COPYRIGHT AND USE OF THIS THESIS

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author’s moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author’s reputation

For further information contact the University’s Copyright Service.

sydney.edu.au/copyright
Pemphigus Vulgaris Disease Activity: the Role of Antibodies to Desmogleins and their Isotype.

Sue-Ching Yeoh

A thesis presented for the degree of Master of Philosophy (Medicine) of the University of Sydney.

2015

Department of Immunopathology, Faculty of Medicine,
University of Sydney, New South Wales

Australia
DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any University; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference has been made in the text.

Sue-Ching Yeoh
ACKNOWLEDGEMENTS

The support of the following clinicians who sent us samples for the study is greatly appreciated: Professor Dedee Murrell, Dr Ming-Wei Lin, Dr Mark Schifter, Dr Anastasia Georgiou, Dr Ben Karim, Dr Carolyn Hawkins, Dr Catherine Toong, Dr Daman Langguth, Dr Monisha Gupta, Dr Samuel Seit. Thank you also to the staff of Immunopathology, Institute of Clinical Pathology and Medical Research, Westmead Hospital for performing the ICSA assays.

Thank you to Dr Karen Byth-Wilson whose expertise and help with data and statistical analysis was greatly appreciated.

Professor David Fulcher, research supervisor and friend – thank you for your patience and guidance.

And finally, my family - Anh-Thong, Emilie and Kira. Thank you for your support, I’m looking forward to spending more “family time” together.
# TABLE OF CONTENTS

| TITLE PAGE | 1 |
| DECLARATION | 2 |
| ACKNOWLEDGEMENTS | 3 |
| TABLE OF CONTENTS | 4 |
| LIST OF TABLES | 7 |
| LIST OF FIGURES | 8 |
| ABBREVIATIONS AND ACRONYMS | 9 |
| ABSTRACT | 10 |
| CHAPTER 1: PEMPHIGUS VULGARIS | 12 |
| 1.1 Introduction | 13 |
| 1.2 Clinical Features | 13 |
| 1.3 Epidemiology | 14 |
| 1.4 Diagnosis | 17 |
| 1.5 Management | 18 |
| 1.6 Prognosis and Outcome | 19 |
1.7 Pathogenesis of Blister Formation

1.8 Desmoglein Compensation Theory

1.9 Anti-dsg-3 IgG Isotypes

1.10 Scoring Systems

1.11 Project Background

1.12 Research Description and Hypothesis

1.13 Aims

CHAPTER 2: MATERIALS AND METHODS

2.1 Patient Recruitment

2.2 Pemphigus Disease Area Index (PDAI)

2.3 Anti-dsg-1 and dsg-3 IgG ELISA

2.4 Indirect Immunofluorescence

2.5 Statistical Analysis

CHAPTER 3: RESULTS

3.1 Patients

3.2 Only anti-dsg-3 IgG4 levels correlated with disease activity at recruitment

3.3 Changes in anti-dsg-3 IgG4 levels reflected changes in disease activity
| 3.4 ICSA titres correlated with anti-dsg-3 IgG levels, and reflected disease activity | 40 |
| CHAPTER 4: DISCUSSION | 45 |
| APPENDICES | 59 |
| REFERENCES | 79 |
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>PAGE NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1: Clinical and Immunohistochemical Variants of Pemphigus</td>
<td>15</td>
</tr>
<tr>
<td>Table 2: Sub-types of pemphigus vulgaris disease in the cohort studied.</td>
<td>37</td>
</tr>
<tr>
<td>Table 3: Summary of previous anti-dsg-3 IgG isotype studies</td>
<td>56</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Hallmarks of PV</td>
<td>17</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Desmoglein Compensation Theory</td>
<td>24</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Quality Control Graphs</td>
<td>33</td>
</tr>
<tr>
<td>Figure 4</td>
<td>$\log_{10}(\text{PDAI}+1)$, plotted against ELISA values for dsg-1 (total IgG) and for dsg-3 (total IgG, subclasses IgG1 and IgG4) at recruitment</td>
<td>39</td>
</tr>
<tr>
<td>Figure 5</td>
<td>$\log_{10}(\text{PDAI}+1)$, plotted against ELISA values for A. anti-dsg-1-IgG; B. for anti-dsg-3-IgG; C anti-dsg-3-IgG1; and D. anti-dsg-3-IgG4, for the 32 patients who had two or more serial samples analysed</td>
<td>42</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Mean within-patient Spearman rank correlations ($r$) between PDAI and ELISA values for dsg-1 (total IgG) and for dsg-3 (total IgG, subclasses IgG1 and IgG4) in 24 patients with three or more assessments during follow-up, and associated 95% confidence intervals</td>
<td>43</td>
</tr>
<tr>
<td>Figure 7</td>
<td>A. Pemphigus vulgaris: The $\log_{10}(\text{PDAI}+1)$, plotted against ICSA titres at recruitment of the 14 patients in the Westmead cohort. B. Pemphigus vulgaris: Relationship between ICSA titres and dsg-3-IgG levels in the Westmead cohort at all stages of follow-up.</td>
<td>44</td>
</tr>
</tbody>
</table>
ABBREVIATIONS AND ACRONYMS

confidence interval - CI

desmoglein – dsg

enzyme-linked immunosorbent assay - ELISA

horseradish peroxidase - HRPO

immunoglobulin – Ig

intercellular cement substance antibodies – ICSA

optical density - OD

pemphigus disease area index - PDAI

pemphigus vulgaris – PV

p-value - p

Spearman rank correlation - r

tetramethylbenzidine - TMB
ABSTRACT
Pemphigus vulgaris (PV) is an autoantibody-mediated blistering mucocutaneous disease driven by pathogenic antibodies to desmoglein-1 and -3 (dsg-1, 3), levels of which correlate with disease activity. This correlation is said to vary with isotype, with anti-dsg-3 IgG4 antibodies predominating in active disease, and IgG1 in remission. However these observations derive from single time point studies, with crude assessments of disease activity. Using the validated PV clinical scoring tool ‘pemphigus disease area index’ (PDAI), the current study sought to re-evaluate the clinical relevance of desmoglein autoantibodies, sub-divided by isotype, in the routine management of patients with PV.

40 PV patients were enrolled, all at various stages of disease activity and treatment. PDAI was measured and studied in relation to levels of anti-dsg-1 IgG and anti-dsg-3 IgG, the latter divided into IgG1 and IgG4 isotypes. 24 of these patients had data from three or more time points over a median time period of 15 months (range: 4.5 – 23 months), and were evaluated for correlation with changes in clinical activity.

At study enrolment, only anti-dsg-3 IgG4 levels were significantly associated with PDAI values but this association was weak (rank correlation r=0.370, p=0.019). During follow-up, within patient changes in PDAI observed across serial samples were significantly associated with serial changes in total anti-dsg-3 IgG levels, with the strength of these associations being similar for both anti-dsg-3 IgG isotypes. The study was therefore unable to confirm the predominance of anti-dsg-3 IgG1 during remission, nor of anti-dsg-3 IgG4 during active disease. Changes in anti-dsg-1 IgG levels did not clearly follow changes in PDAI, although most patients were negative at baseline.

In conclusion, absolute levels of anti-dsg-3 IgG4 correlated weakly with disease activity scores at a given time point, with wide individual variation. The study was unable to confirm the reciprocity of IgG1 versus IgG4 anti-dsg-3 in relation to disease activity in patients with established PV over time, however within patient changes in levels of anti-dsg-3 IgG, irrespective of isotype, seemed clinically useful in following treatment responses.
CHAPTER 1

Pemphigus Vulgaris
1.1 Introduction

Pemphigus is a group of chronic autoimmune diseases (Table 1) characterised by intra-epithelial blistering, resulting in superficial vesicles or bullae that easily rupture, leading to ulceration of mucosal and/or cutaneous sites. The term “pemphigus” is derived from the Greek word “pemphix”, which means bubble or blister. Lesions result from autoantibody binding to specific desmosomal proteins on the surface of the keratinocytes, resulting in loss of cell-cell adhesion, termed acantholysis. Pemphigus vulgaris is the most common and clinically aggressive variant and is associated with significant morbidity and mortality.[1]

1.2 Clinical Features

PV commonly presents with oral lesions up to 70% of cases. These lesions may be the initial and only sign of disease. Cutaneous lesions tend to develop an average of 5 months after oral involvement [2, 3]. Commonly, oral mucosal lesions appear as intact fluid filled vesicles and bullae that rupture easily. These form tender irregular mucosal erosions and ulcers [1]. Any oral mucosal site may be affected however the most common areas include the gingival, buccal and palatal tissues [4]. Most lesions are painful and slow healing, although do not tend to scar. Desquamative gingivitis is often noted in severe cases where the blisters have ruptured leaving areas of peeling tissue with red erosions and ulcers [5]. Lesions may spread to involve the oropharynx and larynx. Mucosal tissues at other sites such as the conjunctiva, oesophagus, and genitalia may also develop lesions. Cutaneous involvement similarly presents as flaccid fluid-filled blisters which rupture easily due to their intra-epithelial nature, resulting in painful erosions. These lesions arise in normal-appearing skin. A positive “Nikolsky sign” is often noted in association with both mucosal and cutaneous lesions, wherein blisters may be induced or expanded when lateral pressure is applied to the tissue.
1.3 Epidemiology

