

1 **Relevance of the basophil high-affinity IgE receptor in chronic urticaria:**

2 **Clinical experience from a tertiary care institution**

3

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59 **ABBREVIATIONS:**

60 Anti-Tg - Anti-thyroglobulin

61 Anti-TPO - Anti-thyroid peroxidase

62 ASST- Autologous serum skin test

63 APST- Autologous plasma skin test

64 ATA- Anti-thyroid antibody

65 CIndU- Chronic inducible urticaria

66 CsTT- Critical stimulation time threshold

67 CSU- Chronic spontaneous urticaria

68 CTT- Critical temperature threshold

69 CU- Chronic urticaria

70 FcεRI- High-affinity IgE receptor

71 HC- Healthy control

72 Ig- Immunoglobulin

73 MFI- Mean fluorescence intensity

74 UAS7- 7-day urticaria activity score

75 UCT – Urticaria Control Test

76 **ABSTRACT**

77 **Background.** The high-affinity IgE receptor (FcεRI) expression on effector cells has
78 been poorly characterized in patients with chronic urticaria (CU) to date.

79 **Objectives.** To investigate the FcεRI expression on blood basophils in a large cohort of
80 CU patients and its potential relationship with relevant features of the disease.

81 **Methods.** Basophil FcεRI expression was measured by flow cytometry in 287 CU
82 patients (192 with Chronic Spontaneous Urticaria and 95 with Chronic Inducible
83 Urticaria) at their initial evaluation in our Department. A control group of healthy non-
84 atopic individuals was included to provide reference data, and the effect of
85 antihistamine and anti-IgE therapy on the basophil FcεRI expression was also evaluated
86 in a cohort of CU patients.

87 **Results.** The median FcεRI expression was found significantly higher in CU patients
88 compared to healthy controls ($p<0.0001$). A positive correlation was found between
89 serum IgE levels and basophil FcεRI expression ($R=0.422$; $p<0.001$). Significantly
90 higher FcεRI levels on basophils were detected in CU patients who presented with
91 concomitant atopic features ($p=0.003$), negative autologous serum skin test ($p=0.002$),
92 negative autologous plasma skin test ($p=0.009$) or undetected levels of anti-thyroid
93 antibodies ($p=0.01$). Baseline FcεRI expression was not related with the activity and
94 duration of the disease, and was not significantly modified during antihistamine
95 therapy; however, it correlated with the clinical response to omalizumab ($p=0.003$).

96 **Conclusion.** Although further multicenter studies are needed to corroborate these
97 findings, the assessment of basophil FcεRI levels might be relevant in daily clinical
98 practice supporting an autoimmune pathogenesis and predicting response to anti-IgE
99 treatment.

100 **HIGHLIGHTS BOX**

101 **1. What is already known about this topic?**

102 The activation of the high-affinity IgE receptor –FcεRI- on basophils and mast cells is
103 crucial for the immediate hypersensitivity responses in subjects with atopic dermatitis,
104 allergic asthma and allergic rhinitis.

105

106 **2. What does this article add to our knowledge?**

107 Basophil FcεRI expression is significantly upregulated in Chronic Spontaneous and
108 Inducible Urticaria. Patients who present negative autologous serum/plasma skin test,
109 undetected levels of anti-thyroid antibodies or satisfactory clinical response to
110 omalizumab exhibit higher FcεRI levels.

111

112 **3. How does this study impact current management guidelines?**

113 Although further multicenter studies are needed to corroborate these findings, the
114 assessment of basophil FcεRI expression might be relevant in daily clinical practice
115 supporting an autoimmune pathogenesis and predicting response to anti-IgE treatment.

