

# Two different Bioclinical profiles of Chronic Urticaria suggested by basophil number and Reactivity

**Keywords:** Basophil Activation Test; Chronic Urticaria; Basopenia; Low IgE level; Basophil reactivity

## Abstract

**Chronic Urticaria (CU):** is an heterogeneous disease supposed to be due to spontaneous release of histamine from unclarified activation of mast cell or basophil through IgE receptor pathway but treatment targeting either histamine or IgE are not always successful. The aim of this study was to explore Basophil phenotyping and functionality to better classify CU.

**Methods:** the prospective, clinical study enrolled 31 CU and 29 age and sex matched controls. Basophils were analyzed by flow cytometry.

**Results and Discussion:** Our CU population demography was very similar to cohorts previously reported. CU were active for 5.58±5.4 years and severe (UAS7 = 25.8±10). Serum IgE were higher than 114kU/L in 36.8% CU vs 12.0% HC. Serum tryptase was higher than 7µg/L in 20%. Basophil represented less than 0.1% of leukocytes in 32.3% CU and even more in case of recent flares. Ex vivo basophil activation was defective in 75.9% of CU vs 31% of HC. However despite an active disease, 41.9% of patients kept a high basophil count and 7 (24.1%) a high reactivity with low serum tryptase and low activation profile suggesting their basophils are not the main effector in the diseases.

**Conclusions:** Monitoring Basophil count and ex vivo reactivity together with the level of IgE should help in suspecting the basophil and IgE involvement or alternative mechanisms of CU. This could lead to a classification of CU in its heterogeneity and help predicting its response to anti-histaminic or anti-IgE therapies.

## Abbreviations

ASST: Autologous Serum Skin Test; FCM: Flow Cytometry; MFI: Median Fluorescence Intensity; Basophils: Basophils; CU: Chronic Urticaria; HC: Healthy Controls; BMI: Body Mass index; tIgE: Total serum IgE; BAT: Basophil Activation Test; EDTA: Ethylene Diamine Tetra Acetic Acid; PBS: Phosphate-Buffered Saline; FITC: Fluorescein isothiocyanate; PE: Phycoerythrin; APC: Allophycocyanin; NS: non-significant; Ig: Immunoglobulin; NR ex vivo Non-reactive (Basophils); FcεRI high affinity receptor for IgE; IL3 Interleukin 3; UAS7: Urticaria Activity Score other 7 days; UCT: Urticaria Control Test

## Introduction

Chronic Urticaria (CU) is characterized by frequent, sudden occurrences of transient itchy wheals and/or angioedema, over a period of at least 6 weeks. Crises occur without any identified allergen or exogenous triggers (spontaneous CU; CSU) unless it is triggered by physical signals (inducible CU; CINDU) such as dermatographism, heat or cold contact, pressure or vibrations, water, sun or neuro-endocrine stress [1-3]. CU is frequently associated with atopy or auto-immunity [4]. CU is more frequent in females for unclear



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reasons. CU can appear at any age and persist for 3-5 years before disappearing. Approximately 1/5 cases persists for more than 5 years [1,2]. More severe the disease is, longer it persists [5].

The diagnosis of CU is essentially clinical. The daily Urticaria Activity Score over 7 days (UAS7) measures the disease activity/severity, while the Urticaria Control Test (UCT) measures the treatment efficacy [3,6]. CU is considered highly active when UAS7 is higher than 27 and is poorly controlled by the treatment when UCT is below 12. CU is frequently associated with obesity (Body Mass Index -BMI- higher than 27) [7] and raise of D-dimer [8, 5]. Obesity is related to the patient age [9-11].

