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Correlations between disease activity, autoimmunity and biological parameters in patients with chronic spontaneous urticaria

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Key words

Chronic spontaneous urticaria; blood basophil count; IgE; autoimmunity; autologous serum skin test.

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Abbreviations
AAbs: autoantibodies
ANA: anti-nuclear antibodies
ASST: autologous serum skin test
BAT: basophil activation test
BHRA: basophil histamine release assays
CBC: complete blood count
CRP: C-reactive protein
CSU: chronic spontaneous urticaria
FceRI: high-affinity IgE receptor
IgE: immunoglobuline E
RF: rheumatoid factor
Tg: thyroglobuline
TPO: thyroperoxydase
UAS7: Weekly Urticaria Activity Score

Summary

Background. *Biomarkers of disease activity/severity and criteria of autoimmune chronic spontaneous urticaria (CSU) are still a matter of debate.* ***Objective.*** *To investigate possible correlations between clinical and biological markers and their associations with: 1) disease activity, 2) resistance to H_1-antihistamines, 3) autoimmunity and 4) autologous serum skin test (ASST) in patients with CSU. To also analyze biological parameter modifications in patients with CSU treated with omalizumab.* ***Materials and methods.*** *Disease activity, H_1-antihistamines response and presence of concomitant autoimmune disease were prospectively recorded in 95 patients with CSU. For 60 of them, ASST was performed. Broad biological analysis were performed.* ***Results.*** *C-reactive protein (CRP) serum levels were higher in H_1-antihistamines unresponders ($p < 0.0001$) and in more active diseases ($p = 0.033$). D-dimer plasma levels were higher in H_1-antihistamines unresponders ($p = 0.008$) and in patients with autoimmune status (concomitant autoimmune disease and/or with autoantibodies) ($p = 0.016$). Total immunoglobuline E (IgE) serum level was lower in patients with positive ASST. Blood basophil counts were lower in patients with CSU and especially in H_1-antihistamines unresponders ($p = 0.023$), in patients with more active disease ($p = 0.023$), with positive ASST ($p = 0.001$), and with autoimmune status ($p = 0.057$). Conversely, under omalizumab, a decrease of CRP ($p = 0.0038$) and D-dimer serum/plasma levels ($p = 0.0002$) and an increase of blood basophil counts ($p = 0.0023$) and total IgE serum levels ($p = 0.0007$) were observed.* ***Conclusions.*** *This study brings additional evidences of interest to investigate IgE, D-dimer serum/plasma levels and basophil blood counts in patients with CSU as they could be correlated to disease activity, response to treatment and/or autoimmunity.*

Introduction

Chronic spontaneous urticaria (CSU) is defined as the spontaneous occurrence of wheals and/or angioedema daily or almost daily for more than 6 weeks. The pathogenesis of CSU has not been fully established although it seems clear that different mechanisms are involved. Mast cells have long been the key cells involved in CSU pathogenesis, however new evidence argues in favor of the involvement of other cells, *i.e.,* basophils, eosinophils, lymphocytes, and neutrophils, as well as the involvement of cytokines, coagulation pathways and autoantibodies (AAbs). Autoimmune diseases, particularly autoimmune thyroiditis and thyroid AAbs, seem more prevalent in patients with CSU (1, 2). Several AAbs have been associated with CSU: IgG against thyroperoxydase (TPO) or thyroglobuline (Tg) (1, 3), IgG against IgE or against high-affinity IgE receptor (FceRI) (4, 5), and IgE directed against autoantigens, such as TPO or interleukin 24 (6, 7). Furthermore, some patients with CSU react to the intradermal injection of their own serum resulting in a positive autologous serum skin test (ASST) (8). Also *in vitro* tests (basophil histamine release assays (BHRA) and basophil activation test (BAT)) showed that some CSU serum factors are able to induce histamine release/basophil activation (5, 9). The above-mentioned factors, alone or combined, are often used to classify patients as autoimmune or non-autoimmune CSU.

In this prospective cohort of patients with CSU, we analyzed correlations between several clinical and biological markers and their associations with: 1) disease activity, 2) response to H_1-antihistamines, 3) autoimmunity, 4) ASST. We also analyzed biological parameter modifications in patients with CSU treated with omalizumab (anti-IgE treatment).

Materials and methods

This prospective study was conducted from September 2013 to December 2018 in the department of Dermatology of the Cliniques universitaires Saint-Luc, in Brussels, Belgium. The study and data collection were conducted with the approval of the institutional ethical committee. Informed consent was obtained from all patients.

Patient selection and clinical data collection

Ninety-five adults and adolescents (≥ 12 years old) with a diagnosis of CSU, confirmed by a dermatologist according to the international Guideline 2013 (10), were included. Only patients with active CSU were selected. Patients with pure chronic inducible urticaria or bradykinin-mediated angioedema were not included in the study. Upon enrolment, medical history, including history of personal or familial atopy (asthma, atopic dermatitis and allergic rhinitis) and autoimmune diseases, as well as previous and current treatments for CSU were recorded. Using a validated tool, the Weekly Urticaria Activity Score (UAS7), assessed disease activity (10). Patients were asked to record their symptoms for seven consecutive days prior to day of inclusion. Patients were classified as follows: severe CSU (UAS7 = 28-42), moderate CSU (UAS7 = 16-27), mild CSU (UAS7 = 7-15), well-controlled CSU (UAS7 = 1-6) and itch-and wheals-free (UAS7 = 0) (11).