116

117 **Keywords:** Basophil, chronic urticaria, FcεRI, FcεRI expression, IgE receptor,
118 omalizumab

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120

121 INTRODUCTION

122 Chronic urticaria (CU) is a common skin condition characterized by the
123 recurrent appearance of itchy wheals and/or angioedema for longer than 6 weeks.¹ It is
124 classified into two subtypes: chronic spontaneous urticaria (CSU), when the lesions
125 occur without an obvious stimulus, and chronic inducible urticaria (CIndU), when
126 symptoms are induced by different triggers, e.g. low temperatures, heat, pressure or
127 exercise.^{1,2} Existing evidence demonstrates that CU symptoms may have major
128 detrimental effects on quality of life, including daily activities and emotional well-
129 being.³

130 The pathophysiology of CU involves the activation and degranulation of effector
131 cells, such as basophils and mast cells, and the subsequent release of pro-inflammatory/
132 pathological mediators that play a key role in the development of CU symptoms.^{4,5} As
133 of yet, it is unclear completely what causes such activation and degranulation. One of
134 the most attractive explanation in most of the patients is the autoimmune mechanism, in
135 which effector cells are activated by immunoglobulin (Ig) E or IgG through the high-
136 affinity IgE receptor, FcεRI, located on the surface of basophils, mast cells and antigen-
137 presenting cells.⁶⁻⁹ Thereby, crosslinking of FcεRI with the complex IgE-autoantigen
138 (Type I autoimmunity) or with just IgG or the complex IgG-IgE (Type IIb
139 autoimmunity) would cause the activation/degranulation of effector cells with the
140 consequent release of preformed mediators and newly synthesized active substances.^{6,10}

141 Despite its supposed importance in the disease pathogenesis, FcεRI expression
142 on effector cells has been poorly characterized in CU patients to date. Therefore, we
143 sought to investigate the FcεRI expression on blood basophils in a large cohort of
144 patients with CSU and CIndU to answer the following questions: (i) Are basophil FcεRI
145 levels increased in CU patients? (ii) Are there clinical features that modulate the FcεRI

146 expression in patients with CSU or CIndU? (iii) Is FcεRI expression modified during
147 treatment in CU patients? and more important, (iv) is the assessment of FcεRI
148 expression relevant in daily clinical practice?

149

150 **PATIENTS & METHODS**

151 Subjects and study design

152 This prospective study included patients with CSU or CIndU referred to the
153 Urticaria Clinic of the Department of Dermatology of Hospital del Mar (Barcelona) during
154 the period from January 2014 to June 2018 (Ethical approval no. 2012/4913/I). Following
155 a systematized clinical protocol, a thorough and structured history (including age, sex,
156 disease duration, disease severity, personal history of atopic features [i.e. atopic dermatitis,
157 allergic rhinitis and/or allergic asthma], presence of angioedema and concomitant subtypes
158 of CU) and laboratory analyses (including total serum IgE levels, thyroid function and
159 levels of anti-thyroid antibodies [ATAs]: anti-thyroid peroxidase [anti-TPO] and anti-
160 thyroglobulin [anti-Tg]) were performed in all patients at the initial evaluation.
161 Additionally, autologous serum skin test (ASST), autologous plasma skin test (APST)
162 and/or standardized inducible testing were performed (when appropriate) as part of the
163 routine study protocol. CIndU diagnosis was based on the patients' clinical history and the
164 results of standardized provocation testing.² As the main objective of the present
165 investigation, peripheral blood samples were obtained from CU patients to measure the
166 FcεRI expression on basophils by flow cytometry. To avoid potential interferences,
167 patients who were under treatment with biologic therapies (including omalizumab), oral
168 corticosteroids and/or other immunosuppressive agents were excluded from the study.
169 Blood samples from a group of healthy controls [HCs] without family and personal history
170 of CU or atopic features were also evaluated to obtain reference data.

