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ORIGINAL ARTICLE

The use of anti-intercellular substance antibody in diagnosing patients with clinical suspicion of pemphigus

Hsiao-Han Wang [1, 2], Yu-Tsung Chen [3], Wei-Yu Chen [4], Ting-Jui Chen [1, 2, *]

[1] Department of Dermatology, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[2] Department of Dermatology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[3] Department of Dermatology, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan
[4] Department of Pathology, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

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ABSTRACT

Background: Pemphigus is frequently diagnosed via indirect immunofluorescence (IIF) or skin biopsy with direct immunofluorescence staining. However, the accuracy of IIF to detect anti-intercellular substance autoantibodies (anti-ICS Ab) has not been evaluated in Asian populations.
Method: IIF was performed on 177 patient samples. Demographic data and underlying diseases were analyzed. Histopathology and direct immunofluorescence results were also reviewed. The false-positive group included 45 patients without pemphigus but with positive ICS Ab results. The other groups included true-negative (116 patients without pemphigus and negative ICS Ab results), true-positive (14 pemphigus patients with positive ICS Ab results), and false-negative (two pemphigus patients with negative ICS Ab results) results. Univariate and multivariate analysis were performed to exam the factors associated with false positivity.
Results: Anti-ICS Ab detected using IIF has been shown in 87.5% of patients with confirmed pemphigus. The specificity of IIF on pemphigus was 72.1%. The positive and negative predictive rates were 23.7% and 98.3%, respectively. The false positive rate was 25.4% ($n = 45/177$). The titers of the false positive results were 1:20, 1:40, and 1:80, in 28, 10, and 7 patients, respectively. The false-positive results also diminished during sequential follow-ups in some patients after treatments. There was higher rates of patients with blood type O (66.7% vs. 36.3%; $p = 0.02$) and bullous pemphigoid (31% vs. 15%; $p = 0.05$) in the false-positive group, compared with the rest of the population. In the adjusted analysis, blood type O was found to be significantly associated with a false-positive IIF results when compared with blood types A and B.
Conclusion: This study provided practical statistics for the accuracy of anti-ICS IIF tests. Due to the high false-positive rate and low positive-predictive rate, the results of anti-ICS IIF for pemphigus diagnosis should be carefully interpreted.

Background

Pemphigus is a potentially fatal autoimmune blistering disease caused by immunoglobulin G (IgG) autoantibodies against desmoglein (Dsg)3 (a 130-kDa protein) and Dsg1 (a 160-kDa protein), leading to the destruction of cell–cell adhesions between stratified keratinocytes.[1,2]

The diagnosis of pemphigus relies on histological findings of intraepidermal acantholysis and immunofluorescence studies documenting the presence of skin autoantibodies, either via direct immunofluorescence (DIF) of perilesional skin, indirect immunofluorescence (IIF), or enzyme-linked immunosorbent assay (ELISA) analysis of patients' sera.[3,4] Positive immunofluorescent intercellular substance (ICS) staining is characterized by the deposition of IgG along the epithelium in a pattern reminiscent of chicken wire.[3,4]

Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Department of Dermatology, Wan Fang Hospital, Taipei Medical University, Number 111, Section 3, Hsing-Long Road, Taipei 116, Taiwan, ROC.
E-mail address: paladerm@yahoo.com.tw (T.-J. Chen).

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However, DIF or ELISA for diagnosing pemphigus may not be available in all medical facilities, and thus, IIF for anti-ICS autoantibodies (anti-ICS Ab) may be the most common clinical test. However, in clinical practice, the interpretation of both positive and negative results of this indirect test may be complicated. The documented sensitivity of anti-ICS IIF in patients with pemphigus ranges from 81% to 90%,[5–8] and the observed variance is because of the use of substrates such as monkey and guinea pig esophagus.[5,6,9,10] In addition, concurrent autoimmune disorders, burns, infections, and even blood types have been reported to lead to false-positive test results in a few studies.[11–14] Although monkey esophagus is a common commercial substrate for anti-ICS Ab screening, the accuracy of this test in Asian populations is currently unknown. In addition, studies have not thoroughly explored whether age, sex, concurrent diseases, or other factors, such as blood type, may interfere with the test results. Furthermore, the potential of IIF persistence is also unknown. Therefore, this study investigated the specificity and sensitivity of anti-ICS and the factors associated with the accuracy of this diagnostic method.

Materials and methods

Patients

Patients tested for serum anti-ICS Ab with IIF between May 2012 and December 2014 at Wan Fang Hospital in Taiwan were subjected to a retrospective review. Given the rarity of pemphigus, all the pemphigus patients in the past decade diagnosed using both IIF and DIF results were included. Demographics, blood type, laboratory data, pathology reports, and medical histories of underlying diseases were collected. These patients underwent the tests to confirm suspicion regarding autoimmune blistering diseases. The diagnosis of pemphigus or another autoimmune dermatosis was based on clinical presentation, histologic findings, IIF and DIF results. Final diagnoses of other dermatological diseases were based on clinical manifestations, lab tests, skin biopsy (if available), and the clinical course with treatment. The false-positive group included 45 patients without pemphigus but with positive ICS Ab results. The other groups included true-negative (116 patients without pemphigus and negative ICS Ab results), true-positive (14 pemphigus patients with positive ICS Ab results), and false-negative (two pemphigus patients with negative ICS Ab results) results. The study was approved by the Taipei Medical University-Joint Institutional Review Board with waiver of informed consent.

