Accepted Article

DR. SABINE ALTRICHTER (Orcid ID : 0000-0001-9955-385X)
DR. VASILIKI ZAMPELI (Orcid ID : 0000-0002-6219-1178)
PROF. MARCUS MAURER (Orcid ID : 0000-0002-4121-481X)

Article type : Original Article: Atopic Dermatitis, Urticaria and Skin Disease

IgMand IgA in addition to IgG autoantibodiesagainst FcεRIα are frequent and associated with disease markers of chronic spontaneous urticaria

Sabine Altrichter[1], Vasiliki Zampeli[1], André Ellrich[1], Ke Zhang[2], Martin K Church[1]and Marcus Maurer[1]

[1] Dermatological Allergology, Allergie-Centrum-Charité, Dpt. of Dermatology and Allergy, Charité- Universitätsmedizin Berlin, Germany

[2] Department of Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

Short title:

autoantibodies to FcεRIαin urticaria

Key words: autoantibodies, autoimmunity, chronic spontaneous urticaria, FcεRIα, IgM

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14412

This article is protected by copyright. All rights reserved

Word count (not including abstracts, figure legends and references): words:3319/3500, tables: 1, figures: 3, supplemental tables: 2, supplemental figures: 2

Corresponding author

Marcus Maurer, MD, Dpt. of Dermatology and Allergy, Allergie-Centrum-Charité/ECARF, Charité-Universitätsmedizin Berlin, Charitéplatz 1 10117 Berlin, Germany

Tel +49 30 450 518 043, Fax +49 30 450 518 972

email: marcus.maurer@charite.de

Abbreviations

Ab	Antibody
ASST	Autologous serum skin test
BAT	Basophile Activation Test
BHRA	Basophil histamine release assay
CRP	C-reactive protein
CSU	Chronic Spontaneous Urticaria
DLQI	Dermatology Life Quality Index
ELISA	Enzyme linked immunosorbent assay
FcεRIα	High-affinity IgE receptor α
MC	Mast cell
SD	Standard deviation
TSH	Thyroid Stimulating Hormone
UAS	Urticaria activity score

Authorship

Sabine Altrichter has coordinated the study, collected patient data, was involved in statistical analysis and drafted the manuscript.

Vasiliki Zampeli was involved in manuscript preparation and proof-reading of the manuscript.

André Ellrich was involved in the statistical analysis and proof-reading of the manuscript.

Accepted Article

This article is protected by copyright. All rights reserved

Ke Zhang performed laboratory tests and was involved in the proof-reading of the manuscript.

Martin Church was involved in manuscript preparation and proof-reading of the manuscript

Marcus Maurer was the overall study coordinator and was involved in manuscript preparation and proof-reading of the manuscript.

Acknowledgement

We want to thank Beate Schinzel for assistance and Gillian Brodie for English proof reading.

This study and the development of our report benefitted from the exchange of ideas and interactions with the members of the GA^{2}LEN network of Urticaria Centers of Reference and Excellence (UCARE; www.ga2len-ucare.com).

Conflict of Interest

All authors have no conflict of interests in relation to this work.

Accepted Article

This article is protected by copyright. All rights reserved

Abstract

Background: IgG-autoantibodiesagainst the high affinity IgE-receptor,FcεRIα**,** contribute the pathogenesis of autoimmune chronic spontaneous urticaria (CSU). However, it is not known if such patients also exhibit IgM or IgA autoantibodies against FcεRIα.

Objectives: To develop an ELISAto assess serum levels of IgG, IgM andIgA autoantibodies against FcεRIα, and to investigateif their presence is linked to clinical features of CSU including the response to autologous serum skin testing (ASST).

Methods: Serum samples of 35 CSU patients (25 ASST-positive) and 52 healthy controlindividuals were analyzed using a newly developed competitive ELISA for IgG, IgM and IgAautoantibodies to FcεRIα.

Results: One in four CSU patients (8/35, 24%) had elevated serum levels of IgG-anti-FcεRIα compared with(3/52, 6%) in healthy controls. More than half of patients had IgM (21/35, 60%) and IgA (20/35, 57%) vs (3/52, 5%) each in healthy controls. ElevatedIgM, but not IgG or IgA autoantibodies, were significantly more frequent in ASST-positive CSU patients (18/25, 72%) compared with ASST-negative patients (3/10, 33%, P =0.022).Also, elevated levels of IgM-anti-FcεRIα, but not of IgG or IgA against FcεRIα, were linked to low blood basophil(r = 0.414, P = 0.021) and eosinophil(r = 0.623, P < 0.001) counts.

Conclusions: Increased serum levels of IgM-anti-FcεRIα are common in patients with CSU and linked to features of autoimmune CSU. The role and relevance of autoantibodies to FcεRIα in CSU can and should be further characterized in future studies, and our novel assay can help with this.

Accepted Article

This article is protected by copyright. All rights reserved

Introduction

Chronic spontaneous urticaria (CSU) is a mast cell–driven skin disease characterized by the recurrence of transient wheals, angioedema, or both for more than 6 weeks.[1] Mast cell degranulation induced via the high affinity IgE receptor (FcεRI) is considered to be one of the most frequent initiating factors in CSU.[2] This is evidenced by the effectiveness of omalizumab (anti-IgE) in reducing the symptoms of the disease.[3] In CSU, FcεRI-dependent mast cell degranulation may occur in either of two ways. First is Type I autoimmunity (aka autoallergy) in which a patient's IgE is directed to autoallergens, such as thyroid peroxidase [4] and interleukin-24.[5, 6] Second is Type II autoimmunity in which autoantibodies to IgE or FcεRI, initiate mast cell activation.[7, 8]Beside these well characterized mechanisms further, yet unidentified, serum derived mast cell activation factors could be involved in the pathomechanism of CSU.[9-11]

The initial suggestion of autoantibodies to IgE or FcεRI arose more than 30 years ago from the autologous serum skin test (ASST) in which a positive response was the development of a wheal at the site of injection of a patient's own serum. [12] Studies by Grattan and colleagues identified these autoantibodies of the IgG class.[8, 13, 14] More recent studies have indicated them to be IgG_1 and IgG_3, which are able to cause degranulation of skin mast cells and activate complement.[15-18]

Whether or not CSU patients exhibit IgM or IgA autoantibodies to FcεRI, is presently unknown as immunoblotting assays with purified IgG [18-20] and ELISAs [15, 21] for the detection of IgG-anti-FcεRI or IgG-anti-IgE are not yet well-established for other antibodies. Althougha rapid and specific dot-blot immunoassay to detect autoantibodies against FcεRIα was introduced by Lee and colleagues in 2014,[22]it is not available for routine clinical use.

In this study, we aimed to develop a simple and practical, yet highly specific, ELISA test to detect autoantibodies against FcεRIα in the serum of patients with CSU. We did this fortwo reasons.First, wewished to facilitate the identification of CSU patients with Type IIb autoimmune urticaria by providing a test that is suitable foruse in routine clinical practice. Second,we wished to analyze the serum of CSU patients for IgM- and

This article is protected by copyright. All rights reserved

IgA-autoantibodies against FcεRIα and assess if their presence is linkedto positiveASST responses, disease activity and other clinical parameters.

Methods

Patients

Serum samples and clinical information was obtained from 35 patients with CSU seen at the Urticaria Center of Reference and Excellence (UCARE) at the Department of Dermatology and Allergy, Charité–Universitätsmedizin Berlin.[23] CSU was diagnosed and its severity assessed according to the recent EAACI/GA²LEN/EDF/WAO guideline for urticaria [24]. Patient blood samples and data were obtained as part of their clinical diagnostic workup, and surplus serum from clinical analyses was stored at –80°C until used for this study. All patients provided prior informed consent on the use of left-over serum as well as their clinical data for research purposes. As all analyses were performed retrospectively and anonymously, additional ethics approval was not needed or obtained. Patients had not taken systemic steroids and H_1-antihistamines in the last two weeks and three days before sampling, respectively.

The control group consisted of 52 serum samples from healthy controlswith a negative history of chronic urticaria, autoimmune disease, or clinically relevant allergic disease. Within this group, a sex- and age-matched healthy subgroup (n=16) was analyzed in detail, to rule out differences due to demographics. Clinical characteristics and demographics of all patients with CSU and healthy controls are presented in Supplemental Table S1.

