Patients at baseline divided on the basis of the final response to omalizumab

Based on omalizumab response, 13 patients were considered early responders, 9 late responders and 10 non responders. The baseline values of the various parameters in the three subgroups are reported in Table 2. No significant difference was evident among the three subgroups for all the parameters tested at baseline (Tables 1 and 2). Concerning comorbidities, among the 13 early responders, 3 were atopic and 1 had thyroiditis; among the 9 late responders, 3 were atopic and 1 had thyroiditis; and among the 10 non-responders, 3 were atopic and 3 had thyroiditis. The duration of CSU was unrelated to omalizumab response.

Patients one-week after the first omalizumab administration divided on the basis of final response

The values of the various parameters one week after the first injection of omalizumab are reported in Table 2. In early responders, already one week after the first omalizumab administration, the levels of D-dimer dropped significantly (244 ng/ml [83-722]) from baseline (393 ng/ml [121-2623]) ($p=0.028$) and were significantly lower than in non-responders (378 ng/ml, [191-1781]) ($p=0.05$). D-dimer level was not able to discriminate between late and non-responders one week after the start of treatment. A similar behavior in early responders was observed for the levels of IgE to FcεRI, which significantly decreased from 612 OD (370-1421) to 314 OD (255-622) after one week and were significantly lower than in non-responders (391 OD, [261-1137]) ($p=0.045$) (Table 1 and Figure 2). Again, no statistically significant difference was observed between late- and non-responders one week after the start of the treatment. Levels of substance P significantly increased in all CSU patients one week after omalizumab administration from 140 [20-1114] to 395 [51-1586] ($p<0.001$), without any difference among the three subgroups. Finally, the three subgroups did not show significant differences in the levels of ECP, MRGPRX2, IgE-anti-FcεRII, IgG-anti-FcεRI and IgG-anti-FcεRII one week after the start of anti-IgE treatment.

Manuscript accepted for publication

Prediction of the final response to omalizumab by different factors at baseline and after one-week therapy.

Logistic regression analysis showed no association of the parameters at baseline with the final response to omalizumab. In contrast, one week after the start of biological therapy, the reduction of IgE anti-FcεRI (figure 3) was significantly associated with a final good response to omalizumab (Figure 4), with an odds ratio (OR) of 0.12 (95% CI: 0.01-1.06). Considering again the IgE anti-FcεRI response after one week of omalizumab therapy, the ROC (receiver operating characteristic) curve showed a good capacity to discriminate between responders and non-responders after three months of therapy as indicated by an area under the curve (AUC) of 0.66 (95% CI: 0.45-0.88) (Figure 5)

DISCUSSION

In the present work we studied several potential mast cell activators in patients with severe CSU with the aim to detect whether differences existed between patients and normal control, between different patients' subgroups, and whether omalizumab-refractory patients could be identified before the start of the treatment.

Concerning D-dimer plasma levels, we confirmed our previous findings that D-dimer represents an excellent marker of severity of CSU, as its levels were significantly more elevated in our patients than in normal controls irrespective of the final clinical response to omalizumab (14). At the same time, we confirmed also that D-dimer level is an excellent marker of disease activity as it strictly parallels the clinical response to CSU treatment (15,16). What was still unknown is the rapid drop of elevated D-dimer in response to anti-IgE treatment, that was already significant as short as one week after the first omalizumab administration. This demonstrates that in CSU the activation of the coagulation cascade is an exquisitely inflammatory process that stops suddenly as soon as the mast cell activation ceases.

Manuscript accepted for publication

In this study, we did not evaluate other potential predictors of response to omalizumab, such as anti-TPO autoantibodies and total IgE, which we had assessed in a previous study (17). That study concluded that thyroid autoimmunity alone cannot serve as a clinical predictor of response to omalizumab, whereas total IgE levels remain the most reliable prognostic marker for omalizumab response in patients with severe CSU (17). Other authors found that the IgG anti-TPO/total IgE ratio may be a good predictor of omalizumab response (18,19). Similarly, the autologous serum skin test (ASST) was not carried out in the patients included in the present work; however, a recent prospective study performed by one of us demonstrated that a positive ASST predicts a slow response to Omalizumab (20).

