High prevalence of autoimmune urticaria in children with chronic urticaria

Luigia Brunetti, MD,[a] Ruggiero Francavilla, MD, PhD,[a] Vito L. Miniello, MD,[a] Michael H. Platzer, MSc,[b] Domenica Rizzi, MD,[a] Maria Letizia Lospalluti, MD,[a] Lars K. Poulsen, PhD,[b] Lucio Armenio, MD, PhD,[a] and Per Stahl Skov, MD, PhD[b]

Bari, Italy, and Copenhagen, Denmark

Background: The etiology of chronic urticaria (CU) in childhood often remains unrecognized. Recently, in adults it has been shown that approximately 40% of patients with CU have autoimmune urticaria (AU); however, no data are available in children.

Objective: To determine the prevalence and possible risk factors for AU in children with CU.

Methods: Ninety-three consecutive children (52 male; median age, 7.8 years) with CU were evaluated for AU by means of autologous serum skin test (ASST) in all and serum-induced basophil histamine release (HR-urticaria test) in 52. All other known causes of CU were excluded as appropriate.

Results: A cause for CU was identified in 44 children (47%), whereas 49 (53%) remained idiopathic. ASST and HR-urticaria test had positive results in 22 of 49 (45%) and in 16 of 31 (52%) children with idiopathic CU compared with 1 of 44 (2%) and 5 of 21 (24%) with CU of a known cause, respectively ($P < .00001$; $P = .09$). Sensitivity, specificity, and positive and negative predictive values of the ASST for diagnosing AU are 78%, 85%, 74%, and 88%. The prevalence of AU in childhood is 31% (15/52; 95% CI, 24%-51%). None of the variables studied were predictive for development of AU.

Conclusion: Our results demonstrate for the first time that children have the same ability as adults to produce functionally active autoantibodies directed against IgE or IgE receptor and that AU occurs in children in as many as 30% of cases. The addition of screening for AU dramatically decreases the rate of the idiopathic form from 52% to 20%. (J Allergy Clin Immunol 2004;114:922-7.)

Key words: *Chronic idiopathic urticaria, childhood, autologous serum skin test, serum induced basophil histamine release, autoimmune urticaria*

Chronic urticaria (CU) is a common skin disorder characterized by recurrent, transitory, itchy wheals with individual lesions lasting less than 24 hours and affecting patients for 6 weeks or longer.[1-3] This condition is thought to affect at least 0.1% of the population,[4] and often it can be severe and difficult to treat.[5]

Abbreviations used

ASST:	Autologous serum skin test
AU:	Autoimmune urticaria
CIU:	Chronic idiopathic urticaria
CU:	Chronic urticaria
HP:	*Helicobacter pylori*
HR-urticaria test:	Serum induced basophil histamine release

The pathogenesis of CU is not completely understood, but mast cell degranulation and histamine release are thought to play a central role.[4] It is known that the binding of an antigen (allergen) to antigen-specific IgE on mast cells and basophils causes cell degranulation resulting in the release of histamine and other vasoactive mediators, responsible of clinical symptoms. However, most patients with CU have no specific allergic trigger for mast cell or basophil activation, and when no cause can be identified, the final diagnosis is chronic idiopathic urticaria (CIU).[1]

Recently it has been shown that, in patients with severe CIU, the intradermal injection of autologous serum elicits an immediate wheal-and-flare response and mast cell degranulation,[1,6] and it is now established that approximately one third of patients with CIU have circulating autoantibodies directed against epitopes in the α-chain of the high-affinity receptor (FcεRI) or against IgE.[6,7] These autoantibodies are functionally active, causing histamine release from basophils of healthy donors and from dermal mast cells *in vitro*.[8] The presence of anti-FcεRI autoantibodies in CIU has been confirmed by Western blot analysis and ELISA in adult series.[8,9]

Few and anecdotal data are published on FcεRI autoantibodies in children with CU.[10] The current investigation was aimed to determine the frequency and pattern of autoantibodies directed against IgE or IgE receptor in children with CU.