Response to H_1-antihistamines was also evaluated and assessed using the UAS7 over several months. Patients were classified as follows: H_1-antihistamines responders (UAS7 ≤ 7 with 1 to 4 tablets daily of H_1-antihistamines); H_1-antihistamines unresponders (UAS7 > 7 with 4 tablets daily of H_1-antihistamines).

Disease duration was defined as the time from the first onset of symptoms to day of inclusion. Recurring episodes of CSU, defined as recurrence of symptoms after at least 6 months of spontaneous remission, were also recorded.

For ASST and blood analyses, patients were considered as untreated, if they had stopped H_1-antihistamines for at least 48 hours (or longer, depending on drug activity of each molecule) (8), anti-leukotrienes and H_2-antihistamines for at least 7 days, and corticosteroids or cyclosporine A for at least 1 month and have never taken omalizumab before inclusion.

Autoimmunity and autologous serum skin test

Patients were also classified according to their "autoimmune status". Autoimmune status was inferred in the case of a personal history of concomitant autoimmune disease or in the presence of at least one type of AAbs (included IgG against Tg and TPO, anti-nuclear antibodies (ANA) and rheumatoid factor (RF)). No autoimmunity was defined as the absence of concomitant autoimmune disease and AAbs.

ASST was performed on 60 untreated patients by the intradermal injection of 50 µL of the patient's own serum into the volar part of the forearm (8). Prick tests with histamine and intradermal injection of normal saline solution served as respectively positive and negative controls. A positive test was defined as the appearance, within 30 minutes, of a red wheal with a diameter of 1.5 mm or greater than the wheal produced by the injection of normal saline solution. Patients were classified as either having a positive or a negative ASST.

Biological tests

Blood analyses include: complete blood count (CBC) with differential, platelet parameters, total IgE serum levels, thyroid function tests, serum levels of IgG against Tg and TPO, ANA, RF, C-reactive protein (CRP) serum levels, complement components (C3, C4), C1-inhibitor, classical complement pathways, protein electrophoresis, and D-dimer plasma levels. Titers were considered positive if IgG anti-Tg > 115 U/ml, IgG anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. For basophil blood counts, the reference range often used is from 0 to 200 or 300/

µL. As this range is very large and start from zero, we used a reference mean for blood basophil counts which was established by the department of Clinical Biology of the Cliniques universitaires Saint-Luc based on healthy controls values.

Omalizumab treatment

For 22 patients treated with omalizumab (Xolair , Novartis, Camberley, UK) at the initial recommended dose of 300 mg every 4 to 5 weeks (12), blood tests were performed before initiation and under omalizumab treatment.

Patients were classified according to their response to omalizumab treatment as follows: complete responders if UAS7 was 0, partial responders if UAS7 fell by at least 10 points (but UAS7 0), and non-responders if UAS7 remained unchanged, rose, or fell by less than 10 points. Based on time to response, patients were classified as early responders, if their UAS7 fell by at least 10 points after one month of treatment. Others were classified as late responders.

Statistical analyses

As some clinical or biological data may be missing for some patients, the number of patients studied for each parameter is always indicated. Data for categorical variables are expressed as frequencies followed in brackets by the number patients positive for this parameter over number of patients studied, and for continuous variables as mean ± standard deviation (SD) with minimum and maximum values in brackets. The Pearson's x^2 test was applied to compare percentages of categorical variables. Mann-Whitney test and Kruskal-Wallis test were used to compare continuous variables between categorical variables. Wilcoxon matched-pairs signed rank test was used to compare paired variables, such as values before initiation and under omalizumab. The Pearson correlation coefficient was used to calculate correlation between continuous variables. In all tests, the level of significance was a two-sided P value of less than 0.05. All statistical analyses were performed, and graphs created using SPSS software Version 24 (SPSS, Chicago, IL, USA) and GraphPad Prism Version 8 (GraphPad Software Inc., USA).

Results

Relevant patient data **(table I)**

Clinical data

This study included 95 patients with CSU, 68 women (71.6%) and 27 men (28.4%). Mean age at inclusion was 45 ± 16 years and mean duration of CSU was 4.7 ± 7 years. Mean age of CSU onset was 40 ± 16 years. Angioedema was associated with wheals in 66 patients (69.5%). Recurring episodes of CSU after at least 6 months of symptom free intervals without treatment were reported in 25.3% of patients (23/91). 58.9% (56/95) were H_1-antihistamines responders and 41.1% (39/95) were H_1-antihistamines unresponders. Concerning disease activity based on UAS7, 24 patients (40.7%) have severe disease, 10 patients (16.9%) have moderate disease, 17 patients (28.8%) have mild disease, 3 patients (5.1%) have well-controlled disease and 5 patients (8.5%) were itch-and wheals-free. ASST was performed on 60 patients and 24 (40%) were positive.

