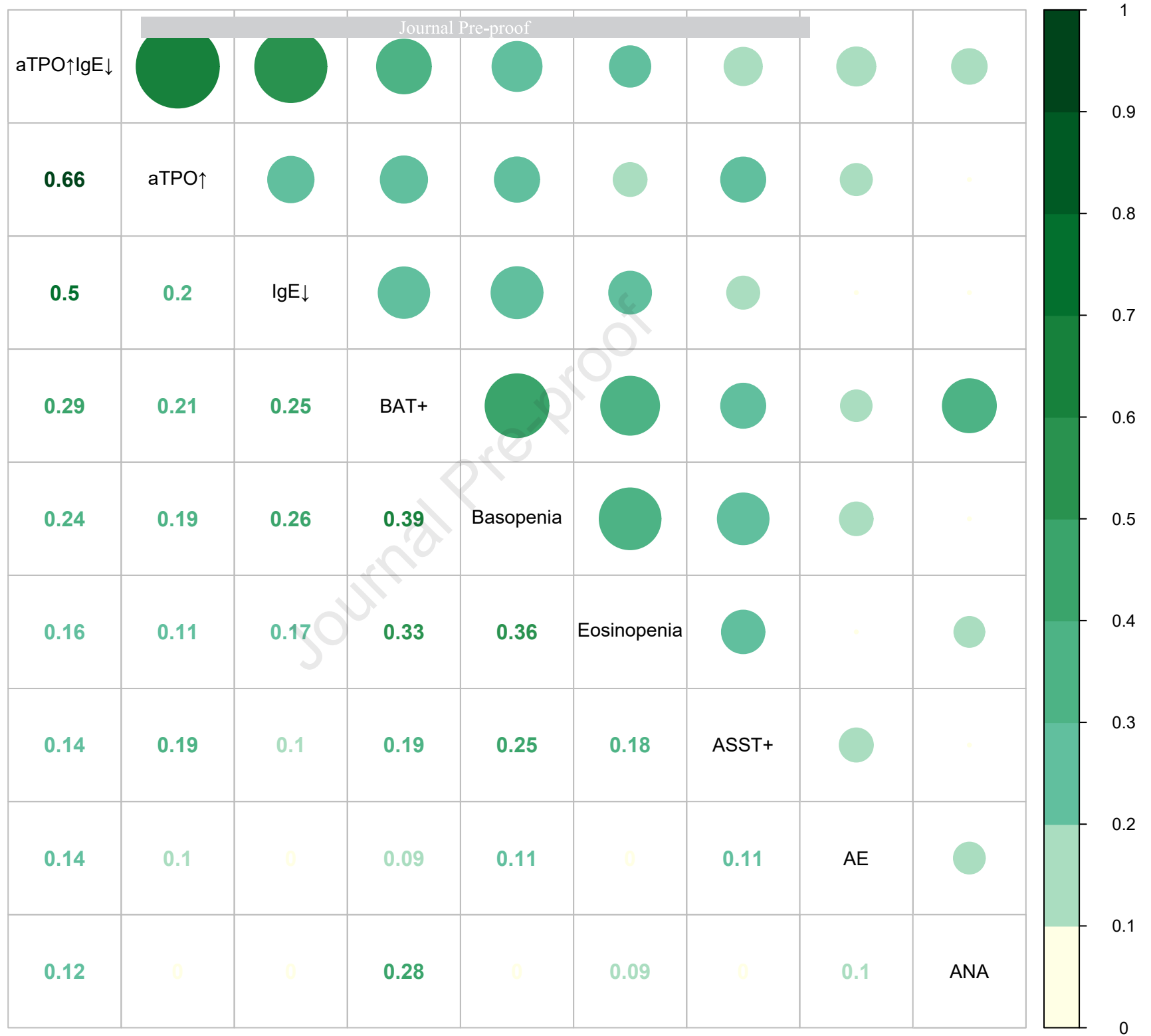
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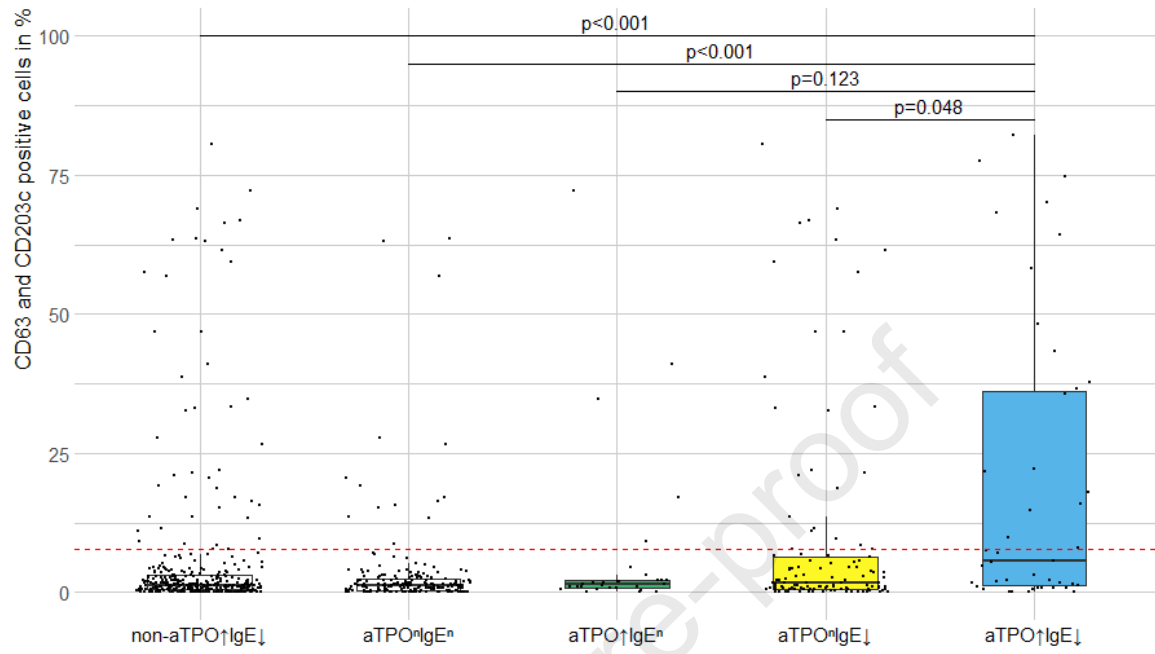
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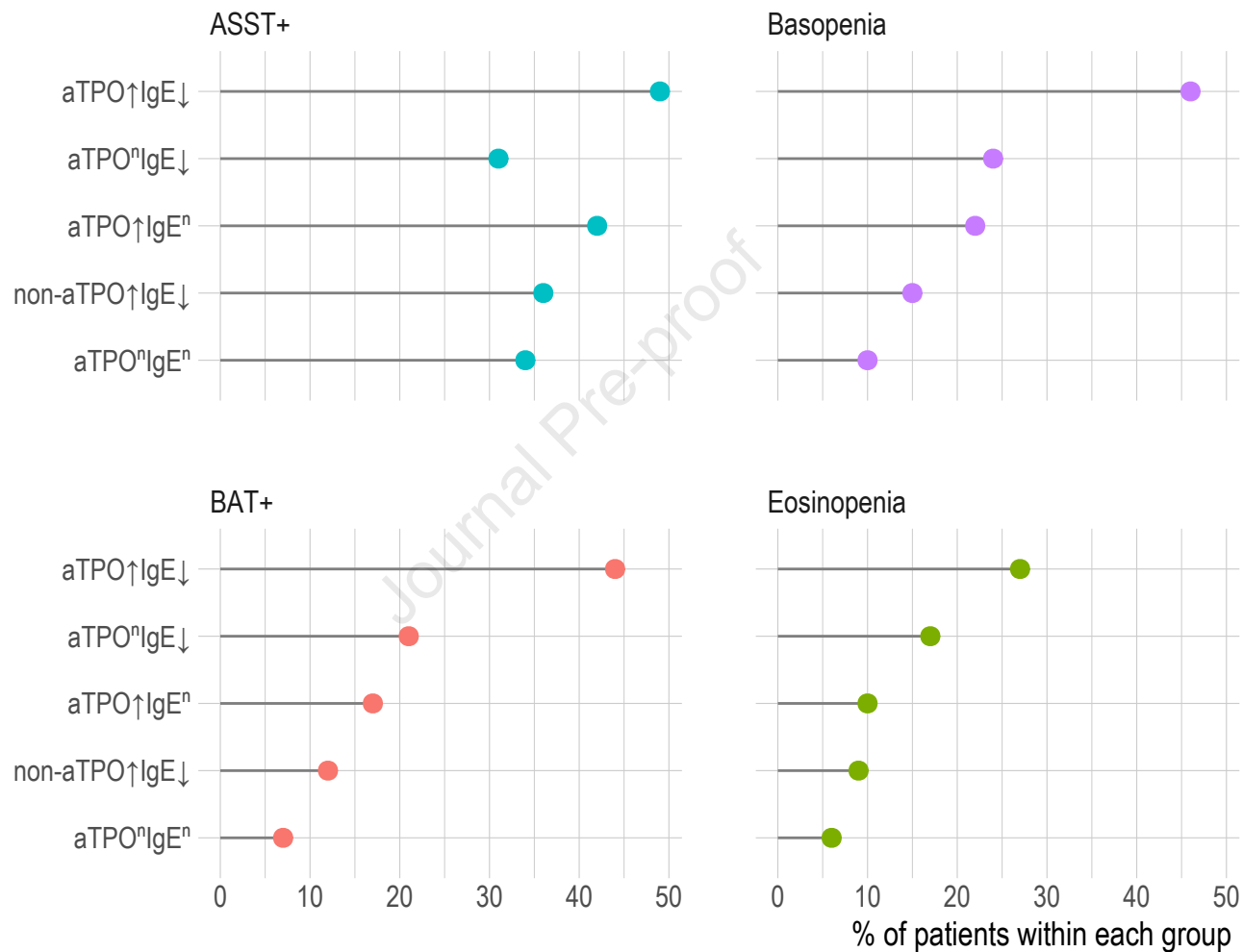