The incidence of PV varies amongst populations from 0.1–0.5 patients per 100 000 population per year [6], with a mean age of onset between 50 to 60 years of age, although there have been reported cases of children and elderly individuals also being affected [7, 8]. There is an approximately equal male to female ratio. PV is more prevalent in Ashkenazi Jews, where the population incidence increases to 1.6-3.2 cases per 100 000 [9, 10] and people of Mediterranean and South Asian background. Within these ethnic groups, there is an association with certain HLA subtypes: HLA-DR4 (DRB1*0402) seen in Ashkenazi Jews and, DR14 (DRB1*1401) and DQB1*0503 in patients of European and Asian backgrounds, respectively [11] Recently, a polymorphism within the pro-apoptotic molecule ST18 has also been reported in association with PV [12].
<table>
<thead>
<tr>
<th>Disease and Subtype(s)</th>
<th>Clinical Presentation</th>
<th>Prognosis/Outcome</th>
<th>Target Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral</strong></td>
<td><strong>Cutaneous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemphigus vulgaris (PV)</td>
<td>Common.</td>
<td>Commonest and most aggressive form of pemphigus: oral mucosal involvement common and often first site of presentation leading to extensive skin involvement.</td>
<td>Fatal if untreated</td>
</tr>
<tr>
<td></td>
<td>Usually the first site involved</td>
<td></td>
<td>Good with treatment</td>
</tr>
<tr>
<td>Pemphigus vegetans</td>
<td>Uncommon and less aggressive clinical variant of PV: presents with large verrucous confluent plaques and pustules localized to flexural areas in the axilla and groin.</td>
<td>Often progresses to pemphigus vulgaris</td>
<td></td>
</tr>
<tr>
<td>of Neumann</td>
<td>Rare</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(in all 3 forms of pemphigus vegetans)</td>
<td>Often begins and ends as typical PV. Needs more intense immune-suppression than seen with PV, with patients troubled by chronic relapses (and remissions).</td>
<td>Frequent relapses (even with treatment)</td>
</tr>
<tr>
<td></td>
<td>Relatively benign, usually very well localised disease.</td>
<td>Prolonged remission (with treatment)</td>
<td></td>
</tr>
<tr>
<td>Pemphigus foliaceus (PF)</td>
<td>All forms of PF are characterised by clinically by superficial cutaneous blisters and erosions are seen clinically and histologically by subcorneal acantholysis.</td>
<td>More benign course than PV, with prolonged remission.</td>
<td>Desmoglein 1</td>
</tr>
<tr>
<td>Pemphigus erythematosus</td>
<td>Rare</td>
<td>Very rare condition with the combined features of pemphigus foliaceus and SLE which manifests with small, flaccid bullae with scaling and crusting confined to sun-exposed skin, with lesions of the face presenting in the typical butterfly pattern seen in SLE.</td>
<td></td>
</tr>
<tr>
<td>(&quot;Senear-Usher syndrome&quot;)</td>
<td>(all 3 forms of pemphigus vegetans)</td>
<td>More benign course than PV, with prolonged remission.</td>
<td></td>
</tr>
<tr>
<td>Endemic pemphigus</td>
<td>PF and FS are identical clinically, histologically, and serologically but differs significantly, epidemiologically, with marked geographic clustering in Brazil, being a diseases of people resident or near the rainforests. Other endemic forms of FS have been reported in Colombia, Peru, and Tunisia. The autoimmune response in FS is thought to be triggered by a putative environmental factor.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td>Description</td>
<td>Presentation</td>
<td>Pathogenesis</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>IgA pemphigus</td>
<td>Rare, characterised by pruritic, flaccid vesicles and/or pustules in annular pattern with central crusting, sometimes hypopyon (sterile leukocytic exudate, seen in the anterior chamber of the eye). Pathogenesis: more directly related to the neutrophilic infiltrate in the epidermis rather than solely to the binding of IgA to target epidermal antigens. DIF: IgA (cf IgG seen in all other forms of pemphigus) deposits in lower epithelium or entire epidermal cell surfaces</td>
<td>Recalcitrant to treatment with corticosteroids</td>
<td>Desmoglein 3</td>
</tr>
<tr>
<td>Subcorneal pustular dermatosis (&quot;Sneddon-Wilkinson disease&quot;)</td>
<td>Subcorneal (beneath the stratum corneum) blister containing neutrophils with epidermal acanthosis and spongiosis, results in superficial fragile blistering.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraepidermal neutrophilic IgA dermatosis</td>
<td>Deeper, intra-epidermal blister containing neutrophils with epidermal acanthosis and spongiosis, results in more marked blistering and consequent ulceration.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraneoplastic pemphigus</td>
<td>Polymorphous skin eruption, consisting of blisters, erosions, and targetoid lesions; severe mucous membrane involvement. DIF: IgG deposits on entire epidermal cell surfaces +/- granular-linear complement autoantibodies to rat bladder epithelium in 75% of cases.</td>
<td>Fatal</td>
<td>Desmoglein 3, Desmoplakin 1, Desmoplakin 2, BP 230, evoplakin, periplakin, others</td>
</tr>
<tr>
<td>Familial benign chronic pemphigus (&quot;Hailey-Hailey disease&quot;)</td>
<td>Autosomal Dominant, it presents a chronic recurrent bullous and vesicular dermatitis of intertriginous areas that is characterized histologically by suprabasal acantholysis. Cause: heterozygous mutations of the ATP2C1 gene leads to a malfunction of the encoded protein hPMR1 - hPMR1 being a high-affinity calcium transport ATPase pump of the Golgi complex. Low levels of intracellular Ca^2+ induces premature keratinocyte proliferation, which may lead to dysfunctional desmosomal proteins and thus abnormal keratinocyte adhesion.</td>
<td>Chronic, relapsing–remitting course</td>
<td>Desmocollin 1</td>
</tr>
</tbody>
</table>

Abbreviations: PV = pemphigus vulgaris; PF = pemphigus foliaceus, SLE = systemic lupus erythematosus, FS = Fogo Sevagem, DIF = direct immune-fluorescence, cf = in contrast, BP = bullous pemphigoid, Ca^{2+} = calcium ion
1.4 Diagnosis

Biopsy of perilesional tissue demonstrates intraepithelial vesicle formation. Intercellular oedema is a relatively early feature, and this is usually accompanied by loss of intercellular attachment in the basal layer. “Clefting” may be noted as the suprabasal keratinocytes cells separate from the basal cells. The basal cells themselves are separated from each other, forming a “tombstone” appearance at the floor of the blister, but these cells remain attached to the basement membrane. Acantholytic (Tzanck) cells may be seen within the vesicle [6, 14]. (Fig 1)

![Hallmarks of PV](image)

**Figure 1. Hallmarks of PV.** Normal epidermis (A). Suprabasal acantholysis in patient with PV (B). Oral lesions and epidermal blistering manifested in PV patients (C) [15].

Direct immunofluorescence (DIF) performed on fresh tissue commonly shows a classical “basket weave” pattern on staining for IgG, reflecting its deposition on the surface of the keratinocytes. IgM and complement components such as C3 are also often present. This pattern is driven by circulating IgG autoantibodies that bind to the surface of epithelial cells (‘intercellular cement substance antibody’, ICSA) may be detected by indirect immunofluorescence (IIF) in 80-90% of patients with PV. Antibody titres may also help guide prognostication and therapy [16], generally reflecting disease activity.
These antibodies are detected using either primate oesophagus or human skin as the substrate, but specificity studies have demonstrated that the main autoantigens are desmoglein 1 and 3 (dsg-1 and -3)[17-20].

This diagnostic specificity has been improved by using purified autoantigens as substrate on enzyme linked immunosorbent assay (ELISA). This assay was developed using human recombinant dsg-1 and dsg-3 proteins, and detects circulating autoantibodies in pemphigus patients [21], and has been shown to be highly sensitive and specific. Studies have shown that ELISA values often correlate with pemphigus disease activity [22]. An ELISA method that utilises antigen ectodomains of dsg-1 and dsg-3 generated in human HEK293 cells for the detection of autoantibodies anti-dsg-1 and anti-dsg-3 has also recently become available [23].

Most studies have suggested that ELISA constitutes a better test for the diagnosis and management of PV. Meta-analysis exploring the diagnostic accuracy of the anti-dsg-3 ELISA concluded that with a mean sensitivity of 97% and a mean specificity of 98.5%, the high diagnostic accuracy of this test afforded greater consistency of diagnosis despite differences in the selection of control groups and defining cut off values between studies [24]. No analysis could be performed comparing IIF and ELISA methods due to the limited number of studies that have utilised both techniques simultaneously. However, analysis of the 6 studies [25-30] that investigated IIF for the diagnosis of PV, reported sensitivity values ranging from 74% to 99.9%, values that were equal to or lower than the analytical sensitivity of ELISA methods [24].

1.5 Management

Prior to the advent of corticosteroids the mortality rate for untreated PV patients ranged from 60% to 90% [10]. Use of steroids and other immune therapies have reduced the rate significantly to 5-15%
However, as with all therapies, there are associated side effects. Currently, the goal of managing PV is to induce and maintain remission, using the lowest dose of medication possible, so as to minimize such side effects.

Corticosteroids remain the primary treatment for PV patients, due to their ability to rapidly and effectively induce remission. The side effects associated with steroid use include weight gain, diabetes, osteoporosis, hypertension, immunosuppression, sepsis, and changes to fluid balance [32]. Long-term management of these patients almost inevitably requires the introduction of a steroid sparing agent, commonly azathioprine or mycophenolate [33-38].

In addition to conventional methods of therapy, there are a number of emerging therapies that have been developed over the years, including intravenous immunoglobulin, plasmapheresis, extracorporeal photochemotherapy, and cholinergic agonists [10, 39-48]. Furthermore, there have been many case reports suggesting the use of rituximab, a monoclonal anti-CD20 antibody [47, 49-64] and TNF-antagonists (i.e. infliximab and etanercept), in suppressing PV [65-69].

1.6 Prognosis and Outcome

The natural course of PV is variable as is the severity of the disease. Most deaths occur during the first few years of disease, and if the patient survives 5 years, the prognosis is good. Management and control of the condition is more straightforward during early, limited disease, whereas widespread disease is generally more difficult to control, hence mortality may be higher if therapy is delayed [70].
Morbidity and mortality are related to the extent of disease, the maximum dose of immunosuppressive therapy required to induce remission, and the presence of other medical co-morbidities[71]. Complications secondary to the use of immunosuppressive therapy may also contribute to the reported mortality rate [71].

Several groups have evaluated laboratory tests for measuring and predicting clinical activity. Levels of desmoglein autoantibodies are reported to fluctuate with disease activity, consistent with their pathogenic role; thus titres of ICSA [16, 35] and levels of anti-dsg IgG [72-75]tend to fall with successful therapy, and rise with relapse.

The incidence and duration of true remission in PV is uncertain[70]. It is unknown whether treatment suppresses disease manifestations, and so must be continuously administered, or whether it induces complete true remission in some patients, allowing therapy to be discontinued. A recent long-term longitudinal study of complete and long-lasting remissions (defined as lesion-free with no systemic therapy for at least 6 months) enlisted 40 patients with PV treated conventionally and followed up for an average of 7.7 years[70]. The authors reported that five patients (5%) died of the disease but complete and long-lasting remissions were induced in 25, 50 and 75% of patients at 2, 5 and 10 years, respectively, after diagnosis [70]. The majority of the remaining patients were in partial remission or had mild disease controlled with a low dose of systemic corticosteroids. The authors concluded that it was possible to induce complete and long lasting remissions in many patients, allowing systemic therapy to be discontinued without a relapse in clinical disease activity[70].

1.7 Pathogenesis of Blister Formation

Patients with PV generate autoantibodies against the desmosomal cadherins, typically dsg-3 and in 50% of cases, also to dsg-1 [14, 20, 76-79], both of which are adhesion molecules found in stratified
squamous epithelium. Dsg-3, a 130kd glycoprotein, is found primarily in the suprabasal layer of the epithelium. This antigen-antibody interaction activates complement, releasing inflammatory mediators and recruiting activated T cells. Damage to the intercellular area induces apoptosis, loss of cell-to-cell adhesion (acantholysis) and eventually intra-epithelial blistering [6].

The pathogenic nature of dsg-3 specific autoantibodies has been studied and supported by observations of various groups who have found that disease activity in PV is mostly correlated with dsg-3 specific IgG titres in the patients’ sera. Blisters have been observed in newborn babies of mothers with active PV as a result of transplacental transmission of maternal dsg-3 specific autoantibodies. In addition, pemphigus-like lesions may be induced in neonatal mice following the passive transfer of purified serum IgG from patients with active disease [80-82].

It is generally accepted that PV can be classified into two subtypes; the mucosal dominant type with mainly mucosal lesions and minimal, if any, skin involvement, and only anti-dsg-3 IgG autoantibodies; and the mucocutaneous type in which patients have mucosal and skin lesions, and anti-dsg-1 in addition to anti-dsg-3 IgG autoantibodies [25]. The clinical manifestations of mucosal PV compared with mucocutaneous PV can be explained by the distinct pattern of distribution of the two target antigens, dsg-1 and dsg-3, within the epithelia, thus determining the site of acantholysis. Dsg-3 is highly expressed in oral epithelium, whereas skin epithelium expresses both dsg-3 and dsg-1. As such, oral involvement tends to be an early sign of the disease; however, once dsg-1 antibodies develop, skin and lesions of other mucosal site develop [6, 20, 22].

In the related disease, pemphigus foliaceous, in which only skin lesions are apparent, antibodies are typically directed against dsg-1 alone [83, 84].
1.8 Desmoglein Compensation Theory

The mechanism by which blisters form in PV is thought to be related to the autoantibody mediated disruption of desmoglein-dependent cell adhesion. However, there is variability in the site of this disruption amongst the pemphigus diseases, with PV patients having relatively deep blisters, whilst the blisters in PF patients are more superficial, despite both conditions being driven by desmoglein antibodies. Distribution of lesions also varies, the lesions in PF being confined to the skin, whilst in patients with PV, some have only oral involvement, whilst others have both skin and mucosal lesions. The “desmoglein compensation theory” attempts to explain this variability. It proposes that dsg-1 and dsg-3 “compensate” for their adhesive function when they are co-expressed in the same cell (Fig. 2)[85-87].

This theory is based on the pattern of intraepithelial expression of dsg-1 and dsg-3. In the skin, dsg-1 is expressed throughout the epidermis, but more intensely in the superficial layers. On the other hand, dsg-3 is found to be expressed in the lower section of the epidermis, concentrated in the basal and suprabasal layers[77]. In mucosae, both dsg-1 and dsg-3 are expressed throughout the epithelium, however dsg-1 is expressed at a much lower level than dsg-3[85].