Biological parameters

Biological tests that could be influenced by treatment (*e.g.*, cell blood counts, platelet parameters, D-dimer plasma levels, IgE and CRP serum levels, complement components) were analysed only in untreated patients. Mean serum IgE levels was 208.2 ± 451.8 kU/L, with 43.7% (31/71) of patients having levels higher than 150 kU/L and 23.9% (17/71) having levels lower than 40 kU/L. Mean D-dimer plasma levels was 1278.2 ± 1939 ng/ml, with 56.3% (40/71) of patients having levels higher than 500 ng/ml. For both IgE and D-dimer levels, the mean values observed were higher than normal ranges and large variability was seen across patients. Blood basophil counts were lower in patients with CSU (30.1 ± 24/µL) (74 patients) compared with reference mean of healthy controls (40.0 ± 17.3/µL) ($p = 0.019$). No significant abnormalities were found in the rest of the CBC, in protein electrophoresis, nor in complement.

Associations with autoimmune disease, autoimmune serology or atopy

One third of the patients (32/94, 34.0%) had clinical history and/or serological markers of autoimmunity, and therefore were considered as having a positive autoimmune status. Indeed, a concomitant autoimmune disease was present in 18.3% (17/93) of patients, mainly thyroiditis (11/17), and AAbs were present in 30.5% (29/95), predominately anti-TPO (20/92). As well, familial history of autoimmune disease was found in 14,6% of cases (13/89). Nearly half of the patients (45/91, 49.5%) had a personal history of atopy (based on anamnesis).

Correlations between disease activity, response to H_1-antihistamines and clinical or biological parameters

Response to H_1-antihistamines was not correlated with clinical parameters such as angioedema, symptomatic dermographism, duration of the disease, age, weight, gender, personal or family history of atopy. In addition, no association was found between H_1-antihistamines response and concomitant autoimmune disease, presence of AAbs, the positivity of ASST nor blood cell counts or total IgE serum levels.

Table I *- Clinical and biological data of the cohort of patients with CSU.*

	N studied	Numbers (%) or mean ± SD (min-max)	Reference value
Sex female	95	68 (71.6%)	
Age at inclusion (years)	95	45.1 ± 16.2 (13.7-91.5)	
Age of onset (years)	95	40.4 ± 16.4 (10.1-86.6)	
Disease duration		4.7 ± 7 years (2 months-38 years)	
Period of remission ≥ 6 months	91	23 (25.3%)	
Angioedema	95	66 (69.5%)	
Symptomatic dermographism	23	15 (65.2%)	
Personal history of atopy	91	45 (49.5%)	
Familial history of atopy	89	39 (43.8%)	
Personal history of concomitant autoimmune disease	93	17 (18.3%)	
Thyroiditis	17	11 (64.7%)	
Vitiligo	17	3 (17.6%)	
Thyroiditis + Vitiligo	17	1 (5.9%)	
Alopecia areata	17	1 (5.9%)	
Idiopathic thrombopenia purpura	17	1 (5.9%)	
Familial history of autoimmune disease	89	13 (14.6%)	
Positivity of ASST	60	24 (40%)	
CRP serum levels (mg/L)	71	7.6 ± 13.3 (1-78)	< 5
D-dimer plasma levels (ng/ml)	71	1278.2 ± 1939 (250-12687)	< 500
Blood cells counts			
leukocytes (x 10^3/µL)	74	7.52 ± 2.94 (3.26-24.12)	(4.0-10.0)
neutrophils (x 10^3/µL)	74	4.77 ± 2.48 (1.31-18.17)	(1.6-7)
lymphocytes (x 10^3/µL)	74	1.92 ± 0.55 (0.74-3.67)	(0.8-5)
monocytes (x 10^3/µL)	74	0.48 ± 0.14 (0.16-0.84)	(0.2-1)
eosinophils (x 10^3/µL)	74	0.14 ± 0.09 (0-0.47)	(80-600)
basophils (x 10^3/µL)	74	0.03 ± 0.02 (0-0.15)	(0-0.2)
platelets (x 10^3/µL)	74	265.43 ± 76.69 (125-694)	(150-350)
Platelet parameters			
mean platelet volume (µm^3)	74	10.6 ± 1 (8.9-13.4)	(9.1-11.9)
PDW (fL)	74	12.5 ± 2.2 (9.4-19.1)	(9.9-15.4)
plateletcrit (%)	74	0.3 ± 0.1 (0.1-0.7)	(0.17-0.35)
ratio large platelet (%)	74	29.5 ± 8.1 (16.5-53.1)	(17.5-42.3)
Total IgE serum levels (kU/L)	71	208 ± 451.8 (2-3656)	< 150
Positivity of AAbs	95	29 (30.5%)	
AAbs anti-Tg	92	15 (16.3%)	
AAbs anti-TPO	92	20 (21.7%)	
ANA	90	9 (10%)	

Table I *- Clinical and biological data of the cohort of patients with CSU.*

		N studied	Numbers (%) or mean ± SD (min-max)	Reference value
Rheumatoid factor		91	2 (2.2%)	
Autoimmune status		94	32 (34.0%)	
H_1-antihistamines	responders	95	56 (58.9%)	
	unresponders	95	39 (41.1%)	
UAS7		59	21.0 ± 12.6 (0-42)	
Activity based on UAS7				
28-42: severe		59	24 (40.7%)	
16-27: moderate		59	10 (16.9%)	
7-15: mild		59	17 (28.8%)	
1-6: well-controlled		59	3 (5.1%)	
0: itch-and wheals-free		59	5 (8.5%)	