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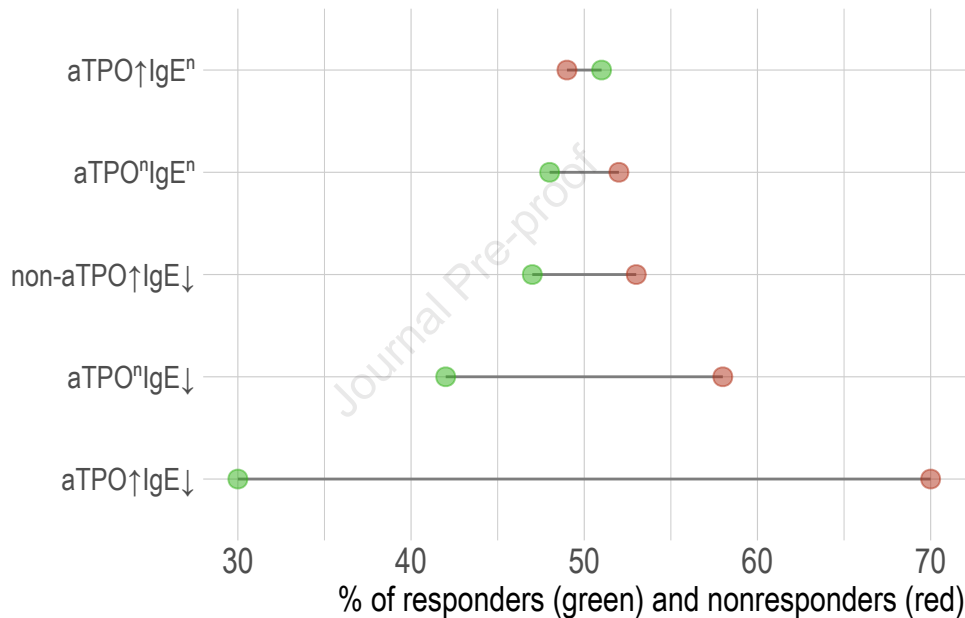
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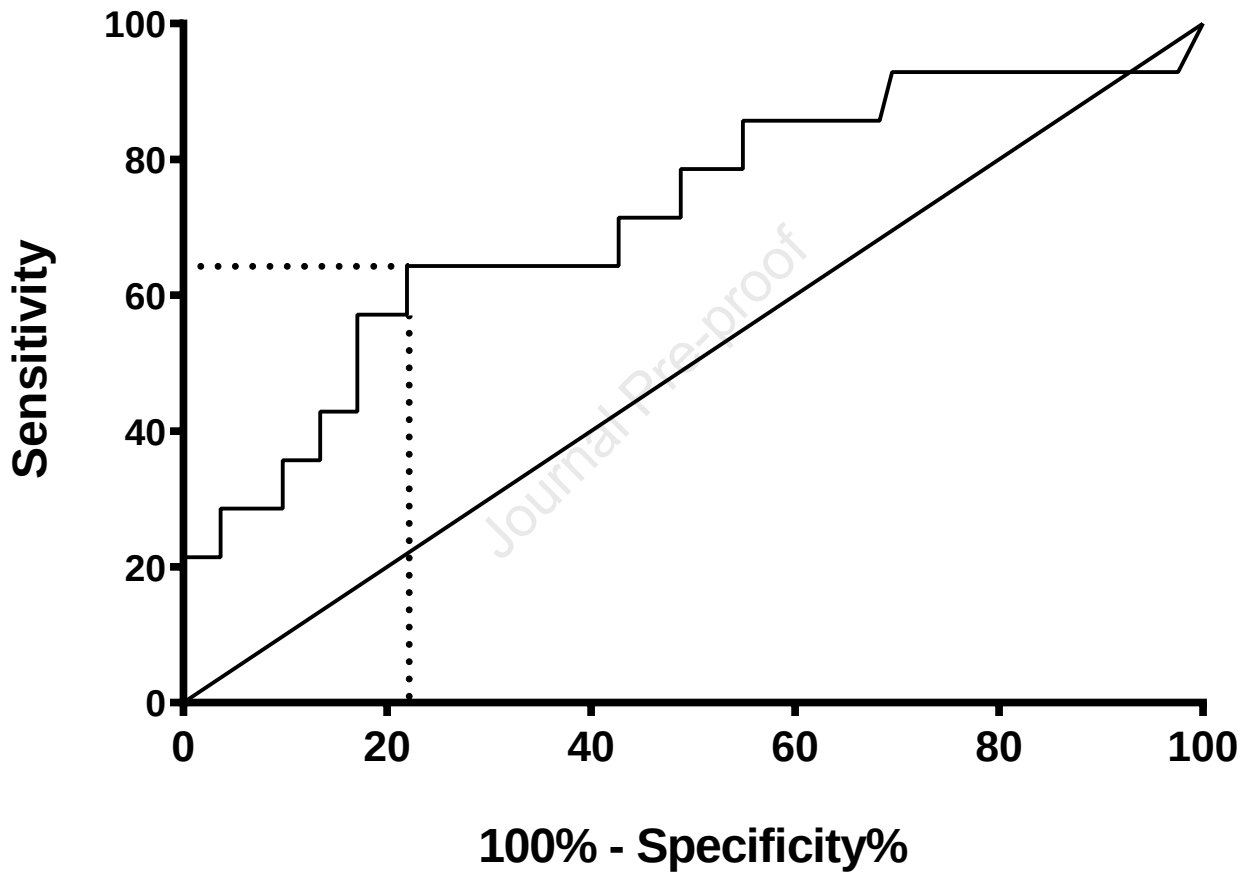
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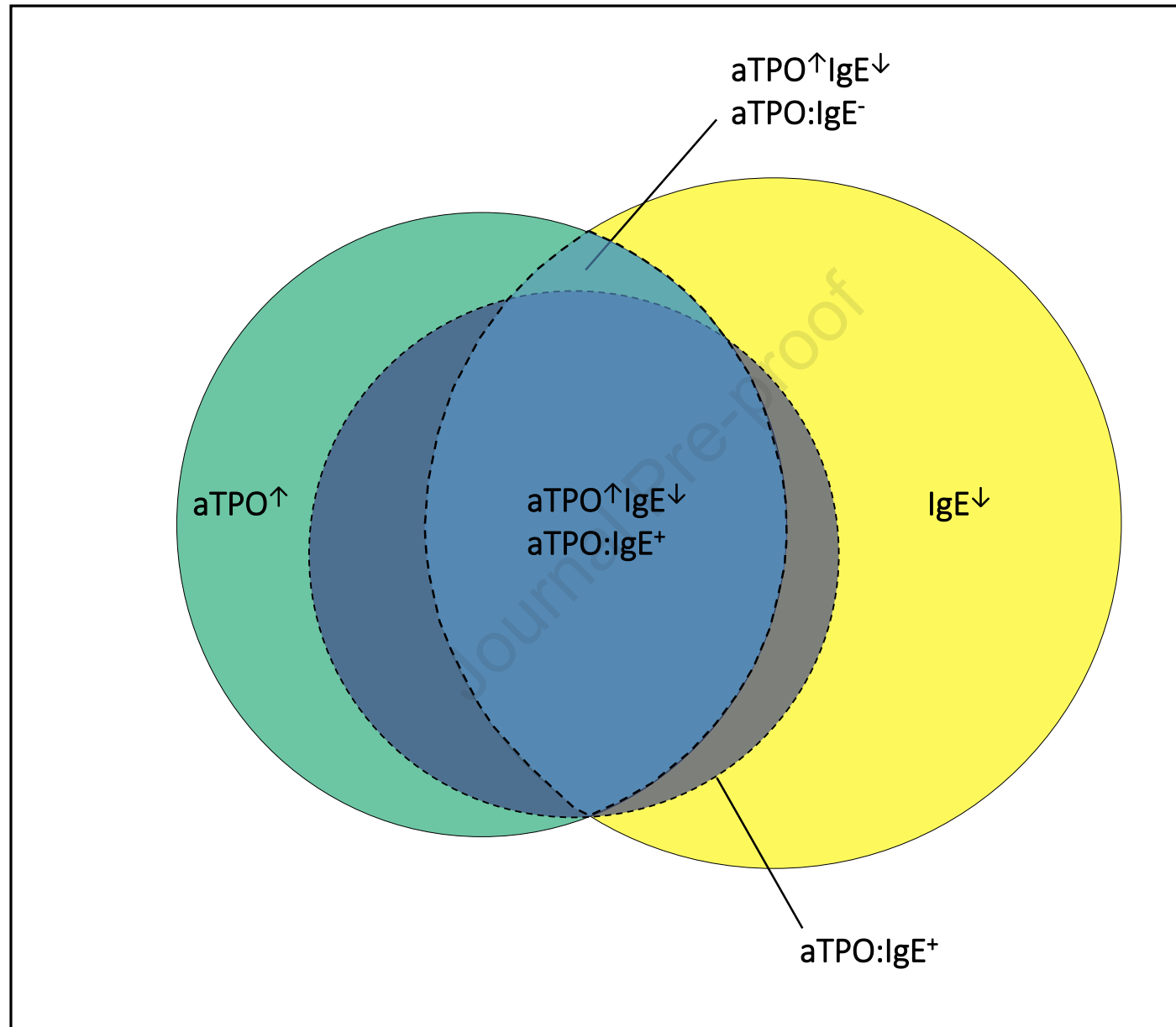