The analysis of the levels of FcεRI IgE in plasma confirms that type I autoimmunity is largely prevalent in CSU patients, irrespective on their response to omalizumab (21,22), although IgE levels gradually decreased from early omalizumab responders to late and non-responders. Omalizumab response is probably largely influenced by the co-occurrence of autoimmune IgG to the high affinity IgE receptor (21,22). In effect, recent studies showed that autoimmune CSU is in most cases associated with auto-allergic immune reactivity, but not vice-versa (23). Interestingly, but not surprisingly, the levels dropped as soon as one week after omalizumab administration, which indirectly confirms the good quality of our data and the rapidity of action of the drug.

The levels of autoimmune IgG to the high affinity IgE receptor (IgG-anti-FcεRI), that are considered responsible for histamine release in patients with autoimmune CSU (3), increased gradually from early omalizumab responders up to late and non-responders. These auto-antibodies were missing in normal controls. Not surprisingly the levels of circulating IgG autoantibodies to the high affinity IgE receptor did not change one week after the first administration of the anti-IgE mAb.

Manuscript accepted for publication

IgG-anti-FcεRII autoantibodies have been considered as part of the type IIb autoimmune process in CSU as activators of eosinophils (24). Interestingly they were detected mainly in late and non-responders to omalizumab although at low levels (table 2).

ECP, a marker of eosinophil activation, was elevated in CSU patients irrespective of the final omalizumab response. This finding confirms our previous observations about eosinophil activation as a turning point in the expression of both tissue factor, that causes the activation of the coagulation cascade by the extrinsic pathway, and of vasoactive substances such as VEGF (12,25,26). Another important point to keep in mind is that ECP is able to activate mast cells via the MRGPRX2 receptor.

Substance P, which was investigated as one of the several substances able to activate mast cells by membrane MRGPRX2 receptor, was not elevated in our patients irrespective on their response to omalizumab. This finding fully confirms the result of an old study by our group (27), although other groups got different results (28). To our surprise, the plasma levels of substance P increased dramatically in the same patients as short as one week after the first administration of omalizumab, irrespectively of the final clinical response to the drug. A similar trend of substance P was previously observed in CSU at 3 and 6 months of therapy in two Turkish studies (29, 30). One could speculate that, since substance P is an activator of mast cells via the MRGPRX2 receptor, the down-regulation of mast cell function may eventually lead to a release of substance P from the receptors they are bound to.

Soluble MRGPRX2 showed uniformly low levels in all CSU subsets, and such levels were even lower than those detected in normal controls. This finding is quite surprising, in view of previous studies reporting a hyper-expression of Mas-related gene X in skin mast cells (31), and the detection of elevated MRGPRX2 serum levels in patients with severe CSU positively correlated with UAS7 (32). Our finding might theoretically suggest that the plasma levels of the soluble form of this receptor are inversely related to the degree of activation of the mast cell or, in other words, that

Manuscript accepted for publication

strongly activated mast cells retain the MRGPRX2 on their surface as a highly expressed membrane receptor. Such levels did not increase one week after omalizumab administration, irrespective of the final response to the drug. It would be interesting to re-measure MRGPRX2 levels in CSU patients stably in clinical remission

In summary, our study confirms the relevance of D-dimer as a non-specific marker of severity and of acute inflammation in a proportion of severely affected patients. It also shows the rapid drop in circulating IgE after omalizumab administration, which may explain the nearly immediate clinical response observed in some CSU patients. On the other hand, the low levels of circulating MRGPRX2 and of SP at baseline were unexpected; particularly impressive was the increase in SP levels as short as 7 days after the first omalizumab administration. Following-up our in-vitro variables for longer that one week would have been ideal, but due to funding shortage this was not possible. IgE anti-FcεRI response at one week might be proposed as one further marker to predict the response to omalizumab. However, this assay is not present in most settings, while other in vivo and in vitro assays such as the autologous serum skin test, total IgE levels, or thyroid autoimmunity are much more commonly available. Although we were not able to detect novel markers of response to omalizumab and larger studies are needed to confirm our findings, we believe that this study, albeit with its evident limitations, may open new pathways in the understanding of the complex immune-pathogenesis of CSU.