It is generally admitted that CU symptoms are due to inappropriate mast cells and basophil degranulation. Accordingly, it was shown that these cells infiltrate the wheal lesions [9,12] and that basophil count is reduced during flares, as demonstrated long before Flow cytometry (FCM) was used [13-15]. Basopenia is believed to be due to local recruitment [16]. Histamine and tryptase raise may be detected in serum during flares [17-19] and non-sedative anti-Histamine (H1) drugs may relieve symptoms, but frequently require high doses. Total serum IgE (tIgE) are usually in the normal range (<114kU/L) unless the patient is atopic. IgE play an important role in mast-cell/basophil homeostasis [20] and basophil degranulation is easily induced ex vivo with anti-IgE or anti-IgE receptor type I (Fcε-RI) antibodies. Anti-IgE biotherapy (namely Omalizumab) has remarkable efficiency in preventing CU's flares in a great majority of the patients [21,22]. Anti-IgE biotherapy is known to prevent the IgE binding to mast cells and basophil membrane Fcε-RI. So, IgE, Fcε-RI, degranulation and release of histamine are considered as critical in inducing CU symptoms but still, CU's physiopathology remains unknown and not all patients are sensitive to anti-H1 or Omalizumab treatments.

CU may be an auto-immune disorder. Indeed, CU is frequently associated with autoimmune diseases like thyroiditis. Furthermore,

Skin Test with Autologous Serum (ASST) may induce wheals possibly due to the presence of auto-antibodies ASST is not used in France for ethical reasons. difficult toe for inter laboratory comparison [23,24]. Several arguments suggest that major targets of autoimmunity in CU are either FcεRI or IgE most of which being bound to their membrane receptors (named Type II Autoimmune CU). Unfortunately there is no reliable method available yet to detect these auto-antibody, except the ASST. Alternatively IgE auto-immunity to organs or tissue have been suspected (named type I auto-immune CU or Auto-Allergy) and IgE anti-thyroperoxidase, thyroglobulin and interleukin-24 have been reported in CU [6, 25-27].

The Basophil Activation Test (BAT) measures basophil degranulation by FCM despite the low concentration of basophils in peripheral blood. Basophils are identified by immunodetection of membrane proteins such as CCR3, CD123 (together with plasmacytoid dendritic cells) or CD203c. CD203c that is basically expressed on basophils and is upregulated during stimulation. Basophil granules express protein p53 (CD63) in their inner side of the membrane, not accessible to immunolabelling of fresh cells. Basophil degranulation induces a fusion of granules with the cytoplasmic membrane and a strong and rapid expression of CD63 that become detectable on the surface of the cells [28]. Usually, less than 5% of basophils spontaneously express CD63. Degranulation is rapidly induced by allergenes in allergic patients. BAT sensitivity is generally improved by IL-3 for diagnosis. using Furthermore, a default in ex vivo basophils reactivity (PR) has been reported, during sample preparation [15,29,30]. Basophil general reactivity is measured by challenging them with Fc symbol

RI or IgE monoclonal antibody. Note that BAT is performed in whole blood and elevated plasma IgE can compete with in anti-IgE stimulation. Basophil reactivity can also be tested with N-formyl-methionyl-leucyl-phenylalanine (FMLP) a strong stimulant of a G protein-coupled receptor (FPR1), independently of Fcε-RI. Generally, anti FcεRI, anti IgE or FMLP induce degranulation of up to 90% of basophils in a dose dependent manner. For unclarified reasons, few patient's basophils have a poor ex vivo reactivity (PR), even when IL-3, as opposed to Good reactive (GR). The "non-responder" term is some time used but is a source of confusion because it is also used for patients who are not sensitive to the treatment. In CU, PR frequency is increased and is related to the disease severity [31] decreasing during remissions [14,32] suggesting some causality link. During flares, some level of basophils activation has been reported on expression of CD69 [33], CD203c [34] or even some spontaneous expression of CD63 [23,33]. An indirect BAT assay has been proposed to reproduce ASST using patient serum and a donor basophils or a mast cell line but this test has poor performances and strongly need for standardization [23,24].

So, IgE, mast cells/basophils and histamine release are considered to be critical in the physiopathology of CU. An inappropriate mast cell degranulation can be intrinsic or extrinsic mechanisms that are not all identified. Several auto-immune mechanisms targeting mast-cell/basophils are suspected but difficult to evidence and only explain part of the cases. As a matter of fact, treatments targeting histamine [35-37] or IgE [38-40] are frequently inefficient. As we regularly explore basophil as part of allergy diagnosis, we thought measuring

basophil reactivity could help in a better understanding of the role of basophil /IgE regarding the heterogeneity of CU.