METHODS

Participants

The Paediatric Immuno-Allergology Clinic of the University of Bari is the tertiary referral center for the diagnosis and follow-up of allergic diseases of childhood in Southern Italy (Puglia, Calabria, and Basilicata), covering an estimated population of 1.5 million children,

From [a]the Department of Biomedicina dell'Età Evolutiva, University of Bari; and [b]the National University Hospital, Laboratory of Medical Allergology, Copenhagen.

Supported by the University of Bari.

Received for publication April 20, 2004; revised June 27, 2004; accepted for publication July 1, 2004.

Reprint requests: Luigia Brunetti, MD, Clinica Pediatrica I, University of Bari, Piazza Giulio Cesare, 11-70125 Bari, Italy. E-mail: l.brunetti@pediatria3.uniba.it.

0091-6749/$30.00

doi:10.1016/j.jaci.2004.07.042

with approximately 350 new cases of urticaria every year. Between August 2000 and October 2002, 680 new cases of urticaria were admitted to the clinic, most of which were acute. Among these, a total of 93 consecutive children (14%; 52 boys and 41 girls; median age, 7.8 years; range, 9 months to 16 years) with CU, defined as recurrent (at least twice a week), short-lived cutaneous wheal with or without accompanying angioedema and occurring for more than 2 months,[1,10] were prospectively enrolled in the study.

Patients younger than 6 months or older than 16 years were excluded. None had chronic diseases or was taking steroids or immunosuppressive therapy at the time of investigation. Antihistamine treatment was stopped at least 15 days before skin testing was performed or serum sample collected.

Familial (atopy, autoimmune diseases), clinical (age at onset, gastrointestinal symptoms, respiratory symptoms), and laboratory (hemoglobin, white cell, neutrophil, lymphocyte, eosinophil, basophil, and monocyte cell count, IgG, IgE, IgA, IgM) data were carefully collected to evaluate their possible role as predictors of autoimmune urticaria.

Procedures

Full past medical history was recorded and a complete physical examination performed. To investigate the possible cause of CU, all children underwent extensive laboratory evaluations, including the following: (1) blood tests for complete blood count, erythrocyte sedimentation rate, blood chemistry, liver function tests, antistreptolysin antibody, serum levels of complement components C3 and C4, free thyroxin, thyroid stimulating hormone, and total serum IgE; (2) autoimmune panel (antinuclear antibody and anti-DNA antibody [if antinuclear antibody–positive], anti–smooth muscle antibodies, antiparietal cell antibodies, antithyroid peroxidase antibodies, antithyreoglobulin antibodies), (3) infective panel (hepatitis B surface antigen, antibody titers for hepatitis B virus and C virus, TORCH [toxoplasmosis, other, rubella, cytomegalovirus, and herpes simplex virus], Epstein-Barr virus, ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* (HP), stool culture, urine analysis and culture, nasal, throat and vulvar swabs for *Streptococcus β-hemolyticus* and *Staphylococcus aureus*); (4) chest and sinus x-rays; (5) skin prick tests for cypress, olive tree, grass, *Parietaria*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria*, dog and cat dandruff, cereals, egg, fish, meat, fruits, legumes, solancee, milk, and soy; and (6) ice cube test for cold-induced urticaria and skin stroking for dermatographism.[11] If any of these tests were abnormal, further evaluations were performed as appropriate.

All children underwent autologous serum skin tests (ASSTs), and serum-induced basophil histamine release (HR-urticaria test) was performed in 52 children for whom serum was available.

Skin testing with autologous serum. ASST was performed while the urticaria was active. Briefly, 50 μL autologous serum, histamine (10 mg/mL in saline; positive control) and 0.9% sterile saline (negative control) were separately injected into the dermis of the volar forearm at gaps of 5 cm between injecting sites. Areas of previous involvement by spontaneous wheals were avoided. The 2 orthogonal diameters of the wheals were measured 30 minutes after the injection. According to Sabroe,[12] a positive skin test was defined as the presence of a serum-induced wheal with a mean wheal diameter at least 1.5 mm greater than that induced by saline (Δ wheal diameter >1.5 mm). In the absence of a positive response to histamine, the ASST was considered not informative and was repeated after at least 24 hours. All ASSTs were performed by a single investigator (L.B.).