Thus, in the presence of anti-dsg-1 IgG, blistering occurs only in the superficial layers of the epidermis, as this is the only area in which dsg-1 is expressed without co-expression of dsg-3 (Fig. 2A); the deeper epidermis remains unaffected due to the adhesive action of dsg-3, thus “compensating” for the loss of functional dsg-1. In other words, despite the anti-dsg-1 IgG binding to the mucosa, no lesions are noted because of the co-expression of dsg-3. This hypothesis is compatible with the clinical findings in PF, where there is formation of only superficial lesions in the skin in absence of mucosal involvement.
By contrast, in the presence of anti-dsg-3 IgG alone, the co-expression of dsg-1 itself is now able to “compensate” for loss of functional dsg-3. Thus there are few if any skin lesions (Fig. 2B). In the mucosae, dsg-1 is unable to compensate for the impaired dsg-3 function because of its low expression, resulting in the clinical predominance of oral lesions, in the absence of skin involvement, in these patients’ PV.

When sera contain both anti-dsg-1 and anti-dsg-3 IgG, the function of both dsg-1 and dsg-3 are disrupted, resulting in both skin and mucosal lesions, typical of patients with the mucocutaneous type of PV (Fig. 2C).
Figure 2. Desmoglein Compensation Theory [76]

The triangles represent the distribution of dsg-1 and dsg-3 in the skin and mucous membranes. PF sera contain only anti-dsg-1 IgG and cause superficial blisters in the skin because dsg-3 functionally compensates for the impaired dsg-1 in the lower part of the epidermis, while these sera do not cause blisters in the mucous membrane because cell-cell adhesion is mainly mediated by dsg-3 (A). Sera containing only anti-dsg-3 IgG cause no or only limited blisters in the skin because dsg-1 compensates for the loss of the dsg3-mediated adhesion, whereas those sera induce separation in the mucous membrane where the low expression of dsg-1 will not compensate for the loss of the dsg-3-mediated adhesion (B). When sera contain both anti-dsg-1 and anti-dsg-3 IgG, the function of both desmogleins are compromised and blisters occur both in the skin and mucous membranes (C).
1.9 Anti-dsg-3 IgG Isotypes

Several groups have analysed the distribution and role of anti-dsg-3 IgG isotypes at discrete stages of disease. In the majority of cases these studies had small numbers of subjects and analysed one, or a very limited set of IgG isotypes, often with contradictory results[88].

There seems to be general consensus that both IgG4 and IgG1 are the most important isotypes in PV [28, 75, 89-94]. Some studies provided evidence for a critical role of dsg-3 specific IgG4 in the development of PV [28, 95-98] concluding that anti-dsg-3 IgG4 antibodies predominate in acute and active disease [28, 75, 89-93, 99], whilst anti-dsg-3 IgG1 autoantibodies predominate in chronic disease and remission [75, 97, 99, 100]. That anti-dsg-3 IgG1 antibodies may be less pathogenic was supported by studies of healthy relatives of PV patients and carriers of PV-related HLA class II alleles, who have low levels of dsg-3-reactive IgG1 but remain disease-free [97, 99, 100]. By contrast, other studies have found that both isotypes remain elevated during remission [75, 88, 99, 101]. Finally, only two groups have explored other immunoglobulin isotypes beyond the IgG subclass, and either observe [94, 99], or do not observe [102] increased levels of antigen-specific IgA and IgE.

These conflicting observations may relate to differences in definitions of disease activity, since studies were performed before a validated disease extent tool was available, and might also have been limited by the study of patients at only a single time point.

1.10 Scoring Systems

In recent years, several groups have addressed the need for a standardised, validated outcome measure for disease assessment in PV [103]. The PDAI was developed by the International Pemphigus Group at a similar time to the Autoimmune Bullous Skin Disorder Intensity Score
(ABSIS), developed by the German Blistering Disease Group. When the two scoring systems were compared, the PDAI had the highest inter-rater and intra-rater intra-class correlation coefficients (ICCs) [104]. A limitation of this study was that most patients had relatively stable disease.

More recently, Kamiya et al. investigated whether pemphigus disease activity correlated with dsg-3 titres over time and found that the titres of the conformational dsg-3 epitopes correlated with the PDAI score [105]. Patsatsi et al. assessed the disease correlation of PV and PF with anti-dsg-1 and anti-dsg-3 ELISA titres using the PDAI and ABSIS systems. They found a statistically significant association between the total PDAI or ABSIS scores and dsg-1 titres in patients with cutaneous involvement, but only moderate correlation between dsg-3 titres and ABSIS or PDAI scores in patients with only mucosal involvement [106].

A third scoring system, the Pemphigus Vulgaris Activity Score (PVAS) was developed and validated by Rahbar et al. The study compared all three systems with ELISA scores for dsg-1 and -3, finding a higher inter-rater reliability for the PDAI compared with the ABSIS and PVAS. The inter-rater reliability remained similar for the PDAI and the PVAS at both high and low dsg titres, whereas for the ABSIS, it was less reliable at low dsg titres. High inter-rater reliability was observed for skin activity and mucosal activity of the PDAI, followed by skin activity of the PVAS, and then oral involvement of the ABSIS. Hence it was agreed that the PDAI was the most useful of the three systems [107].

One advantage of the PDAI is its sensitivity to low numbers of lesions within defined anatomical areas, which results in increased inter-rater reliability. By contrast, the ABSIS and PVAS use items that make the measures less reproducible. The ABSIS uses the low-agreement rule of nines to estimate the percentage of body surface area involvement, which becomes more difficult for limited disease activity. Both the ABSIS and PVAS require evaluation of lesion type and apply it as a
weighting factor, which exaggerates small differences between raters and causes lower inter-rater reliability[103].

1.11 Project Background

Current dogma suggests that pathogenic ICSAs are of the IgG4 isotype in early disease, but IgG1 predominates in remission, however despite this widespread belief, results from many studies have been inconsistent with this view.

The earliest studies of IgG profiles in PV utilised immunofluorescence techniques [90-93]. In general, these groups found that IgG4 was the most prevalent isotype present in PV, followed by IgG1. IgG2 and IgG3 were often present however at a significantly lower level.

More recent studies have employed immunoblotting and ELISA techniques. These methods have the advantage of being able to distinguish between anti-dsg-3 and anti-dsg-1 antibodies [28, 75, 89, 99, 100, 102, 108-110]. Many of these studies again found a predominance of anti-dsg-3 IgG4 and IgG1, with lower or absent levels of IgG2 and IgG3 antibodies in PV patients [25, 28, 89, 97, 102].

There are, however, conflicting results when immunoglobulin levels are compared between patients with active disease and those who are in remission. Several studies found significant predominance of IgG4 during the active phase of PV, and IgG1 during times of disease quiescence[75, 89, 99]. Contrary to this, several groups observed a predominance of anti-dsg-3 IgG4 during remission [90], or reported a decreased, though still detectable, level of IgG4 and IgG1 anti-dsg-3 antibodies, with no subclass switch[102].

The majority of studies did not employ an objective reproducible measure of clinical activity.
1.12 Research Description and Hypothesis

The current project therefore sought to measure anti-dsg-3 IgG isotype changes at various clinical stages of PV activity using ELISA-based methods and a validated PDAI scoring tool, and examining the hypothesis that the use of PDAI scoring systems, along with a carefully calibrated IgG isotype-specific ELISA in static and serial samples from PV patients, will support the contention that PV is characterised by anti-dsg-3 IgG4 antibodies during its active phase, and by IgG1 during remission.

1.13 Aims

This study aimed to measure IgG autoantibodies to dsg-1 and dsg-3, the latter divided by isotype, in a cohort of patients with PV, both at a single time point during established disease, and then serially, using a validated tool for measuring the extent of cutaneous and mucosal disease, in the form of the Pemphigus Disease Area Index (PDAI)[104].
CHAPTER 2

Materials and Methods
2.1 Patient recruitment

Entry into the study required a clinical diagnosis of PV, supported by the typical histopathology changes of suprabasal acantholytic blisters, along with deposition of IgG and usually C3 on the intercellular cement substance by direct immunofluorescence microscopy, and positive circulating autoantibodies to intercellular cement substance as detected by indirect immunofluorescence using primate oesophagus as substrate [94]. Samples were referred for anti-dsg-1 and -3 IgG testing from various tertiary referral hospitals around Australia, including Immunology, Dermatology and Oral Medicine clinics, and were collected between January 2010 and January 2012. Clinicians supplied a PDAI [104] (see appendix) for each sample submitted. Subsequent serum samples were collected at review appointments, along with the updated PDAI.

A total of 40 PV patients from seven centres were studied, involving 187 serum samples (24 with three or more samples, eight with two serum samples, and eight patients with a single sample). The median period of follow-up of the 24 patients with three or more samples was 15 months (range 4.5-23). Patients with various degrees of disease activity and on various immunosuppressive medications were studied, including patients whose disease was restricted to the mucosae and also those with mixed skin and mucosal involvement (Table 1).

Serum was stored at -20°C until analysis. ICSA titre results were sourced from a subset of patients followed in the Oral Immunology outpatient clinic at Westmead Hospital. These data were used to quantify the association between ICSA and anti-dsg-1 and dsg-3 IgG. The study was approved by the Ethics Committee at Westmead Hospital (see appendix)
2.2 Pemphigus Disease Area Index (PDAI)

Disease activity was recorded using a validated scoring system termed the PDAI which captures the extent and severity of disease and has good inter-rater reliability [104]. Each anatomical area is examined and assigned a score based on the number and size of lesions present. A damage component is usually incorporated to record areas affected by PV, such as areas of post inflammatory hyperpigmentation, but was ignored for the purposes of this study. The total possible disease activity scores thus ranged from 0 to 250 points, 120 points for skin activity, 10 points for scalp activity, and 120 points for mucosal activity. Because the observed distribution of PDAI in our population was highly skewed, the transformed variable ‘$\log_{10} (PDAI+1)$’ was used for statistical analysis and graphical representation (See appendix).

2.3 Anti-dsg-1 and dsg-3 IgG ELISA

ELISAs were performed using the Mesacup Desmoglein Test – Dsg 1 and Dsg 3 ELISA kits (MBL International, Japan) according to the manufacturer’s instructions. Briefly, recombinant purified Dsg-1 and- 3 autoantigen-coated wells were incubated for 60 minutes at room temperature with 100µl patient serum diluted with Tris buffer, bovine serum and 0.09% sodium azide to a concentration of 1/101. Manufacturer supplied negative calibrator (normal human serum with Tris buffer, bovine serum and 0.09% sodium azide) and positive calibrators (anti dsg-1 antibody positive human serum with Tris buffer, bovine serum and 0.09% sodium azide and anti-dsg-3 antibody positive human serum with Tris buffer, bovine serum and 0.09% sodium azide , for dsg-1 and -3 ELISA kits respectively) were also included. Wells were washed with a solution of PBS and Tween 20and then bound IgG was detected by adding 100µl of conjugate (horseradish peroxidase conjugated mouse monoclonal anti-human IgG diluted with HEPES and bovine serum albumin to a concentration of
1/101) to each well before incubation at room temperature for 60 minutes. Following a second wash stage, binding of this conjugate was developed by a 30 minute incubation at room temperature with 3,3',5,5'-tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H₂O₂). The reaction was stopped by the addition of 100µl 1.0N sulphuric acid to each well. The optical density (OD) was determined using an automated microplate reader with absorbance read at a wavelength of 450nm (a450). Results were calculated by the following formula:

\[
\text{Unit value (U/ml)} = \left( \frac{a_{450\text{<sample>}} - a_{450\text{<calibrator 1>}}}{a_{450\text{<calibrator 2>}} - a_{450\text{<calibrator 1>}}} \right) \times 100
\]

Where “calibrator 1” was the supplied positive calibrator, and “calibrator 2” was the supplied negative calibrator.

