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Correlation of clinical severity and ELISA indices for the NC16A domain of BP180 measured using BP180 ELISA kit in bullous pemphigoid

Yukiko Tsuji-Abe, Masashi Akiyama*, Yasuko Yamanaka, Toshiro Kikuchi, Kazuko C. Sato-Matsumura, Hiroshi Shimizu

Department of Dermatology, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan

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Summary

Background: Titres of circulating autoantibodies detected by indirect immunofluorescence (IIF) have been used for the diagnosis and evaluation of disease activity in bullous pemphigoid (BP). In BP, the major pathogenic epitope is known to be the non-collagenous extracellular domain (NC16A) of the 180-kDa transmembrane hemidesmosomal protein (BPAG2). Recently, an enzyme-linked immunosorbent assay (ELISA) kit using the NC16A domain recombinant protein (BP180 ELISA kit) has become commercially available to measure the quantities of pathogenic autoantibodies circulating in BP patients.
Objective: To investigate the correlation of clinical severity and ELISA indices in BP.
Methods: Fourteen patients with a typical form of BP and one refractory BP patient who died despite extensive treatment were included in this study. Antibody titres in sera from these patients were measured using BP180 ELISA kit and an analysis of ELISA indices with disease activity was performed.
Results: ELISA indices were significantly reduced after successful therapy, although IIF titres did not always show apparent correlations. In the patient with refractory BP, ELISA indices also showed a good correlation with disease course. ELISA indices measured using the BP180 ELISA kit were well correlated with the disease activity.
Conclusion: This commercially available kit more closely followed disease activities than the IIF titres. The BP ELISA system may be a useful tool to evaluate the disease activity and to assess the effectiveness of the treatment of BP.

* Corresponding author. Tel.: +81 11 716 1161x5962; fax: +81 11 706 7820.
E-mail address: akiyama@med.hokudai.ac.jp (M. Akiyama).

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1. Introduction

Bullous pemphigoid (BP) is an autoimmune blistering disease in which patients have autoantibodies to basement membrane zone (BMZ) components. The 230-kDa intracellular hemidesmosomal protein (BPAG1) and the 180-kDa transmembrane hemidesmosomal protein (BPAG2) have been identified as autoantigens in BP. The major pathogenic epitope is known to be the non-collagenous extracellular domain (NC16A) of BPAG2 [1]. Titres of circulating autoantibodies detected by indirect immunofluorescence (IIF) tests using normal human skin substrates have been used for the diagnosis and evaluation of disease activity. The clinical usefulness of the enzyme-linked immunosorbent assay (ELISA) for the detection of circulating anti-NC16A autoantibodies in the patients with BP was recently reported [2–7]. More recently, an ELISA kit using an NC16A domain recombinant protein (Medical and Biological Laboratories Co. Ltd., Nagoya, Japan) has been made commercially available to accurately measure anti-NC16A pathogenic circulating autoantibodies in BP patients' sera. In this study, using the ELISA kit, we precisely quantified the changes in ELISA indices of 14 BP patients before treatment and after remission as well as the correlation of ELISA indices and disease activity in a refractory BP patient who died, despite extensive treatment.

2. Materials and methods

2.1. Patients and sera: typical BP patients

Twenty-eight serum samples from 14 patients with BP showing typical clinical features were included in this study. Mucosal involvement was completely absent or only slight in all the cases. Out of three cases showing negative results for BP180 ELISA before treatment, two cases had no mucosal involvement and one had very slight oral mucosal lesions. In all the patients, the diagnosis was confirmed by histopathological observation and immunofluorescence study, i.e., subepidermal blister formation was seen histopathologically and direct and indirect immunofluorescence studies revealed positive autoantibody labeling in the basement membrane zone. Indirect immunofluorescent studies using 1.0 M NaCl split-skin as a substrate revealed that the IgG deposition on the basement membrane zone was on the roof side of blister in the three cases with negative BP180 ELISA before treatment. From these findings, epidermolysis bullosa acquisita was excluded in the three cases. All patients were treated successfully with oral prednisolone therapy of 30–50 mg/day with or without azathioprine or a combination therapy using tetracycline and nicotinamide. Sera were obtained for BP180 ELISA before the treatment and after the remission.

2.2. Patients and sera: a refractory BP patient who died despite extensive treatment

Twenty-one sera samples were taken from a patient with severe BP during the disease course. A 64-year-old Japanese male showed typical clinical, histopathological and immunohistopathological features of BP. His BP symptoms did not respond to treatments including oral steroid, oral immunosuppressant, and steroid pulse therapy. The patient died after 21 months of in-patient treatment due to disseminated intravascular coagulation and multiple organ failure. As far as we were able to determine, the patient had no internal malignancy.

2.3. Scoring method for clinical severity and definition of remission

Scoring criteria for clinical severity are defined as follows: 0, no skin lesions (erythema, bullae, erosions); 1, ~20% of lesions of total skin area; 2, ~40% of lesions of total skin area; 3, ~60% of lesions of total skin area; 4, ~80% of lesions of total skin area. Clinical remission was defined as when erythema, bullae and erosions healed completely and only a low dose of oral predonisolone (<5 mg/day) or no treatment was needed to maintain this condition.

2.4. Indirect immunofluorescence (IIF) for circulating autoantibodies

Indirect immunofluorescence was performed using normal human skin as a substrate obtained from surgical operations with fully informed consent. Immunofluorescence staining was performed as previously described [8]. Fluorescence labeling was detected using an epifluorescence microscope. The highest dilution of the sera showing positive fluorescence on BMZ was adopted as the IIF titre.

2.5. ELISA for autoantibodies to NC16a domain of BPAG2 (BP180)

Concentration of IgG autoantibodies in the patients' sera directed against the NC16A domain of BPAG2 were measured using the BP180 NC16A ELISA kit following the kit's instructions. By standard, indirect