HR-urticaria test. One adult donor whose basophils responded to anti-IgE with a release >30% was used in the assay. The basophil leukocytes were prepared by dextran sedimentation (5 mL freshly drawn heparinized blood was added to 1 mL 5.0% dextran and 0.25 mL 0.2 mol/L EDTA (pH 7.0) and allowed to sediment for 45 minutes at 5°C. The basophil-containing layer was washed once with piperazine-1,4-bis(2-ethanesulfonic acid) containing 0.036% human serum albumin (PIPES-HSA) and the cells resuspended in 2 mL PIPES-HSA containing 500 pg/mL recombinant human IL-3 (R&D Systems, Minneapolis, Minn). Samples of 40 μL cell suspension were incubated in filterplates (Whatman, Brentford, United Kingdom) for 60 minutes at 37°C with serum diluted 1:4 and 1:8 in duplicate (final reaction volume, 80 μL). After incubation, all samples were added to 200 μL PIPES-HSA and centrifuged at 2000*g* for 5 minutes at 5°C to remove serum proteins and released histamine. The cell sediment trapped on the filter was lysed by using 20 μL of a 7% $HClO_4$. Then 200 μL PIPES buffer was added and the samples filtered by centrifugation to collect the histamine from the lysed basophils. The histamine content in the filtrate was measured by using the glass fiber method[13,14] according to the manufacturer (RefLab, Copenhagen, Denmark).

Serum-induced histamine release is expressed as a percentage of total histamine content. Spontaneous release in all experiments was less than 10% and was subtracted to determine serum specific release. Serum from healthy individuals (n = 79) induced histamine release as much as 16%, and the HR-urticaria test is therefore considered positive only at a release >16.5%.

The diagnosis of autoimmune urticaria (AU) was made only if the HR-urticaria test had a positive result.[15]

The study was approved by the local ethical committee, and a written informed consent was obtained for all patients from both parents.

Statistical analysis

The statistical analysis was performed by using the Number Cruncher Statistical System 6.0.1 program (NCSS Statistical Software, Kaysville, Utah). Nonparametric Mann-Whitney test was used for comparison of continuous data (Wilcoxon rank-sum test). A regression stepwise model was applied for the identification of the independent predictors of outcome (AU). Spearman rank correlation was used for examining linear association between percentage of histamine release and Δ wheal diameter. Numbers are reported as median with a range or mean ± SD. A P value less than .05 was considered significant.

RESULTS

The extensive work-up allowed us to establish a cause for CU in 44 of 93 children (47%), whereas 49 (53%) remained idiopathic. The causes of urticaria were distributed as follows: physical in 19 (20%), infectious in 17 (18%), allergic in 3 (3%), and more than one cause in 5 (5%). According to the diagnosis, children were then divided into the following 4 diagnostic groups:

(1) CIU (n = 49 (52%); 30 male (61%); median age, 6.1 years; range, 1.2-16.6 years) if all of the aforementioned clinical and laboratory findings were within normal range. HR-urticaria test was performed in 31 patients (63%).
(2) Physical urticaria (n = 19 (20%); 9 male (47%); median age, 6.1 years; range, 1.0-15.6 years) if provoked by cold or pressure or exercise or dermographism. All other findings were within normal range. HR-urticaria test was performed in 7 patients (37%).
(3) Infectious urticaria (n = 17 (18%); 10 male (59%); median age, 5.5 years; range, 3.3-13.2 years) if

TABLE I. Demographic characteristics of children enrolled

	CIU (n = 49)	Physical (n = 19)	Infectious (n = 17)	Miscellaneous* (n = 8)
Age, y†	6.1 (1.2-16.6)	6.1 (1-15.6)	5.5 (3.3-13.2)	9.2 (2.1-11.7)
Female/male	19/30	10/9	7/10	2/6
Age at onset, y‡	3.6 (0.1-12)	4 (0.2-14.4)	4.6 (0.3-9)	3.5 (0.4-11)
IgE sensitization	13 (27%)	2 (10%)	4 (23%)	4 (50%)
Angioedema	11 (22%)	6 (32%)	7 (41%)	3 (38%)
Gastrointestinal symptoms	19 (39%)	1 (5%)	7 (41%)	4 (50%)
Recurrent respiratory infections	3 (6%)	—	—	1 (13%)
Atopy in the family	28 (57%)	7 (37%)	5 (29%)	5 (63%)

*Allergic (n = 3); allergic + physical (n = 2); physical + infective (n = 2); allergic + physical + infective.
†Age at referral in our unit.
‡Age at first presentation of urticaria.