171 In addition, FcεRI levels were assessed in a cohort of CU patients at different time
172 points to investigate the effect of antihistamine and anti-IgE therapy on the basophil FcεRI
173 expression. For antihistamine treatment, FcεRI levels were measured at the baseline
174 evaluation and at least 1 month after the initiation of therapy (non-sedating H1-
175 antihistamines, doses ranging from 1 to 4 times the recommended dose depending on the
176 patient's symptoms severity). Response to therapy was defined as an improvement in the
177 patients' signs and symptoms achieving a 7-day Urticaria Activity Score (UAS7, a
178 composite score of itch severity and hive count over 7 days; range 0–42) ≤ 6 and/or an
179 Urticaria Control Test (UCT, a validated tool for assessing disease control in daily
180 practice; range 0–16) ≥ 12 .^{1,11} On the other hand, as the effect of omalizumab on the FcεRI
181 expression in CSU patients has been extensively studied in recent investigations,^{12,13} we
182 have focused on the analysis of the kinetic of FcεRI levels during anti-IgE therapy in
183 patients diagnosed with pure CIndU. Thus, FcεRI expression was evaluated in CIndU
184 patients who showed unsatisfactory response to antihistamines and were therefore treated
185 with subcutaneous injections of omalizumab 300mg monthly. Basophil FcεRI levels were
186 measured on day 0 and on weeks 4, 8 and 20 of treatment (i.e. prior to the 1st, 2nd, 3rd and
187 6th injections); and response to therapy was evaluated at 6 months of treatment according
188 to the UCT score.

189 Most primary endpoints of the study (e.g. evaluate the basophil FcεRI expression as
190 a potential biomarker of disease activity, disease duration, therapeutic response and for
191 confirming CSU and distinguishing it from HCs) were pre-specified at the initiation of the
192 investigation. Nevertheless, some observations, such as the FcεRI-IgE correlation and the
193 differences in FcεRI expression regarding the “autoimmune” condition in CSU patients,
194 were evaluated after the data collection was completed.

195

196 Basophil cell preparation and flow cytometry for FcεRI expression

197 Flow cytometry analysis was performed following standard procedures.¹²
198 Briefly, 150 µl of anticoagulated blood was incubated on the same day of collection
199 during 20 min at 4°C with an excess of human immunoglobulins to block unspecific
200 binding. Afterwards, blood was stained with anti-CD123-PE (BD Biosciences, San
201 Jose, California) and anti-CD193-APC (Miltenyi Biotec GmbH, Bergisch Gladbach,
202 Germany) to identify basophils and with anti-FcεR1a-FITC (clone CRA1, eBioscience,
203 San Diego, California) or an isotype control to establish the expression of FcεRI on the
204 surface of blood basophils. It should be taking into account that, after fine tuning the
205 technique and evaluating the FcεRI expression using separately anti-CD123-PE and
206 anti-CD193-APC markers, similar basophil FcεRI levels were observed in terms of
207 mean fluorescence intensity (MFI; Figure S1); however, both antibodies were
208 simultaneously used to avoid FcεRI positive dendritic cells. The samples were then
209 lysed and fixed using the FACS Lysing Solution (BD Biosciences) and analyzed by
210 flow cytometry in a FACSCanto using the FACSDiva software. At least 2×10^5 events
211 were acquired. Levels of the basophil FcεRI receptor were expressed as MFI.
212 Instrument settings (e.g. scatter and voltage settings and compensation matrix) and
213 experimental conditions (e.g. antibody clones and dilution) remained constant for all
214 samples throughout the study. To ensure consistency in the analysis, the same
215 investigator processed and analyzed all samples and two independent researchers
216 correlated the levels of basophil FcεRI expression and the clinical scores.

217

218 Serum IgE levels, levels of ATAs and ASST/APST

219 Total IgE and levels of circulating anti-TPO and anti-Tg antibodies were
220 analyzed in serum by chemiluminescence immunoassay technique using the
221 IMMULITE 2000 XPi System (Siemens, Munich, Germany). The ASST was performed
222 in CSU patients as previously described.¹⁴ Briefly, venous blood was taken at the initial
223 evaluation, and samples were centrifuged at 2500 rpm for 10 minutes and the serum
224 separated. For the APST, citrated blood was centrifuged at room temperature to separate
225 the plasma. Afterwards, patients received intradermal injections of 50 μ L of fresh
226 undiluted autologous serum and 50 μ L of autologous plasma on the volar forearm.
227 Similar volumes of 0.9% NaCl saline and 100 mg/mL histamine were used as negative
228 and positive controls, respectively. A positive ASST/APST was considered when the
229 diameter of serum-induced wheal was >1.5 mm compared to the saline-induced response
230 at 30 minutes.

