

Review Article

The use of ELISA in pemphigus

於天庖瘡病中應用酶聯免疫吸附分析法

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ELISA for desmoglein 1 and 3 antibodies using recombinant Dsg 1 and 3 is a new, specific and sensitive technique for diagnosing pemphigus vulgaris and foliaceus. It also provides quantitative data which may later prove to be a valuable tool for monitoring of disease activity.

用酶聯免疫吸附分析法以重組的橋粒芯糖蛋白1和3測定其抗體是一種新方法用以診斷尋常型天庖瘡和落葉型天庖瘡，其特異性和敏感性高。其定量測定更可能成為監察病情的有用指標。

Keywords: ELISA, Pemphigus vulgaris

關鍵詞：酶聯免疫吸附分析法，天庖瘡

Introduction

Pemphigus is a group of autoimmune blistering diseases. There had been abundant evidence that antibodies against components within the desmosomes were responsible for its pathogenesis. The major antigens in pemphigus vulgaris and pemphigus foliaceus are desmoglein 3 (130 kDa) and desmoglein 1 (160 kDa) respectively. They are glycoproteins belonging to the cadherin family and form part of the transmembranous component of the desmosomes.¹ Conventionally, pemphigus

vulgaris and pemphigus foliaceus are distinguished by their differences in clinical and histological features.

Investigation for anti-desmoglein antibodies

Direct immunofluorescence performed on skin biopsies would show epidermal intercellular deposits of IgG and C3 in both pemphigus vulgaris and foliaceus. The presence of the circulating antibodies can be detected by indirect immunofluorescence technique. Patients' serum is incubated with appropriate substrates (e.g. monkey oesophagus, guinea pig or human skin) at serial dilutions. The sections are then washed and incubated with fluorochrome-labelled conjugate against human IgG. Finally, they are examined under fluorescence microscope, with or

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without counter stain, after a second wash. However, this method is time consuming, operator dependent and difficult to perform on large number of samples. The sensitivity also depends on the availability of suitable substrate and ranged from 83-90% in experienced centres.² Monkey oesophagus is more sensitive for the diagnosis of pemphigus vulgaris and human skin is more sensitive for pemphigus foliaceus.³ The use of two substrates, namely monkey oesophagus, (rich in desmoglein 3) and human skin (rich in desmoglein 1) is recommended to improve the sensitivity.²

Immunoblotting technique can be used to characterise the pemphigus antigens according to their molecular weight. Patient's serum and the enzyme labelled anti-human IgG is added to epidermal extract of normal skin. For example, if anti-desmoglein 3 is present, a band would be produced at the 130 kDa area and if anti-desmoglein 1 is present, a band would be produced at the 160 kDa area. Although this technique can be used to identify the target antigens, it is again time consuming and can only produce qualitative results.

ELISA for desmogleins 3 and 1

In recent years recombinant desmoglein 3 and desmoglein 1 had been produced using baculovirus expression and these recombinant desmogleins were used to develop specific ELISA (enzyme linked immunosorbent assay) to detect antibodies against them. The first report of ELISA for diagnosis of pemphigus was in 1997 and it suggested that the method was sensitive and specific.⁴ It also provided a quantitative method for measuring the antibodies levels. The principal of the test is to measure anti-desmoglein-1 and anti-desmoglein-3 antibodies present in the sera by ELISA (Figure 1). Calibrators and patient sera are added to microwells coated with desmoglein 1 and desmoglein 3 antigens, allowing antibodies to react with the immobilised antigens. After washing to remove any unbound serum proteins,

