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Extended diagnostic value of autologous serum skin test and basophil CD63 expression assay in chronic urticaria

Running head: Diagnostic value of functional tests in chronic urticaria

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Conflict of interest

All authors have declared that they have no conflict of interest.

MADAM, Chronic urticaria (CU) is characterised by the occurrence of widespread, short-lived weals, appearing daily or almost daily for at least 6 weeks. [1] There has been strong evidence supporting an autoimmune basis of the disease in approximately 27-50% of CU patients, who have functional autoantibodies against the high affinity IgE receptor and/or IgE. [1] A correct diagnosis of autoimmune CU (ACU) is difficult but important because of the presence of more severe clinical symptoms. Currently, autologous serum skin test (ASST) serves as a screening method, after which more adequate confirmation is gained through functional assays, to identify the ACU group. [2] The basophil histamine release assay and flow cytometric basophil activation tests (BAT) are considered to be the gold standards; however, these tests are not accessible on an outpatient basis. [3,4] ASST is normally performed using undiluted autologous patient sera. [5] To avoid non-specific results in ASST, a 1 : 10 serum dilution has been suggested. [6] Our goal was to investigate whether ASST using diluted instead of undiluted sera would enhance specificity and to detect any correlation between the degree of basophil CD63 expression and clinical severity of the ACU.

We recruited 46 patients with chronic spontaneous urticaria (CU) [mean durations of hives of 32·4 (2-189) months]. The ASST with undiluted sera [5] and BAT detecting basophil CD63 expression [7] were conducted on all patients and on ten nonatopic healthy controls as described. Controls were negative in both tests [CD63: median: 2·72%, IQR (25-75%): 4·260-5·755%]. ASST was also performed by using 1 : 10 and 1 : 100 diluted CU sera in CU patients**.** The urticaria score that was introduced by Breneman et al. [8] was recorded at the time of performing the ASST to assess disease severity (mean total urticaria score index: 10·22 ± 2·39). Correlations between the two methods, the BAT and the ASST performed with undiluted, 1 : 10 and 1 : 100 diluted autologous patient sera were detected.

Out of 46 patients, 34 showed positive ASST using undiluted sera and 30 were regarded as ACU on the basis of CD63 positivity. The statistical analyses demonstrated a significant positive correlation and moderate concordance between the undiluted ASST and BAT (Fig 1a). There were 2 nonspecific negative ASST cases and 6 ASST positive cases with unidentified autoreactivity among the patients when undiluted sera were used.

The 1 : 10 dilution of sera reduced the number of nonspecific positives from 6 to 3, but nonspecific negative results increased dramatically from 2 to 20. The concordance between ASST and CD63 positivity was fair (Fig. 1b), and further decreased to poor by using 1 : 100 diluted sera (Fig. 1c). There were no correlations between the BAT and ASST using diluted patient sera. ASST using undiluted sera showed 93·23% sensitivity and 62·5% specificity, which were calculated using the BAT as a reference. Although the specificity of ASST increased with rising serum dilutions, all of the other diagnostic performance parameters (sensitivity, lilelihood ratios, predictive values) changed unfavourably. As a consequence of these results, we conclude that conducting ASST with 1 : 10 or 1 : 100 diluted patient sera does not aid in the diagnosis of ACU. In the meantime, ACU patients ($CD63^+$) with positive ASST at a 1 : 10 serum dilution showed significantly higher mean total urticaria scores compared with those ($CD63^+$) with negative ASST at the same dilution (Fig. 2a). There was also a significant correlation between the mean total urticaria score index and rate of CD63 positivity in the investigated ACU patients (Fig. 2b). These findings support the utility of ASST uing 1 : 10 diluted sera and the degree of CD63 cell surface expression in the measurement of ACU severity.

In spite of some non-specific results in ASST, it is still the most practical screening test in the diagnosis of ACU. This is also the reason why attempts have been made to enhance the diagnostic value of this method. When the use of plasma was compared to that of serum,

the plasma was not recommended because serum showed higher sensitivity, although they shared similar specificities. [9] In concordance with our results, Godse [10] analysed the effects of serum dilution in ASST on a limited number of patients and found that ASST using undiluted sera was more reliable than diluted sera for the diagnosis of ACU. However, this result was not confirmed by a functional test.

In summary**,** we have validated for the first time on a larger study group by comparing the ASST with the gold standard BAT that for the diagnosis of ACU, ASST using diluted autologous sera does not provide any incremental information. However, both ASST with 1 : 10 diluted sera and the degree of CD63 cell surface expression can be used for determining ACU severity.