The aim of this study was to find Basophil phenotype and functional parameters that could help in better classifying CU. To address that question, we performed large biological study of basophils in a prospective, monocentric, case control study of CU out-patients.

## Materials and Methods

Between March 2016 and September 2017 we performed a prospective observational study on patients with CU and compared them to age and sex matched healthy controls (HC) from the melanoma preventive outdoor clinic in the dermatology department. HC with previous history of inflammatory or allergic skin diseases or urticaria were not included. The diagnosis of urticaria was based on clinical history and physical examination according to European Guidelines guidelines [3]. Patients were invited to participate to the study the first day they were addressed to the clinic. Data were collected on age, gender, IgE levels, and allergy (asthma, atopic eczema, food allergy, and allergic rhinitis). The disease activity was evaluated using UAS7 and UCT scores according to European guidelines [3]. During the same time, age and sex matched healthy controls (HC) who attended the clinic for detection of melanoma, without any history of urticaria were informed of the study. Patients and volunteers who have accepted to sign the informed consent were enrolled and tested the same day.

Serum total IgE (reference value<114kU/L) and serum Tryptase (reference value<11.4kμg/L) were measured using ImmunoCAP<sup>®</sup> (UNICAP 250, Thermo Fisher Scientific, Uppsala, Sweden). Serum total IgE were calibrated between 2 and 5000 kUI/L as referred to the international 75/502 WHO standard) and Tryptase was calibrated between 1 and 200 μg/L. D Dimer values were measured using Vidas D-dimer exclusion II DEX2 (Biomérieux Lyon France). D Dimers reference values were below <500u.

Basophil phenotyping was performed on EDTA anti-coagulated blood the day of enrolment. Basophils were identified using anti-CD123 antibody conjugated with Allophycocyanin (APC)- Alexa 700 (clone SSDCLY107D2, Beckman-Coulter; Fullerton, CA), anti-HLA DR Horizon-V450 (clone L243, BD Biosciences<sup>®</sup> San Jose, CA). Basophil activation was measured using BasoflowEx<sup>®</sup> kit (Exbio, Praha, Czech Republic) adapted as described recently [28]. Briefly, EDTA samples were diluted 1/1 with Hanks saline Buffer complemented with calcium chloride 20μmol/ml (Renaudin laboratory, France) and sodium heparinate 500 UI/ml (Choay; Sanofi-Aventis<sup>®</sup>, France). No IL-3 was added in the test. Basophils were firstly either unstimulated or stimulated with anti-IgE antibody (clone BE5, Exbio, Praha) at 10μg/ml or part of the cohort with anti-Fcε-RI 5 μL (provided in the Bühlmann Laboratories AG kit, Schönenbuch, Switzerland) and with anti-IgE +formyl-methionyl-leucyl-phenylalanin (provided in the Exbio BasoflowEx<sup>®</sup> kit). The samples were stained with anti-CD203c-PE monoclonal antibody (clone NP4D6) and anti-CD63-FITC (clone MEM-259, Exbio, Praha) during the activation time, for 20 minutes in the dark in a 37°C water bath. Erythrolysis was performed using Immunoprep on TQ-PREP Workstation (Beckman-Coulter; Fullerton, CA). At the end, the sample was washed in Phosphate

buffer (PBS, Eurobio, France) and re-suspended in PBS-1% Bovine serum Albumin fraction V (BSA, Eurobio Courtaboeuf France) and analyzed within 4 hours on Navios<sup>™</sup> cytometre using Navios<sup>™</sup> software (Beckman-Coulter).

Data were analyzed on Kaluza<sup>™</sup> software (Beckman-Coulter): cell shape was checked on Forward/side scatter dot plots. Bubbles were excluded on basophil label versus time scatter. Cell doublets were excluded on Forward area under curve/ height scatter. Basophils were then selected on their specific expression of CD203c versus Side Scatter (SSC) and the fractions of activated basophils (CD63<sup>+</sup>) as well as the CD203c/CD63 labelling (MdFI) were measured among this population. Cytometer settings were checked daily using quality controls procedure according to the manufacturer instructions. Compensations of spectral overlaps were set up using single labeling on anti-mouse IgG beads (Versacomp<sup>™</sup> beads, Beckman-Coulter) and calculated using the software application on Navios<sup>™</sup> software.