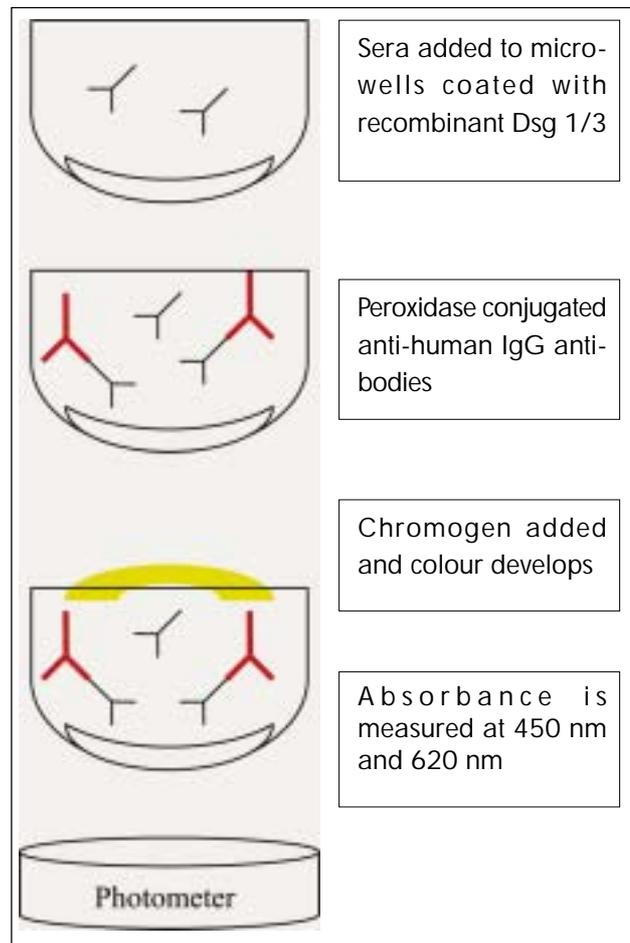


Figure 1. Schematic diagram of ELISA. (Dsg - desmoglein)

horseradish peroxidase conjugated IgG (Fab') is added and incubated. Following another wash step, the peroxidase substrate is added. Stop solution is then added to each well to terminate the enzyme reaction and to stabilise the colour development. The assay can be quantified by measuring the reaction photometrically and plotting the results.

The use of ELISA was evaluated further in recent years. In the study by Amagai, 97.9% of sera from pemphigus foliaceus patients were positive in the desmoglein 1 ELISA and 97.5% of sera from pemphigus vulgaris patients were positive in the desmoglein 3 ELISA.⁵ Only 1.1% and 2.2% of the control sera were positive in desmoglein 1 and desmoglein 3 ELISAs respectively. Harman et al

recruited 82 patients with pemphigus vulgaris and 25 patients with pemphigus foliaceus who had their diagnosis confirmed by clinical features, histology, direct and indirect immunofluorescence.⁶ They also recruited 124 healthy controls and 193 disease controls (with mucous membrane pemphigoid, lichen planus, other bullous and skin diseases). The sensitivity of the desmoglein 3 ELISA for diagnosing pemphigus vulgaris was 95% overall and 100% in untreated patients. The sensitivity of desmoglein 1 ELISA for diagnosing pemphigus foliaceus was 92% overall and 100% in untreated patients. Desmoglein 1 antibodies were detected in 60% of pemphigus vulgaris patients, while no desmoglein 3 antibodies were detected in pemphigus foliaceus patients. The specificity was as high as 98% or more. The overall sensitivity of indirect immunofluorescence was 79% for pemphigus vulgaris and 84% for pemphigus foliaceus respectively in this study.⁶

They concluded that ELISA was a sensitive and specific diagnostic tool for pemphigus. Although the level of sensitivity dropped when patients on treatment were included, the figures were still far better than the overall sensitivity of indirect immunofluorescence.⁶ Using a combination of desmoglein 1 and 3, it can also aid to distinguish pemphigus vulgaris and pemphigus foliaceus. A positive desmoglein 3 result is indicative of pemphigus vulgaris, regardless of the associated desmoglein 1 result. A negative desmoglein 3 and a positive desmoglein 1 indicate a diagnosis of pemphigus foliaceus.

