

EVALUATION OF A FUNCTIONAL ASSAY FOR THE DIAGNOSIS OF AUTOIMMUNE CHRONIC URTICARIA (CU)

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Abstract

CU is a disorder affecting 0.1-1% of the population. Studies demonstrate that 30–50% of CU patients have an autoimmune etiology. Diagnosis of autoimmune CU is difficult on clinical criteria but is supported with the autologous serum skin test (ASST). Our purpose was to develop a laboratory test to aid in CU diagnosis; accordingly we have compared two functional tests to detect autoantibodies relevant to autoimmune CU. Patients from an allergy practice who fit established criteria for CU were selected. Sera were stored at -20°C for up to 30 months. Basophils from a nonatopic donor were incubated with patient plasma, patient serum, buffer and anti-IgE as a positive control. Following ex-vivo challenge, the cells were analyzed for CD63 expression (a basophil activation marker) and the supernatants recovered for histamine analysis. A positive result in each test was based on the mean +2SD of a control population, 12.9% for histamine release and 16.4% for CD63 expression. Validation of the histamine release assay demonstrated an intra assay variability of 8% and an inter assay variability of 5.3%. Both the histamine release and the CD63 expression method were compared to the ASST on stored sera. Sera from ASST+ patients (n=38) caused a mean histamine release of 29.5% compared to 17.6% for ASST- patient sera (n=32) and 4.8% for control serum (n=11). Similarly, ASST+ patient sera (n=24) caused 21.4% of donor basophils to up regulate CD63, compared to 11.9% for ASST- sera (n=7) and 9.0% for control sera (n=11). In this study 58% of ASST+ (n=38) and 22% of ASST- (n=32) patients were positive in the histamine release assay, while 44% of ASST+ (n=24) and 14% of ASST- patients (n=7) were positive in the CD63 assay. There was a high correlation in the identification of positive sera with the two methods. A recent study demonstrated that plasma and serum gave different results in the autologous skin test. Therefore, sera and plasma (n=36) from CU patients were evaluated in the histamine release assay. Sera from CU patients resulted in a higher mean histamine release (26.1%) compared to plasma (15.9%). These data show that sera and plasma are not interchangeable in this assay. Because of the reported association of autoimmune thyroid disease with chronic urticaria, patient sera were also tested for the level of antibodies to thyroglobulin (Tg) and thyroid peroxidase (TPO). 31.3% of patients that were positive on the histamine release assay were positive for either anti-TPO or anti-Tg antibodies; this compares with only 12% of patients who were negative on the histamine release assay. The measurement of antibodies to FcεR and IgE or other serum factors, that activate basophils in this functional assay, can be used to assess autoimmunity in chronic urticaria patients.

Introduction

- CU is estimated to affect 0.1% of the population
- Rarely fatal
- Significant adverse effects on the quality of life
- Average duration of 3-5 years
 - 14% >5 years
 - 70% >1 year
- Drug/Food-specific IgE (generally associated with acute not chronic urticaria)
- Autoimmune- anti-IgE (5-10%), anti-FcεR1α (30-40%), and anti-CD23 (FcεRII)
- ASST- Autologous Serum Skin Test (similar to allergy skin testing with patient serum serving as the allergen)
- Patient serum can be tested *in vitro* using donor basophils to determine if auto-antibodies are present
 - Antibodies against FcεR1α, FcεRII, or IgE Histamine Release or CD63 upregulation

Materials and Methods

• Patients and Controls

- Patients Group 1- (n=31) Patients from an allergy practice who fit established criteria for CU were selected. ASST was performed and serum evaluated for *in vitro* CU testing with CD63 upregulation and histamine release.
- Patients Group 2-(n=36) Patients from an allergy practice who fit established criteria for CU were selected. ASST and APST were performed. Plasma and serum were evaluated for *in vitro* CU testing with histamine release assay.
- Healthy Controls- (n=11) Serum was evaluated for *in vitro* CU testing with CD63 upregulation and histamine release.