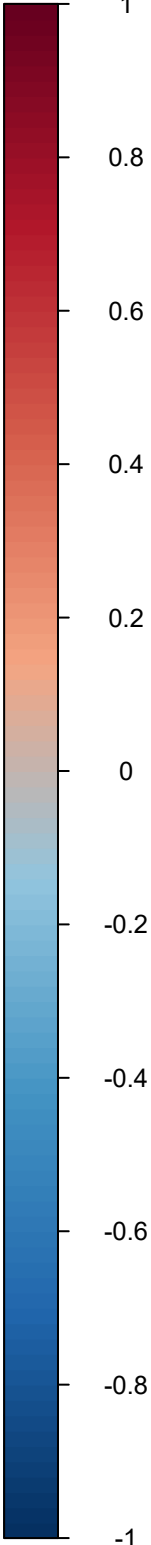
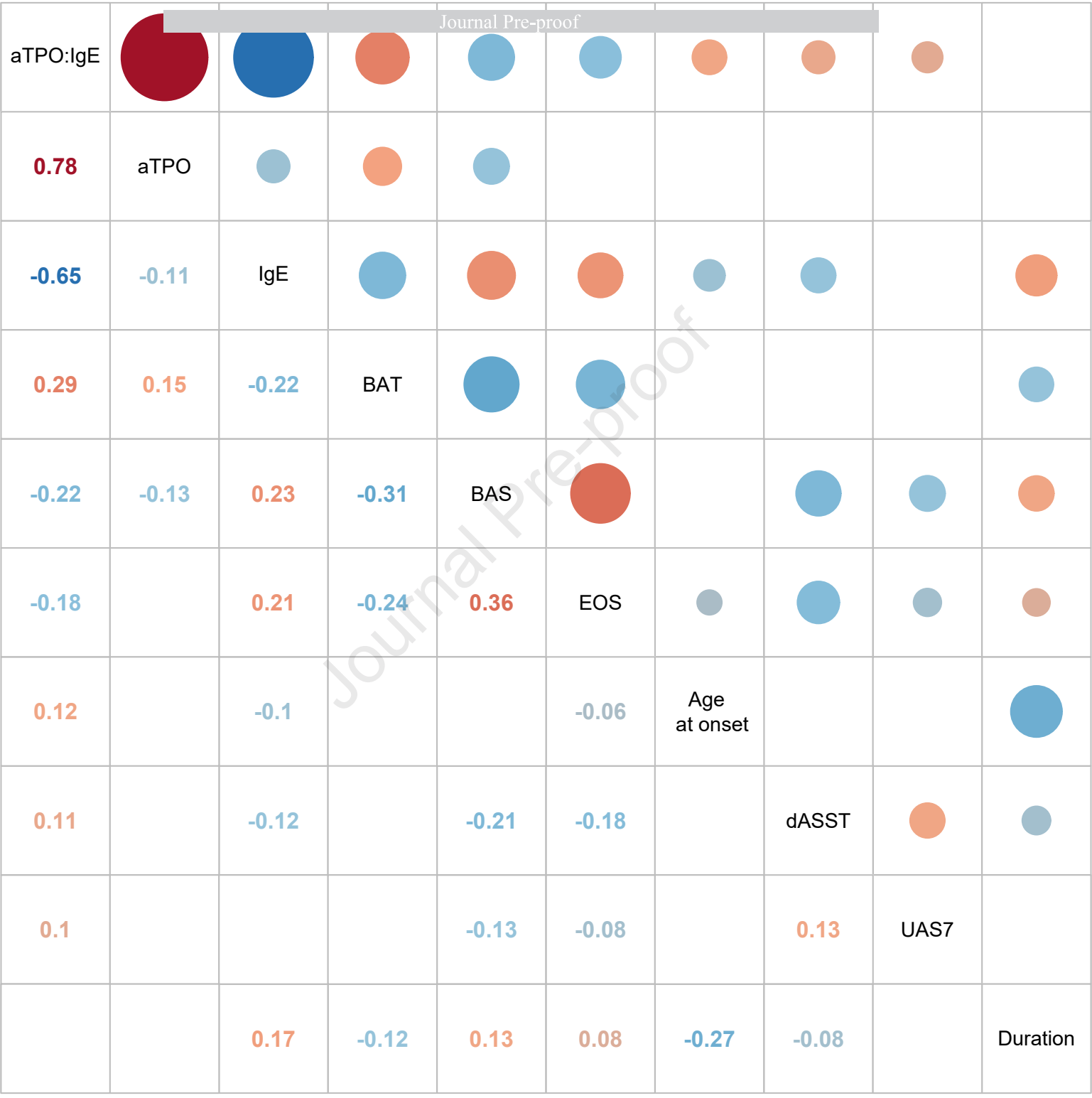












