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Antinuclear antibodies are common and linked to poor response to omalizumab treatment in patients with CSU

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To the Editor:

Chronic spontaneous urticaria (CSU) is a frequent disorder that is strongly associated with autoimmunity(1). This is supported by high rates of autoimmune comorbidities, the age of onset and the female preponderance(1,2). Also, CSU patients have been shown to exhibit functional and relevant mast cell-activating IgG and IgE autoantibodies(1). These autoantibodies are held to be responsible for the development of the urticarial signs and symptoms of most patients with CSU(3). Autoimmune CSU (aiCSU) is linked to more severe disease and poor treatment responses to omalizumab(4,5). Clinical markers of aiCSU and predictors of treatment responses are needed.

Patients with CSU commonly have anti-thyroid autoantibodies, and antinuclear antibodies (ANAs) have also been reported to be elevated in CSU(5). As of now, however, it is unclear how common and relevant increased levels of ANAs are in CSU.

Here, we analyzed a large population of 447 patients with CSU treated at the Urticaria Center of Reference and Excellence (UCARE)(6) of Kayseri City Hospital for the prevalence of increased ANA levels. Also, we compared ANA-positive (ANA⁺) and ANA-negative (ANA⁻) patients for differences in their disease characteristics and response to treatment.

All patients had wheals with or without angioedema; patients with angioedema and without wheals were excluded from the study. This study was approved by the Institutional Review Board and the local ethics committee. Serum samples were analyzed for ANAs by traditional fluorescence microscopy using HEp-2 slides (EUROIMMUN, AG, Lübeck,

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Germany). The cut-off for ANA positivity was set at an antibody titer of 1/100, as per our center's laboratory standard(7). We also assessed demographic and clinical features, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), total IgE, vitamin B12, and anti-TPO (Table I). Overall disease activity was assessed by a physician at baseline and week 12 of omalizumab treatment (300mg/4weeks) in 171 patients, by a 10 cm visual analog scale (VAS). Patients who had a more than 80% improvement and a 20%-80% improvement at week12 were considered complete and partial-responders, respectively (non-responders= VAS improvement<20% at week12). More information on the rationale and methods of this study is in the supplemental repository.

Of 447 patients with CSU, 107 (23.9%) were ANA⁺, and 37% of them showed titers of 1/320 or higher. The rate of ANA positivity was higher in female patients ($p<0.001$) (Table I). ANA⁺ patients with CSU exhibited angioedema more often than ANA⁻ patients (70% vs 53%; $p=0.002$) (Table I). Also, rheumatoid arthritis (10.3% vs 4.4%; $p=0.025$) and Sjögren disease (4.7% vs 0.6%; $p=0.003$) were seen more often in ANA⁺ patients (Table I). In contrast, ANA⁺ and ANA⁻ patients with CSU did not differ in their age, age at onset of CSU, or their disease activity, control, or duration, or any of the other demographic or clinical features assessed (Table I).

ANA⁺ patients with CSU had markedly lower total IgE serum levels than ANA⁻ patients, 110 vs 64 IU/ml on average ($p<0.001$). Also, more ANA⁺ patients had low IgE levels, i.e. total IgE<40 IU/ml (38% vs 23%, $p=0.003$), and more ANA⁻ patients had elevated IgE levels, i.e.>100 IU/ml (53% vs 39%, $p=0.014$). There was a statistically significant, albeit weak negative correlation between ANA titers and IgE levels ($p<0.001$, $r=-0.170$). ANA⁺ patients had higher serum levels of B12 ($p=0.003$, Table I), and there was as statistically significant weak positive correlation between ANA titers and B12 levels ($p=0.002$, $r=0.175$). ANA⁺ patients also were positive for anti-TPO more often than ANA⁻ patients (27% vs 18%, $p=0.049$, Table I). In contrast, ANA⁺ and ANA⁻ patients with CSU showed similar blood levels of markers for inflammation (CRP, ESR).

Of 171 patients treated, 28 (16%) were resistant to omalizumab. More than half of these non-responders (54%) were ANA⁺, and ANA⁺ patients had markedly higher rates of non-response (45% vs 9%, in ANA⁻ patients, $p<0.001$) (Table II). After 12 weeks of omalizumab treatment, ANA⁻ patients showed a mean reduction in disease activity of 95% as assessed by VAS, compared to 33% in ANA⁺ patients. Also, ANA titers and treatment

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responses to omalizumab showed a significant negative correlation ($p < 0.001$, $r = -0.345$). When we used 1:320 as the cut-off for ANA positivity, 1 of 2 ANA⁺ patients were non responders, significantly more than in the ANA⁻ group ($p = 0.01$). In contrast, ANA⁺ and ANA⁻ patients with CSU showed similar rates of resistance to antihistamine treatment (i.e. VAS scores ≥ 7), and antihistamine resistant and non-resistant patients were similar in their rates of ANA positivity (Table II).