Development of an ELISA-based assay for the detection of anti-FcεRIαautoantibodies

An ELISA for IgM, IgG and IgA autoantibodies against FcεRIα was developed and used to test sera of patients with CSU and healthy controls. Recombinant soluble FcεRIα expressed from yeast (MyBiosource, San Diego, CA, USA) was coated at 50 ng/ml onto 96 well microtiter ELISA plates with 0.1 M bicarbonate buffer pH 9.6 overnight at 4°C. The plates were blocked with 10% foetal bovine serum (FBS) in phosphate buffered saline with 0.1% Tween-20 (PBST) for two hours. CSU and healthy control sera (1:100 with 10% FBS-PBST) were pre-incubated with FcεRIα at 100 ng/ml or with respective

This article is protected by copyright. All rights reserved

volume PBST of for one hour at room temperature. Pre-incubated and mock-incubated sera were loaded into the plates in parallel. After two hours of incubation at room temperature, the plates were washed with PBST (0.05% Tween-20) three times, followed by incubation with appropriately diluted AP-labelled goat-anti-human IgG, IgM or IgA (KPL, Gaithersburg, MD, USA) for one hour at room temperature. The plates were washed five times prior to adding the blue phosphatase substrates for colorimetric development and read with a wavelength of 650 nm. The reduction of signal by pre-incubation with FcεRIα was considered to indicate the presence of antibodies to FcεRIα of the respective immunoglobulin group. Since those assays detect the presence of autoantibodies (IgG, IgM, IgA) against FcεRIα via signal differences with and without the pre-incubation with soluble FcεRIα (ratio of signal without and with FcεRIα), its results are largely independent of background signals and lower ratios reflect higher levels of autoantibodies (Supplemental Figure 1).

Selected sera were measured repetitively (ten times) to assess the intra-assay variation-coefficient (Supplemental Figure 2). The normal range of assay results was determined by the use of healthy control sera. No significant differences between the autoantibodies values and ranges were seen when unselected healthy controls or selected age- and sex-matched cohorts were analyzed (Supplemental Table 1). The 95% percentile was identified to give the best discriminative values (Supplemental Table 2) and set as the cut-off for elevated levels of anti-FcεRIαautoantibodies. The 95% percentile cut-off values set with all healthy controls for IgM were determined at a ratio of 0.50, for IgA at 0.49, and for IgG at 0.46.

Routine Laboratory Assessments

Routine laboratory assessments were performed in the central laboratory of the Charité-Universitätsmedizin Berlin. Routine assessments included C-reactive protein (CRP), complete blood count, total serum IgG, IgA, IgM and IgE, electrolyte levels (sodium, potassium), laboratory values of the kidney (creatinine, urea) and liver (aspartate transaminase, alanine transaminase, gamma-GT), thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), thyroid autoantibodies (anti-TPO, anti-TG), and IgG to hepatitis A, B and C. Presence of Helicobacter pylori antigen was analyzed via PCR in the feces.

Accepted Article

This article is protected by copyright. All rights reserved

Autologous Serum Skin Test (ASST)

The ASST was performed as described previously [25]. Reactions were evaluated after 15 and 30 minutes andwheal diameter of at least 1.5 mm greater than that of the control wheal induced by the saline solution was recorded as positive. Because a higher prevalence of IgG-anti-FcεRIα was expected in ASST-positive patients, CSU patients were preselected into 25 ASST-positive and 10 ASST-negative patients.

Assessment of urticaria activity and quality of life

Urticaria activity was assessed using the seven-day urticaria activity score (UAS7). [1]Assessment of disease control over the previous four weeks on signs and symptoms, quality of life impairment, efficacy of treatment and overall disease control was performed using the urticaria control test (UCT).[26, 27]Quality of life was assessed by the dermatology life quality index (DLQI) using the validated “band descriptors” [28]as follows: DLQI: 0-1 = no effect on patient’s life, 2-5 = small effect, 6-10 = moderate effect, 11-20 = very large effect, 21-30 = extremely large effect.

Statistics

D'Agostino & Pearson omnibus normality test was used to test for normal distribution. Parametric data are depicted using mean and standard error of mean values and non-parametric data using median and interquartile (IQR) range.Variation coefficient was calculated for repetitive measurements. For the cut-off determination of IgA, IgM and IgG the 0.05 percentile of the healthy population was calculated. The cut-offs assessed this way resulted in excellent specificity and were close to expert suggestions of a cut-off of 0.5, and therefore considered best practice for the analysis.Binominal variables were analyzed using Chi-square test. Parametric variables were compared using the unpaired t test. Non-parametric continuous variables were compared using the Mann-Whitney test. Correlations between two continuous variables were calculated using Spearman's rank correlation coefficient. $P < 0.05$ was considered to indicate statistical significance.

Results

This article is protected by copyright. All rights reserved

CSU patients have elevated levels of IgG, IgM, and IgAautoantibodies against FcεRIα

Of 35 patients with CSU, 8 (24%) had elevated serum levels of IgG autoantibodies against FcεRIα (IgG-anti-FcεRIα), compared with 6% of the healthy control group (*P* = 0.037, Table 1). Among all tested sera, IgG-anti-FcεRIα levels, on average, were comparable in CSU patients and healthy subjects (CSU median 0.82 [IQR 0.48 - 1.02] vs healthy median 0.79 [IQR 0.67 - 1.01], *P* = 0.661).

More than half of the CSU patients analyzed, 60% and 57%, respectively, had elevated IgM antibodies and IgA antibodies against FcεRIα as compared to 5 % each of healthy controls. The CSU patient cohort also had significantly higher average levels of IgM-anti-FcεRI and IgA-anti-FcεRI (as reflected by lower ratios) compared with healthy controls (*P* ≤ 0.001, Table 1).

Only four CSU patients (11%) did not have elevated levels of anti-FcεRIαautoantibodies of any subclass, and two patients (6%) were positive for all three subclasses. Six of the eight IgG-anti-FcεRIα-positive patients also had elevated levels of antibodies of other subclasses (IgM: n=3; IgA: n=5). A large proportion of CSU patients (n=12; 35%) had both, elevated IgM and IgA antibodies against FcεRIα(Figure 1).

Across all sera tested (CSU patients and healthy control subjects), IgG-anti-FcεRIα levels correlated weakly but significantly with IgA-anti-FcεRIα (r = 0.349, *P* = 0.001), but not with IgM-anti-FcεRIα (r = 0.066, *P* = 0.547). IgM-anti-FcεRIα and IgA-anti-FcεRIα were also significantly correlated, albeit weakly (r = 0.375, *P*< 0.001).

In patients with CSU, a positive ASST is linked to elevated levels of IgM against FcεRIα, but not IgG or IgA against FcεRIα

The analyzed CSU patient cohort contained 25 ASSTpositive (71%) and 10 ASSTnegative (29%) patients. IgG-anti-FcεRIα levels were elevated in four of the ASST-positive (17%) and four of the ASST-negative (40%) patients (*P* = 0.154, Figure 2a). IgA-anti-FcεRIα antibodies were elevated in about half the patients who were ASST-positive (n = 13, 52%) and ASST-negative (n = 6, 60%, p = 0.668) (Figure 2b).In contrast, IgM-anti-FcεRIα were elevated significantly more often in ASST-positive patients (n = 18,

This article is protected by copyright. All rights reserved

72%) compared with ASST-negative patients with CSU (n = 3, 30%; *P* = 0.022) (Figure 2c).

In patients with CSU, IgM against FcεRIα, but not IgG or IgA against FcεRIα, is linked to basopenia and eosinopenia

The presence of elevated levels of IgM-anti-FcεRIα, but not IgG-anti-FcεRIα or IgA-anti-FcεRIα, correlated with low basophil numbers (r = 0.414, *P*= 0.021). IgM-anti-FcεRIα-positive CSU patients had significantly lower blood basophil numbers (median 0.02/nl [IQR 0.01 - 0.03]) as compared to IgM-anti-FcεRIα-negative patients (median 0.22/nl [IQR 0.13 - 0.32] *P*=0.0081, see Figure 3a).

