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Statistical studies of clinical and immunological findings including anti-desmocollin antibodies in 104 cases of paraneoplastic pemphigus studied at Kurume University

Running head: Paraneoplastic pemphigus

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What's already known about this topic?

Paraneoplastic pemphigus (PNP) is an autoimmune bullous disease with polymorphous mucocutaneous lesions associated with either benign or malignant neoplasms. Bronchiolitis obliterans with progressive respiratory failure is a cause of death in PNP.

What does this study add?

Several PNP patients with typical clinical and immunological features did not show

detectable neoplasms. Anti- desmocollins autoantibodies are useful for a diagnosis of PNP.

Summary

Background: Although there are many reports of sporadic paraneoplastic pemphigus (PNP) patients, only a few systemic studies on many PNP patients have been reported.

Objective: To statistically analyze the clinical and immunological findings in large cohort of PNP patients.

Methods: This retrospective study consisted of 104 PNP patients. Clinical and histopathological manifestations, associated neoplasms, complicated diseases, prognosis and results of immunofluorescence, immunoblotting and enzyme-linked immunosorbent assays (ELISA) were analyzed.

Results: Reliable information for mucocutaneous lesions was obtained for 88 of 104 PNP patients. Malignant lymphomas, Castleman disease and solid tumors were associated in this order, while 12 patients had no detectable tumors. Sixteen of 20 patients with bronchiolitis obliterans (BO) showed fatal outcomes. In ELISA, 34 (32.7%) and 82 (78.8%) of 104 PNP patients showed antibodies to desmogleins 1 and 3 (Dsg1/3) respectively, while 15 (14.4%) patients were negative for both Dsg1 and Dsg3. In addition, novel ELISAs for desmocollins (Dscs) showed that 19 (18.6%), 42 (41.2%) and 62 (60.8%) of 102 PNP patients showed antibodies to Dsc1, Dsc2 and Dsc3. We found statistically significant correlations between positive Dsg3 reactivity and genital

lesions, and between positive Dsg3 reactivity and BO.

Conclusions: Overall clinical, histopathological and immunofluorescence results, as well as associated tumors, complicated diseases and prognosis, were consistent to those in previous studies. Intriguingly, 12 PNP patients with typical clinical and immunological features did not show detectable tumors. Anti-Dsc autoantibodies are useful for a diagnosis of PNP.

Keywords: paraneoplastic pemphigus, bronchiolitis obliterans, envoplakin, periplakin, desmocollin.

Introduction

Paraneoplastic pemphigus (PNP) is an autoimmune bullous disease with polymorphous mucocutaneous lesions associated mainly with lymphoproliferative neoplasms.[1] PNP shows clinically severe mucocutaneous lesions which resemble pemphigus vulgaris, erythema multiforme, Stevens-Johnson syndrome and lichen planus.

Associated neoplasms include non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Castleman disease, thymoma, gastrointestinal stromal tumor and undifferentiated sarcomas.[2] Histopathological findings are variable and include acantholytic blisters and dyskeratosis in the epidermis and subepidermal bulla.

Direct immunofluorescence (DIF) reveals IgG deposition to keratinocyte cell surfaces and C3 deposition to epidermal basement membrane zone (BMZ). Indirect immunofluorescence (IIF) detects IgG antibodies reactive with cell surfaces of normal human skin and with transitional epithelium of rat bladder.

The main autoantigens in PNP are plakin family proteins (plectin, desmoplakins I and II, BP230, envoplakin and periplakin) and desmogleins 1 and 3 (Dsg1/3).[3-5] Recently, alpha-2-macroglobulin-like-1 has been identified as the 170 kDa PNP antigen.[6] In addition, enzyme-linked immunosorbent assays (ELISAs) of envoplakin and periplakin have shown to be highly sensitive in the diagnosis of PNP.[7,8]

The main treatments for PNP are systemic corticosteroids and immunosuppressants, whereas intravenous immunoglobulin（IVIG）, plasmapheresis, immunopheresis and photopheresis have also been used.[9,10] Progressive respiratory failure caused by bronchiolitis obliterans (BO) frequently leads to a fatal outcome in PNP.