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FIGURE LEGENDS

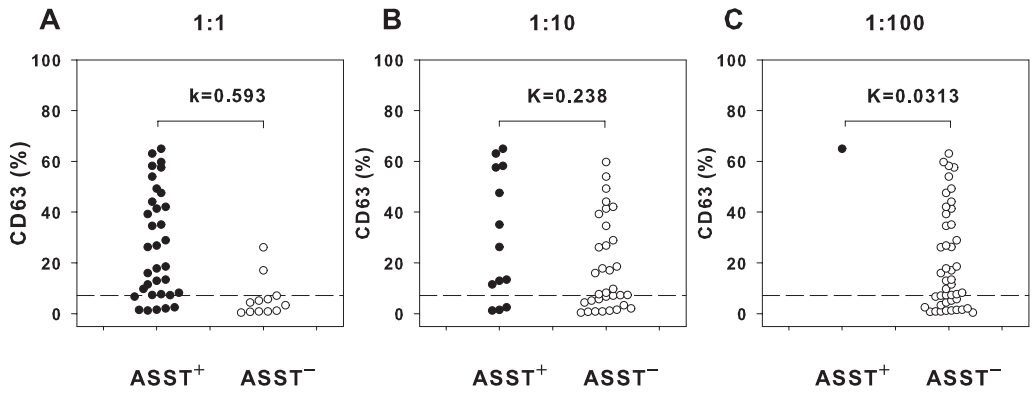
Figure 1. Effect of serum dilution on autologous serum skin test (ASST) performance compared with flow cytometric basophil CD63 expression test (BAT)

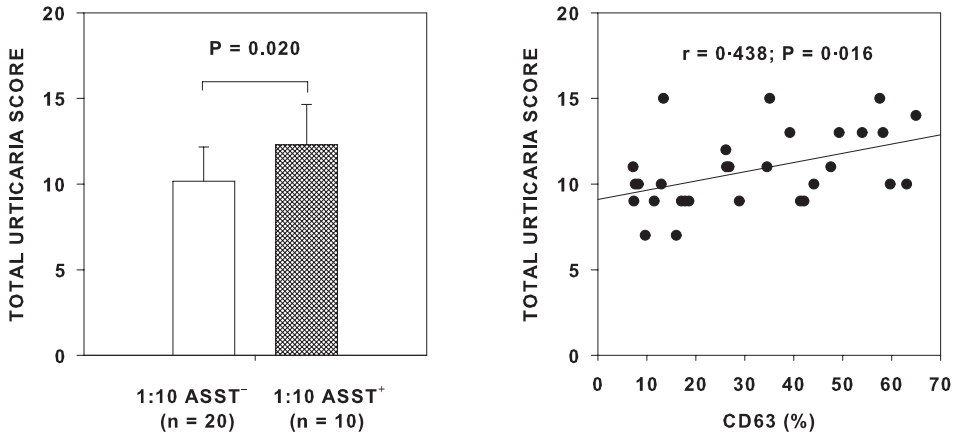
Autologous sera that were obtained from 46 chronic urticaria patients were used in differing dilutions for ASST and in the undiluted form for the BAT (as shown at the top of Fig 1. A: 1 : 1; B: 1 : 10; C: 1 : 100). Horizontal dashed lines represent the cut-off value of 7·175 for basophil CD63 expression that was determined by ROC analysis. **A.** A significant positive correlation (Spearman's rank correlation; r = 0·544, P < 0·001, not shown) and moderate concordance between the undiluted ASST and BAT have been found (Cohen's Kappa test: kappa = 0·593; P < 0·001). **B, C.** With increasing serum dilutions for ASST, the concordance notably decreased between the ASST and CD63 test; at the same time, the number of nonspecific negative cases that was detected by ASST was shown to be enhanced.

Figure 2. Association of urticaria score with ASST positivity using 1 : 10 diluted ACU sera and correlation between basophil CD63 (BAT) expression and urticaria score in ACU patients

A. ASST were performed using 1 : 10 diluted ACU sera on patients who had autoimmune chronic urticaria based on their positive results from the BAT. The mean total urticaria score was significantly elevated in the $ASST^{+}$ patients group compared with the $ASST^{-}$ group (Student's t-test, P = 0·020). **B**. A significant correlation between the degree of CD63 positivity and mean total urticaria score index was found in patients with ACU with undiluted sera (n = 30, Spearman's rank correlation; r = 0·438; P = 0·016).

ASST – autologous serum skin test; ACU – autoimmune chronic urticaria


A
1:1
B
1:10
C
1:100
CD63 (%)
k=0.593
K=0.238
K=0.0313
ASST+
ASST−


TOTAL URTICARIA SCORE
P = 0.020
1:10 ASST−
(n = 20)
1:10 ASST+
(n = 10)
r = 0·438; P = 0·016
CD63 (%)