OD values were translated to Units/mL with reference to a kit calibrator preparation designated to have 100 Units, using a single point standard curve. Quality control was provided by using a negative control (normal serum) as well as a known positive sample, diluted to a value of approximately 20 U/ml. Repeat values were within two standard deviations; assay runs yielding a variation greater than this were discarded, and the run repeated ((Fig 3). In addition, for the assay to be deemed valid, the following manufacturer’s criteria needed to be met:

\[
A_{450} \text{ of Calibrator 1: } \leq 0.100
\]

\[
A_{450} \text{ of dsg-1 (or dsg-3) Calibrator 2: } \geq 0.700
\]

The assay range for the kit according to the manufacturer was 5–150 U/ml, a value exceeding 20 U/ml deemed to be positive. A value exceeding 150 U/ml was re-run in dilution, until a valid result that lay within the working range of the assay was obtained [111].
Figure 3. Quality control graphs.

Quality control was provided by using a negative control as well as a known positive sample, diluted to a value of ~20 U/ml. Repeat values were within two standard deviations; assay runs yielding a variation greater than this were discarded, and the run repeated.
In order to determine the anti-dsg-3 IgG isotypes, this ELISA was modified by using isotype-specific antisera as the detection reagent (mouse anti-human IgG1 or IgG4, labelled with horseradish peroxidase, 1 mg/mL, Invitrogen, Camarillo, California, USA). Conditions were optimised via checkerboard titration, the final working dilution being 1:1000 for anti-IgG1, 1:4000 for anti-IgG4. A positive calibrator for each isotype was established using a strongly positive patient serum sample; this sample was deemed to constitute a value of 100 U/mL, and was aliquotted, frozen and stored at -80°C to be used in each assay run. As above, separate internal controls for anti-dsg-3 IgG1 and IgG4, with a unit value of approximately 20 U/ml, were included, and the run was repeated if the internal control value was outside two standard deviations of the mean (Fig 3). A reference range was established by assaying sera from 20 normal controls, with the cut-off determined by mean plus three standard deviations. From this, a value of >2U/ml was considered positive.

2.4 Indirect Immunofluorescence

Serum samples of patients followed at Westmead Hospital also underwent testing by indirect immunofluorescence, performed by standard methodology. Briefly, serum was diluted 1:10 in phosphate-buffered saline (PBS), and 50 µL was added to a substrate of monkey oesophagus (The Binding Site, UK) and incubated for 30 minutes at room temperature. Slides were then washed with PBS and incubated with anti-human IgG-FITC conjugate (The Binding Site, UK) for 30 minutes, shielded from light. After another wash with PBS, slides were set with mounting medium and read immediately by fluorescence microscopy. A positive ICSA was characterised by linear staining around the cells of the epithelium, an appearance referred to as a "chicken wire" or "snakeskin" pattern. Samples displaying an ICSA pattern were subsequently titrated in four-fold dilutions up to 1:2560.
2.5 Statistical analysis

The statistical software packages SPSS version 20 and SPLPLUS version 8 were used to analyse the data. Two-tailed tests with a significance level of 5% were used throughout. Individual patient profile plots of log-transformed data were used to illustrate the within-patient associations between anti-dsg ELISA results and PDAI. Spearman rank correlation ($r$) was used to quantify the association between variables of interest. Rank correlations calculated using three or more serial measurements within each subject were summarised by the mean of the within subject rank correlations and its 95% confidence interval (CI) and tested for departure from zero using one-sample t tests.
CHAPTER 3

RESULTS
3.1 Patients

A total of 40 PV patients from seven centres were studied and included those whose disease was restricted to the mucosae and also patients with mixed skin and mucosal involvement (Table 2). Patients had various degrees of disease activity and were on various immunosuppressive medications at the time of evaluation.

<table>
<thead>
<tr>
<th></th>
<th>Mucous Membrane only</th>
<th>Mucous Membrane and Skin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2 samples</td>
<td>≥3 samples</td>
<td>1-2 samples</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2. Subtypes of PV disease in the cohort studies. There were no patients whose disease was limited to the skin.

3.2 Only anti-dsg-3 IgG4 levels correlated with disease activity at recruitment

The relationship between levels of serum anti-dsg IgG and disease activity was explored by plotting the $\log_{10} (PDAI+1)$ against ELISA results for anti-dsg-1 (total IgG) and for anti-dsg-3 (total IgG, IgG1 and IgG4) on entry into the study (Fig 4). Anti-dsg-1 IgG levels were positive only in patients with active disease, whereas anti-dsg-3 IgG results were positive in most patients, most likely reflecting the universal mucosal involvement in this cohort (Table 2). A wide variation in anti-dsg-3 IgG values for a given level of disease activity was found (Fig 4B), but no significant rank correlation between either anti-dsg-1 IgG (Fig 4A) nor anti-dsg-3-IgG and disease activity scores (Fig 4B) was found. However, after dividing the anti-dsg-3 specificity by IgG isotype, there appeared to be a weak positive association ($r=0.370, p=0.019$) between levels of anti-dsg-3 IgG4 and disease activity at
enrolment (Fig 4D). However the study was unable to confirm a significant relationship between anti-
dsg-3 IgG1 and disease activity, with no suggestion of a predominance of anti-dsg-3 IgG1 with
falling disease activity scores (Fig 4C).

Given the pathogenic relationships between anti-dsg-1 and skin disease, and anti-dsg-3 and mucosal
disease, the next step was to separate PDAI values into these two component parts, however no
significant rank correlation between anti-dsg-1 IgG values and skin/scalp PDAI scores (r=0.226,
p=0.161), nor between anti-dsg-3 IgG values and mucosal PDAI scores (r=0.101, p=0.535), was
observed.
Figure 4.

Pemphigus vulgaris: The log_{10} (PDAI+1), plotted against ELISA values for A. dsg-1 total IgG; and for B. dsg-3 total IgG; C. dsg-3 IgG1 and D. dsg-3 IgG4; at recruitment of the 40 patients in the full cohort. Spearman rank correlation (r) and associated p-value are shown. Normal range values for anti-dsg-1 IgG and anti-dsg-3 IgG are shaded; the normal range was 0-2 U/mL for anti-dsg-3 IgG1 and 4.
3.3 Changes in anti-dsg-3 IgG reflected changes in disease activity

Notwithstanding the poor correlation between anti-dsg antibody results and PDAI at enrolment (Fig 4), it was important to examine the relationship between and changes in autoantibody levels and changes in disease activity over time. Data was plotted from 32 patients who had two or more anti-desmoglein estimations performed at different time points (Fig 5), and Spearman rank correlations were calculated within each of 24 patients who had had three or more estimations (Fig 6). Within-patient changes in levels of anti-dsg-3 IgG had a moderate to strong statistically significant positive association with changes in disease activity in a majority of patients (Figs 5, 6 r=0.325, p<0.001), with the rank correlation with disease activity being similar for IgG1 and IgG4 isotypes (Fig 6); thus there did not appear to be any clear superiority for any one anti-dsg-3 IgG assay over the others, although the sample size was too small for firm conclusions. The relationship between anti-dsg-3 IgG and disease activity also held true when the PDAI was restricted to just the mucosal component (r=0.410, p=<0.0001).

By contrast, within-patient changes in anti-dsg-1 IgG showed poor association with changes in PDAI, which failed to reach statistical significance (Fig 6, r=0.151, p=0.1395), although patients in our cohort had levels that were largely within the reference range. A similar lack of association applied when within-patient changes in anti-dsg-1 IgG values were compared with changes in just the skin/scalp component of the PDAI scores (r=0.081, p=0.219).

3.4 ICSA titres correlated with anti-dsg-3-IgG levels, and reflected disease activity

In routine practice, ICSA titres are often used to monitor disease activity in pemphigus, hence it was important to determine the overlap between ELISA results and ICSA titres in the assessment of patients with PV in this study. To do this, data from the 14 patients enrolled at Westmead Hospital
were analysed. This confirmed the positive rank correlation between ICSA titre and disease activity at recruitment (Fig 7A, r=0.802, p=0.001), and between the ICSA titre and dsg-3 IgG values (Fig 7B, r=0.680, p<0.001). However since only 12 of these patients had three or more ICSA estimations, meaningful investigations of within-patient rank correlations could not be completed.
Figure 5.

Pemphigus vulgaris: The log_{10}(PDAI+1), plotted against ELISA values for A. anti-dsg-1 IgG; B. for anti-dsg-3 IgG; C anti-dsg-3 IgG1; and D. anti-dsg-3 IgG4, for the 32 patients who had two or more serial samples analysed. Each coloured line represents a single patient. Normal range values for anti-dsg-1 IgG and anti-dsg-3 IgG are shaded; the normal range was 0-2 U/mL for anti-dsg-3 IgG1 and 4.
Figure 6.

Pemphigus vulgaris: Mean within-patient Spearman rank correlations (r) between PDAI and ELISA values for dsg-1 (total IgG) and for dsg-3 (total IgG, subclasses IgG1 and IgG4) in 24 patients with three or more assessments during follow-up, and associated 95% confidence intervals.
Figure 7.

A. Pemphigus vulgaris: The $\log_{10} (PDAI+1)$, plotted against ICSA titres at recruitment of the 14 patients in the Westmead cohort.

B. Pemphigus vulgaris: Relationship between ICSA titres and dsg-3 IgG levels in the Westmead cohort at all stages of follow-up.
CHAPTER 4

DISCUSSION
PV is a classic autoantibody-driven disease in which serum concentrations of pathogenic autoantibodies vary in proportion to disease activity, meaning that total IgG autoantibody levels could be used as an indicator of clinical response to immunosuppression. However there has been varied opinion as to whether IgG isotypes might better reflect this activity. Thus whilst a number of studies have found that anti-dsg-3 IgG4 seems to be the predominant isotype during the active phase of the disease, and anti-dsg-3 IgG1 during remission, other groups have found no clear relation. The present study comprised 40 patients with confirmed PV, 32 of whom were followed over a period of time, and found considerable complexity to this relationship when disease activity was measured carefully using a graded activity scale, and when autoantibody levels were isotyped and carefully standardised.

Three novel observations were made (i) there was poor correlation between anti-desmoglein antibody levels and disease activity at a single time point; (ii) within-patient variations in disease activity was reflected by anti-desmoglein levels; and (iii) there was no obvious isotype-specific effect in this relationship.

1. There was poor to no correlation between the level of anti-desmoglein antibody and the contemporaneous disease activity index, with only the levels of anti-dsg-3 IgG4 reaching significance, albeit weak (Fig 4). Several studies have previously investigated the relationship between clinical disease activity and anti-dsg-3 IgG subclass distribution, with mixed results. Some of this variation could be related to differences in the measures of clinical activity used. Many of these studies grouped their cohort into clinically active or remittent disease [75, 88-90, 100], whilst others further divided the clinically active patients into acute onset and chronic active groups [94, 99]. The disadvantage of this segregated approach is that there is wide variation in the activity of disease within the active groups, with no distinction between patients with mildly active disease and...
those with more widespread activity. Further, definitions of clinical categories varied between groups. Dhandha et al. included patients in the “active” group if their lesions lasted more than a week[88], however other studies divided their cohort into “acute onset” and “chronic active” groups. The former was defined as “de novo development of lesions on previously unaffected mucosal surfaces” in one study[99], however patients participating in another study needed to have cutaneous involvement of less than 3 months[94]. Similar discrepancies between studies were noted for patients included in the “chronic active” group, which was defined as “expansion/persistence of existing blisters on mucosal surfaces” by one study[99], however for patients enrolled in a different study to be included in this disease category group, lesions needed to be present for longer than 3 months [94].

Similarly, the definition of “remission” differed between studies. Dhandha et al. defined this as the “absence of new or established lesions for 1-2 months” [88] however Kricheli et al. only included patients in this category if they had “no clinical symptoms for at least 6 months” [100]. In addition, some groups required that patients in remission have had “no lesions during the 2 or more months prior to study” [94, 99], and yet another study required patients to have “no clinical disease off all treatment for a minimum of 3 years” [75].

