



Development of an Anti-IgE ELISA for Autoantibody Detection in Chronic Autoimmune Urticaria

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Abstract

Introduction: Chronic urticaria (CU) is a disorder affecting 0.1-1% of the population. Previous reports demonstrated that 30-50% of these CU patients have an autoimmune etiology. The autoantibodies have been reported to have specificity for several antigens on the basophil (e.g. IgE, FcεRI or FcεRII.) Our purpose was to develop a test to quantify autoantibodies to IgE to aid in the diagnosis and management of autoimmune CU.

Methods: IgG antibodies specific for IgE were quantified with a solid phase indirect non-competitive ELISA. The test was evaluated with de-identified discard sera from CU patients and normal, healthy controls. Informed consent was obtained. An IgE myeloma protein was coated onto microtiter plates and blocked. Diluted serum was added and bound IgG antibodies detected with alkaline-phosphatase labeled anti-human IgG. The assay calibration curve was based on standards prepared from the humanized monoclonal antibody (Omalizumab). The CU Index™, a functional test for autoantibodies that induce basophil histamine release, was also performed on the sera.

Results: The assay demonstrated high reproducibility (intra- and inter-assay) and good linearity with a mean interdilutional coefficient of variation of 5.8%. The dynamic range of the assay was 15-250 ng/mL. Autoantibodies to IgE were detected in normals and CU patients. The upper 95 % cutoff determined for normal, healthy individuals (n=57) was 60 ng/mL. CU patient sera (n=243) were positive in 11.3% of patients. A few patient sera (4.9%) that were negative on the CU Index™ were observed to be positive for anti-IgE autoantibodies. However, the majority of anti-IgE positive sera were also positive for the CU Index™.

Conclusions: A quantitative assay is now available that can be useful in the detailed evaluation of autoimmunity in CU. Additionally, detection of autoantibodies against IgE may be useful in other diseases such as allergic rhinitis, idiopathic rhinitis, and atopic dermatitis.

Background

- CU is estimated to affect 0.1% of the population
- Rarely fatal, but has significant adverse effects on the quality of life
- Average duration of 3-5 years
- A portion of CU cases have an autoimmune etiology: anti-IgE (5-10%), anti-FcεRIα (30-40%), and anti-CD23 (FcεRII)
- Functional assays will evaluate whether or not an autoantibody is present but do not assess antibody specificity

Materials and Methods

Anti-IgE ELISA

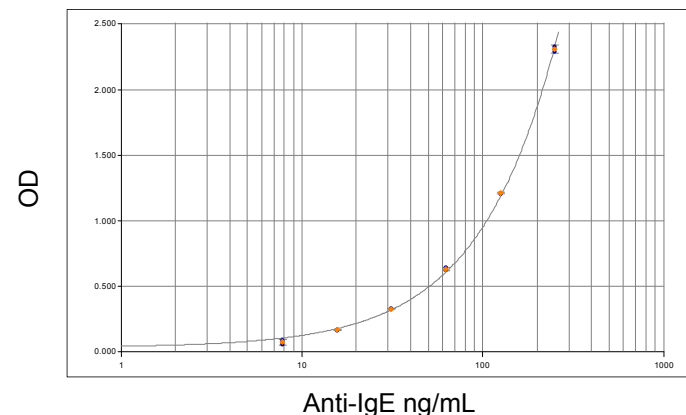
- IgE myeloma coated onto microtiter plates and incubated overnight
- Plates were washed, blocked overnight and dried in a desiccator
- Patient or control serum was added and bound IgG antibodies were detected with alkaline-phosphatase labeled anti-human IgG
- Calibration curve was based on a humanized monoclonal antibody specific for IgE (Omalizumab)

CU Index™

- blood cells (basophils), diluted in stimulation buffer containing IL-3, were incubated with patient serum, negative controls, and positive controls
- The cells were centrifuged and the supernatant was recovered
- Using a quantitative enzyme immunoassay, the histamine released into the supernatant was measured and compared to the total histamine in the basophils
- The cutoff for positive samples was determined using control (non-CU) serum