The cut-off value for the ratio of IgG aTPO to IgE was calculated using the data from the PURIST study and was defined as 2.88 (Figure E1). The ratio of IgG anti-TPO to IgE was positive, i.e. higher than the cut-off value of 2.88 (aTPO:IgE⁺) in 19% of CSU patients (175/922). The age at CSU onset significantly correlated with the ratio of aTPO to IgE, albeit weakly (Table E3). aTPO:IgE⁺ patients were also more likely to be female as compared to aTPO:IgE⁻ patients (92% vs 67%, Table E2). CSU duration was shorter in patients who were aTPO:IgE⁺ as compared to those who were not, and patients with a short disease duration, i.e. one year or less were more likely to be aTPO:IgE⁺ and had a higher aTPO to IgE ratio. Patients who were aTPO:IgE⁺ had higher disease activity as compared to those who were not, and CSU patients with high disease activity, i.e. UAS7 > 27, were more likely to be aTPO:IgE⁺ and had a higher aTPO to IgE ratio. aTPO:IgE showed a weak but significant correlation with disease activity (Table E2, Table E3).

Positive BAT and ASST as well as basopenia and eosinopenia were also more common in aTPO:IgE⁺ vs aTPO:IgE⁻ patients (Figure E3, Table E2, Table E3). Of aTPO:IgE⁺ patients, 43% (27/61) had a positive BAT compared to 12% (28/232) of aTPO:IgE⁻ patients. Relative risk of showing BAT positivity for an aTPO:IgE⁺ patient is 3.532 (95% CI, 2.245 to 5.557). Also, BAT-positive patients had a significantly higher ratio of aTPO to IgE as compared to BAT-negative patients.