The presence of elevated levels of IgM-anti-FcεRIα also showed a clear correlation with the patients' eosinophil numbers (r = 0.623, *P*< 0.001). Again, IgM-anti-FcεRIα-positive CSU patients had significantly lower absolute eosinophil numbers (median 0.11/nl [IQR 0.04 - 0.15]) compared with IgM-anti-FcεRIα-negative patients (median 0.2/nl [IQR 0.12 - 0.32] *P* = 0.0016; see Figure 3b). The presence of elevated levels of IgG-anti-FcεRIα or IgA-anti-FcεRIα was not linked to eosinopenia, and patients who were positive for these autoantibodies did not have reduced blood eosinophil numbers as compared to patients who were not (data not shown).

A weak, but significant correlation of IgM-anti-FcεRIα-positivity, but not IgG-anti-FcεRIα-positivity or IgA-anti-FcεRIα-positivity, was also seen with lymphocyte numbers (r = 0.38, *P* = 0.028).

Indicators of disease activity (UAS7), disease control (UCT) or quality of life (DLQI) in our CSU patient cohort were not significantly different or correlated with any of the anti-FcεRIα subclasses tested. There were also no difference or link to other cells assessed in the blood count, electrolyte levels, CRP levels, kidney or liver markers, thyroid hormones or thyroid autoantibodies or total IgE. Furthermore, IgG, IgM and IgA autoantibodies against FcεRIα were not correlated with total serum IgG, IgM and IgA levels, respectively.

Discussion

Accepted Article

This article is protected by copyright. All rights reserved

In this study, we present a new method for the detection of autoantibodies against FcεRIα. To our knowledge, this is the first report to show that CSU patients, in addition to having IgG autoantibodies, have IgM and IgA autoantibodies against FcεRIα. This new ELISA-based test is inexpensive and easy to perform, suitable for high through-put routine analysis. The assay has proved to be robust in repetitive measurements and circumvents the common problem of serum background signals, by using specific competitive signal inhibition by soluble FcεRIα pre-incubation.

IgG autoantibodies against FcεRIαwere first described in patients with CSU more than 20 years ago [18]. Despite this, no routine testing of these autoantibodies is currently available. Some of the methods used require IgG purification of the serum before detecting via Western blot techniques [18, 19, 29-33], which is time-consuming. Using our newly developed ELISA, the prevalence of elevated levels of IgG autoantibodies against FcεRIαwas 24% in the analyzed CSU patient sera. These results are in line with many other reports in the literature where rates of 25% up to 52% had been reported in CSU patients [18, 19, 29-33].

More interestingly, the majority of our analyzed sera also contained elevated levels of IgM (60%) and IgA autoantibodies (57%) to FcεRIα. Little is known about the prevalence and function of IgM and IgA autoantibodies in patients with CSU. Gruber *et al*. [34] analyzed the prevalence of IgG and IgM autoantibodies directed against IgE in distinct subsets of urticaria, namely CSU and cold urticaria as well as urticaria vasculitis. IgG-anti-IgE antibodies were found in 50% of patients with CSU, in 55% of patients with cold urticaria, and in 50% of patients with urticarial vasculitis. IgM-anti-IgE antibodies with histamine-releasing activity were found exclusively in cold urticaria (22%), but not in patients with CSU.

The ASST is a well-established test to investigate serum autoreactivity in patients with CSU [35] and has been associated with IgG antibodies against the FcεRIα or against IgE (Type IIb autoimmunity) [7, 36]. Also, basophil activation tests and basophil histamine release tests have been shown to be a good surrogate marker for autoimmunity in CSU [7], but are not commonly available as routine analysis and had not been assessed in this study cohort. Surprisingly, in our cohort, ASST-positive patients did not show a higher rate of elevated levels of IgG to FcεRIαcompared with ASST-negative patients. This

This article is protected by copyright. All rights reserved

finding is somewhat contradictory those of other groups, who reported up to 6-fold higher rates of IgG autoantibodies to FcεRIαin ASST-positive patients compared to ASST-negative CSU patients [29, 33]. However, our cohort had overall small numbers, especially in the ASST-negative CSU patient group, which limits the interpretation of our results. Furthermore, other studies used different methods for IgG antibody detection, which might explain, in part, the large differences reported among different studies. In previous reports it had been shown that the IgG autoantibodies in urticaria patients belong predominately to the IgG1 and IgG3 subtype and, to a lesser extent, IgG4[15, 37], who have complement fixing and basophil activation abilities. Asero and colleagues has shown that >100kDa serum fractions (that include IgG) are able to degranulate basophils and that at least in some cases complement seems essential for histamine-releasing activity of serum from patients with CIU[38]. In our study we have not further discriminated the IgG subtypes or complement fixation ability.

One of the most interesting findings of our study is that elevated levels of IgM-anti-FcεRIα are markedly more common in ASST positive patients (72%) compared with ASST negative patients (30%) (Figure 2b).

Antibodies involved in autoimmunity usually belong to the IgG and IgM subclasses. In CSU, Grattan *et al*. showed that the IgM fraction of three CSU sera led to some histamine release from healthy donor basophils, but the specificity of these IgM antibodies remains elusive[8]. To our knowledge, there are no identified IgM autoantibodies with known specificity in patients with CSU in the literature. As IgG autoantibodies to IgE or to FcεRIα exist in CSU, the presence of IgM autoantibodies to IgE or to FcεRIα is feasible and could play a significant role in the pathogenesis of CSU. Their presence might explain ASST reactivity in IgG-anti-FcεRIα and IgG-anti-IgE negative CSU patients, especially as there were only a few patients (n=3) who were double positive for IgG and IgM autoantibodies.Specific IgM is thought to precede IgG formation[39]and thus, IgM positive CSU patients might become IgG positive in the course of their disease. Longitudinal studies following CSU patients over longer time periods assessing IgG or other autoantibodies are currently missing.

Additionally, IgA autoantibodies to FcεRIα were also frequently elevated (57%) in our CSU cohort. Of note, all of the 4 patients in our cohort who tested positive for

This article is protected by copyright. All rights reserved

Helicobacter antigen displayed IgA autoantibodies (data not shown), and five out of eight IgA positive patients had IgG antibodies against hepatitis A, but none of the seven IgA negative patients, whose serological hepatitis were available (Fisher exact; $P = 0.007$), indicating that present or past gastrointestinal or mucosal associated infections could be a trigger for the development of IgA autoantibodies. These autoantibodies showed a correlation with IgM autoantibodies, and more than one third (35%) of the CSU patients were double positive for both autoantibodies. In contrast to the IgM autoantibody titers, there was no significant difference between the ASST positive and negative CSU patient cohorts (Fig. 2c)

FcεRIα is not only expressed on mast cells, but also on basophils [40] and in some inflammatory diseases, also on eosinophils.[41, 42] In CSU, basopenia has been shown to be correlated with disease activity and is restored upon successful treatment with omalizumab.[43, 44] In a subgroup of CSU patients, eosinopenia can also be observed.[45] Serum-induced basophil activation, which can be observed with many CSU sera samples, is thought to be due to autoantibodies against IgE or FcεRIα, leading to basopenia [46]. In our study, IgM, but not IgG autoantibodies against FcεRIα, were significantly correlated with basopenia and eosinopenia. IgM, like some IgG subclasses, is a complement fixing antibody that can directly activate the complement system with subsequent cell destruction,[47] possibly leading to the observed low cell count.

In our CSU patient cohort, the presence of autoantibodies of any class was not associated with higher disease activity, disease burden or less disease control. It has been reported that the presence of IgG autoantibodies against FcεRIα is linked to more severe symptoms and a poor response to conventional antihistamine therapy, as well as a slower response to anti-IgE treatment.[19, 30, 48] In a large multicenter trial using this new assay for IgG-anti-FcεRIα determination, along with many other autoimmune markers, higher UAS7 scores in autoimmune type II CSU patients were seen, but not in other scorings.[7] We cannot explainwhy we did not see this correlation in our study but the small sample size or the preselection of patients may have been relevant. Of note, there was also no correlation with the total serum IgG, IgM or IgA values, which strengthens the idea that the detected autoantibodies are rather specific.

Accepted Article

This article is protected by copyright. All rights reserved

The overall limitations of this study are the retrospective analysis and the limited number of preselected patients from a specialized tertiary care center. Nevertheless, we were able to demonstrate, for the first-time, the presence of new immunoglobulin class antibodies against FcεRIα and the association with autoimmune disease features. Further limitations are given by the fact that this test assesses the occurrence of the autoantibodies, but does not directly answer the question of functional relevance of these autoantibodies. Here further studies are needed to prove this.