### Statistical Analysis

Correlations were analyzed using linear regressions, Student T and chi<sup>2</sup> tests from Excel (Microsoft Corporation, Redmond, WA). Comparison between groups was tested with the non-parametric

Mann-Whitney or Kruskal-Wallis tests, while categorical variables were assessed using the Chi-Square or Fisher's exact tests using GraphPad<sup>™</sup> software.

### Ethical issue

All subjects submitted a written informed consent form at the time of their enrollment into the present study. This biological study was performed blindly on anonymous blood samples collected for diagnosis purpose in accordance to 2011-814 bioethical French law.

### Results

#### Our CU population demography was very similar to previously reported cohorts (Table 1)

Thirty one patients with CU were enrolled in the study, 20 were females (65%) and 11 males, with a mean age of 48.4±17.5 years (from 19 to 85). CU patients were compared to 29 HC of which 18 (62%) were females and 11 males with a mean age of 50.1±17.0 (from 23 to 83) years. Demographic and clinical features are summarized in Table I. The patients claimed to have the disease active for 5.58±5.4 years; 21 patients (77.4%) had CU for more than 1 year, up to 21. The UAS7 obtained from 19 patients only, reflected that CU was severe.

**Table 1:** Basophil specific criteria in Chronic Urticaria as compared to healthy donors.

	Healthy (n=29)	Urticaria (n=31)	PR CU (n=14/31)	GR CU (n=7/29)	p=
Age (years)	50.1±17.0 [23 to 83]	48.4±17.5 [19 to 85]	51.9±17.1	38.4±17.3	NS/0.10
Female nb (%)	18 (62.1%)	20 (64.5%)	68.2%	57.1%	
CU duration (years)		5.58±5.4	3.3±2.7	5.0±3.8	
UAS7 (n=19)		25.8±10	25.3±8.4	27.0±15.3	
UCT		3.7±2.9	3.3±2.7	5.0±3.8	NS
Flares ongoing		11 (35%)	9 (40.9%)	1 (14.3%)	
Spontaneous CU		16 (56.1%)	12 (54.5%)	5 (71.4%)	
Dermographism		14 (45.2%)	10 (45%)	2 (28.6%)	
Allergy	3 (10%)	4 (12.9%)			
BMI (kg/m <sup>2</sup> )	24.3±4.51	27.5±5.22	27.4±4.8	27.6±7.7	<b>0.0171</b>
BMI > 25	8 (28.6%)	18 (62.1%)	12 (57.1%)	4 (66.6%)	<b>0.0111</b>
D-dimer (u)#	241 ; [ 136 to 5651]	373 [ 120 to 10 010]	416 [120-3515]	347 [161-1040]	NS /NS
D-dimer >800u	3 (11.5%)	7 (28%)	6 (38%)	1 (17%)	NS
Total serum IgE (kU/L) [range]	74.5±124 [2-611] (n=25)	149±178 [5 – 621] (n=19)	160.2±190 (n=15)	4.5±1.6 (n=2)	<b>NS/0.0067</b>
tlgE >114kl/L	3 (12.0%)	7 (36.8%)	6 (40%)	0	<b>0.0515</b>
tlgE < 40kl/L	14 (56.0%)	5 (26.3%)	3 (20%)	2 (100%)	<b>0.0489</b>
Tryptasemia (µg/L) [range]	4.7±1.8 [2.2 – 9.6]	5.8±4.2 [1.8-19.9]	6.6±4.6	3.0±1.0	NS/0.0049
Tryptasemia > 7	1 (3.8%)	5 (20.0%)	5 (26%)	0	*0,0735
Basopenia <0.1% of leukocytes	2 (6.9%)	10 (32.3%)			0.0141#
Basal expression of CD203c	6.22±5.27	7.07±5.50	7.07±5.50		
Basophil stimulation					
% PR	9/25 (31%)	22/29 (75.9%)			
%CD63+					
under anti-IgE	42.3±25.5	18.8±18.0%	10.2±10.0	45.9±6.0	<0.001
under anti-IgER	17.6±20.2 (n=9)	10.4±16.2% (n=16)	6.4±12.6	27.8±21.0	NS15
under anti-IgE + fMLP	50.0±19.3 (n=8)	32.0±18.2% (n=7)	32.0±18.2	nd	0.085