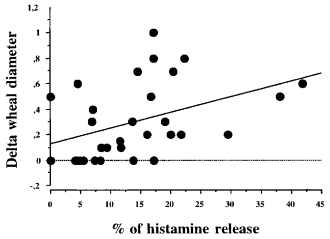
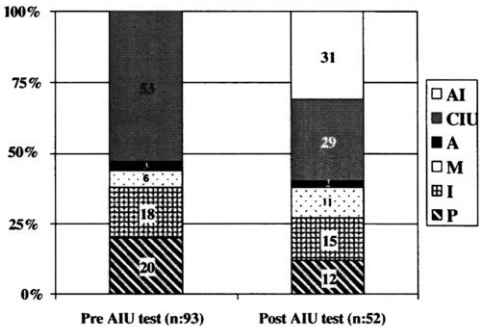


FIG 1. Significant positive upward linear correlation between percentage of histamine release and Δ wheal diameter in children with chronic urticaria (Spearman rank correlation, $r = 0.4$; $P < .007$).



CIU: Chronic idiopathic urticaria; AI: Autoimmune urticaria; A: Allergic urticaria; M: Multifactorial urticaria; I: Infective urticaria; P: Physical urticaria.

FIG 2. Etiology of chronic urticaria before and after the inclusion of the test for autoimmune urticaria in childhood.

caused by Epstein-Barr virus (n = 7), *S aureus* (n = 6), *S β-hemolyticus* (n = 4), or HP (n = 2). HR-urticaria test was performed in 9 patients (53%).

(4) Miscellaneous urticaria (n = 8 (10%); 6 male (75%); median age, 9.2 years; range, 2.1-11.7 years) caused by either allergy (n = 3) or more than one cause (n = 5). HR-urticaria test was performed in 5 patients (63%).

Patients in the different diagnostic categories were not different in demographic characteristics (Table I).

Correlation between ASST and HR-urticaria test

Overall, 52 children (32 male; median age, 6.8 years; range, 1.3-16.6 years) underwent both ASST and HR-urticaria test.

Autologous serum skin test had a positive result in 22 of 49 children with CIU (Δ wheal diameter, 4 ± 1.5 mm), compared with 1 of 44 children with CU of a known cause (45% vs 2%; $P < .00001$). In particular, no children with physical or infectious urticaria had a positive ASST result, compared with children with CIU ($P < .001$ and $P < .002$, respectively). Only 1 patient in the miscellaneous urticaria group (allergic + physical) was ASST-positive (Δ wheal diameter, 5 mm).

Serum-induced basophil histamine release was positive in 16 of 31 children with CIU (mean percent histamine release, 21.8 ± 4.9) compared with 5 of 21 children with CU of a known cause (52% vs 24%; $P = .09$). In particular, HR-urticaria test was positive in 3 children with miscellaneous urticaria (allergic + physical + infective, allergic + physical, and allergic urticaria; n = 1 in each group; mean percent histamine release, 17.2 ± 1.1) and in 2 children with physical and infectious urticaria (n = 1 in each group; percent histamine release, 16 and 18, respectively).

Both tests were either negative or positive in 43 of the 52 patients; therefore, the concordance between the tests is 83%. In detail, there were 5 ASST-positive patients who were HR-urticaria test–negative and 4 ASST-negative patients who were HR-urticaria test–positive. The sensitivity, specificity, and positive and negative predictive values of the ASST for the identification of anti-FcεR1 antibodies are 78%, 85%, 74%, and 88%, respectively. Finally, we have found a weak positive correlation between the percentage of histamine release and the Δ wheal diameter (Spearman rank correlation, $r = 0.4$; $P < .007$; Fig 1).

The number of patients with CIU with a positive reaction to 1 or both tests was calculated to be 67%. Thus, the inclusion of ASST and HR-urticaria test in the work-up for CIU reduces the rate of idiopathic forms from 52% to 29%. Fig 2 shows how the etiology of CU dramatically changes if we include AU in the initial work-up.