Funding: The present study did not receive any funding.

Contributions: RA: Conceptualization, Project administration, Supervision, Writing original draft, Review & Editing. PC and SF: Data curation, Investigation. ML and VC: Methodology, Validation. MC: Conceptualization, Project administration, Supervision, Review & Editing.

Conflict of Interest Disclosure: All the authors declare the absence of any conflict of interest regarding the present manuscript.

Manuscript accepted for publication

REFERENCES

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy. 2022 Mar;77(3):734-766. doi: 10.1111/all.15090.
2. Asero R. Omalizumab in severe chronic urticaria: are slow and non-responders different? Eur Ann Allergy Clin Immunol. 2021; 53(6): 263-266. doi: 10.23822/EurAnnACI.1764-1489.167.
3. Schoepke N, Asero R, Ellrich A, Ferrer M, Gimenez-Arnau A, E H Grattan C, et al. Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria: Results of the PURIST Study. Allergy. 2019 Dec;74(12):2427-2436. doi: 10.1111/all.13949.
4. Schmetzer O, Lakin E, Topal FA, Preusse P, Freier D, Church MK, Maurer M. IL-24 is a common and specific autoantigen of IgE in patients with chronic spontaneous urticaria. J Allergy Clin Immunol. 2018; 142(3): 876-882. doi: 10.1016/j.jaci.2017.10.035.
5. Kolkhir P, Muñoz M, Asero R, Ferrer M, Kocatürk E, Metz M, et al. Autoimmune chronic spontaneous urticaria. J Allergy Clin Immunol. 2022; 149(6): 1819-1831. doi: 10.1016/j.jaci.2022.04.010.
6. Baldo BA. MRGPRX2, drug pseudoallergies, inflammatory diseases, mechanisms, and distinguishing MRGPRX2- and IgE/FcεRI-mediated events. Br J Clin Pharmacol. 2023 Jul 10. doi: 10.1111/bcp.15845.
7. Kuhn H, Kolkhir P, Babina M, Dull M, Frischbutter S, Fok JS, et al. Mas-related G-protein-coupled X2 and its activators in dermatologic allergies. J Allergy Clin Immunol. 2021; 147(2): 456-469. doi: 10.1016/j.jaci.2020.08.027.

Manuscript accepted for publication

8. Elieh-Ali-Komi D, Metz M, Kolkhir P, Kocatürk E, Scheffel J, Frischbutter S, et al. Chronic urticaria and the pathogenic role of mast cells. Allergol Int. 2023; 72(3): 359-368. doi: 10.1016/j.alit.2023.05.003.
9. Asero R. Mechanisms of histamine release from mast cells beyond the high affinity IgE receptor in severe chronic spontaneous urticaria. Immunol Lett 2024; 265:1-4. doi: 10.1016/j.imlet.2023.11.008.
10. Puxeddu I, Pistone F, Pisani F, Levi-Schaffer F. Mast cell signaling and its role in urticaria. Ann Allergy Asthma Immunol. 2024; S1081-1206(24)00242-4. doi: 10.1016/j.anai.2024.04.023.
11. Asero R, Cugno M, Tedeschi A. Eosinophils in chronic urticaria: supporting or leading actors? World Allergy Organ J. 2009 Sep;2(9):213-7. doi: 10.1097/WOX.0b013e3181bb965f.
12. Giménez-Arnau AM, De Montojoye L, Asero R, Cugno M, Kulthanan K, Yanase Y, et al. The Pathogenesis of Chronic Spontaneous Urticaria: The Role of Infiltrating Cells. J Allergy Clin Immunol Pract. 2021; 9(6): 2195-2208. doi: 10.1016/j.jaip.2021.03.033.
13. Yanase Y, Takahagi S, Ozawa K, Hide M. The Role of Coagulation and Complement Factors for Mast Cell Activation in the Pathogenesis of Chronic Spontaneous Urticaria. Cells. 2021 Jul 12;10(7):1759. doi: o10.3390/cells10071759.
14. Asero R, Marzano AV, Ferrucci S, Genovese G, Cugno M. Baseline D-dimer plasma levels correlate with disease activity but not with the response to omalizumab in chronic spontaneous urticaria Allergy. 2019;74(12):2538. doi: 10.1111/all.13936.
15. Asero R, Marzano AV, Ferrucci S, Cugno M. D-Dimer Plasma Levels Parallel the Clinical Response to Omalizumab in Patients with Severe Chronic Spontaneous Urticaria. Int Arch Allergy Immunol. 2017;172(1):40-44. doi: 10.1159/000453453.