\*NS; #Chi<sup>2</sup> or Fisher's test, chi<sup>2</sup> test otherwise Student T test. DDimers: median and [range]

Basophils/Lympho ratio of % number of Basophils on number of lymphocytes x100. Basophils <1% as % of all leukocytes. D-Dimer compared between patients treated or not with Steroids. tlgE = total Serum IgE; BMI = Body Mass Index; UAS7 Urticaria Activity Score other 7 days; UCT Urticaria Control Test. Basophils Poor Reactivity when anti-IgE stimulation induced less degranulation (CD63+) of less than 30% of Basophil.



The UCT score was reported by all but one patients and was lower than 11 in 29 out of 30 patients, and below 5 in 63.3% of the patients. Among 19 patients who provided both UCT and UAS7 scores, 84.2% had a high UAS7 >27 and a low UCT<5.

Urticaria flares were induced (CINDU) in 15 patients, all presenting dermographism and 4 of them having crisis triggered: one by pressure, one by cold air, one by cold water and one by hot air or stress. CINDU and spontaneous CU (CSU) had similar distributions of age or sex, CU duration and BMI, UAS7 or UCT scores. One CSU patient had a history of allergy to food while among CINDU patients, one was allergic to pollens only, one to bee venom and one, whom flares were triggered by cold water, was allergic to animal fur and pollens. Comparatively, 3 HC had an allergy. Allergic CU patients had similar ages, BMI, IgE, basal serum tryptase and basophil status as patients without allergy. None of allergic patients bothered reporting their UAS7 questionnaire. Because of their low number, the whole CU (CSU, CINDU, with or without allergy) group was analyzed altogether unless it is mentioned. Ten patients were not treated before they enrolled in the study. Seven patients were taking 1 or 2 antihistamine tablets per day; 4 more were taking 3 tablets and 5 more were taking 4 tablets per day. Furthermore, 5 patients had taken steroids at some time of the disease (all CSU), 1 had been treated with methotrexate and 2 with montelukast.

CU patients had BMI ( $27.5 \pm 5.22 \text{ kg/m}^2$ ) significantly higher than HC ( $24.3 \pm 4.51$ ,  $p=0.0171$ , Table 1). Among CU patients, 62.1% had a BMI >25 compared to 28.6% of HC ( $\chi^2 p=0.0111$ ) and 34.5% had a BMI >30 compared to 14.3% of HC ( $p=0.077$ ). D-dimer values were widely distributed in the two groups and 28% of CU had D-dimer higher than 800u as compared to 11.5% of HC (NS). D-dimer values were not correlated to the UAS7 or UCT scores or to the BMI (Table 2). Patients with a BMI >30 tended to have higher D-dimer (median:  $680 \pm 739$ ) compared to patients with lower BMI ( $326 \pm 857$ , NS); tended to be older ( $52.4 \pm 19.5$  compared to  $45.8 \pm 17.4$  years, NS) and having a longer disease duration ( $7.5 \pm 6.57$  compared to  $2.81 \pm 3.56$  years,  $p=0.059$ ), but a lower disease activity (UAS7 score:  $23.5 \pm 10.7$  compared to  $26.2 \pm 10.1$ ; NS). The UCT score, tIgE level, serum

tryptase, basophil count and basophil reactivity were not different between patients with moderate or very high BMI. Serum tIgE levels were elevated (>114 kU/L) in 7 out of 19 CU patients tested (36.8%) but only in 3 of 25 HC (12.0%;  $\chi^2 p=0.0515$ ).