231

232 Statistical analysis

233 Descriptive statistics were performed for each variable, using median, range and
234 percentiles 25th (P25) and 75th (P75) for quantitative variables, and absolute (n) and
235 relative (%) frequencies for categorical variables. Mann-Whitney *U* test was used to
236 compare the Fc ϵ RI receptor expression between patients with CSU, CIndU and HCs.
237 Pearson's correlation was used to evaluate the association of Fc ϵ RI receptor expression
238 with serum IgE levels, blood basophil count, disease duration and scores of disease
239 severity. Paired samples T-test and Wilcoxon signed-rank test were used to evaluate
240 changes in Fc ϵ RI levels during treatment with antihistamines and omalizumab,
241 respectively. All analyses were carried out with the SPSS 22.0 statistical package, and a *p*-
242 value < 0.05 was considered statistically significant.

243

244 **RESULTS**

245 Demographics and FcεRI expression in the study population

246 During the study period, 287 CU patients were referred to our Clinic and were
247 therefore included in the analysis. Of these, 192 (66.9%) patients suffered from CSU
248 predominantly and the remaining 95 (33.1%) from pure CIndU (54 cold urticaria, 15
249 symptomatic dermographism, 10 solar urticaria, 7 cholinergic urticaria, 7 delayed
250 pressure urticaria, 1 contact urticaria and 1 aquagenic urticaria). In addition, 46 HCs
251 were included to obtain reference data. Clinical and demographics features of the study
252 population are summarized in Table I.

253 Regarding basophil FcεRI levels, the median (P25-P75) FcεRI expression was
254 found significantly higher in CU patients compared to HCs (9033 [5864- 13630] of MFI
255 vs. 4743 [2771- 7580] of MFI, respectively; $p < 0.0001$; Figure 1). However, among CU
256 patients, no significant differences regarding the FcεRI expression were found in
257 patients with CSU compared to those with pure CIndU (9234 [5934- 13534] of MFI vs.
258 8932 [5566- 13919] of MFI, respectively; $p = 0.826$; Figure 1). It should be also
259 mentioned that no significant differences were observed regarding total serum IgE
260 levels and basophil count between these two groups of patients (CSU vs. pure CIndU;
261 Table I). However, CU subjects showed significantly lower blood basophil numbers
262 than HCs ($p = 0.005$).

263

264 FcεRI expression and clinical and laboratory variables

265 Some clinical and laboratory variables had significant association with basophil
266 FcεRI expression in our cohort of CU patients. Thus, subjects who presented with
267 concomitant atopic features showed significantly higher FcεRI levels than those without
268 personal history of atopic dermatitis, allergic rhinitis and/or allergic asthma (median

269 [P25-P75] FcεRI expression: 11534 [6561-15649] of MFI vs. 8583 [5438- 13109] of
270 MFI, respectively; $p= 0.003$). Likewise, higher basophil FcεRI levels were detected in
271 CSU patients with negative ASST compared to those with positive ASST (median [P25-
272 P75] FcεRI expression: 10684 [7352-16150] of MFI vs. 8061 [1301- 12726] of MFI,
273 respectively; $p= 0.002$; Figure 2). A similar trend was found regarding the APST result
274 (median [P25-P75] FcεRI expression: 10403 [6992-15515] of MFI in APST negative
275 patients vs. 7903 [1243- 13601] of MFI in APST positive patients; $p= 0.009$; Figure 2).
276 FcεRI expression also differed among CU patients depending on the levels of
277 circulating ATAs, with lower FcεRI levels in patients with elevated ATA levels (i.e.
278 >35 UI/mL of anti-TPO and/or >40 UI/mL of anti-Tg; median [P25-P75] FcεRI
279 expression: 6442 [1621-11141] of MFI vs. 9396 [6261-13793] of MFI, $p= 0.010$; Figure
280 2).