Autoimmune status: concomitant autoimmune disease and/or AAbs.
H_1-antihistamines response: H_1-antihistamines responders (UAS7 7 with 1 to 4 tablets daily of H_1-antihistamines); H_1-antihistamines unresponders (UAS7 > 7 with 4 tablets daily of H_1-antihistamines).
Weekly Urticaria Activity Score (UAS7) was recorded by patient for seven consecutive days prior to sampling day. Patients were classified according UAS7 as follows: severe CSU (UAS7 = 28-42), moderate CSU (UAS7 = 16-27), mild CSU (UAS7 = 7-15), well-controlled CSU (UAS7 = 1-6) and itch-and wheals-free (UAS7 = 0).
Blood analyses that can be influenced by treatment (blood cells, platelet parameters, D-dimer plasma level, IgE and CRP serum levels, complement components) were recorded for untreated patient.
Titers for AAbs were considered positive if anti-Tg >115 U/ml, anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. Cut-off for CRP serum level, for D-dimer plasma level and for total IgE serum level detection were respectively 1 mg/L, 250 ng/mL and 2 kU/L.

Conversely, in H_1-antihistamines unresponders, CRP serum levels ($p < 0.0001$) (**figure 1 A**) and D-dimer plasma levels ($p = 0.009$) (**figure 1 B**) were significantly higher than in H_1-antihistamines responders. Moreover, CRP serum levels and D-dimer plasma levels were positively correlated ($p < 0.0001$).
Disease activity (based on UAS7) was positively correlated to CRP serum levels ($p = 0.033$) (**figure 1 C**) and negatively correlated to blood basophil counts ($p = 0.023$) (**figure 1 D**). Correlation between D-dimer plasma levels and UAS7 did not reach significance ($p = 0.069$).
Disease activity was not associated with clinical parameters nor with the rest of CBC values, total IgE serum level nor with platelet parameters.

Correlation between autoimmune or autoreactive factors and biological parameters

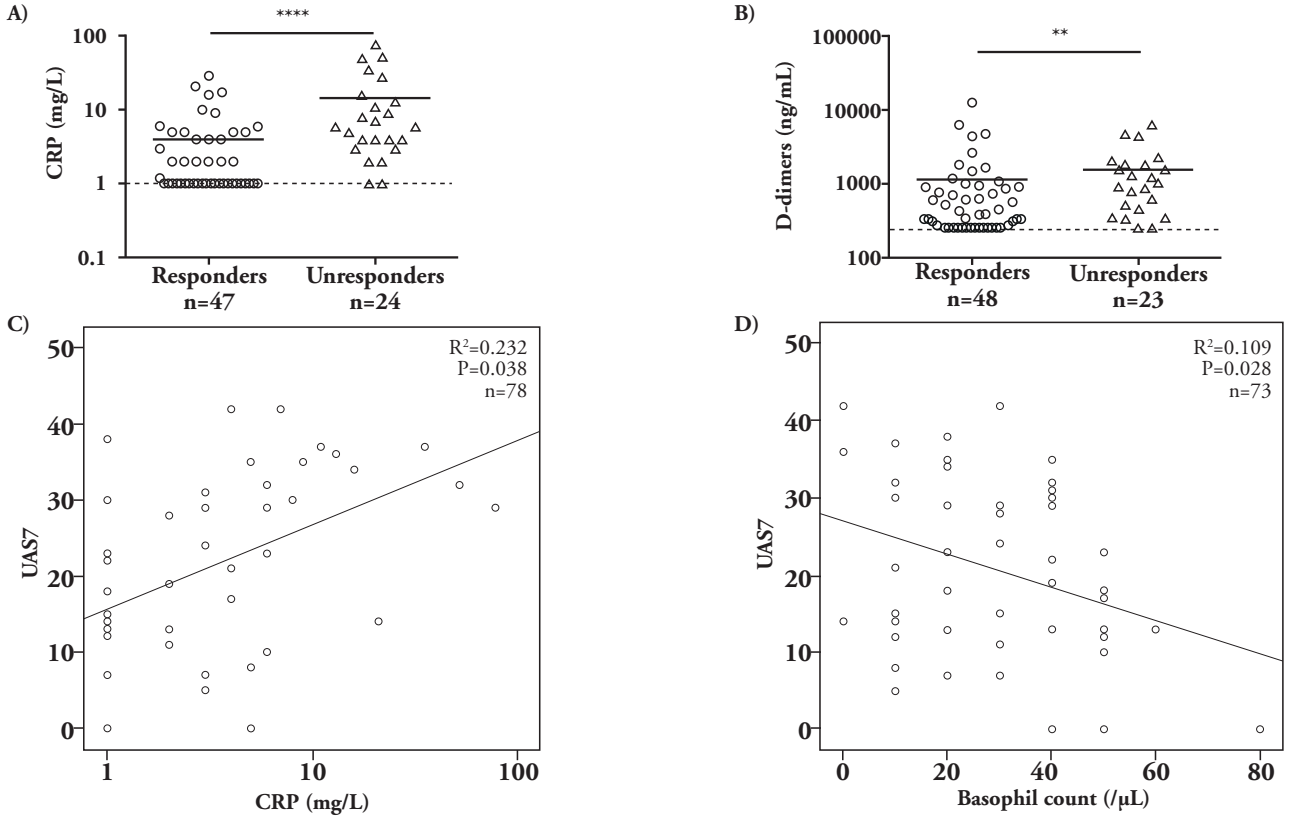
An association between positive ASST results and autoimmune status (defined as the presence of concomitant autoimmune disease and/or AAbs) was observed ($p = 0.037$).