The advantage of the previously validated PDAI, as used in the present study, is that a clear, objective and precise measure of clinical disease activity can be taken for each patient at each time point, allowing for more detailed comparison between time points. Thus, a wide spectrum of disease activities is measurable by the PDAI, allowing a closer evaluation of the relevance of autoantibody levels at any degree of clinical severity. Whilst expecting a stronger association between disease activity and anti-desmoglein antibodies, casual observation of Fig 4 reveals that this was far from the case; rather, a wide scatter of antibody levels was seen for any level of disease activity. This particularly applied to the anti-dsg-3 isotypes, although less can be made of the anti-dsg-1 antibody
levels due to low numbers of positives, itself reflecting the predominantly mucosal disease in the patients in our study (Table 2). Nevertheless, the small number of patients who did have a positive anti-dsg-1 antibody had very active disease. Overall, however, our findings point to wide individual variability in absolute levels of anti-desmoglein antibodies and resultant disease.

2. The second major finding here was that within-patient changes in autoantibody levels during follow-up did indeed reflect changes in disease activity as measured by the PDAI. The within-patient rank correlations between PDAI values and anti-dsg-3 IgG levels were significantly greater than zero and indicated moderate levels of association (average r~0.5) (Fig 6). In other words, whilst the absolute level of autoantibody generally correlated poorly with measures of disease activity (Figs 4&5), changes in anti-dsg-3 IgG levels within individual patients were far more useful in reflecting changes in disease activity. Although the same did not apply to anti-dsg-1 IgG, the paucity of anti-dsg-1 IgG positive patients may have reduced our ability to detect an association, since others have found a positive correlation in both PV and PF [73, 112].

3. Perhaps the most important finding here was that there appeared to be no isotype effect in these serial studies, with similar within-patient rank correlations for total anti-dsg-3 IgG, anti-dsg-3 IgG1 and anti-dsg-3 IgG4 (Fig 6). These derivations therefore contrasted with a number of other groups who suggested that anti-dsg-3 IgG4 predominates with recent onset of disease, whilst IgG1 predominates in remission [75, 89-92, 97, 113]. Thus, whilst it was expected that a trend to falling levels of anti-dsg-3 IgG4 and rising levels of anti-dsg-3 IgG1 as disease activity decreased would be observed, no such effect was apparent (Fig 5).

This discrepancy may relate to methodological differences. The earliest studies of IgG profiles utilised immunofluorescence techniques[90-93]. In fact, the majority of early studies utilising
immunofluorescence techniques concluded that IgG4 was the most prevalent isotype present in PV, followed by IgG1. IgG2 and IgG3 were often present however at a significantly lower titre. For example, David et al. included a total of 27 patients, 13 of whom were in the active phase of disease, and 14 who were classified as in clinical remission of at least 6 months duration (either off treatment or on a maintenance dose of oral steroids), using DIF of fresh skin biopsies of their readout. This group found that IgG1 and IgG4 were the most common isotypes in activity and remission; IgG1 was found in 100% of active patients and 50% of patients in remission. IgG4 was noted in 85% of patients with active disease decreasing to 79% of remittent patients. They concluded that IgG1 was the most sensitive indicator for active disease and that IgG4 was the most common isotype found in remission. It should be noted that this study was performed on skin biopsy samples, rather than oral mucosae. In addition, this was a single time point study, with no serial samples [90].

By contrast, Jones et al. used IIF to analyse serum samples from 8 PV patients at a single time point. They found IgG4 to be present in all samples. In addition, IgG1 was noted in 90% of samples at a seemingly lower titre to IgG4. No information regarding the patients’ stages of disease was included[91]. Yamada et al. also found that IgG4 was dominant in active PV. This group utilised both DIF and IIF and included 16 PV patients, presumably during the active phase of disease, as samples were collected prior to the commencement of therapy. Similar to previous studies, this group only analysed a single time point, finding that IgG4 was present in 100% of samples, and IgG1 in 41%[93]. Other groups used IIF to measure autoantibody levels of the various anti-dsg IgG subclasses [28, 90, 91, 93, 94, 100]. Unfortunately, but lack of standardisation of titrations across these studies, make direct comparison between groups difficult.

The disadvantage of both DIF and IIF is that the methods are only qualitative (ie either positive or negative) or at best only semi-quantitative. This is particularly the case with DIF [90, 93, 94], where
it is difficult to measure fluorescence objectively, with authors grading intensity as negative, weakly positive or strongly positive. Such studies are difficult to reproduce, making comparison between studies very difficult.

More recent studies have employed Western blotting[28, 75, 99, 100, 113] to measure anti-dsg-3 IgG isotypes in patient serum. Immunoblotting measures the presence or absence of each IgG isotype, but again does not provide a quantitative result. This technique also reveals epitopes in denatured proteins, and therefore it is possible that some conformational epitopes of the PV antigen may not be detected. One of the earliest studies employing this technique was conducted by Bhol et al., who enrolled 27 PV patients with active PV and 13 PV patients in remission, along with a large number of healthy controls, samples being taken at a single time point. The authors found that both IgG1 and IgG4 were present during active disease, and lower titres of autoantibody, mainly IgG1, were noted during remission. This led them to suggest that IgG1 was probably a non-pathogenic antibody given its continued presence during clinical quiescence and that IgG4 was most likely the pathogenic antibody in PV[75].

Kricheli et al. included 25 PV patients as well as 55 healthy first-degree relatives and 56 healthy controls. Of the PV patients, 13 had active disease and 12 were in clinical remission, defined as the absence of clinical symptoms for at least 6 months. The main objective of this study was to compare the distribution of IgG subclasses in PV patients and their healthy relatives, however the isotype distribution within the PV group was also reported. In this single time point study, investigators found no statistically significant difference between active patients and those in remission for anti-dsg-1 and -3, irrespective of the four subtypes. Similar to the immunofluorescence studies, the authors only analysed samples from a single time point. The authors attributed the heterogeneity of
results from previous studies to the small number of patients and differences in their clinical status, as well as laboratory methodology[100].

A larger study by Spaeth et al. used immunoblot to analyse single time point serum samples from 41 PV patients and 10 healthy controls. Of the PV patients, 15 of these had acute onset disease, including 9 with mucocutaneous lesions; 18 were in the chronic active phase, which included 5 patients with mucocutaneous disease; and 8 patients in remission. The authors concluded that IgG4 was the major IgG subtype in both acute onset and chronic active PV. Dsg-reactive IgG1 and/or IgG3 were detected in the majority of sera from patients in remission. They concluded that the titre of total IgG reactivity against dsg-3, including both IgG1 and IgG4 isotypes, might represent useful markers to monitor disease activity[99].

The most recent studies investigating autoantibody isotype profiles in PV have utilised ELISA. Futei et al. included 30 PV patients, at “active, mild or moderate stage”; which was otherwise not defined. Sera from different time points of active, moderate and mild or remission stages were also obtained from a subset of 6 of the PV patients. Disease activity was assessed subjectively by the description of clinical findings gleaned from the clinical records. The authors reported ELISA results using OD values, and found anti-dsg-3 IgG4 in all samples, and anti-dsg-3 IgG1 in 83% of samples. In the serial samples, the authors observed that IgG4 was the predominant subclass throughout the course of disease with no subclass shift observed. In the majority of patients, IgG4 titres showed a certain degree of fluctuation with disease activity. IgG1 was positive in most patients, however only one case showed prominent fluctuations with disease activity. The authors suggested that the higher sensitivity of the subclass ELISAs, compared with the total IgG ELISA, may have been important in generating these results. They attributed this higher sensitivity to the use of an extra stage of detection, consisting of anti-IgG subclass antibody as a second antibody and, in their case, anti-mouse IgG
antibody as a third antibody. This high sensitivity may have therefore been useful to detect low levels of PV antibody. This study also used IIF to analyse the sera of 12 PV patients, with the majority of samples being positive for IgG1 and IgG4, with the latter showing stronger staining[102].

Hacker et al. used various laboratory techniques including ELISA to analyse the serum from 17 PV patients, 7 of who had mucosal disease, and 10 with mucocutaneous involvement at a single time point. However no record of the clinical activity of these patients was made at the time of serum sampling. ELISAs were performed using the same commercially available kit as the present study, and modified for subtype studies, but all ELISA results were reported as OD values. This group found that the majority of patients with mucosal disease produced both anti-dsg-3 IgG1 and IgG4. Two patients produced only IgG4, and one patient produced IgG3 as well as IgG1 and 4. Patients with generalized disease produced both anti-dsg-1 and- 3 antibodies[28].

In-house ELISA was utilized by Ayatollahi et al. who included single time point serum from 17 highly active PV patients who had extensive skin erosions and oral cavity involvement, as well as 20 patients in remission. They found that anti-dsg-3 IgG1 and IgG4 were significantly elevated in active patients compared with healthy controls. In addition, anti-dsg-3 IgG1 was significantly elevated in remission compared to controls. As with previous studies, ELISA results were reported in terms of OD[89].

A more recent large study by Nagel et al. enrolled 93 PV patients: 37 of whom had acute onset disease,42 with chronic active disease, and 14 patients in remission. Most patients had mucocutaneous involvement (54%), with the remained divided roughly equally between those with mucosal or cutaneous-predominant lesions. Samples were taken at a single time point, used in-house ELISAs, and reported results in terms of OD. The authors noted that in their study populations,
patients with acute onset PV showed the highest concentrations of anti-dsg-3 IgG4, which were significantly lower in patients in remission[94].

The most recent study by Dhandha et al. included a total of 71 patients with PV, with 169 samples. Patients with non-transient lesions (ie lesions lasting more than a week) were classified as having active disease, and those with an absence of new or established lesions for 1-2 months were classified as in remission. Contrary to other studies that suggested that the IgG1/G4 ratio was significantly elevated in remittent disease when compared to active disease, allegedly resulting from a faster decline of IgG4 than G1 in remission, this study showed a greater decrease in the amplitude of anti-dsg-3 IgG4 than IgG1, leading to a drop in the relative amount of anti-dsg-3 IgG4 versus IgG1, but not in a reversal of the IgG1/IgG4 ratio between disease phases. This study included a large patient population with serial samples, however did not use a validated clinical scoring system. ELISA index values were used rather than OD to report results, along with appropriate positive and negative controls allowing for comparable results from different assay time points [88].

Most studies that utilised ELISA[28, 75, 88, 89, 94] reported results in terms of OD. In one study[28], results were only classified as either positive or negative based on an OD cut off. As OD is not a standardised calibrated reference, there is inherent inaccuracy with no clear way of comparing the reported results between various study groups. To overcome this inaccuracy, all ELISAs performed in the current study were calibrated by a standard reference serum, which remained constant throughout all assays. All assay results were reported in a numerical value (U/ml) which is calibrated to allow accurate comparison between samples. This in turn, allows more meaningful analysis of within-patient serial samples.
The majority of studies that have investigated anti-dsg-3 IgG isotype distribution in PV have only analysed serum at a single time point. Those studies (Futei et al. and Dhandha et al.) that included serial samples did not observe a subclass shift between active disease and remission[88, 102], consistent with the findings of the present study. Also, similar to the present study, these studies [88, 102] noted fluctuations in anti-dsg-3 IgG1 and IgG4 titres that follow the clinical course of the disease. These studies found that IgG4 was the predominant isotype during both active and remittent phases, with fluctuations in this titre reflecting the course of the disease more closely that IgG1 in the majority of their cohort. This led to the authors to conclude that anti-dsg-3 IgG4 titres were a better indicator of disease activity than any other isotype. This conclusion was not supported by the present study, where no single isotype was found to be a superior indicator of disease activity. This conflicting observation could possibly be explained by the difference in study methodology and the way in which the results were analysed. Futei et al. divided the study cohort into three groups; active, moderate, and mild or remission, serial samples were collected from only six subjects[102]. Therefore, the study of anti-dsg-3 IgG isotype titre fluctuations correlating to disease activity was limited by relatively small sample size. Dhandha et al. divided their cohort into two broad disease categories; active and remittent disease. The breakdown of disease severity in the active group was not stated clearly[88]. Neither of these studies used a standardised scoring system to assess disease activity, thereby affecting the ability to accurately correlate disease activity with the isotype titres.