281 We also investigated whether the basophil FcεRI expression in CU patients
282 could be associated with total serum IgE levels and/or blood basophil count. A positive
283 correlation was found between IgE levels and the FcεRI expression ($R= 0.422$; $p<$
284 0.001 ; Figure 3). Conversely, no association was detected between blood basophil count
285 and FcεRI levels ($R= 0.095$; $p= 0.132$).

286

287 FcεRI expression and disease activity and duration

288 Disease activity was evaluated by using the UAS7 in CSU patients and
289 appropriate threshold tests in CIndU patients (e.g. the critical temperature threshold
290 [CTT] and the critical stimulation time threshold [CsTT] assessed by the TempTest® 3.0
291 in patients with cold urticaria).¹⁵⁻¹⁷ In this case, the basophil FcεRI expression was not
292 found associated with disease activity in CSU patients ($R= 0.114$; $p= 0.156$), or with the
293 CTT ($R= 0.062$; $p= 0.708$) and the CsTT ($R= 0.010$; $p= 0.953$) in patients with cold

294 urticaria. Regarding CU prognosis, disease duration, defined as the time from symptoms
295 onset to the initial evaluation, was also not found associated with the basophil FcεRI
296 expression in CU patients ($R= 0.031$; $p= 0.613$).

297

298 FcεRI expression and therapeutic response

299 FcεRI levels were evaluated in 60 subjects (47 CSU and 13 CIndU) during
300 antihistamine therapy (median [range] number of months on therapy before follow-up
301 measurement: 3 [1-21] months). In this group of patients, FcεRI expression was not
302 significantly modified during treatment ($p= 0.118$; Figure 4a). Furthermore, no
303 significant differences were observed regarding the baseline FcεRI expression in
304 responders and non-responders to antihistamines ($p= 0.787$). On the other hand, in the
305 14 patients diagnosed with pure CIndU who received treatment with omalizumab (9
306 cold urticaria, 3 solar urticaria and 2 symptomatic dermographism), a significant drop in
307 the basophil FcεRI expression was observed after the first injection (median [P25–P75]
308 reduction from baseline at 4 weeks: 86.4% [83.7–93.6]; $p= 0.003$; Figure 4b), and such
309 reduction was maintained throughout the whole treatment (median reduction from
310 baseline at weeks 8 and 20: 90.3% and 88.0% respectively). At 6 months of anti-IgE
311 therapy, 11 (78.6%) patients achieved significant clinical improvement ($UCT \geq 12$),
312 while 3 (21.4%) subjects were considered to have poorly controlled disease ($UCT < 12$).
313 Interestingly, these omalizumab “non-responders” showed very low baseline FcεRI
314 levels (median [range] FcεRI expression: 2547 [1172-3778] of MFI in “non-responders”
315 vs. 13591 [7982-18512] of MFI in “responders”).

316

317 **DISCUSSION**

318 FcεRI is a molecular complex expressed on the surface of mast cells, basophils,
319 antigen-presenting cells and eosinophils, and its activation appears to be critical for the
320 immediate hypersensitivity response that is characteristic of allergic diseases.¹⁸
321 Thereby, FcεRI expression has been found significantly upregulated in subjects with
322 atopic dermatitis, allergic asthma and allergic rhinitis compared to healthy non-atopic
323 individuals.^{19,20} Likewise, the results obtained from the present large cohort study
324 demonstrate that FcεRI expression on circulating basophils is also substantially
325 increased in patients with active CSU and CIndU. Although the exact functional
326 significance of elevated FcεRI expression on effector cells in allergic conditions is not
327 completely understood, accumulated evidence suggests that these receptors could
328 enhance their roles as effector cells in allergic inflammation.¹⁹ It has been also
329 postulated that elevated FcεRI expression might profoundly alter the spectrum of
330 allergen-presenting cells available to present allergens to T cells,²⁰ and that FcεRI
331 down-regulation may be followed by an increase in the threshold above which
332 degranulation of effector cells is triggered.¹³ Taken together, our observations support
333 the involvement of FcεRI on the complex inflammatory response that occurs in patients
334 with CSU and CIndU, and also support the assumption that circulating basophils play
335 an important role in the pathophysiology of CU.