In conclusion, this study reports a new and easy method for the detection of IgG, IgM, and IgA autoantibodies against FcεRIα. Elevated levels of IgM and IgA class autoantibodies against FcεRIα were more frequent than those of IgG. The detection of these new autoantibody classes in CSU and, in the case of IgM, the association with clinical features such as the ASST and basopenia adds to our understanding of the role of autoantibodies in CSU.

References

1. Zuberbier T, Aberer W, Asero R, Abdul Latiff AH, Baker D, Ballmer-Weber B, et al. The EAACI/GA(2)LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. *Allergy* 2018;**73**(7):1393-1414.
2. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: What we know and what we do not know. *J Allergy Clin Immunol* 2017;**139**(6):1772-1781 e1771.
3. Chang TW, Chen C, Lin CJ, Metz M, Church MK, Maurer M. The potential pharmacologic mechanisms of omalizumab in patients with chronic spontaneous urticaria. *J Allergy Clin Immunol* 2015;**135**(2):337-342.
4. Altrichter S, Peter HJ, Pisarevskaja D, Metz M, Martus P, Maurer M. IgE mediated autoallergy against thyroid peroxidase--a novel pathomechanism of chronic spontaneous urticaria? *PLoS One* 2011;**6**(4):e14794.

Accepted Article

This article is protected by copyright. All rights reserved

5. Schmetzer O, Lakin E, Topal FA, Preusse P, Freier D, Church MK, et al. IL-24 is a common and specific autoantigen of IgE in chronic spontaneous urticaria. *J Allergy Clin Immunol* 2017:In Press.
6. Lakin E, Church MK, Maurer M, Schmetzer O. On the Lipophilic Nature of Autoreactive IgE in Chronic Spontaneous Urticaria. *Theranostics* 2019;**9**(3):829-836.
7. Schoepke N, Asero R, Ellrich A, Ferrer M, Gimenez-Arnau A, Grattan CEH, et al. Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria (aiCSU): Results of the PURIST Study. *Allergy* 2019.
8. Grattan CE, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;**21**(6):695-704.
9. Grattan CE, Hamon CG, Cowan MA, Leeming RJ. Preliminary identification of a low molecular weight serological mediator in chronic idiopathic urticaria. *Br J Dermatol* 1988;**119**(2):179-183.
10. Bossi F, Frossi B, Radillo O, Cugno M, Tedeschi A, Riboldi P, et al. Mast cells are critically involved in serum-mediated vascular leakage in chronic urticaria beyond high-affinity IgE receptor stimulation. *Allergy* 2011;**66**(12):1538-1545.
11. Cugno M, Tedeschi A, Frossi B, Bossi F, Marzano AV, Asero R. Detection of Low-Molecular-Weight Mast Cell-Activating Factors in Serum From Patients With Chronic Spontaneous Urticaria. *J Investig Allergol Clin Immunol* 2016;**26**(5):310-313.
12. Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A serological mediator in chronic idiopathic urticaria--a clinical, immunological and histological evaluation. *Br J Dermatol* 1986;**114**(5):583-590.
13. Grattan CE. Histamine-releasing autoantibodies in chronic urticaria. *Skin Pharmacol* 1991;**4 Suppl 1**:64-70.
14. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;**328**(22):1599-1604.
15. Fiebiger E, Hammerschmid F, Stingl G, Maurer D. Anti-FcepsilonRIalpha autoantibodies in autoimmune-mediated disorders. Identification of a structure-function relationship. *J Clin Invest* 1998;**101**(1):243-251.

Accepted Article

This article is protected by copyright. All rights reserved

16. Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, Raap U, et al. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy* 2013;**68**(1):27-36.

17. Church MK, Kolkhir P, Metz M, Maurer M. The role and relevance of mast cells in urticaria. *Immunol Rev* 2018;**282**(1):232-247.

18. Fiebiger E, Maurer D, Holub H, Reininger B, Hartmann G, Woisetschlager M, et al. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest* 1995;**96**(6):2606-2612.

19. Sabroe RA, Fiebiger E, Francis DM, Maurer D, Seed PT, Grattan CE, et al. Classification of anti-FcepsilonRI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol* 2002;**110**(3):492-499.

20. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;**107**(6):1056-1062.

21. Zuberbier T, Henz BM, Fiebiger E, Maurer D, Stingl G. Anti-FcepsilonRIalpha serum autoantibodies in different subtypes of urticaria. *Allergy* 2000;**55**(10):951-954.

22. Lee MF, Lin TM, Liu SW, Chen YH. A rapid method of detecting autoantibody against FcepsilonRIalpha for chronic spontaneous urticaria. *PLoS One* 2014;**9**(10):e109565.

23. Maurer M, Metz M, Bindslev-Jensen C, Bousquet J, Canonica GW, Church MK, et al. Definition, aims, and implementation of GA(2) LEN Urticaria Centers of Reference and Excellence. *Allergy* 2016;**71**(8):1210-1218.

24. Zuberbier T, Aberer W, Asero R, Abdul Latiff AH, Baker D, Ballmer-Weber B, et al. The EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. The 2017 revision and update. *Allergy* 2018;**73**(7):1393-1414.

25. Staubach P, Onnen K, Vonend A, Metz M, Siebenhaar F, Tschentscher I, et al. Autologous whole blood injections to patients with chronic urticaria and a positive autologous serum skin test: a placebo-controlled trial. *Dermatology* 2006;**212**(2):150-159.

26. Weller K, Groffik A, Church MK, Hawro T, Krause K, Metz M, et al. Development and validation of the Urticaria Control Test: a patient-reported outcome instrument for

Accepted Article

This article is protected by copyright. All rights reserved

assessing urticaria control. *J Allergy Clin Immunol* 2014;**133**(5):1365-1372, 1372 e1361-1366.

27. Ohanyan T, Schoepke N, Bolukbasi B, Metz M, Hawro T, Zuberbier T, et al. Responsiveness and minimal important difference of the urticaria control test. *J Allergy Clin Immunol* 2017;**140**(6):1710-1713 e1711.

28. Hongbo Y, Thomas CL, Harrison MA, Salek MS, Finlay AY. Translating the science of quality of life into practice: What do dermatology life quality index scores mean? *J Invest Dermatol* 2005;**125**(4):659-664.

29. Ulambayar B, Chen YH, Ban GY, Lee JH, Jung CG, Yang EM, et al. Detection of circulating IgG autoantibody to FcepsilonRIalpha in sera from chronic spontaneous urticaria patients. *J Microbiol Immunol Infect* 2017.

30. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-FcepsilonRI or anti-IgE autoantibodies. *J Am Acad Dermatol* 1999;**40**(3):443-450.

31. Ferrer M, Kinet JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-Fc(epsilon)RIalpha (alpha-subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;**101**(5):672-676.

32. Grattan CE. Autoimmune urticaria. *Immunol Allergy Clin North Am* 2004;**24**(2):163-181, v.

33. Baioumy SA, Esawy MM, Shabana MA. Assessment of circulating FCepsilonRIa in Chronic Spontaneous Urticaria patients and its correlation with clinical and immunological variables. *Immunobiology* 2018.

34. Gruber BL, Baeza ML, Marchese MJ, Agnello V, Kaplan AP. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol* 1988;**90**(2):213-217.

35. Konstantinou GN, Asero R, Maurer M, Sabroe RA, Schmid-Grendelmeier P, Grattan CE. EAACI/GA(2)LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 2009;**64**(9):1256-1268.

36. Baioumy SA, Esawy MM, Shabana MA. Assessment of circulating FCepsilonRIa in Chronic Spontaneous Urticaria patients and its correlation with clinical and immunological variables. *Immunobiology* 2018;**223**(12):807-811.

Accepted Article

This article is protected by copyright. All rights reserved

37. Soundararajan S, Kikuchi Y, Joseph K, Kaplan AP. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J Allergy Clin Immunol* 2005;**115**(4):815-821.

38. Asero R, Tedeschi A, Lorini M, Salimbeni R, Zanoletti T, Miadonna A. Chronic urticaria: novel clinical and serological aspects. *Clin Exp Allergy* 2001;**31**(7):1105-1110.