Results

Anti-IgE ELISA Calibration Curve



Dynamic range 15-250 ng/mL

Results

Anti-IgE ELISA Reproducibility

Table 1. Intra-Assay Precision of the Anti-IgE ELISA

Sample ID	#1	#2	#3	#4
	29.1	116.5	24.5	117.2
	31.5	110.3	24.3	115.1
	28.7	117.6	24.0	110.0
	27.0	113.7	32.8	108.6
	29.1	109.9	26.3	106.5
	35.9	111.9	25.2	114.3
			21.3	106.6
			32.9	109.8
mean	30.2	113.3	26.4	111.0
st dev	3.1	3.2	4.2	4.0
% CV	10.3%	2.9%	16.0%	3.6%

Table 2. Inter-Assay Precision of the Anti-IgE ELISA

Sample ID	#1	#2	#3	#4
	38.4	153.0	30.2	152.5
	36.8	130.0	30.9	108.7
	32.1	122.9	27.8	101.7
	30.2	113.3	26.4	111.0
mean	34.4	129.8	28.8	118.5
st dev	3.9	16.9	2.1	23.0
% CV	11.2%	13.0%	7.2%	19.4%

Anti-IgE ELISA Linearity

Table 3. Linearity of the Anti-IgE ELISA

dilution	#5		#6		#7		#8	
	ng/mL	ng/mL* dilution	ng/mL	ng/mL* dilution	ng/mL	ng/mL* dilution	ng/mL	ng/mL* dilution
neat	298.6	298.6	128.6	128.6	116.5	116.5	235	235
1:2	157.9	315.8	57.7	115.4	56.3	112.6	121	242
1:4	85.7	342.8	33.6	134.4	29.4	117.6	62	248
1:8			14.5	116	16.1	128.8	31	248
MEAN		319.1		123.6		118.9		243.3
ST DEV		22.3		9.4		7.0		6.2
IDCV		7.0%		7.6%		5.9%		2.5%

Results

Anti-IgE levels in controls and CU patients

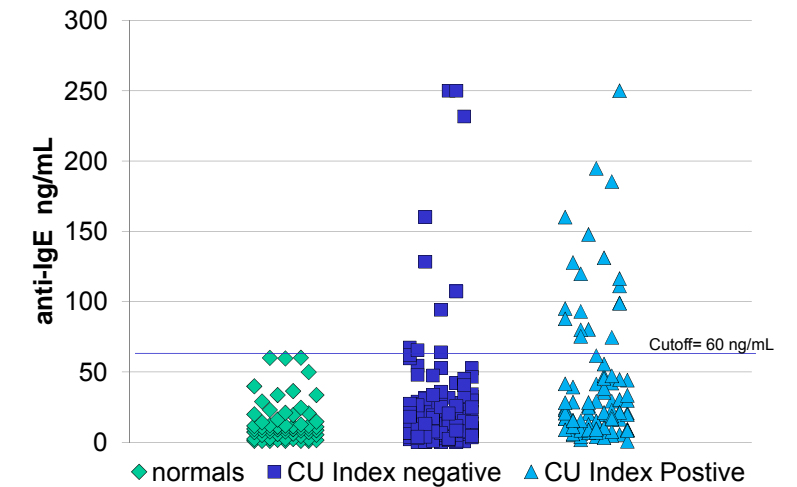


Table 4. Percentage of Patients with positive (>60 ng/mL) anti-IgE results

normals (Non-CU) n=62	All CU Patients n=243	CU Index™ Negative n=138	CU Index™ Positive n=105
3.2%	11.3%	4.9%	20.6%

Conclusions

- A quantitative assay is available to measure IgG autoantibodies to IgE
- Healthy (non-CU) individuals do have detectable levels of anti-IgE
- A proportion of CU patients display elevated anti-IgE levels
- A higher percentage of CU Index™ positive samples contain elevated anti-IgE

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