336 Several lines of evidence also support a regulatory role for serum IgE in the
337 expression of its high-affinity receptor on human mast cells and basophils.²¹ Thereby, a
338 very strong association (correlation coefficient close to 1) has been found between
339 serum IgE levels and FcεRI expression on effector cells in a great variety of disease
340 states, particularly in atopic individuals, but also in other IgE-driven conditions like
341 hyper-IgE syndrome or helminth infestation.^{19,22} Although the basis for this correlation
342 has not been elucidated in detail, it has been suggested either that there are similar

343 regulatory mechanisms to both IgE levels and IgE receptor or that IgE itself upregulates
344 or stabilizes surface expression of the receptor leading to elevated expression in allergic
345 diseases.²¹ However, such association has not been previously evaluated in patients with
346 CSU and CIndU. According to our results, in these conditions, which are not considered
347 classic allergen-driven diseases, this potential association IgE- FcεRI expression seems
348 to be weaker ($R=0.422$), suggesting that there must be other regulatory mechanisms
349 with a significant influence on the FcεRI levels in CU patients. In addition, it does not
350 appear that certain CU features, such as the activity/severity or the duration of the
351 disease, may play an important role in the regulation of FcεRI expression on effector
352 cells.

353 Previous studies have indicated that approximately 30-60% of CU patients may
354 have an autoimmune etiology on the basis of various laboratory and clinical
355 evidence.^{6,9,23} Such autoimmune background is supported by the identification of
356 circulating autoantibodies against FcεRI or (less commonly) IgE that may induce
357 activation of basophils and mast cells, secretion of histamine and recruitment of
358 inflammatory cells.^{6,24} The detection of such autoantibodies may have a complex
359 methodology with variable sensitivity and specificity and, as of yet, is not fully
360 implemented in routine clinical practice.²⁴ Accordingly, several tests have been
361 proposed to evaluate such autoimmune mechanism in CU patients. Among them, two of
362 the most accessible and used in daily practice are the ASST/APST and the detection of
363 circulating ATAs, since accumulated evidence demonstrates that autoantibodies to
364 FcεRI are more frequently identified in patients with positive ASST and/or elevated
365 ATA levels,^{24,25} which may be in line with our findings. Thus, according to our results,
366 patients with negative ASST/APST or undetected ATAs showed significantly higher
367 basophil FcεRI levels, suggesting that these autoantibodies against FcεRI (or other

368 functional autoantibodies that bind to the FcεRI receptor) that are presumably present in
369 patients with autoimmune CU could interfere in the measurement of the basophil FcεRI
370 expression, reducing their levels detected by flow cytometry. Thereby, although there
371 was an overlap of values between both group of patients, it could be said that the
372 assessment of the basophil FcεRI expression may help distinguishing CU individuals
373 according to the potential pathogenic mechanism of their disease. These observations
374 could have important implications for clinicians, since CU patients with an autoimmune
375 etiology may present distinctive clinical features and patterns of therapeutic response.²⁶⁻
376 ²⁸ Further research is needed to see whether the FcεRI measurement can distinguish
377 autoimmune patients without this overlap.