#### A great part of patients shows biological signature compatible with basophil – IgE involvement

In 10/31 (32.3%) CU patients, the basophil count was low. Because basophils are rare events among peripheral leukocytes (<1%), we could not get highly reliable values for technically reasons. Among cells expressing CD123, the mean number of basophils, that expressed CD123 but not HLA DR-, was significantly lower in CU ( $969 \pm 711$  events) compared to HCs ( $1458 \pm 754$  events,  $p=0.0192$ ) while plasmacytoid Dendritic Cells (pDC), that express CD123 and HLA DR, were in similar numbers ( $438 \pm 478$  in CU vs  $404 \pm 290$  in HC, NS). Consistently, the mean ratio of basophil over lymphocyte counts was lower ( $1.37 \pm 0.98\%$ ) in CU compared to HC ( $2.25 \pm 1.87$ ,  $p=0.0421$ ). More simply, basophils represented less than 0.1% of leukocytes named Basopenia (Bp), in 10/31 (32.6%) CU compared to 2/29 (6.9%) HC ( $\chi^2 p=0.0141$ ). The difference was particularly high in females CU patients, (40%) compared males (18%;  $\chi^2 = 0.0004$ ).

In accordance with the hypothesis of local recruitment, basopenia was more frequent in patients who reported recent flares. Patients with Bp reported a longer history of CU ( $7.8 \pm 6.8$  years) compared to patients with a normal basophil count ( $1.0 \pm 1.1$  years;  $p=0.0174$ ) and they tended to be older ( $51.1 \pm 20.3$  vs  $41.4 \pm 16.4$  years old). Bp was observed in 30% of patients with D-dimer higher than 800u but in 10% of patients with D-dimer lower than 800U ( $\chi^2 p=0.0493$ ). Bp was not related to the BMI or UAS7 and UCT scores. However, Bp patients tended to have lower IgE ( $89 \pm 45.3$  vs  $239.6 \pm 200.8$ ) and higher CD203c expression ( $5.97 \pm 1.95$  vs  $4.77 \pm 1.09$ ) suggesting some degree of basophils activation. Furthermore, in two cases, Bp was associated with a raised serum tryptase ( $18.3 \pm 2.2 \mu\text{g/L}$ ), a very long story of the disease (>10 years) and a higher expression of CD203c (MdFI:  $7.23 \pm 3.42$ ) on basophils suggesting a very active disease.

Basophil capacity for degranulation was frequently deficient in CU (Table 1). Under stimulation with anti-IgE antibody, a poor response of basophils (PR) was observed in 22/29 (75.9%) CU but in 6/25 (31%) HC ( $\chi^2 p=0.0031$ ). Because anti-IgE stimulation in whole blood competes with serum IgE that was elevated in a few patients, we confirmed this defect in response by stimulating basophils with an anti-Fcε-RI antibody in a part of patients. Anti-Fcε-RI induced degranulation of  $6.4 \pm 12.6\%$  of basophils in 13 PR as compared to  $17.6 \pm 12.1\%$  in 9 HC. Some kits provided and anti-IgE completed with fMLP as positive control. Stimulation of basophils with an anti-IgE + fMLP, induced a degranulation of  $32.0 \pm 18.2\%$  of basophils in 7 UC compared to  $50.0 \pm 19.2\%$  in 8 HC ( $p=0.085$ ). PR patients tended to be older and had a longer CU history, a higher UAS7 and a lower UCT; their tIgE levels were lower and their serum tryptase higher ( $p=0.0030$ ).

#### On the other hand, some patients lacked evidence of peripheral basophil involvement

Indeed, 14/31 (45.1%) CU patients kept a high number of basophils (>0.50% of leukocytes) despite they had a very active disease. They had low serum tIgE levels and low serum tryptase ( $4.71 \pm 2.33 \mu\text{g/L}$ ), low expression of CD203c on resting basophils (MdFI:  $5.64 \pm 3.97$ ).