Manuscript accepted for publication

16. Baskurt D, Sarac E, Asero R, Kocatürk E. D-dimer levels decline after immunosuppressive treatment rather than anticoagulant treatment in severe autoimmune chronic spontaneous urticaria. Eur Ann Allergy Clin Immunol. 2024 Jan;56(1):42-44. doi: 10.23822/EurAnnACI.1764-1489.272
17. Asero R, Ferrucci SM, Calzari P, Consonni D, Cugno M. Thyroid Autoimmunity in CSU: A Potential Marker of Omalizumab Response? Int J Mol Sci. 2023 Apr 19;24(8):7491. doi: 10.3390/ijms24087491. PMID: 37108654; PMCID: PMC10139159.
18. Brás R, Esteves Caldeira L, Bernardino A, Costa C. Anti-TPO IgG/Total IgE Ratio: Biomarker for Omalizumab Response Prediction in Chronic Spontaneous Urticaria. Int Arch Allergy Immunol. 2023;184(9):866-869. doi: 10.1159/000532021. Epub 2023 Aug 9. PMID: 37557083.
19. Keskinkaya Z, Kaya Ö, Işık Mermutlu S, Öğretmen Z. Predicting the Speed of Response to Omalizumab in Chronic Spontaneous Urticaria. Int Arch Allergy Immunol. 2024;185(8):786-793. doi: 10.1159/000538291. Epub 2024 Apr 16. PMID: 38626739.
20. Palladino A, Villani F, Pinter E, Visentini M, Asero R. The autologous serum skin test (ASST) predicts the response to anti-IgE treatment in Chronic Spontaneous Urticaria patients: a prospective study. Eur Ann Allergy Clin Immunol 2024 Mar 14. doi: 10.23822/EurAnnACI.1764-1489.337.
21. Asero R, Marzano AV, Ferrucci S, Lorini M, Carbonelli V, Cugno M. Co-occurrence of IgE and IgG autoantibodies in patients with chronic spontaneous urticaria. Clin Exp Immunol. 2020 Jun;200(3):242-249. doi: 10.1111/cei.13428.
22. Maronese CA, Ferrucci SM, Moltrasio C, Lorini M, Carbonelli V, Asero R, et al. IgG and IgE Autoantibodies to IgE Receptors in Chronic Spontaneous Urticaria and Their Role in the Response to Omalizumab. J Clin Med. 2023 Jan 3;12(1):378. doi: 10.3390/jcm12010378.