378 Given the emergence of new therapies in CU, there is a growing interest to look
379 for objective biomarkers that reliably predict the effectiveness of a specific therapeutic
380 intervention. Thus, many clinical and laboratory parameters have been claimed to
381 correlate with the response to therapy in CU during the last years.^{29,30} However there are
382 no validated biomarkers in clinical practice for this disease to date. In the particular case
383 of omalizumab, previous data from our group and Metz *et al* demonstrated that the fast
384 clinical improvement achieved during omalizumab therapy in CSU patients is
385 associated with a rapid and sharp reduction in the FcεRI expression on effector cells that
386 is maintained throughout the whole treatment.^{12,13,31} Furthermore, recent investigations
387 have shown that CSU patients who do not respond to omalizumab therapy have lower
388 baseline FcεRI levels on basophils compared to responder patients,^{12,32} suggesting that
389 this biomarker may represent a potential predictor of omalizumab response in CSU.
390 Similarly, in the present study, we have observed that this trend can also be applied in
391 CIndU patients, since non-responders to omalizumab showed almost undetectable
392 baseline FcεRI levels. The reason why these patients show very low basophil FcεRI

393 expression is not yet fully understood. Genetic variability or some masquerading factors
394 could be plausible explanations, since previous studies have shown that experimental
395 molecular changes in the subunits of this receptor can affect its expression on the cell
396 surface.³³ Although further multicentre prospective studies are needed to confirm such
397 observations, these findings might have an important impact in daily practice, allowing
398 physicians to early identify patients who will not benefit from omalizumab therapy.

399 Regarding antihistamines, previous data have also suggested the existence of a
400 possible link between certain aspects related to the IgE receptor and the response to this
401 treatment in CU patients. Thus, for example, Guo *et al* identified a single nucleotide
402 polymorphism in the *FCER1A* gene (a gene that encodes the α -chain of the Fc ϵ RI) that
403 might be associated with the therapeutic efficacy of non-sedating antihistamines in
404 Chinese patients with CSU.³⁴ However, to our knowledge, the effect of antihistamine
405 therapy on the Fc ϵ RI expression has not been previously evaluated. In this sense, we
406 could not find an association between Fc ϵ RI levels and response to antihistamine
407 therapy in our cohort of CU patients, since basophil Fc ϵ RI expression was not
408 significantly modified during treatment and no differences regarding this parameter
409 were observed between responder and non-responder patients.

410 Some limitations of the present study should be pointed out. The analysis was
411 done based in data from real clinical practice and therefore, some comparisons may not
412 have reached statistical significance probably due to a small sample size and
413 insignificant power to show statistical differences (a prior sample calculation was not
414 addressed). A matching procedure for some variables (e.g. age and sex) was not used
415 for the selection of the control group. The overlap of 25-75 ranges between
416 ASST/APST positive (autoimmune) and negative patients, even though the median
417 values were significantly different, may limit the blood basophil Fc ϵ RI expression

418 clinical relevance. Further studies in larger patient populations are needed to see how
419 distinct the two populations are, to determine the amount of overlap and to establish a
420 cut-off value for FcεRI expression that might provide an optimal sensitivity and
421 specificity. Although the evolution of the basophil FcεRI expression appears to be
422 similar as in CSU patients, the limited number of patients with CIndU treated with
423 omalizumab in the present study does not allow drawing firm conclusions about the
424 baseline FcεRI expression as a biomarker of omalizumab response.

425 In summary, FcεRI expression on blood basophils is significantly upregulated in
426 subjects with CSU and CIndU compared to healthy non-atopic individuals. The
427 association between serum total IgE levels- basophil FcεRI expression in CU is weaker
428 than in other classic allergic diseases, suggesting that there must be other regulatory
429 mechanisms with an important influence on the FcεRI levels in this disease.
430 Furthermore, significantly higher FcεRI levels might be detected in CU patients who
431 present with concomitant atopic features, negative ASST/APST or undetected levels of
432 ATAs. Although FcεRI expression would not provide information regarding CU
433 activity and prognosis, its assessment might be relevant in daily clinical practice,
434 helping physicians to identify those patients with the suggested autoimmune
435 pathogenesis (driven by IgE or IgG autoantibodies against FcεRI). More extensive
436 studies would be useful to confirm this observation and to define the clinical value of
437 FcεRI expression in blood basophils as a potential biomarker of omalizumab response.