aTPO:IgE⁺ patients showed lower rates of response to sgAH treatment, 36%, as compared to 47% of aTPO:IgE⁻ patients (p=0.058, Table E2, Table E3). Of sgAH non-responders, 30% were aTPO:IgE⁺ versus 21% of sgAH responders (p=0.035).

The combination of high IgG anti-TPO and low total IgE levels (aTPO[↑]IgE[↓]) was strongly associated with the ratio (Table E1). Most aTPO[↑]IgE[↓] patients (92%) had an aTPO to IgE ratio higher than the cut-off (Figure E2) and showed similar rates of positive BAT, ASST, eosinopenia and/or basopenia (Table E1-3). However, only 63% aTPO:IgE⁺ patients were aTPO[↑]IgE[↓] positive. Forty-two aTPO:IgE⁺ patients had very high TPO but normal IgE values and 22 aTPO:IgE⁺ patients had normal TPO and very low IgE (<9 IU/mL). Most of these patients (>66%) were negative for one or more aiCSU markers, i.e. BAT, ASST, eosinopenia and basopenia.

Suppl. Figures Legend

Figure E1. A cut-off value based on receiver operating characteristic (ROC) curve analysis of ratio IgG anti-TPO to total IgE in patients with chronic spontaneous urticaria. Diagnostic accuracy for the distinction of CSU patients and healthy controls was analysed by ROC curve including the area under the curve (AUC=0.716) and the confidence limits for this curve (CI:0.556–0.876). With the ratio of IgG anti-TPO to total IgE cut-off of >2.88 ng/ml, the selected sensitivity was 64% and the resulting specificity was 78% (displayed as dotted line in the graph). Based on data from the PURIST study (Schoepke et al. Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria: Results of the PURIST Study. Allergy. 2019; 74: 2427– 2436).

Figure E2. Most patients with chronic spontaneous urticaria with high IgG anti-TPO and low IgE levels have above cutoff ratios of the two and vice versa. Venn diagram shows 47% (428) of 918 CSU patients who had elevated IgG anti-TPO (aTPO $\uparrow$), low total IgE (IgE $\downarrow$), or both (aTPO $\uparrow$ IgE $\downarrow$). Most aTPO $\uparrow$ IgE $\downarrow$ patients (92%) had an aTPO to IgE ratio higher than the cut-off of 2.88 (aTPO:IgE $^+$) and vice versa (63%). The rate of aTPO:IgE $^+$ patients was much lower in non-aTPO $\uparrow$ IgE $\downarrow$ patients (8%), aTPO ^{N} IgE $\downarrow$ patients (12%), aTPO $\uparrow$ IgE ^{N} patients (34%) and aTPO ^{N} IgE ^{N} patients (0%). As for aTPO:IgE $^+$ patients, none were aTPO ^{N} IgE ^{N} , 24% were aTPO $\uparrow$ IgE ^{N} , and 13% were aTPO ^{N} IgE $\downarrow$. aTPO $\uparrow$: patients with high levels of IgG anti-TPO; IgE $\downarrow$: patients with low levels of IgE; aTPO $\uparrow$ IgE $\downarrow$: double positive CSU patients, i.e. with low IgE and high IgG anti-TPO; aTPO:IgE $^+$: patients with the ratio of anti-TPO to IgE of >2.88; aTPO $\uparrow$ IgE $\downarrow$ aTPO:IgE $^+$: patients positive for both aTPO $\uparrow$ IgE $\downarrow$ and aTPO:IgE $^+$.

Figure E3. Correlogram of CSU patients' demographic, clinical and laboratory parameters. Pooled data were analyzed (Table E3). The size of the circles and shading in the correlation plot represent the degree of association. The significance level was set to less than 0.05. Nonsignificant correlations were excluded. Correlations between continuous variables are measured by Spearman rho. Positive correlations are displayed in red and negative correlations in blue color. aTPO:IgE: aTPO to IgE ratio; aTPO: IgG anti-TPO levels; IgE: total IgE levels; BAT: basophil activation test; BAS: basophil count; EOS: eosinophil count; dASST: diameter of wheal of autologous serum skin test; UAS7: urticaria activity score over 7 days.

Table E1. Characteristics of CSU patients in terms of total IgE and IgG anti-TPO values