Manuscript accepted for publication

23. Xiang YK, Kolkhir P, Scheffel J, Sauer M, Vera C, Frischbutter S, et al. Most Patients with Autoimmune Chronic Spontaneous Urticaria Also Have Autoallergic Urticaria, but Not ViceVersa. J Allergy Clin Immunol Pract. 2023 Aug;11(8):2417-2425.e1. doi: 10.1016/j.jaip.2023.02.006.
24. Puccetti A, Bason C, Simeoni S, Millo E, Tinazzi E, Beri R, et al.. In chronic idiopathic urticaria autoantibodies against Fc epsilonRII/CD23 induce histamine release via eosinophil activation. Clin Exp Allergy. 2005; 35(12):1599-607. doi: 10.1111/j.1365-2222.2005.02380.x.
25. Cugno M, Marzano AV, Tedeschi A, Fanoni D, Venegoni L, Asero R. Expression of tissue factor by eosinophils in patients with chronic urticaria. Int Arch Allergy Immunol. 2009;148(2):170-4. doi: 10.1159/000155748.
26. Tedeschi A, Asero R, Marzano AV, Lorini M, Fanoni D, Berti E, Cugno M. Plasma levels and skin-eosinophil-expression of vascular endothelial growth factor in patients with chronic urticaria. Allergy. 2009 Nov;64(11):1616-22. doi: 10.1111/j.1398-9995.2009.02069.x.
27. Tedeschi A, Lorini M, Asero R. No evidence of increased serum substance P levels in chronic urticaria patients with and without demonstrable circulating vasoactive factors. Clin Exp Dermatol 2005 Mar;30(2):171-5. doi: 10.1111/j.1365-2230.2005.01732.x
28. Metz M, Krull C, Hawro T, Saluja R, Groffik A, Stanger C, Staubach P, Maurer M. Substance P is upregulated in the serum of patients with chronic spontaneous urticaria. J Invest Dermatol. 2014 Nov;134(11):2833-2836. doi: 10.1038/jid.2014.226. Epub 2014 May 20.
29. Ozaydin-Yavuz G, Yavuz IH, İnalöz HS, Boyvadoglu C. Omalizumab is not just an anti-immunoglobulin E. J Dermatolog Treat. 2022 Sep;33(6):2858-2861. doi: 10.1080/09546634.2022.2089326.

Manuscript accepted for publication

30. Boyvadoglu C, Ulusal H, Taysı S, Ozaydin-Yavuz G, Yavuz IH, Korkmaz P, et al. Effects of Omalizumab on Serum Levels of Substance P, Calcitonin Gene-Related Peptide, Neuropeptide Y, and Interleukin-31 in Patients with Chronic Spontaneous Urticaria. Mediators Inflamm. 2023; 2023:8087274. doi: 10.1155/2023/8087274
31. Fujisawa D, Kashiwakura J, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. J Allergy Clin Immunol. 2014; 134(3): 622-633.e9. doi: 10.1016/j.jaci.2014.05.004.
32. Cao TBT, Cha HY, Yang EM, Ye YM. Elevated MRGPRX2 levels related to disease severity in patients with chronic spontaneous urticaria. Allergy Asthma Immunol Res. 2021 May;13(3):498-506. doi: 10.4168/aair.2021.13.3.498.

Manuscript accepted for publication

Table 1. Demographic and clinical features of chronic spontaneous urticaria (CSU) patients.

	CSU patients (n = 32)	Healthy controls (n = 20)
Age - Median (range)	56 (21-87)	54 (20-81)
Male/Female	11/21	7/13
UAS-7 - Median (range)	31 (20-42)	
Duration of disease – median years (range)	4 (2-7)	
Angioedema (n / total)	17 / 32	
Atopy (n / total)	9 / 32	
Thyroiditis (n / total)	5 / 32	
Eosinophils n/μL– Median (range)	100 (40-800)	100 (30 -350)