39. Janeway CAJ, Travers P, Walport M, Shlomchik MJ. Immunobiology. *Garland Publishing.* 2001;**5th Edition, ISBN 978-0-8153-3642-6. (via NCBI Bookshelf)**.

40. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2010;**125**(2 Suppl 2):S73-80.

41. Messingham KN, Holahan HM, Frydman AS, Fullenkamp C, Srikantha R, Fairley JA. Human eosinophils express the high affinity IgE receptor, FcepsilonRI, in bullous pemphigoid. *PLoS One* 2014;**9**(9):e107725.

42. Gounni AS, Lamkhioued B, Delaporte E, Dubost A, Kinet JP, Capron A, et al. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? *J Allergy Clin Immunol* 1994;**94**(6 Pt 2):1214-1216.

43. Ferrer M. Immunological events in chronic spontaneous urticaria. *Clin Transl Allergy* 2015;**5**:30.

44. Gericke J, Ohanyan T, Church MK, Maurer M, Metz M. Omalizumab may not inhibit mast cell and basophil activation in vitro. *J Eur Acad Dermatol Venereol* 2015;**29**(9):1832-1836.

45. Kolkhir P, Church MK, Altrichter S, Stahl Skov P, Hawro T, Frischbutter S, et al. Eosinopenia, in chronic spontaneous urticaria, is associated with high disease activity, autoimmunity and poor response to treatment *J Allergy Clin Immunol Pract* 2019;**In Press**.

46. Rauber MM, Pickert J, Holiangu L, Mobs C, Pfutzner W. Functional and phenotypic analysis of basophils allows determining distinct subtypes in patients with chronic urticaria. *Allergy* 2017;**72**(12):1904-1911.

47. Parwaresch MR, Horny HP, Lennert K. Tissue mast cells in health and disease. *Pathol Res Pract* 1985;**179**(4-5):439-461.

48. Gericke J, Metz M, Ohanyan T, Weller K, Altrichter S, Skov PS, et al. Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria. *J Allergy Clin Immunol* 2017;**139**(3):1059-1061.

Accepted Article

This article is protected by copyright. All rights reserved

Legends

Table 1: Patients with CSU more frequently display elevated levels of autoantibodies of different subclasses against FcεRIα. For IgG-anti-FcεRI, IgM-anti-FcεRI, and IgA anti-FcεRI (all assessed by the use of a competitive ELISA), subjects with values below the cut-off of 0.46, 0.59 and 0.49 were considered positive, respectively. Rates of patients or healthy controls with elevated autoantibody levels, i.e. values below the cut-off, are given in the upper panel, median and interquartile ranges of ratios are shown in the lower panel of each Ig subclass.

Aabs		**CSU patients** n = 35	**Healthy controls** n = 52	**P value**
IgG	**IgG-anti-FcεRIα positive** Number (%) of patients	**8 (24%)** n = 34[#]	**3 (6%)**	**0.037**
	IgG-anti-FcεRIα (ratio) median (q1; q3) [range]	**0.82** (0.48; 1.02) [0.19 - 1.61] n = 34[#]	**0.79** (0.67; 1.01) [0.4; 1.37]	**0.661**
IgM	**IgM-anti-FcεRIα positive** Number (%) of patients	**21 (60%)**	**3 (6%)**	**< 0.001**
	IgM-anti-FcεRIα (ratio) median (q1; q3) [range]	**0.47** (0.37; 0.98) [0.01 - 1.39]	**0.88** (0.74; 0.99) [0.19 - 1.79]	**0.001**
IgA	**IgA-anti-FcεRIα positive** Number (%) of patients	**20 (57%)**	**3 (6%)** n = 51[#]	**< 0.001**
	IgA-anti-FcεRIα (ratio) median (q1; q3) [range]	**0.47** (0.24; 0.76) [0 - 1.77]	**0.87** (0.74; 1.03) [0.39 - 1.66] n = 51[#]	**< 0.001**

n is given if not all patients of the group were included.

q1 25% percentile

q3 75% percentile

[#] single sample missing due to a technical problem

This article is protected by copyright. All rights reserved

Accepted Article

Figure 1: Venn diagram of co-occurrence of anti-FcεRIα antibodies of the three different subclasses. Numbers in the graph give the number of single, double or triple positive CS.

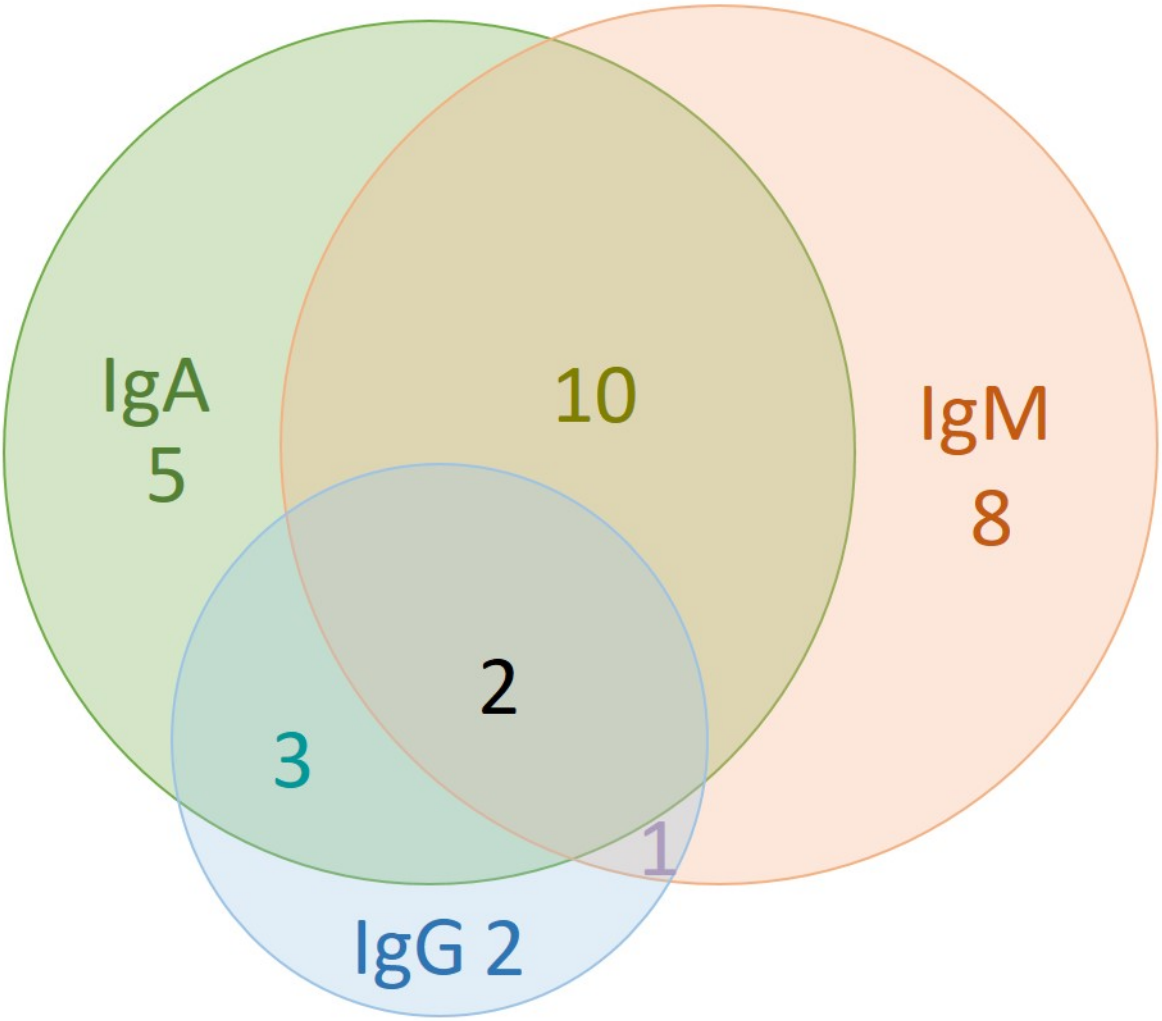
Figure 2: Autoantibodies to FcεRIα in the ASST positive (n = 24) and ASST negative (n = 10) CSU patient subgroups. All three panels of this figure show ratios of individual patients (every dot represents one patient). Low ratios reflect high autoantibody titers. In addition, median and interquartile ranges are shown for the ASST subgroups for a) IgG-anti-FcεRIα, b) IgA anti-FcεRIα, c) IgM-anti-FcεRIα. Cut-offs of the individual assays are depicted as dotted line and areas of elevated levels are depicted in light gray.