In addition to confirming the high prevalence of ANA in CSU, we show that these autoantibodies may be relevant for patients with CSU and importantly linked to their disease. ANA⁺ patients with CSU are more likely to have angioedema and comorbid rheumatological autoimmune diseases. A previous study showed that rheumatoid arthritis and Sjögren disease are more common in patients with CSU(8). Our study suggests that this is indeed the case, and that CSU patients can be screened for both of these conditions by assessing ANAs.

Our finding that ANA⁺ patients have low IgE fits the notion that CSU is driven by IgG autoantibodies in some patients, i.e. patients with aiCSU(1). ANA, together with anti-TPO, which we found to be linked to ANA, may help to identify patients with aiCSU(5). Our study also shows that being ANA⁺ and being ANA⁻ does make a difference when it comes to the effects of omalizumab on CSU. This finding is backed by several recent reports that CSU patients with autoimmune features show less favorable responses to omalizumab(9).

This is a monocentric study, and our patients had severe and difficult to treat CSU. One in three patients was refractory to antihistamine treatment. This may be the reason of the higher rate of ANA positivity as compared to previous studies. For additional considerations on the interpretation and relevance of our findings, please refer to the supplemental repository.

In conclusion, patients with CSU often have ANAs, and this is linked to differences in clinical features, laboratory markers and treatment responses to omalizumab. Our findings support further studies of ANA as a biomarker and to assess these autoantibodies for their relevance in CSU.

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Conflict of interest: None

Tables

Table I. Clinical and laboratory characteristics of CSU patients who do or do not have positive ANA titres

	Patients with CSU		
	ANA+	ANA-	p
Age in years ^a , mean ($\pm$ SD)	39.48 ($\pm$ 16.58)	38.6 ($\pm$ 14.3)	0.607 ¹
Age at onset of disease in years ^a , mean ($\pm$ SD)	33.9 ($\pm$ 16.4)	34.2 ($\pm$ 14.5)	0.877 ¹
Duration of disease in months ^a , median (IQR)	24 (8-96)	24 (9-60)	0.860 ³
Female ^a , n (%)	92 (86%)	223 (66%)	<0.001²
Angioedema (in addition to wheals) ^a , n (%)	75 (70%)	181 (53%)	0.002²
Atopy (positive history) ^b , n (%)	46 (43%)	147 (43%)	0.977 ²
Smoking (positive history) ^c , n (%)	26 (25%)	101 (31%)	0.187 ²
Asthma (comorbidity) ^b , n (%)	21 (20%)	58 (17%)	0.552 ²
Hypertension (comorbidity) ^a , n (%)	10 (9%)	37 (11%)	0.651 ²
Diabetes mellitus (comorbidity) ^a , n (%)	8 (8%)	46 (14%)	0.094 ²
Thyroid disease (comorbidity) ^a , n (%)	26 (24%)	60 (18%)	0.128 ²
Psychological disorder (positive history) ^d , n (%)	37 (35%)	99 (29%)	0.301 ²
History of drug allergy (positive history) ^b , n (%)	43 (40%)	104 (31%)	0.068 ²
History of food allergy (positive history) ^e , n (%)	34 (33%)	102 (33%)	1.000 ²
Infection as a trigger of CSU ^e , n (%)	5 (5%)	35 (11%)	0.055 ²
Positive family history of CU ^f , n (%)	23 (22%)	65 (20%)	0.717 ²
Nocturnal pruritus with disturbed sleep ^f , n (%)	63 (59%)	185 (57%)	0.673 ²
Rheumatoid arthritis ^a , n (%)	11 (10.3%)	15 (4.4%)	0.025²
Sjögren ^a , n (%)	5 (4.7%)	2 (0.6%)	0.003²
Total IgE in (IU/ml), median (IQR) ^g	64 (21-160)	110 (44-236)	0.001¹
ESR (mm/h), median (IQR) ^h	11 (5-18)	8 (4-16)	0.128 ¹
CRP (mg/L), median (IQR) ⁱ	3.3 (3.0-7.5)	3.3 (3.0-6.8)	0.765 ¹
B12 level (pg/ml), median (IQR) ^j	246 (183-338)	199 (155-294)	0.003¹
IgG-anti-TPO (IU/ml) (+) ^a	27 (27%)	58 (18%)	0.049²

Abbreviations: ANA, antinuclear antibody; SD, standard deviation; CSU, ESR erythrocyte sedimentation, chronic spontaneous urticaria; IQR, interquartile range (lower quartile – upper quartile). ¹T-test for parametric variables; ²Chi²-test; ³Mann-Whitney U test. Number of patients for whom information was available: ^an=447, ^bn=446, ^cn=429, ^dn=445, ^en=416, ^fn=430. ^gn=427, ^hn=406, ⁱn=401, ^jn=308

Table II. In CSU, ANAs are linked to poor therapeutic responses in patients treated with omalizumab, but not antihistamines

	Treatment response to omalizumab*			
	CR (n=110)	PR (n=33)	NR (n=28)	p
ANA (+) patients, n=33	12 (36%) ^a	6 (18%) ^b	15 (45%) ^b	<0.001 ¹
ANA (-) patients, n=138	98 (71%) ^a	27 (20%) ^b	13 (9%) ^b	

	Treatment response to antihistamine therapy**		
	Responder (n=278)	Non-responder (n= 173)	p
ANA (+) patients, n=107	72 (67.3%)	35 (32.7%)	0.145 ¹
ANA (-) patients, n=340	202 (59.4%)	138 (40.6%)	

Abbreviations: CSU, chronic spontaneous urticaria; CR, complete responder; PR, partial responder, NR, non responder; ¹Chi²-test.