Manuscript accepted for publication

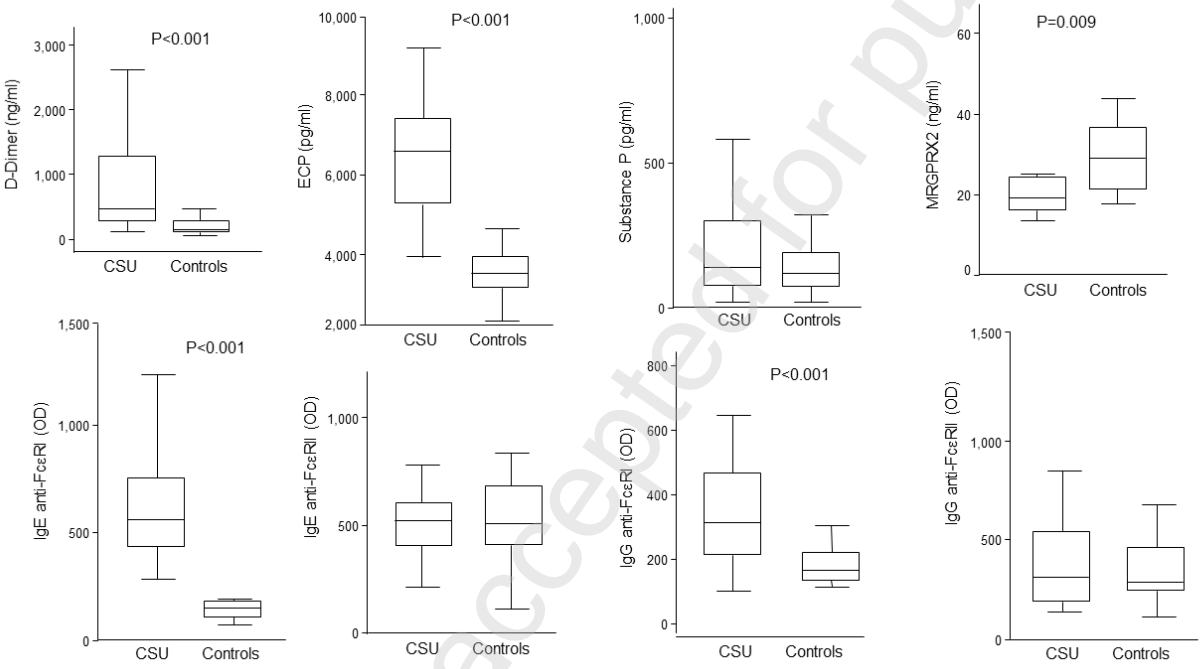
Table 2. Values of parameters involved in the pathogenesis of CSU at baseline and after one-week therapy with omalizumab in 32 CSU patients divided according to the final response to omalizumab.

	BASELINE			ONE-WEEK THERAPY		
	Early responders	**Late responders**	**Non responders**	**Early responders**	**Late responders**	**Non responders**
D-Dimer - ng/ml	393 (121-2623)	370 (157-4530)	378 (191-1781)	244 (83-722) *$	350 (179-3784)	378 (191-1781)
ECP - pg/ml	6392 (3987-8394)	7323 (4766-8797)	6595 (3960-9181)	5918 (3262-7832)	6900 (3461-8202)	5613 (3839-7860)
Substance P - pg/ml	171 (52-581)	235 (46-1114)	119 (20-321)	319 (51-909) $$	400 (70-1586) $$	591 (134-1060) $$
MRGPRX2 - ng/ml	15.8 (10.4-53.7)	16.0 (11.7-76.4)	18.0 (11.2-40.3)	15.1 (6.5-23.6)	17.9 (11.1-69.8)	18.5 (10.0-36.9)
IgE anti-FceRI - OD	612 (370-1421)	557 (283-1043)	475 (400-1399)	314 (255-622) **$$	398 (230-566)	391 (261-1137)
IgE anti-FceRII - OD	497 (353-688)	560 (201-667)	521 (207-1306)	521 (344-651)	575 (192-700)	572 (218-1287)
IgG anti-FceRI - OD	282 (102-583)	304 (121-888)	417 (161-882)	268 (78-565)	263 (100-738)	484 (93-890)
IgG anti-FceRII - OD	282 (154-668)	356 (175-754)	377 (144-1618)	279 (137-676)	372 (168-709)	234 (153-1391)

Manuscript accepted for publication

FIGURE LEGENDS

Figure 1. Plasma levels of D-dimer, eosinophil cationic protein (ECP), substance P, soluble mas-related G-protein coupled receptor member X2 (MRGPRX2), IgE and IgG anti-FCεRI and anti-FCεRII in 32 patients with severe chronic spontaneous urticaria (CSU) at baseline and 20 healthy controls. Results are expressed as median values, interquartile ranges (boxes), and 5th and 95th percentiles (whiskers).



Manuscript accepted for publication

Figure 2. Upper part: plasma levels of IgE anti-FCεRI and D-dimer in patients with severe chronic spontaneous urticaria at baseline and one-week after the first omalizumab administration divided on the basis of final response. Lower part: clinical response to omalizumab, expressed as urticaria activity score on 7 days (UAS7).