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442

443

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545 **FIGURE LEGENDS**

546 **Figure 1:** Box-whiskers plots presenting median, interquartile range and maximum and
547 minimum of basophil FcεRI levels in all CU patients (n=287), patients with CSU
548 (n=192), patients with CIndU (n=95) and healthy controls (n=46). *** $p < 0.0001$

549 **Figure 2:** Box-whiskers plots presenting median, interquartile range and maximum and
550 minimum of basophil FcεRI levels in CU patients with positive and negative results of
551 the autologous serum skin test (ASST) and the autologous plasma skin test (APST) and
552 elevated and non-elevated levels of anti-thyroid antibodies (ATA).

553 **Figure 3:** Correlation between basophil FcεRI expression and total serum IgE levels
554 [represented in logarithmic scale] in CU patients.

555 **Figure 4:** Evolution of the basophil FcεRI expression (median, interquartile range)
556 during treatment with (A) antihistamines and (B) omalizumab in CU patients.

557 **SUPPLEMENTARY FIGURE LEGENDS**

558 **Supplementary Figure 1:** Comparison of the basophil gating strategy. (A) Anti-
559 CD123-PE and (C) anti-CD193-APC allows the identification of basophils among the
560 cells contained in the respective gate. (B & D) High-affinity IgE receptor expression on
561 basophils gated in A and C, respectively.

Table I. Clinical and demographic features of the study population.

Clinical variables	CU patients (n= 287)	CSU patients (n= 192)	CIndU patients (n= 95)	Healthy controls (n= 46)
Female sex, <i>n (%)</i>	210 (73.2)	144 (75.0)	66 (69.5)	35 (76.1)
Age, <i>years (range)</i>	45 (4-88)	46 (4-88)	43 (9-79)	51 (21-68)
Concomitant atopic features, <i>n (%)</i>	65 (22.6)	46 (24.0)	19 (20.0)	0 (0)
Angioedema, <i>n (%)</i>	57 (19.9)	46 (24.0)	11 (11.6)	0 (0)
Thyroid impairment, <i>n (%)</i> [‡]	38 (13.2)	28 (14.6)	10 (10.5)	-
Elevated levels of ATAs, <i>n (%)</i>	31 (10.8)	27 (14.1)	4 (4.2)	-
Disease duration, <i>months (range)</i>	48 (2-480)	40 (2-480)	60 (2-360)	-
Median value of UAS7 (<i>range</i>)	NA	21 (0-42)	NA	-
Median value of CsTT, <i>min (range)</i> ^{††}	NA	NA	3.0 (0.5-5.0)	-
Median value of CTT, <i>°C (range)</i> ^{††}	NA	NA	14 (4-26)	-
Concomitant CIndU, <i>n (%)</i>	NA	57 (29.7)	NA	0 (0)
Positive ASST, <i>n (%)</i> ^{**}	NA	78 (62.9)	NA	-
Positive APST, <i>n (%)</i> ^{**}	NA	56 (45.2)	NA	-
Total serum IgE levels, <i>kU/L (range)</i>	100.0 (1.0-4700.0)	103.0 (1.0-1855.0)	78.9 (2.6-4700.0)	-
Blood basophil count, <i>10³/μl (range)</i>	0.03 (0.01-0.14)	0.03 (0.01-0.14)	0.04 (0.01-0.10)	0.05 (0.02-0.09)

Abbreviations: ASST, autologous serum skin test; APST, autologous plasma skin test; ATAs, anti-thyroid antibodies; CIndU, chronic inducible urticaria; CsTT, critical stimulation time threshold; CSU, chronic spontaneous urticaria; CTT, critical temperature threshold; CU, chronic urticaria; IgE, immunoglobulin E; NA, not applicable; UAS7, 7-day urticaria activity score.

‡ Defined as the alteration of the thyroid-stimulating hormone serum levels

†† With respect to the 54 patients with cold urticaria

** For clinical reasons, ASST and APST were only assessed in 124 CSU patients