The present study therefore constitutes the most comprehensive of those published so far. Here, PV patients were assessed using a previously validated disease activity score (PDAI) allowing serial measurements to be correlated with serial measurements of the patient’s antibody levels. This allows for smaller differences in clinical activity to be recorded and correlations between changes in disease activity and fluctuations in autoantibody titres to be analysed, and firm conclusions to be made, namely that although anti-dsg-3 IgG levels correlated poorly with disease activity at a single time
point, they did reflect within-patient disease course. Furthermore, no single isotype is superior to another in indicating clinical activity.
Table 3. Summary of previous anti-dsg-3 IgG isotype studies

<table>
<thead>
<tr>
<th>Author</th>
<th>PV</th>
<th>Categories</th>
<th>Serial Samples</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>David 1989</td>
<td>27</td>
<td>13 active, 14 remission</td>
<td>No</td>
<td>DIF IIF</td>
<td>• Anti-dsg-3 IgG1 and IgG4 most common in activity and remission.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IgG1 most sensitive indicator for active disease.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IgG4 most common isotype in remission</td>
</tr>
<tr>
<td>Jones 1988</td>
<td>8</td>
<td>No information</td>
<td>No</td>
<td>IIF</td>
<td>• Anti-dsg-3 IgG4 present in all samples.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Lower titres of IgG1 in 90% of samples</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IgG1 not present in 10% of samples</td>
</tr>
<tr>
<td>Yamada 1989</td>
<td>16</td>
<td>16 active</td>
<td>No</td>
<td>DIF IIF</td>
<td>• Anti-dsg-3 IgG4 present in 100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IgG1 present in 41%</td>
</tr>
<tr>
<td>Bhol 1994</td>
<td>40</td>
<td>27 active, 13 remission</td>
<td>No</td>
<td>Western blot</td>
<td>• Anti-dsg-3 IgG1 and IgG4 present in active disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Lower titres of both, but mainly IgG1, in remission</td>
</tr>
<tr>
<td>Kricheli 2000</td>
<td>25</td>
<td>13 active, 12 remission</td>
<td>No</td>
<td>IIF Western blot</td>
<td>• No difference in anti-dsg-3 isotypes between active and remission groups</td>
</tr>
<tr>
<td>Spaeth 2001</td>
<td>41</td>
<td>15 acute onset, 18 chronic active, 8 remission</td>
<td>No</td>
<td>Western blot</td>
<td>• Anti-dsg-3 IgG4 dominant in acute onset and chronic active groups.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• IgG1 detected in majority of patients in remission</td>
</tr>
<tr>
<td>Futei 2001</td>
<td>30</td>
<td>Active, Moderate, Mild or remittent</td>
<td>Yes 6 patients</td>
<td>IIF ELISA</td>
<td>• Anti-dsg-3 IgG4 found in all samples, and predominant throughout disease</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Disease Stage</td>
<td>Test Used</td>
<td>Assays Used</td>
<td>Findings</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>---------------</td>
<td>-----------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>Hacker 2002</td>
<td>14</td>
<td>No information</td>
<td>No</td>
<td>IIF Western blot ELISA</td>
<td>Anti-dsg-3 IgG1 and IgG4 present in majority of patients with mucosal disease</td>
</tr>
<tr>
<td>Ayatollahi 2004</td>
<td>37</td>
<td>17 active, 20 remission</td>
<td>No</td>
<td>ELISA</td>
<td>Anti-dsg-3 IgG1 and IgG4 elevated in active patients compared with controls, IgG1 elevated in remission compared with controls</td>
</tr>
<tr>
<td>Nagel 2010</td>
<td>93</td>
<td>37 acute onset, 42 chronic active, 14 remission</td>
<td>No</td>
<td>IIF DIF ELISA</td>
<td>Anti-dsg-3 IgG4 found in highest concentrations in active onset patients, Significantly lower levels found in remission</td>
</tr>
<tr>
<td>Dhandha 2012</td>
<td>71</td>
<td>Active, Remission</td>
<td>Yes</td>
<td>ELISA</td>
<td>The ratio of anti-dsg-3 IgG4 to IgG1 titres decreased between active and remittent phases, No reversal in the ratio of these two isotypes between disease phases</td>
</tr>
</tbody>
</table>
It should be noted that the present study was not specifically designed to compare new-onset, versus chronic and remittent disease, which typical focus of earlier studies, but rather examined all stages of disease activity based on PDAI. This approach thus addressed a more ‘real-world’ management question.

Finally, on a theoretical basis, the relationship between decreasing disease activity scores and decreasing total anti-dsg-3 IgG (Figs 5B &6), would argue against the proposition that a rising subtype (such as anti-dsg-3 IgG1) might predominate during disease remission, where one would expect stable anti-dsg-3 levels, with alterations in the relative proportions.

The sub-study of patients who had had ICSA performed in a central diagnostic laboratory confirmed a correlation with disease activity scores (Fig 7A), and with anti-desmoglein levels (Fig 7B), suggesting that ICSA remains a valid laboratory test for assessing treatment responses. Nevertheless, the test is only semi-quantitative, making the anti-dsg ELISAs arguably more useful in this regard. There was however a wide range of anti-dsg-3 IgG levels for a given ICSA titre (Fig 7B), and this, along with a smaller sample size in the ICSA cohort, might explain the discrepancy between the lack of significant association between PDAI and anti-dsg-3 IgG levels at enrolment (Fig 4) despite significant rank correlation with ICSA titre (Fig 7).

In conclusion, the present study has demonstrated the utility of monitoring changes in anti-dsg-3 IgG levels, in following response of PV to treatment. There appeared to be limited utility for measuring anti-dsg-3 IgG4 levels in predicting clinical activity at a single time point, and no benefit in differentiating anti-dsg-3 by isotype in following disease response over time. These findings should assist the interpretation of anti-desmoglein antibody results in the clinical management of patients with PV.
APPENDICES
Antibodies to desmoglein-1 and 3

Introduction:
Pemphigus is a group of severe autoimmune diseases characterized by blistering and ulceration of the skin and mucous membranes. The two main types of pemphigus are pemphigus vulgaris (PV) and pemphigus foliaceus (PF), in which pathogenic IgG autoantibodies are directed against desmosomal transmembrane glycoproteins and a number of other autoantigens. However, the major targeted antigens are desmoglein (dsg) 3 and dsg-1.

How to Interpret the Test:
The mucosal type of PV is characterised by an autoimmune response directed just against dsg-3 (Table 1). In contrast, both dsg-1 and dsg-3 are targeted by autoantibodies in the mucocutaneous type of the disease, whilst patients with PF classically show autoantibodies to dsg-1 alone. Although these are general rules, there are a number of exceptions, and diagnosis should also take into account clinical features and histology.

There is strong evidence that levels of antibody to the desmogleins correlate with disease activity. This is particularly the case for anti-dsg-1 in PF and mucocutaneous PV, where remission of disease is usually accompanied by a falling antibody level to complete negativity. On the other hand, anti-dsg-3 levels tend to fall with treatment, but often do not become negative, even with clinical remission. However, persistent positivity or rising levels tend to predict disease relapse.

About the Test:
Antibodies to dsg-1 and -3 are measured by enzyme-linked immunosorbent assay (ELISA) using recombinant human dsg-1 and -3. The test provides a quantitative measure of patient antibody levels, with 20 Units/mL being the upper limit of normal for both tests.

When to Order:
Anti-dsg-1 and -3 antibodies should be considered in patients positive for intercellular cement substance antibody (ICSA) to assist the clinical and histopathological differentiation of PV versus PF, and in predicting cutaneous manifestations in PV.

Once the diagnosis is established, anti-dsg-1 and -3 antibodies may be used to monitor the response to therapy and in prediction of clinical disease relapse.

Sample Requirements:
The test may be run on 1-2 mL of serum, eg from 6 mL of clotted blood.
Transport at room temperature ASAP. If delayed more than 2 hours, send at 4°C.

As the test is a research assay, it will only be done on the understanding that a completed request form including Pemphigus Disease Activity Index (attached) will be faxed to the Immunopathology department (02-9891-5889) prior to processing of the sample.

<table>
<thead>
<tr>
<th></th>
<th>dsg-1</th>
<th>dsg-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mucosal PV</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mucocutaneous PV</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1. Clinical Interpretation of anti-dsg-1 & -3 antibodies

Information:
- Dr Sue-Ching Yeo
  Tel: (02) 9845-6933
  Fax: (02) 9891-3889
  Email: suechingyeoh@gmail.com
- A/Prof David Fulcher
  Tel: (02) 9845-6933
  Fax: (02) 9891-3889
  Email: david.fulcher@sydney.edu.au
Please send all request forms to:

**Dr Sue-Ching Yeoh**  
Immunopathology, Level 2, ICPMR  
Westmead Hospital, Darcy Road  
Westmead NSW 2145

Fax: 9891 3889  
Email: suechingyeoh@gmail.com  
Ph: 02 9845 6933

<table>
<thead>
<tr>
<th>Requesting clinician:</th>
<th>Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Institution:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact number:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Anti-Dsg-1 & 3 request form**

<table>
<thead>
<tr>
<th>Sex:</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Male</td>
</tr>
<tr>
<td>○ Female</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Pemphigus (and if known):</td>
</tr>
<tr>
<td>○ Pemphigus:</td>
</tr>
<tr>
<td>○ Pemphigus vulgaris</td>
</tr>
<tr>
<td>○ Pemphigus foliaceus</td>
</tr>
<tr>
<td>○ Paraneoplastic pemphigus</td>
</tr>
<tr>
<td>○ Other, specify:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current treatment:</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Steroids: Agent:</td>
</tr>
<tr>
<td>○ Azathioprine:</td>
</tr>
<tr>
<td>○ Methotrexate:</td>
</tr>
<tr>
<td>○ Mycophenolate:</td>
</tr>
<tr>
<td>○ Cyclophosphamide oral:</td>
</tr>
<tr>
<td>○ Cyclophosphamide IV:</td>
</tr>
<tr>
<td>○ Rituximab:</td>
</tr>
<tr>
<td>○ Other: (specify):</td>
</tr>
<tr>
<td>○ Other: (specify):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose:</th>
</tr>
</thead>
</table>

**Clinician’s comments:**

......PTO: Pemphigus disease area index (PDAI)
### Pemphigus Disease Area Index (PDAI)

<table>
<thead>
<tr>
<th>Skin Anatomical Location</th>
<th>Activity: Erosion/Blisters or new erythema</th>
<th>Damage: Post-inflammatory hyperpigmentation or erythema from resolving lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of lesions if &lt; 3</td>
<td>0 absent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 present</td>
</tr>
<tr>
<td>0: Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: 1–3 lesions, up to one &gt;2 cm in any diameter, none &gt; 6 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2: 2–3 lesions, at least two &gt; 2 cm diameter, none &gt; 6 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3: &gt;3 lesions, none &gt; 6 cm diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5: &gt;3 lesions, and/or all at least one &gt; 6 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10: &gt;3 lesions, and/or at least one lesion &gt; 16 cm diameter or entire area</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Ears                      |                                          |                                                                          |
| Nose                      |                                          |                                                                          |
| Rest of the face          |                                          |                                                                          |
| Neck                      |                                          |                                                                          |
| Chest                     |                                          |                                                                          |
| Abdomen                   |                                          |                                                                          |
| Back, buttocks            |                                          |                                                                          |
| Arms                      |                                          |                                                                          |
| Hands                     |                                          |                                                                          |
| Legs                      |                                          |                                                                          |
| Feet                      |                                          |                                                                          |
| Genitals                  |                                          |                                                                          |
| Total skin                | /120                                      | /12                                                                      |

| Scalp                     |                                          |                                                                          |
| Scalp                     | Erosion/Blisters or new erythema         |                                                                          |
|                          | Number of lesions if < 3                 |                                                                          |
|                          | 0: Absent                                 |                                                                          |
|                          | 1: in one quadrant                        |                                                                          |
|                          | 2: two quadrants                          |                                                                          |
|                          | 3: three quadrants                        |                                                                          |
|                          | 4: affects whole skull                    |                                                                          |
|                          | 10: at least one lesion > 6 cm            |                                                                          |
| Total Scalp (0–10)        | /10                                       | /1 |

| Mucous Membrane           |                                          |                                                                          |
| Anatomical Location       | Erosion/Blisters                         |                                                                          |
|                          | Number of lesions if < 3                 |                                                                          |
|                          | 0: Absent                                 |                                                                          |
|                          | 1: 1 lesion                               |                                                                          |
|                          | 2: 2–3 lesions                            |                                                                          |
|                          | 5: >3 lesions or 2 lesions > 2 cm         |                                                                          |
|                          | 10: entire area                           |                                                                          |
| Total Mucosa              | /120                                      |                                                                          |