Positivity of ASST was correlated with angioedema ($p = 0.005$) as 87.5% (21/24) of patients with positive ASST had angioedema in contrast to 52.8% (19/36) with negative ASST.
Blood basophil counts (**figure 2 A**), blood monocyte counts, and mean total IgE serum levels (**figure 2 B**) were lower in patients with positive ASST (respectively $p = 0.001$, $p = 0.019$ and $p = 0.016$). However, other clinical and biological parameters were not correlated with ASST results.
D-dimer plasma levels were higher ($p = 0.016$) (**figure 2 C**), and blood basophil counts lower ($p = 0.057$) (**figure 2 D**) when an autoimmune status was present. Blood platelet counts and plateletcrit were also higher in patients with autoimmune status (respectively $p = 0.010$ and $p = 0.002$). Other clinical and biological parameters were not correlated with autoimmune status.

Omalizumab: before initiation and under treatment

For 22 patients, biological parameters were compared before initiation and under omalizumab. 11 patients were complete responders, 8 partial responders and 3 non-responders to omalizumab. Among responders, 17 patients were early responders

Figure 1 - (A) *Positive correlation between CRP serum levels and H_1-antihistamines response. Responders: 4.0-5.7 mg/L (1-29); unresponders: 14.7-19.9 mg/L (1-78).* ***(B)*** *Positive correlation between D-dimer plasma levels and H_1-antihistamines response. Responders: 1144-2093 ng/mL (250-12687); unresponders: 1558.5-1574.3 ng/mL (250-6260).* ***(C)*** *Positive correlation between CRP serum levels and disease activity (UAS7).* ***(D)*** *Negative correlation between blood basophil counts and disease activity (UAS7). Untreated patients. UAS7 recorded seven consecutive days before the blood sample.*



Each symbol represents one patient. Solid horizontal line represents mean.
P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05.
Cut-off for CRP serum level and for D-dimer plasma level detection were respectively 1 mg/L and 250 ng/mL (dotted lines).

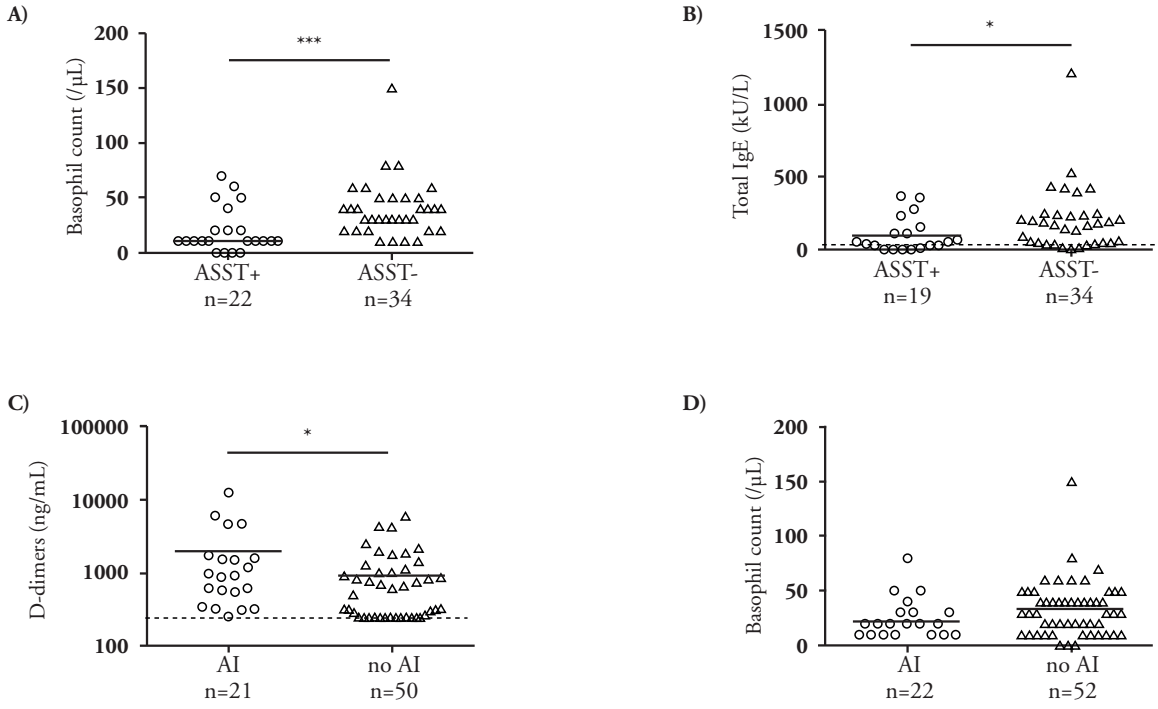
and 2 were late ones. All data concerning patients before initiation and under omalizumab treatment are reported in **table II**. Significant reductions in CRP serum levels (p = 0.0038) and D-dimer plasma levels (p = 0.0002) were observed under omalizumab treatment, whereas increases were observed in blood basophil counts (p = 0.0023) (**figure 3**) and total IgE serum levels (p = 0.0007). Blood basophil counts increased after omalizumab in 13/19 patients, with a mean increase of 113% (20-200%). No differences for the rest of CBC and platelet parameters were observed.