**Table 2:** Chronic Urticaria profiles according to the Basophil count.

Basos count	<0.10% (n=10)	0.10 – 0.50% (n=7)	>0.50% (n=14)
Age (years)	51.120.3	41.4±16.4	49.6±16.9
Disease duration (years)	7.78±6.80 (p=0.0174)	1.00±1.10	3.92±4.10 (NS)
UAS7 (severe >15; n=19)	25.2±8.67	33.3±10.3	23.3±11.3
UCT score (poor efficacy <12)	4.40±3.24	2.29±1.89	4.08±3.18
BMI (kg/m²)	27.3±4.21	27.3±7.03	27.4±5.43
D-dimers (ref <500u)	600 [161-3515]	350 [316-2270]	337 [120-1523]
Tryptasemia (μg/L)	7.72±6.67	5.64±1.60	4.87±2.35
tIgE (kU/L)	89.3±45.3	239.5±260.8	133.1±169.4
Basos evts (% leukocytes)	0.04±0.02	0.29±0.11	0.79±0.34
CD203c MdFI 4.7±2.9	5.26±1.98	4.36±1.61	4.94±1.38
%CD63+ anti-IgE	15.9±19.7	18.5±14.3	18.1±17.2
Poor Reactive Baso	7 (77.8%)	6 (85.7%)	9 (75%)

CU Patients with basopenia and high Basos count are compared with patients having Basos count in normal ranges

These patients tended to be older (49.9+16.3 years) with a long disease history (4.5+4.4 years) compared to patients who had a normal or low basophil count. Their CU was more frequently associated with dermatographism (57%) compared to patients with normal (40%) or low (43%) basophil counts. They had similar levels of BMI, D-dimer, UAS7, UCT scores although they tended to take more tablets of anti-H1.

Also, part of CU patients kept GR and low serum tryptase (2.95+1.00µg/L; compared to PR;  $p=0.0049$ ), even lower than HC (6.05+5.86;  $p=0.028$ ). GR patients generally had high basophil counts with low expression of CD203c (4.94+1.38) but this group did not completely overlap with the group of patients who had high basophil count. These GR patients tended to be younger (38.4+17.3) than PR patients (51.9+17.1, NS) despite they had a longer disease history (5.0+3.8 years) and a high UAS7 score (27.0+15.3). CU was inducible in 2/7 (29%) of GR as compared to 11/22 (50%) PR (NS). IgE dosage, available in only 2 GR patients, was remarkably low (<10kU/L).

## Discussion

This is the first description of French patient-control group of patients with CU, in the real life. The population we observed appeared to be very similar in age, higher frequency of females, frequent dermatographism and overweight distribution as other populations described in other international [36,37,41-45] or French [46,47] studies. The mean duration of the disease was quite long but this does not mean it was due to a delayed diagnosis as most of patients already have had 2 or 3 lines of treatment and were addressed to the local reference center because of difficulties in controlling the disease. This probably also explains why most cases were severe (high UAS7, low UCT scores). As usually reported, CU was more frequent in females for unclear reasons and we did not find differences between females or males in terms of age, overweight, disease severity, IgE levels, serum tryptase or basophil reactivity except that basopenia was more frequent in females. It is a pity we miss information on possible auto immunity because ASST is not used in France and there are no standardized ex vivo alternative test that could replace it yet [48].

Like other studies, we also found a frequent association of CU with overweight [10, 36,37,41-47]. Not surprisingly, the overweight was associated with age and disease duration as previously reported [49]. Our data cannot tell if obesity was anterior and eventually favoring CU or if it could have been a consequence of the disease chronic inflammation. Hormonal disorders have been suggested in epidemiological studies of CU [50] but gain of weight could also be related to the chronic inflammation. Indeed, the production of inflammatory cytokines has been associated with dysregulation of appetite regulators like lipocalin-2 or adiponectin in CU [51,52]. In any case, we did not evidence association between obesity and CU severity in accordance to the previous report [11]. In our study, we observed a very large variability of D-dimer levels in CU as well as in HC. This is why we have reported results in median and range values instead of mean and standard deviation. D-dimer is a marker of coagulation disorder linked to the metabolic syndrome and chronic inflammation [5,8,49,53,54]. In our experience, high levels of D-dimer was not related to overweight but tended to be higher in PR patients suggesting an effect of the disease activity and probably the inflammatory status although anteriority in steroid treatment can also play a role in it.