Total Activity Score: [ ]   Total Damage Score: [ ]
SWAHS HUMAN RESEARCH ETHICS COMMITTEE
(NEPEAN AND WESTMEAD CAMPUSES)

QUALITY IMPROVEMENT/
QUALITY ASSURANCE/
AUDIT

SUBMISSION
CHECKLIST &
APPLICATION FORM
SWAHS QA SUBMISSION FLOWCHART

Steps to assess whether your project requires ethical review by the SWAHS Human Research Ethics Committee (Nepean and Westmead Campuses) or sign off by Department Head

Proposals for Quality Assurance (QA) Projects that require HREC review

Apply the checklist on Page 2 of this application to identify any ethical risks or issues that require HREC review and approval

QA/QI/Audit review by HREC identified

Applicant still unclear if project comprises any ethical risks or issues that require HREC review and approval – To seek clarification from QA committee by submitting documents for consideration

You will be required to complete the SWAHS QA/QI/Audit Application Form for Department Head signature and submit to the HREC for review

Proceed with project once HREC approval is obtained

No QA/QI/Audit review by HREC identified

Complete the attached Checklist and SWAHS QA/QI/Audit Application Form and submit to the Department Head for sign-off

Proceed with project upon signed endorsement from the Department Head
Quality Assurance / Quality Improvement / Audit Checklist (the checklist)

To decide whether or not a proposed study is a Quality Assurance / Quality Improvement / Audit or research project, the following checklist should be completed.

**Notes:** 1. The project can only proceed if participants have provided informed consent, or if the activity is consistent with the National Privacy Principle 2.1. If all questions from 2 to 12 in the checklist are answered 'NO', then the proposal should be forwarded to Sydney West Area Health Service Scientific Advisory Committee for consideration as a Quality Assurance / Quality Improvement / Audit study. In submitting Quality Assurance / Audit projects, please include a copy of this completed checklist. If any question in addition to 1 and 13 is answered YES, then the project most likely represents a Research study and would therefore need be submitted as such to the Sydney West Area Health Service Research Office for full consideration by the Human Research Ethics Committee. If responses to all questions from 2 to 13 are 'NO', then no ethical risks have been identified with this project and no HREC review is required, proceed to Department Head ‘sign off’.

<table>
<thead>
<tr>
<th>Consent</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Has the participant provided informed consent, or is the activity consistent with the National Privacy Principle 2.1 (a)?</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Is there any real or perceived undue pressure placed on participants in the recruitment procedures? (Is the approach to participate made by the researcher who is also the treating clinician?)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risks and Burden</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Does the proposal pose any risk for the participant beyond those of their routine care? (Includes physical, psychological, spiritual, and social harm or distress, e.g. stigmatization or discrimination)</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Does the proposal impose a burden on participants beyond that experienced in their routine care? (Includes intrusiveness, discomfort, inconvenience or embarrassment, e.g. persistent phone calls, additional hospital visits, or lengthy questionnaires)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Privacy and Confidentiality</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5. Will the proposed Quality Assurance / Audit activity be conducted by a person who does not normally have access to the participant’s (i.e. patient’s) record for clinical care or a directly related secondary purpose? (Involvement of a clinical student who is a member of the clinical team/setting or authorized Quality Assurance / Audit officer is acceptable. A student external to the clinical team will require consideration by the HREC).</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Does the proposal risk the privacy of the participant beyond that experienced in the provision of routine care? (Provided the researcher reviewing the records is bound by legislation or a professional code of ethics and the use is directly-related for secondary purposes and is within the expectations of the participant, this question can be answered in the negative).</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Does the proposal risk breaching the Anti-Dag-1 &amp; Dog-2 isotype-specific assay in Pemphigus vulgaris</td>
<td>Yes</td>
</tr>
</tbody>
</table>
8. Is it possible for the participants and, where applicable the institution (in multi-institution studies), to be identified?

9. Does the proposal involve any clinically significant alteration to the routine clinical care provided to the participants? (Application and evaluation of new technology not previously used requires HREC approval).

10. Does the proposal involve randomization, the use of a control group or a placebo? (Comparison of standard routine procedures/interventions with the outcomes of published or prior treatment results is acceptable if there is no randomization involved in participants receiving such procedures/interventions).

11. Does the proposal seek to gather information beyond that collected in routine clinical care? (Includes observations, blood samples, additional investigations etc. Genetic or other studies that seek information on family members, relatives or contacts as well as the patient require HREC approval).

12. Does the proposed activity potentially infringe the rights, privacy or professional reputation of carers, health care providers or institutions? (Consideration should be given to potential legal implications and relevant State or Territory legislation with respect to legal privilege for a Quality Assurance / Audit body).

13. Will the proposal generate data that are likely to lead to publication in peer-reviewed or professional journals?

Additional comments for consideration: Attach list if required.
QUALITY ASSURANCE / QUALITY IMPROVEMENT/ AUDIT DETAILS

Complete all sections below.

Notes: 1. If all of the questions on the above checklist excepting question 1 can be answered “no”, then the proposal does not need consideration by the HREC - in this case, submit the following information for approval by the Department Head. 2. If any question in addition to 1 and 13 is answered YES, then the project most likely represents a Research study and would therefore need to be submitted as such to the Sydney West Area Health Service Research Office for full consideration by the Human Research Ethics Committee. 3. Alternatively, if questions 2-12 are answered “no” and question 13 is answered yes, then complete this form and submit to the HREC for consideration as a QA proposal.

<table>
<thead>
<tr>
<th>Full title of project:</th>
<th>Anti-Dsg-1 &amp; Dsg-3 isotype-specific assay in Pemphigus vulgaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short title:</td>
<td>Anti-Dsg-1 &amp; Dsg-3 isotype-specific assay in Pemphigus vulgaris</td>
</tr>
<tr>
<td>List of personnel involved:</td>
<td>A Prof David Fulcher, Dr Sue-Ching Yeob, Roger Silvestrini</td>
</tr>
<tr>
<td>Duration of project:</td>
<td>Start date: 1/2/2010   Finish Date: 31/12/2016</td>
</tr>
<tr>
<td>Site(s) where research is to be conducted:</td>
<td>Immunopathology, ICPMR</td>
</tr>
<tr>
<td>Department(s) where project is to be conducted:</td>
<td>Immunopathology, ICPMR</td>
</tr>
</tbody>
</table>

1. SUMMARY/OUTLINE
   Please provide an outline/summary of the proposed activity and methodology: Include the following information in brief (provide more detail in attached Plan):
   - Pemphigus vulgaris (PV) is an uncommon autoimmune blistering disease characterised by antibodies (Ab) to the intercellular cement substance of skin. These antibodies, detectable by indirect immunofluorescence, are part of the routine diagnostic panel in Immunopathology, ICPMR. However the autoantigens are desmoglein-3 (Dsg-3), and to a lesser extent Dsg-1, and ELISAs are available for their specific detection. No one in Australia is currently offering these assays, and we wish to establish this service for the first time using a commercially available kit.
   - Such assays detect total IgG against Dsg-1 & 3, yet there is some evidence that IgG4 correlates better with disease activity, whilst IgG1 predominates in remission: such studies however were performed by blotting and have not been used in indirect immunofluorescence assays nor by ELISA.
   - We wish to adapt both assays (indirect immunofluorescence and ELISA) by using IgG1 & IgG4-specific detection reagents, as well as the usual total IgG, to determine whether they provide a better test of disease activity. To do this, we have designed a purpose-directed request form that asks for specific clinical information from which we can gauge the level of pemphigus disease activity (Journal of Investigative Dermatology (2009) 129, 2404–2410).
   - We also wish to engineer a cell line expressing Dsg-3 and use it as a substrate for antibody detection using flow cytometry, so that all isotypes can be detected simultaneously and potentially more cheaply.
   - Routine samples sent for Dsg-1/3 ELISA will be processed in the usual way, but also by using the subclass-specific assay, and the level of clinical activity will be compared with the quantitative results. This study will then inform us as to whether these subclass-specific results provide a better test for disease diagnosis and monitoring, and possibly improve the diagnosis and management of this condition.

2. PRIVACY
   It is necessary for you to complete this part of the application form in order to ensure that you comply with the Health Records and Information Privacy Act (NSW). This enables the HREC to properly assess the protocol under the Act; and ensures the HREC meets its statutory obligations to report to the Privacy Commissioner on its activities under the Act. The Department Head must also be satisfied that you comply with the Privacy Act.
2.1 Is there a requirement for the researchers to collect, use, or disclose information of a personal nature (either identifiable or potentially identifiable) about individuals without their consent? (Tick which applies)

- From Commonwealth departments or agencies? Yes ☐ No ☑
- From State departments or agencies? Yes ☐ No ☑
- From other third parties, such as non-government organisations? Yes ☐ No ☑

If you ticked yes to one or more of the above boxes, please state what information will be sought and how many records will be accessed.

9.9 Is there a requirement for the researchers to collect, use, or disclose personal health information about individuals without their consent which is identifiable or potentially identifiable?

Once the sample of known blood group has been collected, it will be de-identified.

☐ YES – go to question 2.3.

☐ NO – go to 2.6

2.3 Indicate the reason(s) why de-identified information cannot be used (Tick which applies)

☐ The project involves linkage of data

☑ Scientific deficiencies would result if de-identified information was used. Please provide details.

Other. Please provide details.

The study relies critically on correlation of the test result with disease activity, including sequential assays, hence de-identification is not possible. The clinical data sought (see attached) provides a more detailed assessment of disease activity, but in nature is not conceptionally different to clinical information provided routinely on a laboratory request form.

2.4 Why is it impracticable to obtain the consent of the individual to the collection, use or disclosure of their health information? (Tick which applies)

☑ The size of the population involved in the research.

☐ The proportion of individuals who are likely to have moved or died since the health information was originally collected.

☐ The risk of introducing potential bias into the research, thereby affecting the generalisability and validity of the results.

☐ The risk of creating additional threats to privacy by having to link information in order to locate and contact individuals to seek their consent.

☐ The risk of inflicting psychological, social or other harm by contacting individuals with particular conditions in certain circumstances.

☐ The difficulty of contacting individuals directly when there is no existing or continual relationship between the organisation and the individuals.

☐ The difficulty of contacting individuals indirectly through public means, such as
2.5 Explain why the collection, use or disclosure of this information is in the public interest, and why the public interest in the project substantially outweighs the public interest in the protection of privacy.

The results may well generate new and better ways of monitoring disease activity in this uncommon condition, and therefore will be in the public interest. As clinical information is usually provided on a pathology request form for this condition in any case, the study involves requesting more specific data than usual, and hence we believe does not have substantive privacy implications. The tests can all be performed on volumes of serum usually submitted for this assay, and hence the patients will not be inconvenienced by the collection of extra serum.

2.6 Will a study code be generated? If using potentially identifiable or identifiable information to code participant records, this must be included in the Consent Form.

Yes □ No □

if yes, please give details

2.7 Will the subjects be video/audio taped or will any other electronic medium be used?

Yes □ No □

2.8 How will the investigators protect the privacy of the participants and their personal details specifically relating to all patients that attend SWAHS sites (eg a locked filing cabinet)?

Request forms will be stored in a locked filing cabinet, and data extracted from them kept on a single password-protected PC, backed up to flash drives also stored locked in a different office.