Although the differentiation between pemphigus vulgaris and foliaceus can usually be made without detection of anti-desmoglein antibodies, ELISA could be used as an aid in the occasion difficult case. There had been reports on transition of pemphigus vulgaris into foliaceus in a patient over 9 years period. The change in her phenotype was mirrored in the level of desmoglein 3 and 1.⁷

The ELISA had several other advantages in addition to high sensitivity and specificity. It

allowed samples to be tested in duplicates and multiple samples to be tested without wasting materials and reagents. The assay was simpler in contrast to immunoblotting, required only 5 µl of serum and could be completed within one day, allowing the analysis of large numbers of samples in a relatively short time. The enzyme reaction in ELISA when conjugated with anti-IgG antibodies produced a colour change that could be measured by absorbance which was indirectly related to the quantity of antibodies. So it provides objective, reproducible and quantitative data.⁶

Monitoring disease activity

Early studies suggested that antibodies titres measured by indirect immunofluorescence could be used as a marker of disease activity. However, later studies gave conflicting results on the reliability of this correlation, especially whether it could be used as guide to therapy.^{8,9} This may partly be due to the semi-quantitative and subjective nature of the technique. With the development of ELISA, this question had been addressed again. In a study by Harman et al, 80 pemphigus vulgaris and 24 pemphigus foliaceus patients were classified to quiescent, mild, moderate or severe disease by a single investigator.⁹ 96-wells ELISA plates coated with recombinant proteins of extracellular domain of either desmoglein 1 or 3 were used. Antibodies against desmoglein 3 and 1 in patients' serum were detected by a standard method and expressed as a percentage of a positive control which arbitrarily contained 100 units of desmoglein antibodies. The results showed that the oral disease severity was related to desmoglein 3 antibody levels and skin disease severity to desmoglein 1 antibodies level, regardless of diagnosis. A 10-unit increase in desmoglein 1 ELISA value was associated with a 44% chance of having a greater skin disease score in pemphigus foliaceus cases (CI 20-73%, $P < 0.0005$) and 33% in pemphigus vulgaris cases (CI 23-44%, $P < 0.005$). A 10-unit increase in desmoglein 3 ELISA

value was associated with a 20% chance of having a greater oral disease score in pemphigus vulgaris cases (CI 11-29%, $P < 0.0005$). There was no evidence of any effect of desmoglein 1 antibodies on the severity of oral disease nor desmoglein 3 on skin disease.⁹ This was also consistent with the 'desmoglein compensation theory', which explained the difference in phenotype between pemphigus foliaceus, mucosal pemphigus vulgaris and mucocutaneous pemphigus vulgaris (Figure 2).¹⁰ As desmoglein 1 is expressed more in upper epidermis, acantholysis occurs in superficial dermis. The mucous membrane and lower epidermis is spared due to the co-expression of desmoglein 3 in these sites. In pemphigus vulgaris, acantholysis occurs in the mucous membrane due to the distribution of desmoglein 3. If both desmoglein 3 and 1 antibodies are present, there tend to be more severe mucocutaneous disease.

The presence of desmoglein 1 antibodies in pemphigus vulgaris patients predicted the phenotype as well as a more severe disease subgroup.¹¹ It was suggested that the antibodies to desmoglein 1 may be the result of epitope spreading. However, some vulgaris patients did not develop desmoglein 1 antibody despite long standing disease. Proportion of patients with desmoglein 1 antibodies were the same whether they had treatment or not in the 79 patients studied.¹¹ Genetic factors may be important in determining the autoantibody profile as proportion of desmoglein 1 subjects was higher in patients of Indian origin compared with white northern Europeans.¹¹

Despite the correlation, there were definitely patients with high ELISA titres but clinically quiescent disease. This may be due to non-pathological immunoglobulins (different subclass or bind to epitopes that do not trigger disease) or treatments. For patients with active disease but low ELISA values, it is possible that there were pathogenic antibodies to other epitopes which was not detected.⁹ Preliminary data also suggested that ELISA values were parallel to disease activity