Figure 3: The presence of elevated levels of IgM antibodies against FcεRIαis linked to low basophil (3a) and eosinophil numbers (3b). Ratios of individual patients are displayed as dots, median and interquartile ranges are shown for the anti- FcεRIα IgM positive (n = 20) and negative subgroups (n = 11).

Accepted Article

This article is protected by copyright. All rights reserved

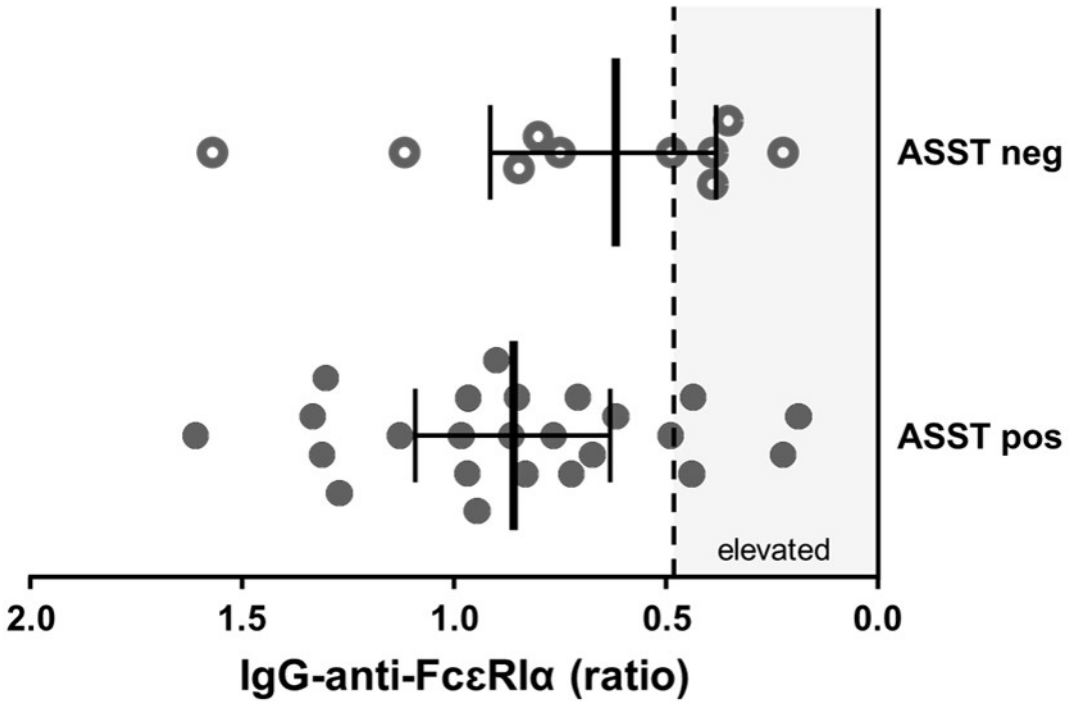
Accepted Article



all_14412_f1.jpg

This article is protected by copyright. All rights reserved

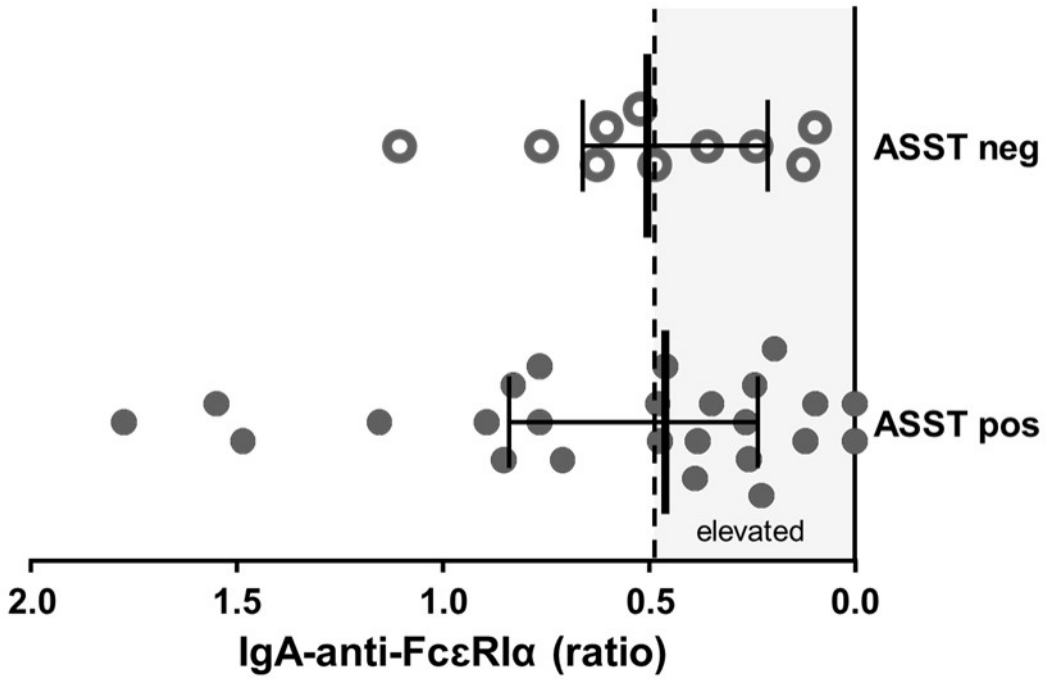