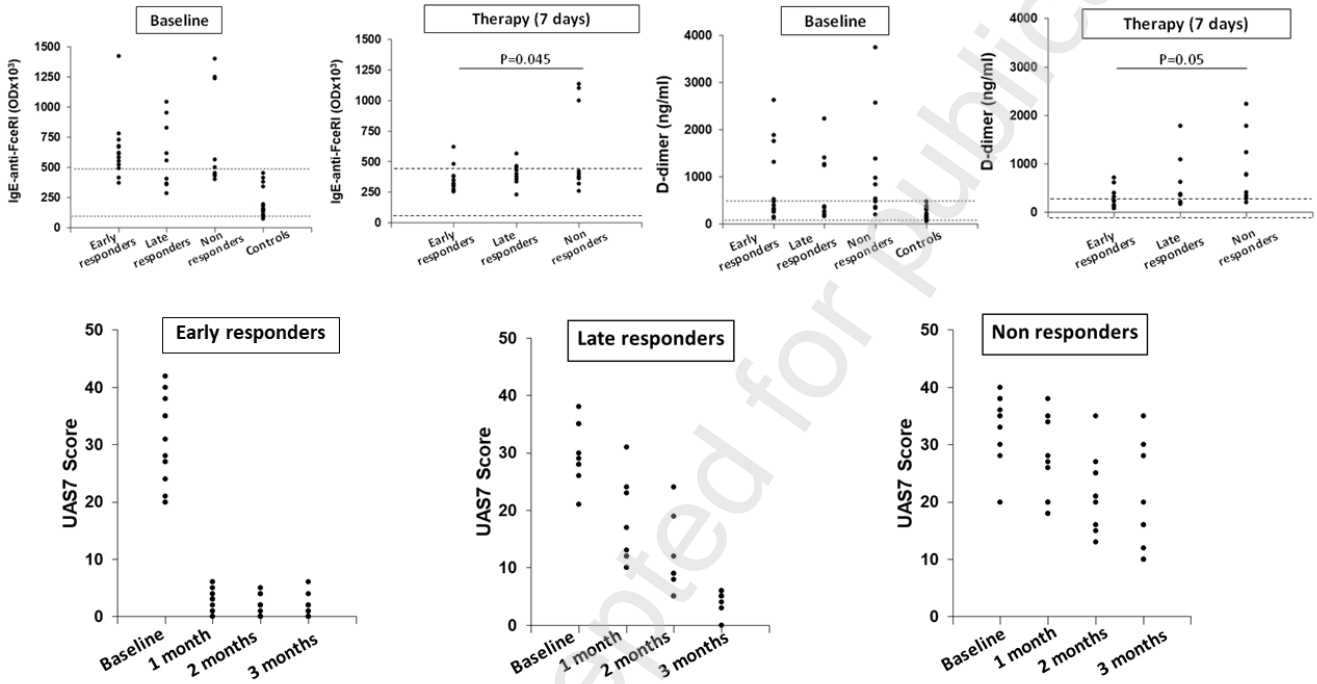
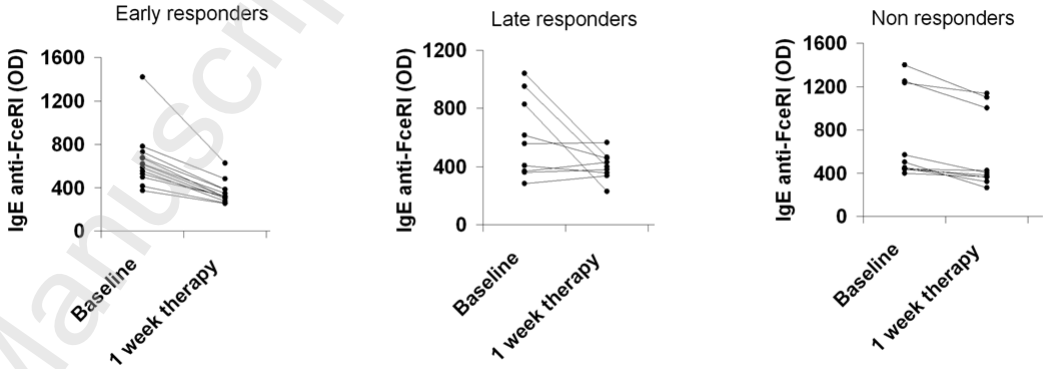
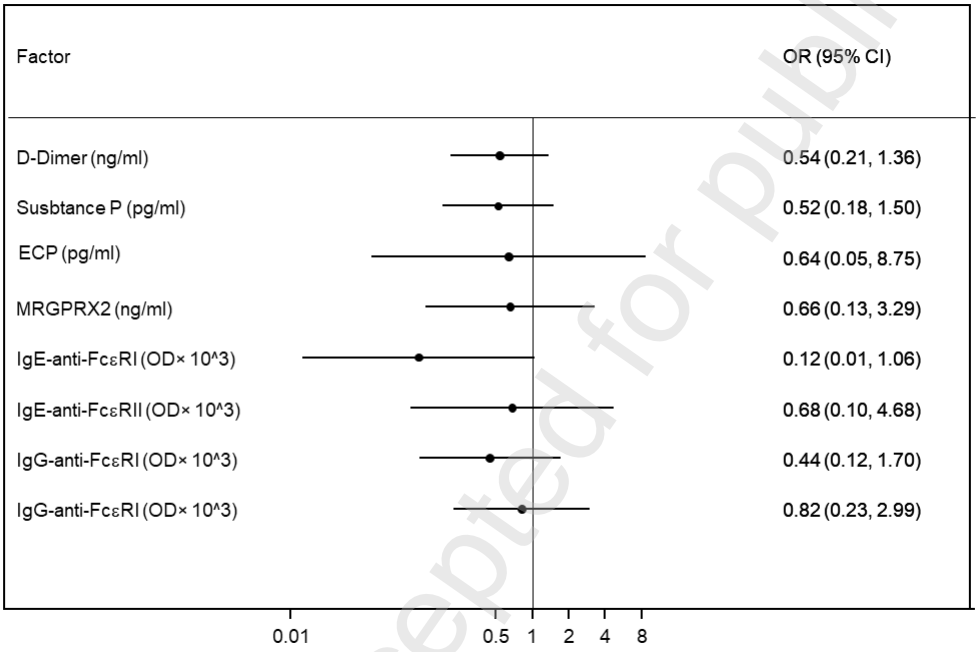