IIF study

Patients' sera were collected at Wan Fang Hospital. IIF was performed by incubating serial serum dilutions with a commercial kit [ImmuGlo™ Anti-Skin Antibody (IC/BMZ) Test System manufactured by IMMCO Diagnostics, Inc.] at the Taipei Institute of Pathology—a qualified laboratory that provides pathology testing techniques that meet international standards and collaborates as a referral institute with many hospitals in Taipei. Bound IgG-class antibodies were detected by incubating monkey esophagus substrate sections with fluorescein-labeled, antihuman IgG. Reactions were observed under a fluorescence microscope equipped with appropriate filters. The presence of anti-ICS Abs was demonstrated by the appearance of apple-green fluorescence on the respective histologic structures. The titer was reported as the final dilution ($\geq$1:20) at which the serum yielded a positive staining pattern. Data were all confirmed by two professional technicians.

A false-positive result was defined as a positive IIF result for anti-ICS Ab but a failure of the histo- or immuno-pathological evidence or clinical course to indicate pemphigus rather than other diseases. Patients in the "true negative" group had both negative IIF results and a clear exclusion of pemphigus because of other suggestive diagnoses and a lack of evidence of pemphigus.

Statistics

The summarized statistical results of the baseline characteristics of all patients are expressed as percentages for categorical data and means ± standard deviations for continuous variables. Means were compared using an independent *t*-test analysis of variance. Categorical data were assessed using the Chi-square test. Sensitivity, specificity, and positive and negative predictive values were also determined. A multivariate logistic regression was used to assess the factors associated with false positivity. A p-value of <0.05 was considered statistically significant. PASW Statistics for Windows, Version 18.0. (Chicago: SPSS Inc. Released 2009) was used for statistical analysis.

Results

A total of 177 patients, including 101 women (57.1%) and 76 (42.9%) men were enrolled in the study (Table 1). The mean age was 58.4 ± 21.4 years. The rates of A, B, O, and AB blood types in this cohort were 26.5%, 25.9%, 44.1%, and 3.3%, respectively. Sixteen patients were diagnosed with pemphigus [14 pemphigus vulgaris (PV) and two pemphigus foliaceus (PF)], and 161 patients were eventually diagnosed with other dermatologic disorders. Fifty-five percent of the patients underwent concomitant IIF for the detection of antibasement membrane zone (anti-BMZ) autoantibodies indicative of bullous pemphigoid (BP), and 19.2% (34/177) of the patients were diagnosed with BP. Nonautoimmune-related blistering and spongiotic dermatitis were diagnosed in 67.2% (119/177) of the patients.

IIF detected anti-ICS Ab in 27.9% (45/161) of the patients without pemphigus. The false-positive IIF results reported titers of 1:20, 1:40, and 1:80 in 28 (62.2%), 10 (22.2%), and seven (15.6%) patients, respectively (Table 1). False-negative IIF results were also observed in two patients diagnosed with PV and PF, respectively. The sensitivity, specificity, and positive and negative predictive values of the IIF test for pemphigus were 87.5% [95% confidence interval (CI): 61.6–98.5%], 72.1% (95% CI: 64.4–78.8%), 23.7% (95% CI: 13.6–36.6%), and 98.3% (95% CI: 94.1–99.8%) using a standard cut-off titer of 1:20. These four values were 68.8% (95% CI: 41.3–88.9%), 89.4% (95% CI: 83.6–93.7%), 39.3% (95% CI: 21.5–59.4%), and 96.6% (95% CI: 92.3–98.9%) with the cut-off point at 1:40. With a cut-off titer taken at 1:80, the sensitivity decreased to 56.3 (95% CI: 29.9–80.3%) and the rest of the indicants were 95.6% (95% CI: 91.3–98.2%), 56.3% (95% CI: 29.9–80.3%), and 95.7% (95% CI: 91.3–98.2%), respectively.

The persistence of false-positive IIF test results was unknown according to literature. In our study, four patients had false-positive ICS staining at a titer of 1:20 (two patients with urticaria and angioedema, and two with eczema and underlying sicca syndrome). Another patient who was eventually diagnosed with bullous scabies presented with a false-positive staining titer of 1:40, and one patient with eczema and underlying sicca syndrome presented with a false-positive staining titer of 1:80. The follow-up IIF test results of these patients were all negative at 3–6 months after treatment and stabilization of the skin conditions.

The patients were divided into two groups according to their IIF test results: the "false-positive" group ($n = 45$) and the "others" group ($n = 132$). These groups did not differ significantly in terms of the mean age and sex distribution (Table 1). The "false-positive" group had a significantly higher proportion of patients with blood type O than the "others" group (66.7% vs. 36.3%; $p = 0.022$). More

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