Accepted Article



all_14412_f2a.jpg

This article is protected by copyright. All rights reserved

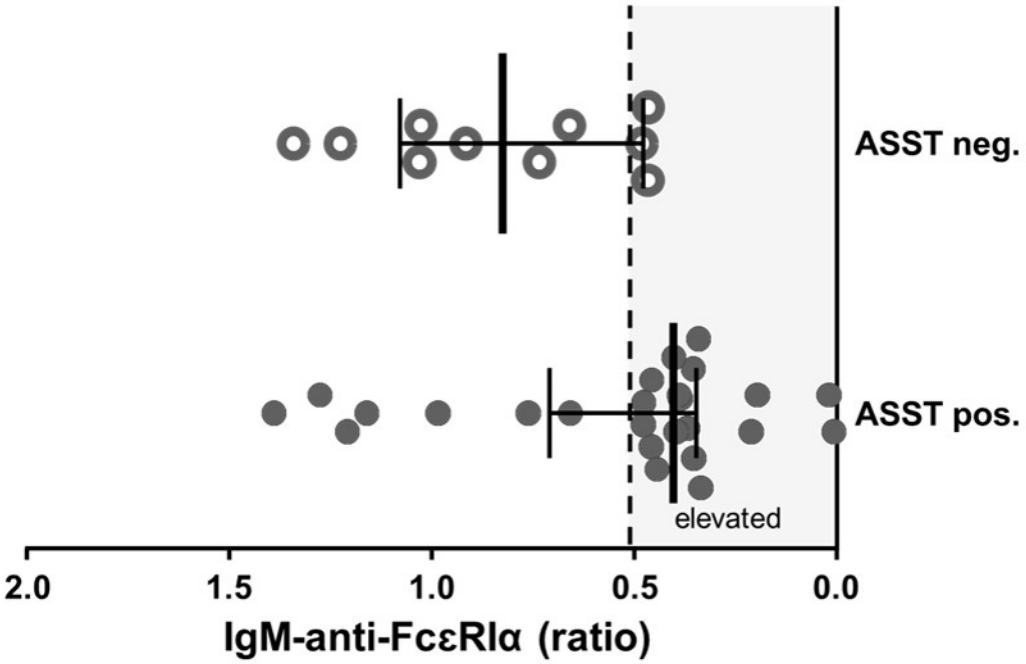
Accepted Article



all_14412_f2b.jpg

This article is protected by copyright. All rights reserved

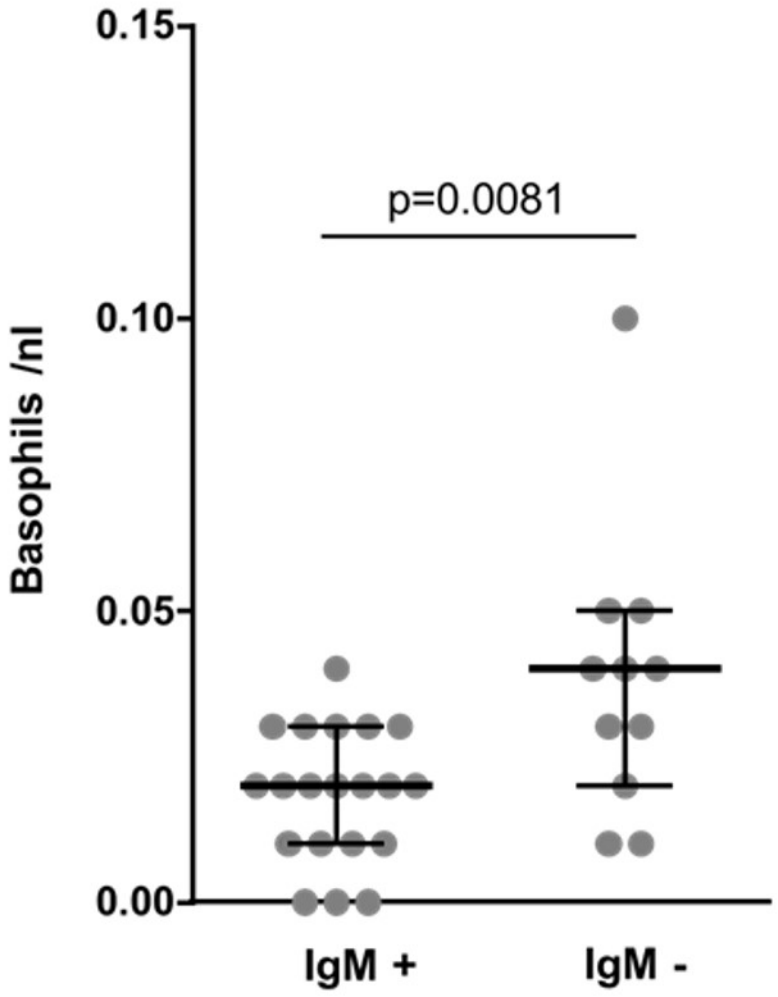
Accepted Article



all_14412_f2c.jpg

This article is protected by copyright. All rights reserved

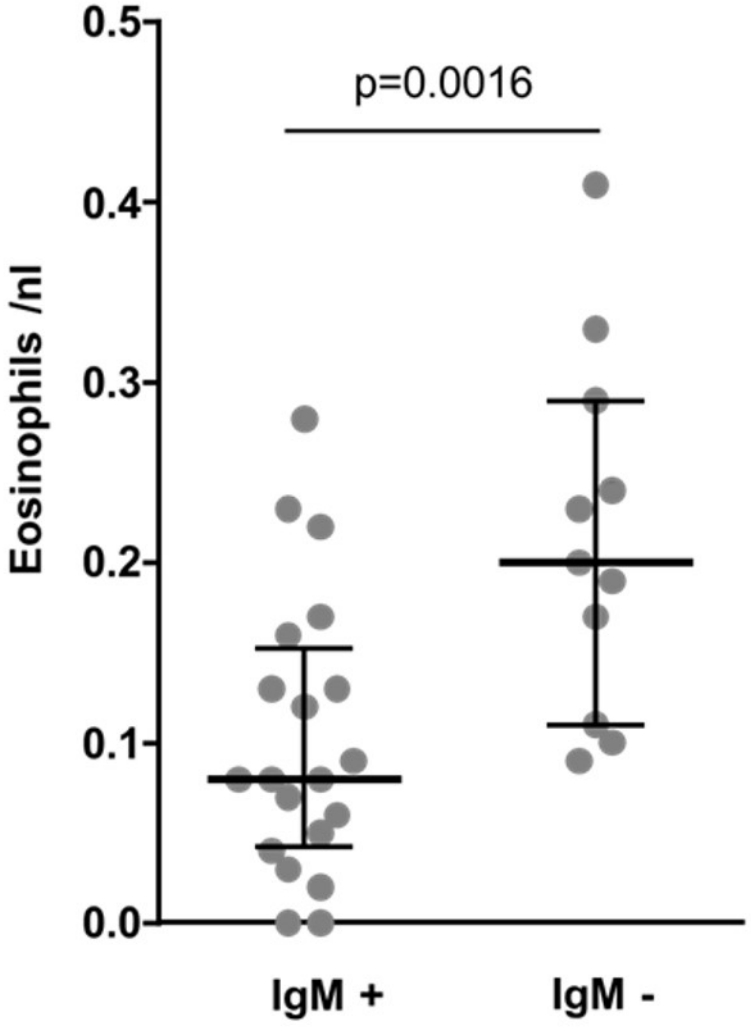
Accepted Article



all_14412_f3a.jpg

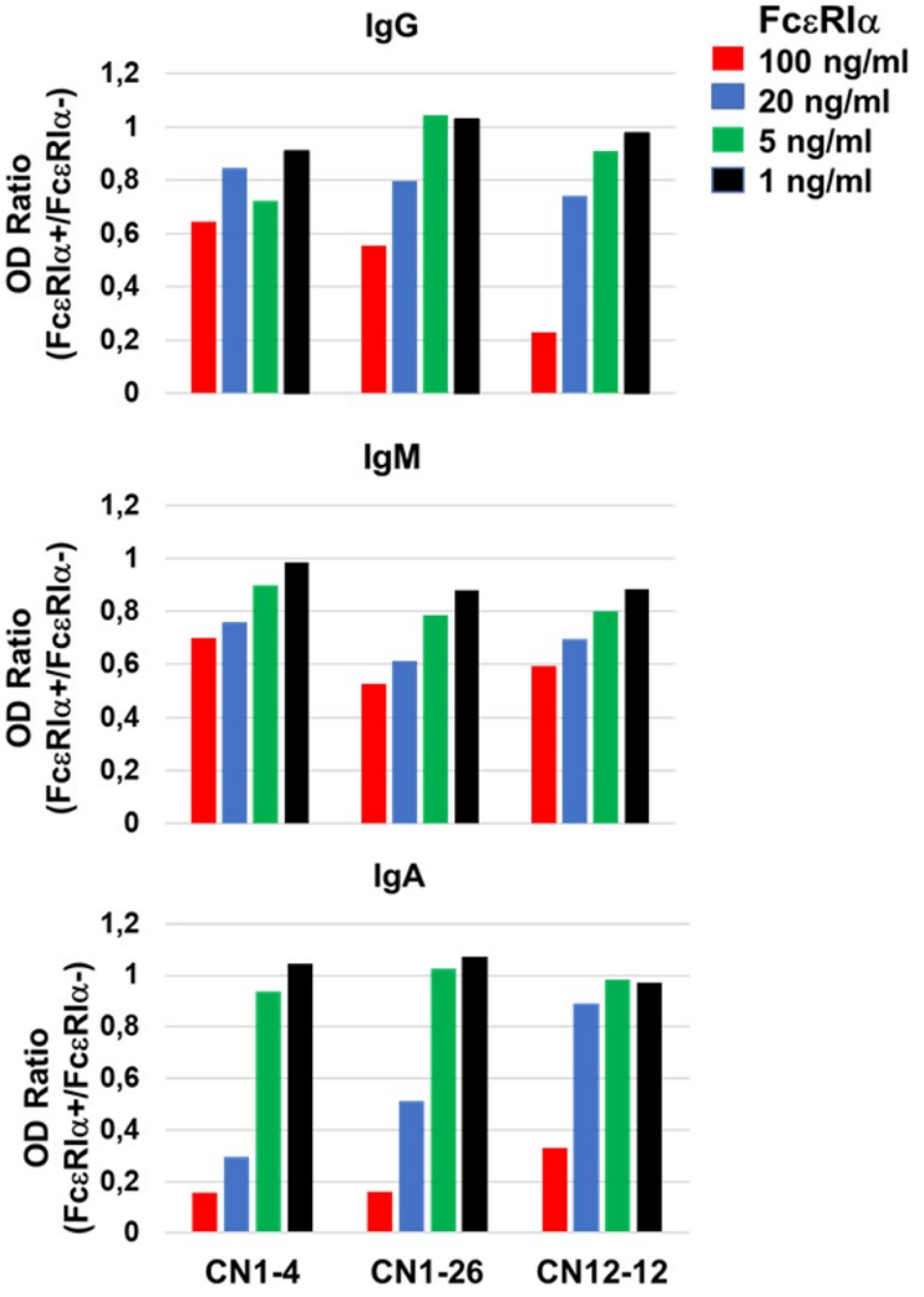
This article is protected by copyright. All rights reserved