TABLE II. Characteristics of patients with CIU vs AU

	CIU (n = 31)	AU (n = 21)	*P*
Age, y*	6.8 (1.2-13.7)	7.1 (1.9-16.6)	.8
Female/male	12/19	9/12	.9
Age at onset, y†	4.8 (2.3-9.2)	3.9 (1.2-7.8)	.4
IgE sensitization	7 (22%)	8 (38%)	.4
HP infection	2 (6%)	—	.5
Gastrointestinal symptoms	9 (29%)	2 (10%)	.2
Recurrent respiratory infections	9 (29%)	2 (10%)	.2
Atopy in the family	15 (48%)	12 (57%)	.6
Autoimmunity in the family	4 (13%)	5 (24%)	.5
White cell count (10^3/L)	7372 ± 3565	7507 ± 2019	.5
Basophils (10^9/L)	0.025 ± 0.034	0.016 ± 0.033	.4
Eosinophils (10^9/L)	0.23 ± 0.33	0.12 ± 0.13	.2
Monocytes (10^9/L)	0.23 ± 0.24	0.36 ± 0.21	<.04‡
IgG (IU/mL)	1014 ± 217	929 ± 319	.3
IgA (IU/mL)	123 ± 41	143 ± 94	.5
IgE (IU/mL)	409 ± 455	420 ± 748	.4
IgM (IU/mL)	80 ± 32	117 ± 19	<.02‡

*Age at referral in our unit.
†Age at first presentation of urticaria.
‡P = .5 and .3 after Bonferroni correction.

Predictors of AU

Comparing children with AU and CIU, 2 variables were identified by univariate analysis as predictors of autoimmune urticaria: (1) monocyte count (358.2 ± 212.6 vs 225.2 ± 236.9; $P < .04$) and (2) serum IgM (117 ± 19.3 vs 80.2 ± 32.4; $P < .02$). However, none was confirmed after Bonferroni correction for multiple comparisons (Table II).

Prevalence of AU in children

With the assumption that only children positive to HR-urticaria test without any other known cause for CU had autoimmune urticaria, the lower limit of prevalence in this pediatric cohort would have been of 31% (15/52; 95% CI, 24%-51%). If we add the 5 children who have a positive HR-urticaria test but have a cause that could be responsible of their CU, then the higher limit of prevalence of this disorder is 40% (21/52; 95% CI, 30%-57%).

DISCUSSION

Chronic urticaria is defined as the daily occurrence of short-lived wheals for at least 6 weeks.[1,10] Although this condition is well recognized in adults, little is known about CU in children. However, the data derived from the few published series have shown that in more than 70% of the cases, no cause can be identified leading to the diagnosis of CIU.[16-18] The pathogenesis of this condition is still unclear, but there is now emerging evidence supporting an autoimmune phenomenon related to the presence of circulating functional histamine-releasing autoantibodies reactive against the α-subunit of the high-affinity IgE receptor.[1] Studies in adults have clearly demonstrated that antibodies against the FcεRIα are detectable in 25% to 45% of causes of CIU,[1,19-21] but no data are available in children. Many issues need to be addressed about the prevalence of these autoantibodies, the concordance between ASST and the histamine release test, and the possible clinical or biochemical features suggestive of autoimmune urticaria.

We report for the first time the data on a large series of consecutive children with a verified diagnosis of CIU (all children underwent an extensive diagnostic work-up to exclude known causes of CU) who had been systematically studied for AU by ASST and HR-urticaria test. We have shown that the inclusion of ASST and/or HR-urticaria test as part of the initial work-up in children referred for CU decreases the rate of the idiopathic form by approximately 25%, and that autoimmunity should be considered one of the major pathogenetic determinants of CU because autoantibodies against the FcεRIα can be found in as many as 40% of cases. Moreover, Fig 2 shows that the contribution of AU to the definition of the idiopathic subgroup is almost halved, as previously shown in adult series.[15] It is noteworthy that autoantibodies are also found in children with known eliciting agents (physical, allergic, or infective), and this raises the suspicion that autoimmunity more than other causes may contribute to determining and maintaining CU.