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Parameters	Cohorts	CSU patients, total n	Comparison of four groups						Comparison aTPO ⁺ IgE ⁺ vs all	
			aTPO ⁺ IgE ⁺ (1)	aTPO ^N IgE ⁺ (2)	aTPO ⁺ IgE ^N (3)	aTPO ^N IgE ^N (4)	p-value*	Pairwise*	non-aTPO ⁺ IgE ⁺	p-value
Age in years, median (IQR); n	Russian	353	47 (31-58.2); 66	42.0 (32-55.2); 74	50.0 (30.5-58.0); 57	36 (30.0-49.7); 156	0.015	ns	39 (30-55); 287	0.133
	German	765	47 (31.2-59.5); 56	44 (30-54); 163	47 (36.5-54); 65	41 (30-50); 481	0.011	ns	42 (30-50); 709	0.138
	All	1,118	47.0 (31.0-58.2); 122	43 (31.5-55); 237	48.0 (34-56.2); 122	39 (30-50); 637	<0.001	1,2,3 vs 4	41 (30-53); 174	<0.001
Female, % (n/total)	Russian	355	91.0 (61/67)	73.3 (55/75)	84.2 (48/57)	64.1 (100/156)	<0.001	1 vs 2,4	70.5 (203/288)	0.001
	German	765	94.6 (53/56)	76.1 (124/163)	83.1 (54/65)	65.1 (313/481)	<0.001	1 vs 2,4	69.3 (491/709)	<0.001
	All	1,120	92.7 (114/123)	75.2 (179/238)	83.6 (102/122)	64.8 (413/637)	<0.001	1 vs 2,4	69.6 (694/997)	<0.001
CSU duration in years, median (IQR); n	Russian	351	0.79 (0.48-2.06); 66	0.83 (0.5-2.5); 73	2.0 (0.87-8.2); 56	1.5 (0.58-5.0); 156	0.001	3 vs 1,2	1.4 (0.5-5); 285	0.016
	German	724	1.5 (1-6); 55	1.5 (1-4); 158	3 (1-10); 63	2.5 (1-7); 448	0.002	2 vs 3,4	2 (1-7); 669	0.501
	All	1,075	1.0 (0.67-5.0); 121	1.25 (0.7-3.0); 231	2.3 (1-9); 119	2 (1-7); 604	<0.001	1,2 vs 3,4	2 (1-6); 954	0.001
Age at disease onset in years, median (IQR); n	Russian	351	45.1 (30-57); 66	41.5 (29.95-54.2); 73	37.0 (25.8-55.5); 56	32.7 (26-46.7); 156	0.008	1,2 vs 4	35.8 (27-51.7)	0.046
	German	723	40.5 (24.7-53); 55	39.8 (27.4-49.6); 158	37 (27.5-48.6); 63	34 (24-45); 447	0.008	2 vs 4	36 (25-46.5); 668	0.317
	All	1,074	41 (26.5-56.4); 121	40 (29-51); 231	37 (27-52.4); 119	33.6 (24.4-45); 603	<0.001	1,2 vs 4	36 (25.5-48); 953	0.016
UAS7, median (IQR); n	Russian	126	13.5 (4-26); 20	6 (0-14); 32	2 (0.5-14.5); 13	9 (2-15.5); 61	0.177	–	7 (0-14.2); 106	0.048
	German	664	20 (8-26); 47	15 (8-26.2); 146	19 (10.7-28); 58	15 (9-23); 413	0.151	–	16 (8-23); 617	0.570
	All	790	16 (7-26); 67	14 (5-23); 178	18 (6-25); 71	15 (7-22); 474	0.476	–	15 (7-23); 723	0.455
Presence of angioedema, % (n/total)	Russian	355	80.6 (54/67)	73.3 (55/75)	71.9 (41/57)	67.3 (105/156)	0.240	–	69.8 (201/288)	0.077
	German	728	70.4 (38/54)	45.2 (71/157)	42.2 (27/64)	47.0 (213/453)	0.006	1 vs 2,3,4	46.1 (311/674)	0.001
	All	1,083	76.4 (92/121)	54.3 (126/232)	56.2 (68/121)	52.2 (318/609)	<0.001	1 vs 2,3,4	53.2 (512/962)	<0.001
Comorbid inducible urticaria, % (n/total)	Russian	329	25.9 (15/58)	25.7 (18/70)	37.7 (20/53)	34.5 (51/148)	0.322	–	32.8 (89/271)	0.299
	German	742	5.7 (3/53)	13.8 (22/160)	19.0 (12/63)	20.6 (96/466)	0.022	ns	18.9 (130/689)	0.016
	All	1,071	16.2 (18/111)	17.4 (40/230)	27.6 (32/116)	23.9 (147/614)	0.038	ns	22.8 (219/960)	0.113
Ratio of IgG anti-TPO to total IgE	Russian	353	28.9 (6.35-95.8); 65	0.09 (0.02-0.67); 75	2.1 (0.52-9.18); 57	0.02 (0.01-0.07); 156	<0.001	all	0.06 (0.01-0.35); 288	<0.001
	German	565	24.2 (6.7-95.2); 56	0.6 (0.31-1.2); 110	1.46 (0.5-3.3); 65	0.06 (0.03-0.12); 334	<0.001	1 vs all; 2 vs 4; 3 vs 4	0.12 (0.04-0.41); 509	<0.001
	All	918	25.7 (6.4-95.0); 121	0.47 (0.13-0.97); 185	1.5 (0.58-6.2); 122	0.05 (0.02-0.11); 490	<0.001	all	0.4 (0.1-2.1); 797	<0.001
Positive ratio of IgG anti-TPO to total IgE	Russian	353	96.9 (63/65)	6.7 (5/75)	41.2 (24/57)	0.0 (0/156)	<0.001	all	10.1 (29/288)	<0.001
	German	565	85.7 (48/56)	15.5 (17/110)	27.7 (18/65)	0.0 (0/334)	<0.001	1 vs all; 2,3 vs 4	6.9 (35/509)	<0.001
	All	918	91.7 (111/121)	11.9 (22/185)	34.4 (42/122)	0.0 (0/490)	<0.001	all	8.0 (64/797)	<0.001
BAT, %, median (IQR); n	German	387	5.8 (1.0-36.5); 43	1.7 (0.6-6.5); 113	1.6 (0.8-2.7); 29	1.2 (0.4-2.5); 202	<0.001	1,2 vs 4; 1 vs 2	1.3 (0.5-3.1); 334	<0.001
BAT+ (>7.77%), % (n/total)	German	387	44.2 (19/43)	21.2 (24/113)	17.2 (5/29)	6.9 (14/202)	<0.001	1 vs 2,4; 2 vs 4	12.5 (43/344)	<0.001
C-reactive protein, median (IQR); n mg/L	Russian	295	3.5 (1.4-10.3); 51	2.25 (1.0-6.2); 62	2.9 (0.62-5.37); 52	2.75 (1.34-6.47); 130	0.436	–	2.7 (1-6.2); 244	0.170
	German	739	2.3 (0.85-5.1); 54	1.9 (0.6-5.6); 159	1.9 (0.85-6.4); 61	2.2 (0.7-5.4); 465	0.974	–	–	0.862
	All	1,034	2.7 (1.2-7.1); 105	2.1 (0.8-5.6); 221	2.0 (0.7-6.1); 113	2.0 (0.8-5.8); 595	0.572	–	2.2 (0.8-5.8); 929	0.167
Basophils, x10 ⁹ /L median (IQR); n	Russian	153	0.0 (0.0-0.01); 29	0.01 (0.0-0.06); 37	0.01 (0.0-0.03); 26	0.01 (0.1-0.06); 61	0.067	–	0.01 (0-0.05); 124	0.018
	German	741	0.01 (0.0-0.02); 54	0.02 (0.01-0.03); 158	0.02 (0.01-0.03); 62	0.02 (0.01-0.04); 467	<0.001	1 vs 2,3,4; 2 vs 4	–	<0.001
	All	894	0.01 (0.0-0.02); 83	0.02 (0.01-0.03); 195	0.02 (0.01-0.03); 88	0.02 (0.01-0.04); 528	<0.001	all (apart 2 vs 3)	0.02 (0.01-0.04); 811	<0.001
Basopenia, <0.01 x 10 ⁹ /L, % (n/total)	Russian	153	55.2 (16/29)	32.4 (12/37)	38.5 (10/26)	37.7 (23/61)	0.281	–	36.3 (45/124)	0.062
	German	741	40.7 (22/54)	22.2 (35/158)	14.5 (9/62)	6.4 (30/467)	<0.001	1 vs all	10.8 (74/687)	<0.001
	All	894	45.8 (38/83)	24.1 (47/195)	21.6 (19/88)	10.0 (53/528)	<0.001	1 vs 2,3,4	14.7 (119/811)	<0.001
Eosinophils,	Russian	205	0.09 (0.02-0.17); 36	0.1 (0.07-0.17); 47	0.11 (0.1-0.2); 35	0.14 (0.1-0.2); 87	0.001	1,2 vs 4	0.12	0.007