Figure 3. Plasma levels of IgE anti-FcεRI at baseline and after one week from the first administration of omalizumab in patients with severe chronic spontaneous urticaria divided on the basis of final response to the drug.



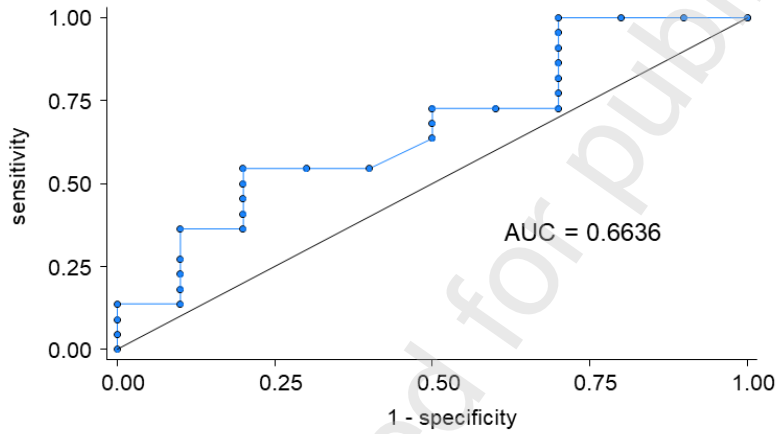
Manuscript accepted for Publication

Figure 4. Evaluation of the association between the response of the various parameters after one week from the first administration of omalizumab and the final clinical response to the drug. Logistic regression analysis shows that IgE anti-FcεRI levels after one week of therapy were significantly associated with a final good response to omalizumab, with an odds ratio (OR) of 0.12 (95% CI: 0.01-1.06).



Manuscript accepted for publication

Figure 5. Receiver operating characteristic (ROC) curve relative to the IgE anti-FcεRI response after one week of omalizumab therapy, showing a good capacity to discriminate between responders and non-responders as indicated by an area under the curve (AUC) of 0.66 (95% CI: 0.45-0.88).



Manuscript accepted for publication