Neither clinical, nor biological parameters (including ratio of these parameters under omalizumab and before initiation) were associated with omalizumab response or with delay of response. However, the number of patients in each group was insufficient to have a statistically significant analysis.

Discussion

Since nearly 30 years, several lines of evidence argue for an autoimmune basis of CSU, or at least in a subgroup of them. However, the way to distinguish autoimmune and non-autoimmune CSU is still a matter of debate (13, 14). In this study, we focused on correlations between several biological parameters, concomitant autoimmune disease and/or presence of AAbs (included IgG anti-Tg, IgG-TPO, ANA and RF) and positivity of ASST. We found a relatively high incidence of autoimmune disorders and AAbs (autoimmune status) in patients with CSU. One

Figure 2 - (A) *Lower blood basophil counts in patients with ASST + than with ASST -. ASST +: 19.6-20.6/µL (0-70); ASST-: 39.7-26.6/µL (10-150).* ***(B)*** *Lower total serum IgE levels in patients with ASST + than ASST -. ASST +: 103.4-120.2 kU/L (2-368); ASST -: 207.3-225.5 kU/L (10-1216).* ***(C)*** *Higher D-dimer plasma levels in patients with autoimmunity than patients without autoimmunity. AI: 2050-2952 ng/ml (256-12687); nonAI: 953. 9-1205 ng/ml (250-6207).* ***(D)*** *Lower blood basophil counts in patients with autoimmunity than patients without autoimmunity. AI: 22.7-18.8/µL (0-80); nonAI: 33.3-25.4/µL (0-150). ASST + and ASST -: patients with positive or negative ASST respectively. AI and noAI: patients with autoimmune status (concomitant autoimmune disease and/or positive for at least one AAbs) and patients without autoimmune status (no concomitant autoimmune disease, no AAbs).*



Each symbol represents one patient. Solid horizontal line represents mean.
P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05.
Cut-off for total IgE serum level and D-dimer plasma level detection were respectively 2kU/L and 250ng/mL (dotted lines).

third of the patients had concomitant autoimmune disease and/or AAbs, mainly autoimmune thyroiditis and IgG against TPO. Moreover, a familial history of autoimmune disease was also found in 14,6% of patients. Recently, Schoepke *et al.* showed that autoimmune CSU (defined by the presence of IgG anti-IgE or anti-FceRI, a positive BAT and a positive ASST) have significantly higher IgG against TPO than patients with non-autoimmune CSU (15). In our cohort, we found a correlation between positive ASST and presence of concomitant autoimmune disease and/or AAbs. This association was not always found in previous studies, a discrepancy which could be explained by the fact that we got interest for both; presence of concomitant autoimmune disease and AAbs (16-19). The proportion of positive ASST in our cohort of patients with CSU (40%) is consistent with previous reports (30% to 50%) (8, 20). Presence of concomitant autoimmune disease and/or AAbs or positivity of ASST was not correlated with disease activity nor with H_1-antihistamines response. In the literature, this association between ASST and disease activity remains controversial (16, 20-22). In line with previous reports, angioedema was more frequent in patients with positive ASST in our cohort (17, 18). This study put forward that personal and familial autoimmune disease history as well as autoimmune serology, especially IgG against TPO, are easy to get and could be interesting to record in patients with CSU.

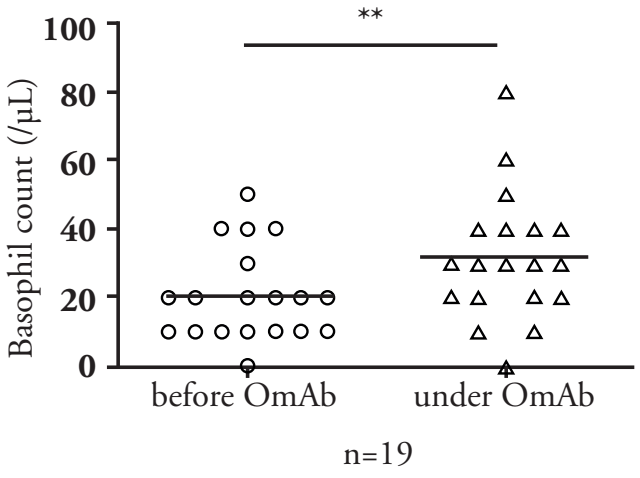
As several studies had postulated that CRP and IgE serum levels or D-dimer plasma level could be considered as biomarkers of CSU or CSU activity, we have measured them and looked for