• Skin Testing

- ASST /APST- 50 mL of serum (ASST) or plasma (APST) and was injected ID into the forearm
 - Histamine served as the positive control and saline as the negative control in both tests
- Immediately after injection the size of the wheal in mm was recorded
- After 15 minutes the size of the reaction in mm was recorded for all injections
- For certain individuals readings were also taken at 30 minutes
- A positive result was recorded when the difference, in the wheal reaction size, between the saline and serum/plasma sites was greater than 3 mm

• *In vitro* Autoimmune CU testing

- **CD63 upregulation-**
 - blood cells (basophils), diluted in stimulation buffer containing IL-3, were incubated with patient serum, negative controls, and positive controls
 - Cells were stained with antibodies against IgE and CD63 and analyzed on the Beckman Coulter FC500
- **Histamine Release-**
 - 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

• Thyroid Autoimmune disease

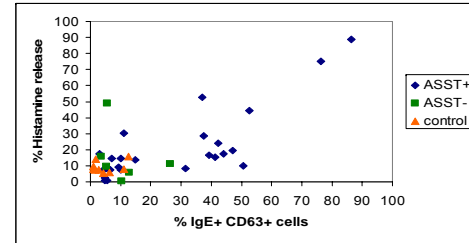
- **Anti-TPO-** Levels of serum antibodies measured on the Immulite 2000; normal values are less than 35 IU/mL.
- **Anti-Tg-** Levels of serum antibodies measured on the Immulite 2000; normal values are less than 40 IU/mL.
- **TSH-** Levels of TSH in serum measured using the Rapid TSH kit on the Immulite 2000; normal values are between 0.4 and 4.0 mIU/mL.

Skin Test Results

	ASST+	ASST-	APST+	APST-
Group 1 n=31	n=24 77%	n=7 23%	NA	NA
Group 2 n=36	n=13 36%	n=22 61%	n=15 42%	n=18 50%

In Vitro Histamine release correlates with CD63 upregulation

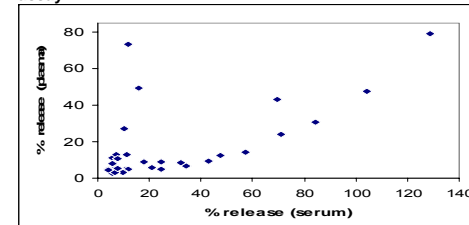
Sera from CU patients (n=31) and healthy controls was used in both the CD63 upregulation and histamine release assay. Results between the two assays showed a high degree of correlation and positivity.



% positive	Healthy controls n=11	All CU patients n=31	ASST+ n=24	ASST- n=7
CD63 upregulation	0%	38%	44%	14%
Histamine Release	0%	45%	55%	14%

Serum is the best specimen for the *In Vitro* Histamine release test

Sera and plasma from CU patients (n=36) were used in the histamine release assay. Results between the two specimens showed serum to be the best specimen for this assay.



Mean Histamine Release	All CU patients n=36	ASST+ n=13	ASST- n=23
Serum	26.1%	34.7%	21.2%
Plasma	15.9%	22.2%	12.4%

Correlation between CU and Thyroid Autoimmune Disease

Sera from CU patients (n=41) was used to measure both histamine release and anti-thyroid antibodies. Results demonstrated that a higher percentage of CU patients that are Histamine Release test positive have anti-thyroid antibodies.

		% Anti- TPO +	% anti-Tg +	High TSH
CU patients Histamine Release +	n=16	31.3	18.8	0
CU patients Histamine Release -	n=25	12.0	0	12.0
Healthy controls (historic)	8	8	5	0

Conclusions

- *In Vitro* histamine release assay is more sensitive than CD63 upregulation for detecting autoimmune CU
- Serum is the best specimen for use in the histamine release assay
- CU patients that are positive in the Histamine Release Assay have a higher prevalence of thyroid auto-antibodies
- The sensitivity and the specificity between the Histamine Release Assay and ASST are similar to previous reports

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