x10 ⁹ /L, median (IQR); n									(0.09-0.23); 169	
										<0.001
	All	953	0.09 (0.04-0.2); 90	0.11 (0.07-0.18); 208	0.12 (0.09-0.2); 98	0.17 (0.1-0.26); 557	<0.001	1,2,3 vs 4	0.15 (0.09-0.23); 863	<0.001
Eosinopenia, <0.05 x 10 ⁹ /L, % (n/total)	Russian	205	30.6 (11/36)	19.1 (9/47)	2.9 (1/35)	10.3 (9/87)	0.004	1 vs 3,4	11.2 (19/169)	0.003
	German	748	24.1 (13/54)	16.1 (26/161)	14.3 (9/63)	5.7 (27/470)	<0.001	1,3 vs 4	8.9 (62/694)	<0.001
	All	953	26.7 (24/90)	16.6 (35/208)	10.2 (10/98)	6.5 (36/557)	<0.001	1 vs 3,4	9.4 (81/863)	<0.001
Eosinopenia + basopenia, % (n/total)	Russian	152	21.4 (6/28)	8.1 (3/37)	3.8 (1/26)	4.9 (3/61)	0.052	–	5.6 (7/124)	0.007
	German	740	18.5 (10/54)	10.8 (17/158)	6.5 (4/62)	2.1 (10/466)	<0.001	1 vs 4	4.5 (31/686)	<0.001
	All	892	19.5 (16/82)	10.3 (20/195)	5.7 (5/88)	2.5 (13/527)	<0.001	1 vs 3,4	4.7 (38/810)	<0.001
ASST positivity, % (n/total)	Russian	147	61.3 (19/31)	45.2 (14/31)	50.0 (13/26)	44.1 (33/59)	0.450	–	54.3 (63/116)	0.123
	German	761	42.6 (23/54)	28.2 (46/163)	40.0 (26/65)	21.5 (103/479)	<0.001	1 vs 4	24.8 (175/707)	0.004
	All	908	49.4 (42/85)	30.9 (60/194)	42.9 (39/91)	24.0 (129/538)	<0.001	1 vs 2,4	27.7 (228/823)	<0.001
ANA positivity, % (n/total) (≥1:80)	German	488	57.9 (22/38)	41.7 (43/103)	41.9 (18/43)	33.6 (102/304)	0.02	1 vs 4	36.2 (163/450)	0.008
NSAID hypersensitivity, % (n/total)	Russian	266	22.0 (11/50)	14.8 (9/61)	13.2 (5/38)	14.5 (17/117)	0.607	–	14.4 (31/216)	0.181
Efficacy of sgAHs in standard doses, % (n/total)	Russian	349	30.3 (20/66)	41.9 (31/74)	50.9 (28/55)	48.1 (74/154)	0.064	–	47 (133/283)	0.014

sgAHs: second generation antihistamines; BAT: basophil activation test; ANA: antinuclear antibodies; NSAID: nonsteroidal anti-inflammatory drugs; ASST: autologous serum skin test; IQR: interquartile range; aTPO^IIgE^L: IgG anti-TPO high and total IgE low; aTPO^NIgE^L: IgG anti-TPO normal and total IgE low; aTPO^IIgE^N: IgG anti-TPO high and total IgE normal or high; aTPO^NIgE^N: IgG anti-TPO normal and total IgE normal or high. *The “p-value” column shows a p-value for difference in proportions between groups. In the “pairwise” column statistically significant differences between groups are included (p-values are not shown). For some parameters chi-square test of homogeneity or Kruskal-Wallis test are statistically significant (the “p-value” column), but none of the pairwise comparisons for these parameters are statistically significant. This can happen for a number of reasons. One possible reason is that the Bonferroni adjustment can lead to a conservative test (i.e., it is harder to detect a statistically significant difference than it should be).