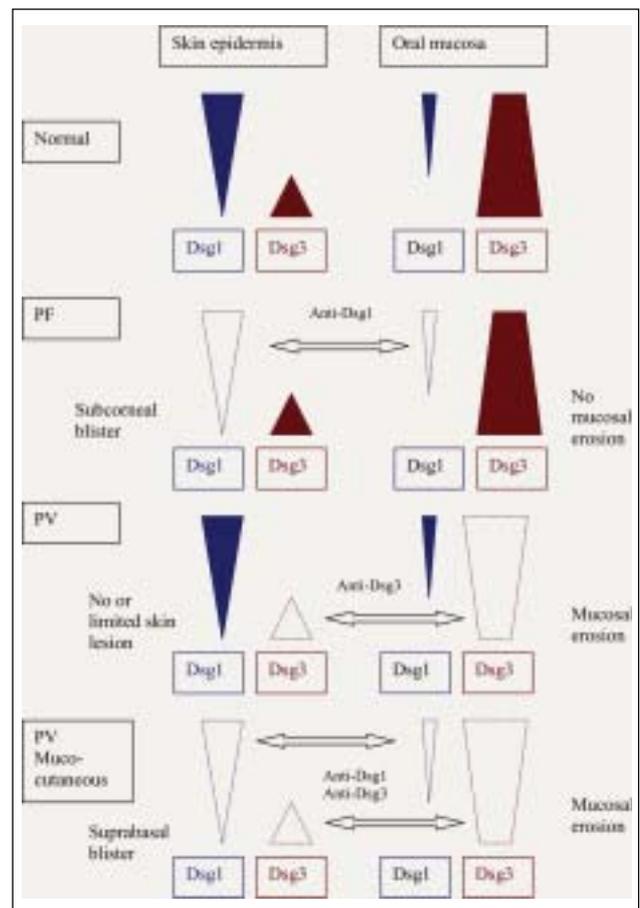


Figure 2. Schematic diagram of desmoglein compensation theory.

(unpublished data, Harman and Black). With adequate normal and disease controls in a population, studies are now being carried out to see whether ELISA values may also reflect the differences between different patients.

Other studies also produced similar conclusions. It was also suggested that if the antibodies level was very high, the reaction may saturate. Appropriate dilution of the serum will be needed to give a linear relationship and can provide serological information on disease activity.¹² There is no data on the time lag between changes in antibody titres and clinical symptoms and signs. Sometimes increase in antibody titres may precede clinical deterioration and this should alert the clinician in management of the patient.

The cost of testing a single sample in both the desmoglein 1 and 3 ELISAs was UK£13-18 (excluding labour, shipping costs and consumables), depending on the number of samples tested per batch.⁹ The manufacturer listed certain limitations of the test: the test should be interpreted in conjunction with clinical evaluation of the patient along with other diagnostic procedures, performance of these assays in the paediatric population has not been established. The assay performance characteristics have not been established for matrices other than serum. A positive result indicates the presence of antibodies to recombinant desmoglein-1 or desmoglein-3 and does not specifically identify a certain type of pemphigus and a negative result does not rule out the presence of all pemphigus disease.¹³

ELISA had also been used to confirm the transplacental transfer of antibodies by measuring the desmoglein 3 antibodies level in serum from mother with pemphigus vulgaris, cord blood and the neonate.¹⁴ Currently ELISA for other conditions are being developed such as BP 180 antigen for the use in bullous pemphigoid.

In Hong Kong, the number of pemphigus patients in each individual centre is limited. If ELISA for desmoglein is to be introduced, it would probably be more cost effective to have a regional centre to collect all serum samples and perform the tests as a batch. It would also be necessary to obtain data on normal control within the population. The frequency of test needed in each individual patient for disease monitoring will depend on the severity of his/her condition and clinical grounds.

Summary

The ELISA for desmoglein 1 and 3 antibodies appears to be a highly specific and sensitive technique for diagnosing pemphigus vulgaris and foliaceus. The quantitative data provided may also prove to be a valuable tool for

monitoring disease activity and even in guiding patient management. However, it should be emphasised that the patients and treatments should be assessed primarily by clinical improvement and only secondarily by laboratory data.

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