Table II *- Clinical and biological data for patients treated with omalizumab. Comparison before initiation and under omalizumab.*

	All omalizumab patients				
	N studied	**Number (%) or mean ± SD (min-max)**			
Sex female	22	17 (77.3%)			
Age (years)	22	42.7±14.2 (13.7-70.2)			
Weight (kilogrammes)	4	77.3 ± 23.8 (61-112)			
Angioedema	22	16 (72.7%)			
Duration of disease (years)	22	4.5 ± 7.9 (0.5-38.3)			
Period of remission ≥ 6 months	22	2 (9.1%)			
Autoimmune status	22	9 (40.9%)			
Positivity of ASST	10	4 (40%)			
	Before omalizumab initiation		**Under omalizumab**		
	N studied	**number (%) or mean ± SD (min-max)**	**N studied**	**number (%) or mean ± SD (min-max)**	**P-value**
UAS7	18	31.4 ± 7.4 (17-42)	12	6.3 ± 11.4 (0-38)	
Activity based on UAS7					
28-42: severe	18	14	12	1	
16-27: moderate	18	4	12	1	
7-15: mild	18	0	12	2	
1-6: well-controlled	18	0	12	2	
0: itch-and wheals-free	18	0	12	6	
CRP serum levels (mg/L)	18	18 ± 21.9 (1-78)	18	5.1 ± 5.4 (1-23)	0.0038
D-dimer plasma levels (ng/ml)	17	1668 ± 1795 (250-6260)	17	397.1 ± 307.8 (250-1248)	0.0002
Leukocytes (x 10^3/µL)	19	7.9 ± 2.4 (4.7-12.2)	19	7.4 ± 2.1 (4.3-12)	0.35
Neutrophils (x 10^3/µL)	19	5.2 ± 2.1 (2.4-9.1)	19	4.6 ± 1.6 (2.4-8.2)	0.10
Lymphocytes (x 10^3/µL)	19	2.1 ± 0.7 (0.7-3.7)	19	2.1 ± 0.7 (0.6-3.8)	
Monocytes (x 10^3/µL)	19	0.5 ± 0.2 (0.2-0.7)	19	0.5 ± 1.1 (0.3-0.7)	0.25
Eosinophils (x 10^3/µL)	19	0.1 ± 0.08 (0-0.3)	19	0.1 ± 0.06 (0-0.2)	0.83
Basophils (x 10^3/µL)	19	0.02 ± 0.01 (0-0.05)	19	0.03 ± 0.02 (0-0.08)	0.0023
Platelets (x 10^3/µL)	19	282 ± 58.2 (196-378)	19	275.7 ± 39.3 (216-359)	0.75
Mean platelet volume (µm^3)	19	10.5 ± 0.9 (9.1-12.6)	19	10.7 ± 0.8 (9.1-12)	0.15
Total IgE serum levels (kU/L)	18	137.4 ± 121.9 (2-425)	18	458.6 ± 420.7 (2-1243)	0.0007
Response to omalizumab					
complete responders			22	11 (50%)	
partial responders			22	8 (36.4%)	
non responders			22	3 (13.6%)	
Time to respond to omalizumab					
early responders			19	17 (89.5%)	
late responders			19	2 (10.5%)	

Autoimmune status: concomitant autoimmune disease and/or AAbs.
Weekly Urticaria Activity Score (UAS7) was recorded by patient for seven consecutive days prior to sampling day.
Titers for AAbs were considered positive if anti-Tg >115 U/ml, anti-TPO > 34 U/ml, ANA > 1:160, and RF > 1:40. Cut-off for CRP serum level, for D-dimer plasma level and for total IgE serum level detection were respectively 1 mg/L, 250 ng/mL and 2 kU/L.

Figure 3 *- Elevation of blood basophil counts under omalizumab treatment. Before omalizumab initiation: 20.5 ± 13.5/µL (0-50); under omalizumab: 31.6 ± 18.6/µL (0-80).*



Each symbol represents one patient. Solid horizontal line represents mean. P-value: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, blank p > 0.05. Before OmAb: patients before beginning of omalizumab treatment; under OmAb: patients under omalizumab.

correlations with each other, and with clinical data in this prospective study of 95 CSU patients.

CRP serum level has been proposed as a biomarker of disease activity in patients with CSU, however results are contradictory (23, 24.) Our findings show that CRP (mean CRP 7.7 mg/L) was higher in CSU patients with more active disease and in H_1-antihistamines unresponders. However, to our opinion, CRP is not an interesting biomarker in CSU due to its relatively low levels compared with others inflammatory diseases and due to possible fluctuations by intercurrent phenomena which are not always reported especially in retrospective studies. CRP is more useful in the differential diagnoses of CSU, a high CRP level, not reducing under treatment, could be a sign of an auto-inflammatory syndrome.