Table E2. Characteristics of CSU patients in terms of ratio IgG anti-TPO to total IgE

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Parameters	Cohorts	CSU patients, n	IgG anti-TPO:IgE ⁺	IgG anti-TPO:IgE ⁻	p-value
Age in years, median (IQR); n	Russian	351	46 (33-58); 91	38.5 (30-54.7); 260	0.054
	German	568	49 (35-60); 83	40 (29-50); 485	<0.001
	All	919	48 (33-58); 174	40 (30-52); 745	<0.001
Female, % (n/total)	Russian	353	91.3 (84/92)	68.2 (178/261)	<0.001
	German	568	92.8 (77/83)	68.6 (334/485)	<0.001
	All	921	92.0 (161/175)	68.6 (512/746)	<0.001
CSU duration in years, median (IQR); n	Russian	349	0.92 (0.5-2.5); 90	1.3 (0.5-4.2); 259	0.055
	German	547	1.75 (1-7.2); 82	2 (1-6.5); 465	0.362
	All	896	1.21 (0.67-5); 172	2 (1-6); 724	0.007
Age at disease onset in years, median (IQR); n	Russian	349	38.9 (27.7-56.4); 90	35.7 (27.0-52.0); 259	0.124
	German	546	42.3 (26.7-53.7); 82	34 (24-45.4); 464	0.001
	All	895	40.8 (27.2-55.6); 172	34.7 (25-48); 723	<0.001
UAS7, median (IQR); n	Russian	126	10 (2.5-24.5); 25	7 (0-14); 101	0.058
	German	487	21 (7-28); 71	14.5 (7-22); 416	0.062
	All	613	18 (7-27); 96	13 (6-21); 517	0.035
Presence of angioedema, % (n/total)	Russian	353	77.2 (71/92)	70.1 (183/261)	0.195
	German	563	60.5 (49/81)	58.2 (281/482)	0.700
	All	916	69.4 (120/173)	62.4 (464/743)	0.088
Comorbid inducible urticaria, % (n/total)	Russian	329	31.3 (26/83)	31.7 (78/246)	0.948
	German	551	8.5 (7/82)	13.9 (65/469)	0.187
	All	880	20.0 (33/165)	20.0 (143/715)	1.0
BAT in %, median (IQR); n	German	293	5.7 (1.2-35.6); 61	1.3 (0.5-3.3); 232	<0.001
BAT positivity (>7.77%), % (n/total)	German	293	42.6 (27/61)	12.1 (28/232)	<0.001
C-reactive protein in mg/L, median (IQR); n	Russian	294	3.5 (1.6-12); 74	2.5 (1.0-6.1); 220	0.045
	German	543	2.3 (1.1-5.1); 80	1.8 (0.5-5.4); 463	0.191
	All	837	2.75 (1.3-6.6); 154	2.1 (0.7-5.6); 683	0.011
Basophils, x10 ⁹ /L, median (IQR); n	Russian	153	0.01 (0.0-0.02); 47	0.01 (0.0-0.05); 106	0.054
	German	548	0.01 (0.0-0.02); 78	0.02 (0.01-0.04); 470	<0.001
	All	701	0.01 (0.0-0.02); 125	0.02 (0.01-0.04); 576	<0.001
Basopenia (<0.01 x 10 ⁹ /L), % (n/total)	Russian	153	44.7 (21/47)	37.7 (40/106)	0.418
	German	548	34.6 (27/78)	8.9 (42/470)	<0.001
	All	701	38.4 (48/125)	14.2 (82/576)	<0.001
Eosinophils, x10 ⁹ /L, median (IQR); n	Russian	205	0.01 (0.06-0.17); 58	0.12 (0.09-0.22); 147	0.026
	German	554	0.08 (0.03-0.18); 79	0.16 (0.1-0.25); 475	<0.001
	All	759	0.12 (0.04-0.17); 137	0.15 (0.1-0.24); 622	<0.001
Eosinopenia (<0.05 x 10 ⁹ /L), % (n/total)	Russian	205	20.7 (12/58)	12.2 (18/147)	0.123
	German	554	27.8 (22/79)	6.7 (32/475)	<0.001
	All	759	24.8 (34/137)	8.0 (50/622)	<0.001
Eosinopenia + basopenia, % (n/total)	Russian	152	15.2 (7/46)	5.7 (6/106)	0.053
	German	547	19.2 (15/78)	3.2 (15/469)	<0.001
	All	699	17.7 (22/124)	3.7 (21/575)	<0.001
ASST positivity, % (n/total)	Russian	146	56.3 (27/48)	44.9 (44/98)	0.197
	German	564	34.9 (29/83)	25.2 (121/481)	0.062
	All	710	42.7 (56/131)	28.5 (165/579)	0.001
ANA positivity, % (n/total) (≥1:80)	German	410	52.6 (30/57)	43.1 (152/353)	0.177
NSAID hypersensitivity, % (n/total)	Russian	266	20.9 (14/67)	14.1 (28/199)	0.185
Efficacy of sgAHs in standard doses, % (n/total)	Russian	347	35.6 (32/90)	47.1 (121/257)	0.058

sgAHs: second generation antihistamines; BAT: basophil activation test; ANA: antinuclear antibodies; NSAID: nonsteroidal anti-inflammatory drugs; ASST: autologous serum skin test; aTPO:IgE⁺: CSU patients with the ratio of anti-TPO to IgE of >2.88; aTPO:IgE⁻: CSU patients with the ratio of anti-TPO to IgE of ≤ 2.88.

Table E3. Correlation between ratio IgG anti-TPO to total IgE and clinical and laboratory parameters

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Parameters	Cohorts, r, p-value, n		
	Russian	German	All
Age, years	0.156 0.003 351	0.133 0.001 568	0.139 <0.001 919
CSU duration, years	-0.091 0.089 349	-0.077 0.071 547	-0.063 0.060 897
Age at disease onset, years	0.139 0.009 349	0.125 0.003 546	0.122 <0.001 895
UAS7	0.114 0.206 126	0.071 0.119 487	0.099 0.014 613
IgG anti-TPO, kU/L	0.867 <0.001 353	0.658 <0.001 567	0.775 <0.001 920
Total IgE, kU/L	-0.543 <0.001 353	-0.799 <0.001 569	-0.653 <0.001 922
BAT, %	–	0.291 <0.001 293	–
ASST, wheal diameter	–	0.112 0.016 463	–
C-reactive protein, mg/L	0.063 0.284 294	0.035 0.410 543	0.029 0.408 837
Basophils, x10 ⁹ /L	-0.209 0.010 153	-0.269 <0.001 548	-0.255 <0.001 701
Eosinophils, x10 ⁹ /L	-0.188 0.007 205	-0.257 <0.001 554	-0.223 <0.001 759

n: number of CSU patients; r: Spearman rho; sgAHs: second generation antihistamines; BAT: basophil activation test; ASST: autologous serum skin test; UAS7: urticaria activity score over 7 days