We analyzed serum tryptase as a marker of mast cell activation. Raise of serum tryptase is very helpful in diagnosis of acute urticaria and mast cell disorders. In our study, serum tryptase was rarely elevated above the reference range in CU and was not correlated to the disease severity. However, we could see that basal serum tryptase, was still a bit higher in CU as compared to age, sex matched HC in accordance with previous M Ferrer's report [17]. In fact, the reference ranges that are usually admitted, have been proposed by the manufacturer of the dosage system but are certainly too broad. Indeed, usual basal serum tryptase we routinely detect are closer to the level we observed in our HC group. Considering the threshold of 7 as a reference range, we observed that serum tryptase was more frequently high in CU, and was more frequently high in patients who reported recent flares just before they came to the clinics suggesting it reflects some residual mast cell activation.

Beside the clinical data, we looked for biological parameters theoretically related to the physiopathology that could help in characterization of CU and although no basophil biological monitoring is actually recommended in the international guidelines for the diagnosis of CU. And indeed, we found that signs of involvement of basophils and IgE were altered at least in part of patients. Basopenia is compatible with basophil recruitment and consumption in the tissue and basophil renewal can decline with the duration of the disease [14,15,55,56]. Interestingly, Eosinopenia has also been reported in CSU and has been associated with autoimmunity (ASST), low IgE, basopenia and poor response to Omalizumab [57]. So basopenia is an informative parameter to consider in monitoring CU activity (if not severity) and is easy to get in routine differential leukocyte count in accordance with Borzova [58]. Peripheral basophils show some level of activation evidenced by a raise of CD203c expression but this was limited in blood, probably because the cell activation is mostly restricted to the tissue. Frequent challenges of basophils can explain some level of exhaustion and lack of ex vivo reactivity that Sabroe called "desensitization" [25,59]. Alternatively, PR can be explained in part by a lack of peripheral basophils maturity due to an increased turn over.

Mast cell activation can explain the occasional raise of serum tryptase. This was rarely observed, probably because the disease history is so chaotic that it is difficult to get testing at the peak of production in outpatients.

CU has been associated with a background of atopy that can explain the high level of serum tIgE. However, if the eventual Anti-IgE auto-immunity that eventually induces spontaneous degranulation without requiring allergen [60], most probably also binds to soluble IgE, making immune complexes rapidly removed from serum. This can explain IgE depletion [61] while anti-FcRI auto-immunity induces degranulation [61,62], but without interfering with soluble IgE [63-66]. It is then not surprising to observe low IgE levels in some patients but this can also depend on the chaotic appearance of flares raising once more the high heterogeneity of the biological status collected at some limited time points.

Daily dosages of tIgE in healthy population show a large variability and are mainly considered as pathological when tIgE are above a threshold (>114kU/L) that was defined by the major manufacturer of the dosage system. Thus, dosage of IgE is rarely explored in its lower range where its clinical significance is generally neglected. Our results

suggest that considering lowering level of IgE could be of interest in monitoring CU. Furthermore, one can question the potential benefit of treatment with anti-IgE biotherapy in case serum tIgE are very low. On the other hand, CU with high levels of IgE may be compatible with a possible type I auto-immunity in which IgE may play a role and high IgE has been shown to be associated with a good efficacy of Omalizumab treatment of CU [39,67].

But our results also show the lack of evidence for basophile involvement in some cases of CU. This suggests that CU could be related to alternative release of vasoactive mediators, independent of the IgE and Fcε-RI activation pathway as previously shown [66,68]. It is of interest to mention that CU has been observed in patients with very rare primary deficiency in IgE [69]. This would explain some failure of anti-histaminic treatment. We cannot say if this is compatible with the type I auto immune mechanism of CU as we unfortunately lack of diagnostic tools for mechanism.

## Conclusion

In conclusion, we report basophil monitoring in a prospective case-control study of Chronic Urticaria diagnosed in a middle size teaching hospital and our results show that our epidemiological data are very similar to other studies reported. Our biological data show that total serum IgE and serum tryptase dosages, basophil count and basophil ex-vivo reactivity could bring precious information in classifying CU according to different possible physio-pathological mechanisms and could eventually anticipate the potential response to biotherapy.

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