Several studies have shown 1) higher D-dimer plasma levels in patients with CSU compared with HCs, 2) higher D-dimer plasma levels in more active disease, as well as 3) normalization of D-dimer plasma levels during remission (25, 26). In our cohort, D-dimer plasma levels were higher in H_1-antihistamines unresponders and to a lesser extent, in more active disease. Interestingly, we also found that patients with autoimmune status had higher D-dimer plasma levels than those without autoimmunity. Furthermore, in line with previous reports, we found a significant decrease of D-dimer plasma levels under omalizumab treatment. Baseline D-dimer plasma levels do not predict the response to omalizumab (27-29), whereas the decrease of D-dimer plasma levels under omalizumab treatment seem to be a marker of good response to treatment (30). Measurement of D-dimer plasma levels could be added to clinical tools, such as UAS7, to evaluate activity/severity. Concerning response to treatment, as well as relation between D-dimer plasma levels and autoimmunity, further studies are needed.

Mean total IgE serum levels have often been studied in patients with CSU and have been proposed as a biomarker of disease activity, however, results remain contradictory (31, 32). In our study, the mean total IgE serum levels of untreated patients were in normal range or little high (208.2 ± 451.8 kU/L, except in atopic patients for which is high), and less than half of patients had total IgE serum levels higher than normal reference value. Total IgE serum levels didn't correlate with disease activity nor with response to H_1-antihistamines. Interestingly total IgE serum levels were lower in patients with positive ASST (mean 103.37 ± 120.25 kU/L) compared with negative ASST (mean 207.32 ± 225.51 kU/L) ($p = 0.016$). This finding is in line with recent publications showing that very low total IgE and positive ASST have been relat ed with poor/no response and with slow response to omalizumab respectively (27, 33-36). Moreover, Schoepke *et al.* put forward that autoimmune CSU have significantly lower total IgE serum levels than non-autoimmune CSU (15). Low baseline IgE has been described as a marker of poor response (27, 34, 37). However this has not been confirmed by all studies (38, 39). Interestingly, a recent paper suggest that total IgE levels can be used as predictors of response to omalizumab only in nonatopic CSU patients, actually they showed that the atopic status modify the ability of IgE to predict the response to the treatment (40).

Several authors have discussed a possible main role of basophils. Indeed, in our study, we found that blood basophil counts were significantly lower in patients with CSU compared with healthy controls. Moreover, blood basophil counts correlated with disease activity. Basophils are probably recruited into the skin during wheal formation, as evidenced by an abundance of basophils in skin samples (41, 42), and low blood basophil counts in patients with chronic urticaria (43, 44). Furthermore, in our study, blood basophil counts were significantly lower in patients with positive ASST, and also tended to be correlated with presence of concomitant autoimmune disease and/or AAbs. This correlation has previously been poorly investigated and with controversial results (21, 43, 45, 46). Nevertheless, expression of activation markers, such as CD203c and CD63, has been found to be higher in blood basophils of CSU patients with positive ASST compared to patients with negative ASST (45, 47). It is tempting to speculate that in patients with positive ASST, basophils are implicated and activated in a more important way, and thus reduced in blood due to recruitment into the skin. Mechanisms implicated in basophils activation/recruitment in skin are actually unknown, however according to previous findings, AAbs could be indirectly implicated.

Additionally, under omalizumab treatment, we observed a significant increase in blood basophil counts, suggesting that omalizumab blocks this basophil shift from the bloodstream into the skin. Unfortunately, our cohort of patients was too small to identify differences between responders/unresponders and fast/slow responders to omalizumab. In the same line, previous authors have already observed increased blood basophil counts in correlation with improvement on treatment or remission (48, 49). However, data concerning blood basophil counts in patients with CSU under omalizumab treatment are scarce (50-53).
Consistence of this study is to have analyzed several biomarkers, evaluated in untreated patients, as well as a series of clinical parameters in a prospective cohort. Limitations are mainly due to the fact that correlations between clinical/biological parameters and omalizumab response (or delay of response) were not possible due to the small number of patients in each group. Secondly, concerning autoimmune CSU investigations, we haven't performed functional tests as BAT and BHRA nor IgG anti-FcεRI/IgE measurement.

Conclusions

To conclude, in this prospective study, we found a relatively high incidence of concomitant autoimmune disease and AAbs. D-dimer plasma level is higher in H_1-antihistamines unresponders and in patients with autoimmune status. Total IgE serum levels were lower in patients with positive ASST compared with negative ASST. We found lower blood basophil counts in patients with CSU compared with healthy controls. Moreover, this finding was more significant in patients with positive ASST and to a lesser extent in patients with autoimmune status. Moreover, under omalizumab, blood basophil counts and total IgE serum levels increased and conversely D-dimer plasma levels decrease.
Our study brings additional evidences over the utility of those clinical and biological parameters to investigate in patients with CSU as they could be related to disease activity, response to treatment or autoimmunity.

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Conflict of interests

L.d.M. reports medical investigator/advisor and educational activities for Novartis. A-S.D. reports educational activities for Novartis. A.G.A. acted as medical advisor for Uriach Pharma, Genentech, Novartis, FAES, GSK, Sanofi. A.G.A reports research Grants supported by Uriach Pharma, Novartis, Grants from Instituto Carlos III- FEDER and educational activities for Uriach Pharma, Novartis, Genentech, Menarini, LEO- PHARMA, GSK, MSD, Almirall, and Sanofi. M.B. reports medical investigator/advisor and educational activities for Novartis.

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