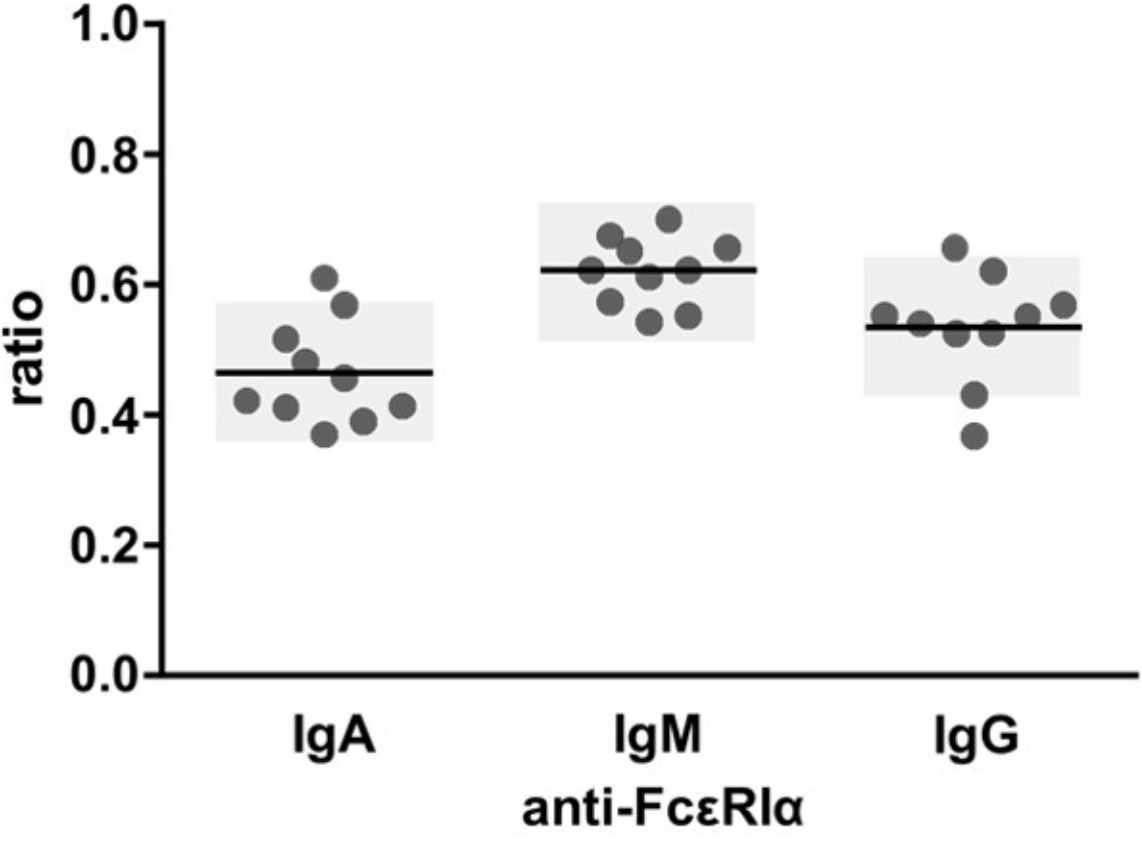
Accepted Article



all_14412_f3b.jpg

This article is protected by copyright. All rights reserved


IgG
IgM
IgA
FcεRIα
100 ng/ml
20 ng/ml
5 ng/ml
1 ng/ml
OD Ratio
(FcεRIα+/FcεRIα-)
1,2
1
0,8
0,6
0,4
0,2
0
CN1-4
CN1-26
CN12-12


1.0
0.8
0.6
0.4
0.2
0.0
ratio
IgA
IgM
IgG
anti-FcεRIα

Supplemental Material

Supplemental Table S1: Clinical characteristics of study participants. Healthy controls as displayed as either all healthy controls or just a subgroup of age and sex matched healthy controls. No significant differences between the two healthy control groups had been seen regarding Aabs results of any class.

	CSU patientsn = 35	**Total healthy controls** n = 52	**Age- and sex-matched healthy controls** n = 16
Age mean +- sd median (q1 , q3) [range]	**40.9**±15.2 **43** (29; 52) [13 - 82]	**26.4**±9.4 **22** (21; 33.8) [19 - 51]	**37.4**±9.3 **38.5** (31.3; 45.5) [22 - 51]
Sex M:F	**11:24**	**31:21**	**6:10**
Duration of disease (years) mean +- sd median (q1 , q3) [range]	**5.0**±6.8 **2** (1; 5.8) [0.1 - 22]	-	-
UASday (0-6 points) mean +- sd median (q1 , q3) [range]	**2.6**±1.8 **3** (1; 4) [0 - 6]	-	-
UAS7 (0-42 points) mean +- sd median (q1 , q3) [range]	**17.6**±11 **18** (9; 25) [0 - 40]	-	-
ASST negative : positive*	**10:25**	**15:1** (n = 16)	**15:1** (n = 16)
IgG-anti-Fc£RIα positive number (%) of patients	**8 (24%)** n = 34#	**3 (6%)**	**0 (0%)**
IgG-anti-Fc£RIα (ratio) median (q1; q3) [range]	**0.82** (0.48; 1.02) [0.19 - 1.61] n = 34#	**0.79** (0.67; 1.01) [0.4; 1.37]	**0.79** (0.64; 0.93) [0.5 – 1.15]
IgM-anti-Fc£RIα positive number (%) of patients	**21 (60%)**	**3 (6%)**	**0 (0%)**
IgM-anti-Fc£RIα (ratio) median (q1; q3) [range]	**0.47** (0.37; 0.98) [0.01 - 1.39]	**0.88** (0.74; 0.99) [0.19 - 1.79]	**0.84** (0.62; 0.93) [0.52 - 1.06]

IgA-anti-Fc£RIα positive number (%) of patients	**20 (57%)**	**3 (6%)** n = 51#	**1 (6%)**
IgA-anti-Fc£RIα (ratio) median (q1; q3) [range]	**0.47** (0.24; 0.76) [0 - 1.77]	**0.87** (0.74; 1.03) [0.39 - 1.66] n = 51#	**0.79** (0.64; 0.93) [0.48 - 1.15]

* wheal to autologous serum ≥1.5mm compared to saline
n is given in the table, if data are not obtained from all patients
single sample missing due to a technical problem
q1 25% percentile
q3 75% percentile

Supplemental Table S2: Cut-off determination.Using total healthy controls (n=52 for IgG and IgM; n=51 for IgA) the 95% percentile of the ratio was calculated, where 95% of the healthy controls had values above the cut-off. Furthermore sensitivity, specificity and positive and negative predictive value of those cut-offs are shown.

	IgA anti-FcεRIα	**IgM anti-FcεRIα**	**IgG anti-FcεRIα**
95% percentile	0.49	0.50	0.46
Sensitivity	0.57	0.60	0.23
Specificity	0.94	0.94	0.94
Positive predictive value	0.87	0.88	0.73
Negative predictive value	0.76	0.78	0.64

Supplemental Figure S1: Ten repetitive measurements of the same sample gave comparable results. Single results are shown as individual dots; black lines indicate the mean and the grey areas show the variation of 10% in each direction. The exact variation coefficient of the IgA measurements was 29.4%, for IgM 8.4% and for IgG 15.7%.

Supplemental Figure S2: Dose-dependent inhibition of the anti-FceRIα OD ratio by FcεRIα preincubation in the competitive ELISA. Using three different sera (CN1-4, CN1-26, CN12-12) with different levels of autoantibodies directed against FcεRIα, the competitive ELISA was performed with pre-incubation concentrations of FcεRIα ranging from 1 to 100ng/ml.

Supporting Information

Animated methods and results from APPEAL-1 can be viewed at:
https://youtu.be/oEcEU81j2kg