Currently, several tests have been suggested for the diagnosis of autoimmune urticaria.[15] Our choice to use 2 functional assays such as the ASST and HR-urticaria test relies on the need to compare an *in vivo* clinical, easy-to-perform test with the gold standard for the detection of autoantibodies. We have not examined the presence of autoantibodies by Western blot analysis, as suggested by others.[9] This was partly a result of shortage of serum from a significant number of the children, but also of the fact that autoantibodies have been demonstrated in serum from individuals without any forms of urticaria. Histamine release seems therefore to be the *in vitro* method that, at least in adults, reflects the presence of functional autoantibodies against IgE or the IgE receptor.

We have found a good concordance between ASST and HR-urticaria test, with the sensitivity and specificity of the ASST test for detecting autoantibodies of 78% and 85%, even greater than the figures reported in adults.[22] Differences between ASST and basophil histamine release may depend on histamine-releasing factors other than autoantibodies, such as complement and low-molecular-weight components specific for skin mast cells. Finally there are interindividual differences in the ability of donor basophils to release histamine on incubation with patient sera. In the current study, we have selected basophils from a donor on the basis of the best concordance between serum induced histamine release and ASST (unpublished results demonstrated a concordance of 75% between histamine release and ASST in adults).

Discordance between the 2 tests may be easily explained by the fact that a positive ASST test in the absence of *in vitro* evidence of autoantibodies may reflect a low autoantibodies titers or the presence in the serum of

histamine releasing substances,[1,20,21] whereas a negative ASST test with a positive HR-urticaria test may reflect the injection of the autologous serum in a refractory site secondary to a recent wheal.[12] Another factor relevant for the outcome of ASST in children is age, as described regarding skin prick test to both histamine and allergen.[12] A stratification of ASST responses in children would therefore be relevant to perform in the current study, but the number of positive reactions within each age group is too low to perform a valid statistical analysis.

Autologous serum skin test is cheap, is easy to perform, and, if performed as appropriate,[12] has good sensitivity and even better specificity at detecting autoantibodies also in children; therefore, it can be used as a predictive clinical test to diagnose AU, especially in places where the basophil histamine-releasing test is not available.

The main concern is when to consider a child affected by autoimmune urticaria. Indeed, in contrast with adults, there is little experience with this condition, and it is possible that the cut-off value of HR-urticaria test in children might be different from that of adults. Moreover, the HR-urticaria test and ASST identify 2 different phenomena. The first shows the presence of autoantibodies, whereas the latter shows that serum is able to induce histamine release from skin mast cells, without knowing whether this is induced by autoantibodies in all cases.[23] To distinguish between autoantibodies against IgE and the IgE receptor, previously published protocols assessed basophils of healthy low and high IgE donors.[19] Our own unpublished pilot studies using basophils from different donors showed that the serum-induced histamine release was not depending only on the level of serum IgE. A future alternative to using basophils from high and low IgE donors might be to use intact and IgE deprived cells from the same donor. However, further studies are needed to establish the optimal cell source. We have therefore chosen to use only one source of basophils, as discussed.

We believe that both tests provide important information when assessing a child with CU other than AU, the definition of which we reserve for children with autoantibodies.

When we have tried to assess whether it was possible to suspect AU on the basis of clinical and laboratoristic data, we could not find any difference between the autoimmune and nonautoimmune groups except for monocyte count and serum IgM; however, the significance was lost after correction for multiple testing. The lack of clinical and laboratoristic differences between CU with and without autoantibodies indicates that the reaction to a cause rather than the cause itself plays a major role in the clinical presentation. In contrast with adult studies, none of the children with AU had another concomitant autoimmune disease,[24] signs of thyroid autoimmunity,[25] celiac disease,[26,27] or HP infection.[28] We are aware that the likelihood of having more than one autoimmune disease increases with age, and therefore, it is possible that the absence of associated autoimmune conditions depends on the pediatric age of our series; however, the follow-up of this cohort of children will clarify the issue.

We have determined that the prevalence of AU in children is not less than 30%. The achievement of a correct diagnosis is of paramount importance, especially in the case of AU, in which plasmapheresis[29] or immunosuppressive treatment[30] may be indicated. These treatments should be used only in the presence of a definitive diagnosis.