*Response to omalizumab treatment was assessed by measuring disease activity at baseline and week12 of omalizumab treatment by visual analog scale (VAS). Patients who had a more than 80% improvement and a 20%-80% improvement at week12 were considered complete and partial-responders, respectively (non-responders= VAS improvement<20% at week12).

**Resistance to antihistamine was assessed by use of the VAS post treatment with VAS scores ≥7 taken to reflect resistance to treatment.

In patients with chronic spontaneous urticaria, antinuclear antibodies are common and linked to poor response to omalizumab treatment

Study patients and study conduct

In this study, we analyzed 447 patients with CSU between January 2014 and December 2018.

Assessment of antinuclear antibodies

Staining of cytoplasmic structures and mitosis were accepted as “positive ANA” because of possible clinical importance (1). Positive slides were evaluated for their intensity of fluorescence using a five-point scale (+ to +++++). + corresponds to ANA 1/100 positive; ++ corresponds to >1/100, <1/320 positive ANA; +++ corresponds to $\geq$ 1/320, <1/1000 positive ANA; ++++ corresponds to $\geq$ 1/1000, <1/3200 positive ANA, and +++++ corresponds to $\geq$ 1/3200 positive ANA (1, 2). All ANA+ patients underwent a rheumatological diagnostic workup.

Clinical characterization of patients

Patients were assessed for their onset and duration of disease, age at onset of disease, comorbidities ie. hypertension, diabetes mellitus, thyroid disease, occurrence of angioedema (co-existed or appeared during the course of CSU) and history of atopy, i.e. history and/or presence of asthma, allergic rhinitis/sinusitis, atopic conjunctivitis or atopic dermatitis. We also assessed, by use of a questionnaire, patients for comorbid food and drug hypersensitivity, a history of smoking, a family history of CU in the first and/or second grade relatives, history of psychological disorder, infection as a trigger of CSU and nocturnal pruritus with disturbed sleep by asking whether exist or not.

Assessment of laboratory markers in CSU patients

Total IgE levels were measured by quantitative analysis by the nephelometric method (BNTM II System, Siemens, Germany). CRP measurements were also performed nephelometrically (Siemens BN I), and blood sedimentation rate was measured with the Westergren method (Alifax). Serum anti-TPO and vitamin B12 levels were determined with chemiluminescent immunoassays (Beckman-Coulter, Woerden, the Netherlands).

Treatment regimen and assessment and definition of omalizumab treatment responses

Patients were treated in line with the current EAACI/GA²LEN/EDF/WAO guideline for urticaria (3). The first- and second-line treatment of our patients was standard-dosed and up to fourfold standard-dosed antihistamine therapy, respectively.

Patients who were unresponsive to four-fold AH treatment during routine follow-up visits, were considered anti-histamine resistant, and omalizumab therapy was initiated. At baseline, week 4 and week 12, the activity of disease was determined by the use of the VAS.

Statistical Analysis

Parametric variables were presented as means and standard deviations, non-parametric variables were presented as medians and interquartile ranges (lower and upper quartiles).

Kolmogorov Smirnov test and histograms analyses were used to determine whether continuous variables were normally distributed. Levene's test was used for the evaluation of homogeneity of variances. Number of cases and percentages were used for categorical variables. Two independent groups of parametric variables were compared using Student t test. For non-parametric variables Mann Whitney U test was administered. Three independent groups of non-parametric variables Kruskal Wallis test was administered. Post-hoc analyses were done. Categorical data were analyzed by Chi-square or Fisher's exact test, where applicable. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. P values of 0.05 or less were considered to indicate statistically significant differences.

Results

The most common fluorescence patterns were speckled (27%), dense fine speckled (27%), and nucleolar (11%). As expected, the rate of ANA positivity was higher in female patients with CSU ($p<0.001$)

The current study has limitations and strengths. This is a monocentric study, and our patient population may have a selection bias towards patients with severe and difficult to treat CSU. The strengths of this study include the large number of patients investigated and that we assessed them for immunological and clinical markers as well as treatment responses to antihistamines and omalizumab. Moving forward, our results should be confirmed and complemented by future global multicenter studies, for example within the network of GA²LEN urticaria centers of reference and excellence (4).

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