2.9 Storage & Security of Information relating to all SWAHS sites. Please complete the following:

| Security of data storage: | As detailed in 2.8 |
| Location of stored data: | Immunopathology office, ICPMR |
| Format of stored data: | Paper records and spreadsheet/database |
| Duration data will be kept: | At least 7 years after publication of any data arising, or after completion of the study |
| Method of destruction of data: | Zero-level deletion of electronic data, and shredding of paper-based data |
2.10 Does the project involve the transfer within or outside Australia of a subject’s personal information? (e.g. date of birth, initials, name and/or address being transferred in a serious adverse event form or a Case Report Form)

Yes ☐ No ☐

If yes, complete the following table

<table>
<thead>
<tr>
<th>Specify type of information (as detailed above)?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where and how will the information be transferred from and to whom?</td>
</tr>
</tbody>
</table>

| How will the patients’ information remain confidential during the transfer process? |

2.11 Please confirm that information which identifies individuals or from which an individual’s identity can be reasonably ascertained, WILL NOT be published in any generally available publication?

Yes ☐

Confirmed published data will not identify participants. As the publication of identifiable data is not permissible under the Act.

3. SUPPORTING DOCUMENTS

In addition to this completed checklist and application form attach the following supporting documents:

- Brief plan (1-2 pages) demonstrating how collecting this data will inform/improve clinical practice and what steps will be taken to disseminate data within a quality improvement framework (mandatory)
- Data collection form (mandatory)
- Surveys, questionnaires (if applicable)
- Advertisements (if applicable)
- Participant information sheets (if applicable)
- $50 application fee (internal submission) or $55 (incl GST) for external submission

Please submit 3 x copies of each document (collated in bundles as follows) together with either cheque for $50 ($55 for external applications) or Internal QA/Audit Application Submission Fee form:

- Application form
- Checklist
- Study plan
- Data Collection Form
- Surveys, questionnaires, participant information sheets etc (if applicable)
Declaration by Principal Investigator

I have reviewed the proposed activity with the checklist provided and the activity is classified as quality assurance. It does therefore not require a full research application to the SWAHS HREC.

I understand should any changes be made to the original activity as outlined above that I should contact the Research Office for advice on whether or not a full research application would be subsequently required. I will also advise the Research Office of any changes in writing so these may be reviewed.

<table>
<thead>
<tr>
<th>PRINCIPAL INVESTIGATOR’S NAME:</th>
<th>A/Prof David Fulcher</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIGNATURE:</td>
<td></td>
</tr>
<tr>
<td>DATE:</td>
<td>7/1/2010</td>
</tr>
<tr>
<td>MAILING ADDRESS:</td>
<td>Immunopathology, Level 2, ICPMR, Westmead Hospital, Westmead. 2145</td>
</tr>
<tr>
<td>CONTACT NUMBERS:</td>
<td>02-9846-6933</td>
</tr>
<tr>
<td>EMAIL ADDRESS:</td>
<td><a href="mailto:david_fulcher@wmi.usyd.edu.au">david_fulcher@wmi.usyd.edu.au</a></td>
</tr>
</tbody>
</table>

Declaration by Department Head

I certify that:

- I am familiar with this project and endorse its undertaking as a QA project;
- The resources required to undertake this project are available;
- The personnel involved have the skill and expertise to undertake this project appropriately.

<table>
<thead>
<tr>
<th>DEPT HEAD NAME:</th>
<th>Sujatha Fernando</th>
</tr>
</thead>
<tbody>
<tr>
<td>(or delegated representative)</td>
<td></td>
</tr>
<tr>
<td>SIGNATURE:</td>
<td></td>
</tr>
<tr>
<td>DATE:</td>
<td>7/1/2010</td>
</tr>
</tbody>
</table>

Please ensure the declarations page is completed prior to submission to the Research Office.
Internal QA/Audit Application Submission Fee
Transfer Form

(For Sydney West Area Health Services internal submissions only)

One copy to be submitted with QA / Audit application documentation

QA / Audit Project Title:

Anti-Dsg-1/3 antibodies in pemphigus vulgaris: the role of IgG4 versus IgG1 subtyping in disease monitoring

QA / Audit Application Fee

Please arrange to charge the amount of $ 50 (GST Inclusive) to Cost Code

___380907___-___3611___ in payment of the QA / Audit Application

submission fee for the above project.

Chief Investigator’s Name ___A/Prof David Fulcher______________

Signature __________________________ Date ___7/1/2010___

__________________________________________

Authorised by:

Department Manager ______Sujatha Fernando______________

Signature __________________________ Date ___7/1/2010___
Anti-Dsg-1 & Dsg-3 isotype-specific assay in Pemphigus vulgaris

Background

Pemphigus vulgaris (PV) is an uncommon autoimmune disease characterised by antibodies to the intercellular cement substance of skin. Patients develop extensive cutaneous blisters and erosions of the mucous membranes, which left untreated, can be fatal. There are strong racial influences, with Ashkenazi Jews and people of Mediterranean and Indian origin being particularly susceptible (1). Much of this susceptibility is related to specific HLA class II alleles, including DRB1*0402 and DQB1*0503 (2). Intercellular cement substance antibodies (ICSA) are thought to interfere with the formation of desmosomes present in epithelial surfaces, resulting in loss of epithelial integrity and ‘acantholysis’, i.e. the breaking apart of a cell from its neighbours.

There are four autoantigens targeted; desmoglein-3 (dsg-3) in most patients, desmoglein-1 (dsg-1) in about 50%, and some patients also recognise α9 acetylcholine receptor and pemphaxin (3, 4). There is considerable support for the belief that these antibodies are intrinsically pathogenic, including the correlation between titre and disease activity, the finding that transplacental transport of antibodies from mothers with active pemphigus results in temporary blisters in newborns, and that lesions can be induced by passive transfer of patient IgG into mice (5). Thus PV constitutes a disease that is almost exclusively autoantibody-mediated in its pathogenesis, although the immune dysregulation that underlies the production of anti-Dsg-3 autoantibodies is poorly understood. Such a breakdown must involve failure of both B- and T-cell tolerance mechanisms, given the critical importance of T-cell help in activating B cells to inducing high-affinity class-switched antibody, and such a role for CD4 T cells is supported by both the HLA associations and the direct demonstration of autoreactive T cells which respond to Dsg-3-derived peptides (6).

Diagnosis requires the demonstration of antibodies to the intercellular cement substance, currently performed by indirect immunofluorescence using monkey oesophagus as the substrate. This assay is time-consuming and labour-intensive. It is also susceptible to a significant number of false-positive reactions. Titres are said to correlate with disease activity, although determining the precise end-points by titration is crude and inexact. These problems could potentially be overcome by using an ELISA based on purified Dsg-1 and -3 as the autoantigen. Amenable to automation, such an assay also provides an objective value for the level of autoantibody, has proven to perform very well in the diagnostic setting (graph below) and which is reported to correlate better with disease activity.
Currently, given the uncommon nature of the diagnosis, no laboratory has set up this ELISA in Australia, and clinicians are required to transport specimens overseas to gain access to these results. Finally, studies have suggested that IgG4 isotype anti-Dsg-3 antibodies are the best correlates with disease activity (7) using an immunoblot assay, although this has not been explored using the more readily accessible ELISA.

Aims

1. To establish the ICPMR as a referral centre for the testing of anti-Dsg-1 & 3 antibodies by ELISA;
2. To type anti-Dsg-3 antibodies by isotype (IgG1 vs IgG4), correlate with isotype-specific ICSA results, and correlate both with disease activity indices on the dedicated request form;
3. To explore the use of a cell line engineered to express Dsg-3 as a substrate for a flow cytometry-based isotype-specific assay.

Proposal

Currently no laboratory in Australia has set up an ELISA for anti-Dsg-1 & 3, yet verbal communications with dermatologists, immunologists and oral medicine physicians has indicated that there is a strong need for this assay, and that specimens would be forth-coming if such a service were offered. This provides a unique opportunity for the Immunopathology unit of the ICPMR to fill this void and strengthen its reputation for innovation and service provision. It is therefore proposed that the above Aims be addressed in the following ways:

1. A commercial kit, consisting of ELISA plates coated with recombinant Dsg-1 and Dsg-3, is available from Corgenix, which has been trialled in this department previously. This kit will be purchased and used according to the manufacturer’s instructions. Practitioners ordering the test will need to complete a specialised application form (appendix) in which the patient’s diagnosis and disease activity is objectively recorded. This will allow us to assess the performance of the kit in terms of diagnosis and disease activity.

2. The Dsg-3 wells will adapted in-house to allow differentiation based on isotype-specific reagents (anti-IgG1 and anti-IgG4), and these results, correlated with disease activity scores obtained as outlined in 1, will allow for the first time the correlation between isotype-specific autoantibody levels by ELISA and disease activity scores.

3. An amendment to the above immunoassays will involve the generation of a cell line engineered to express membrane-bound Dsg-3 by DNA transfection (similar to the creation of
the HEp-2000 cell line for detection of anti-SSA antibodies). This cell line could then form the substrate for detection of anti-Dsg-3 antibodies by incubating stably transfected cells with patient serum, then detecting bound anti-Dsg-3 IgG1 and IgG4 antibodies simultaneously using fluorochrome-conjugated secondary reagents and calibrated against fluorescent bead standards. This approach has the advantage of allowing detection of conformational epitopes which may lost during binding to the ELISA plates, and could show better sensitivity and correlation with disease activity.

Outcomes
This will be an ongoing project performed by Sue-Ching Yeoh as part of her PhD studies, which has a very strong potential to generate important results in terms of optimal management of patients with pemphigus vulgaris. The assays will be billed and resulted as per the current item numbers, although this will not cover the development costs of the specialised assays outlined. At the completion of the project, we will have determined the optimal approach to measuring these antibodies which could then be offered as mainstream diagnostic tests.

References
Our ref: HREC2010/2/5.3(3108) QA

11 February 2010

A/Prof David Fulcher
Department of Immunopathology
ICPMR

Dear Professor Fulcher

QA Project: Anti-Dsg-1 & Dsg-3 isotype-specific assay in Pemphigus vulgaris

Your request to undertake the above protocol as a Quality Assurance project was reviewed by the Westmead Scientific Advisory QA Committee and the Secretary of the SWAHS Human Research Ethics Committee. We are satisfied your proposal meets the criteria for quality assurance and it is therefore approved.

Please send a copy of the results of the study to the SWAHS Research Office when they become available.

Yours sincerely,

[Signature]

Dr Jim Hazel
Secretary
Sydney West Area Health Service
Human Research Ethics Committee
We, the authors of the journal publication:


grant permission to Dr Sue-Ching Yeoh to include “Table 4. Clinical and Immunohistochemical Variants of Pemphigus” in her Master of Philosophy thesis titled “Pemphigus Vulgaris Disease Activity: the Role of Antibodies to Desmogleins and their Isotype.”

Mark Schiffter

Sue-Ching Yeoh

Hedley Coleman

Anastasia Georgiou

19/8/2015
I, the author of the thesis:

Delva, E., *Determining the cellular mechanisms involved in pemphigus vulgaris-induced desmoglein 3 internalisation and desmosomal disassembly*, in *Biochemistry, Cell and Developmental Biology* 2009. Emory University: USA.

grant permission to Dr Sue-Ching Yeoh to include "Figure 2.1 - Hallmarks of Pemphigus" in her Master of Philosophy thesis titled "Pemphigus Vulgaris Disease Activity: the Role of Antibodies to Desmogleins and their Isotype."

Emmanuella Delva  

Date 8/19/15
REFERENCES


107. Rahbar, Z.D., M; Mirshams-Shahshahani, M; Esmaili, N; and K.A. Heidari, N; Hejazi, P; Ghajarzadeh, M; Chams-Davatchi, C, Pemphigus Disease Activity Measurements. Pemphigus Disease Area Index, Autoimmune Bullous Skin Disorder Intensity Score, and Pemphigus Vulgaris Activity Score. JAMA Dermatol, 2014.