REFERENCES

1. 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;328:1599-604.
2. Greaves MW. Chronic urticaria. N Engl J Med 1995;332:1767-72.
3. O'Donnell BF, O'Neill CM, Francis DM, Niimi N, Barr RM, Barlow RJ, et al. Human leucocyte antigen class II associations in chronic idiophatic urticaria. Br J Dermatol 1999;140:853-8.
4. 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;40:443-50.
5. O'Donnell BF, Lawlor F, Simpson J, Morgan M, Greaves MW. The impact of chronic urticaria on the quality of life. Br J Dermatol 1997;136: 197-201.
6. Grattan CEH, 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;21:695-704.
7. 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;90:213-7.
8. Sabroe RA, Francis DM, Barr RM, Greaves MW. Anti-FcεRI autoantibodies and basophil histamine releasability in chronic idiopathic urticaria. J Allergy Clin Immunol 1998;102:651-8.
9. Greaves MW. Chronic urticaria. J Allergy Clin Immunol 2000;105: 664-72.
10. Greaves MW. Chronic urticaria in childhood. Allergy 2000;55:309-20.
11. Kobza Black A, Lawlor F, Greaves MW. Consensus meeting on the definition of physical urticarias and urticarial vasculitis. Clin Exp Dermatol 1996;21:424-6.
12. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. Br J Dermatol 1999;140:446-52.
13. Skov PS, Mosbech H, Norn S, Weeke B. Sensitive glass microfibre based histamine analysis for allergy testing in washed blood cells. Allergy 1985;40:213-8.
14. Nolte H, Storm K, Schiotz PO. Diagnostic value of a glass fibre based histamine analysis for allergy testing in children. Allergy 1990;45:213-23.
15. Grattan CE, Sabroe RA, Greaves MW. Chronic urticaria. J Am Acad Dermatol 2002;46:645-57.
16. Harris A, Twarog FJ, Geha RS. cronich urticaria in childhood: natural course and etiology. Ann Allergy 1983;51:161-5.
17. Volonakis M, Katasarou-Katasari A, Stratigos J. Etiologic factors in childhood chronic urticari. Ann Allergy 1992;69:61-5.
18. Ehlers I, Niggemann B, Binder C, Zuberbier T. Role of nonallergic hypersensitivity reactions in children with chronic urticaria. Allergy 1998;53:1074-7.
19. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. J Invest Dermatol 1996;106: 1001-6.
20. Tong LJ, Balakrishnan G, Kochan JP, Kinet JP, Kaplan AP. Assessment of autoimmunity in patients with chronic urticaria. J Allergy Clin Immunol 1997;99:461-5.
21. Ferrer M, Kinet JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-Fc(epsilon)RIalpha (alpha-subunit) in chronic urticaria. Allergy Clin Immunol 1998;101:672-6.
22. 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;110:492-9.

23. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. J Allergy Clin Immunol 2001;107:1056-62.
24. Gaig P, Garcia-Ortega P, Enrique E, Richart C. Successful treatment of chronic idiopathic urticaria associated with thyroid autoimmunity. J Invest Allergol Clin Immunol 2000;10:342-5.
25. Ryhal B, DeMera RS, Shoenfeld Y, Peter JB, Gershwin ME. Are autoantibodies present in patients with subacute and chronic urticaria? J Investig Allergol Clin Immunol 2001;11:16-20.
26. Levine A, Dalal I, Bujanover Y. Celiac disease associated with familial chronic urticaria and thyroid autoimmunity in a child. Pediatrics 1999; 104:e25.
27. Meneghetti R, Gerarduzzi T, Barbi E, Ventura A. Chronic urticaria and coeliac disease. Arch Dis Child 2004;89:293.
28. Bakos N, Hillander M. Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria. Int J Dermatol 2003;42:613-5.
29. Grattan CE, Francis DM, Slater NG, Barlow RJ, Greaves MW. Plasmapheresis for severe, unremitting, chronic urticaria. Lancet 1992; 339:1078-80.
30. Grattan CE, O'Donnell BF, Francis DM, Niimi N, Barlow RJ, Seed PT, et al. Randomized double-blind study of cyclosporin in chronic "idiopathic" urticaria. Br J Dermatol 2000;143:365-72.