In 2004, Anhalt et al. suggested the five criteria to define PNP,[2] (1) painful stomatitis and polymorphous cutaneous eruption with lesions that may be blistering or lichenoid or may resemble erythema multiforme or drug eruption, (2) histopathological findings that reflect the variability of the cutaneous lesions, showing acantholysis and lichenoid or interface changes, (3) DIF demonstrating deposition of IgG and complement in epidermal cell surfaces, and often granular/linear complement deposition along BMZ, (4) serum autoantibodies that bind the cell surfaces of skin and mucosae in a pattern typical for pemphigus, but additionally bind to simple, columnar and transitional epithelia, and (5) the serum autoantibodies reacting with Dsg1 and Dsg3, as well as plakin family proteins, including desmoplakins I and II, envoplakin, periplakin, bullous pemphigoid antigen 1 and plectin. However, many reported PNP patients did not satisfy these criteria.

Thus, although several case studies have been reported, only a few systemic studies have been performed with reference to PNP. In this study, we collected and

retrospectively studied 104 PNP patients for clinical and histopathological findings and the results of various immunological tests, and performed extensive statistical analyses.

Material and methods

This study was approved by the Ethical Committee of Kurume University. We searched all PNP-suggestive patients, who were treated in our hospital or had been consulted from other hospitals between January 1997 and April 2013. We have reported that envoplakin and periplakin were the major PNP autoantigens with high diagnostic significance.[11] Therefore, in this study, we selected PNP patients, who satisfied the two inclusion criteria; (1) envoplakin and periplakin were positive by immunoblotting (IB) of normal human epidermal extract and (2) severe lesions were present on at least one mucous membrane. Eventually, this study enrolled 104 patients, who showed almost typical clinical, histopathological and immunological features of PNP. All sera were stored at -30℃ or -80℃, and aliquots with 0.1% sodium azide as a preservative were kept at 4℃ during the experiments.

We first clinically analyzed the patients in terms of age, symptoms, clinical course, associated neoplasms, complications, treatments and prognosis. Then, we examined their histopathological and DIF features. As for serological tests, we performed IIF of

normal skin, 1M NaCl-split normal human skin and rat bladder using standard methods. IB analyses of normal human epidermal extract was performed as described previously.[12] IgG ELISAs for Dsg1 and Dsg3 were performed using commercially available kits (MBL, Nagoya, Japan).[13] The cut-off index values were 14.0 for Dsg1 and 7.0 for Dsg3. In addition, recently developed ELISAs of mammalian recombinant proteins of human desmocollin 1 (Dsc1), Dsc2 and Dsc3 were also performed.[14] In this study, our cut-off values were calculated as mean+3 SD, and were 0.2 for Dsc1, 0.07 for Dsc2 and 0.12 for the Dsc3.

We statistically compared differences in various clinical features and immunological results by using the Mann-whitney Rank Sum test, Student t-test, Chi-square test and Pearson's correlation using SigmaPlot 12.0 soft (Hulinks, Inc, Tokyo, Japan). P values less than 0.05 were considered statistically significant.

Results

Ages, genders, presence of BO and clinical outcomes, as well as all immunological results, for all 104 PNP patients, are shown in Table S1.

Patient background

The 104 patients were comprised of 34 (32.7%) males and 59 (56.7%) females, whereas gender was not reported for the remaining 11 (10.6%) patients. The patient's age was available in 92 patients, and was ranged 11-83 years with an average of 56.7 years. Regarding complications, 7 patients had myasthenia gravis, 6 patients had hypertension, 3 patients had type II diabetes mellitus and one each had hypothyroidism, pulmonary emphysema, optic neuritis and lung tuberculosis. Twenty patients were complicated with BO during the course of PNP.

Mucocutaneous lesions

All 104 PNP patients were reported to have severe lesions on at least one mucosa. Reliable information for mucocutaneous lesions was obtained for 88 of the PNP patients (Table 1). Eighty-two (93.2%) of 88 patients with description had oral lesions, while the remaining 6 patients were free from oral lesions. Twenty-four (27.3%) of 88 PNP patients had only mucosa lesions. Thirty-three, 9 and 28 patients had ocular, nasal and genital lesions, respectively.

Fifty-nine (67.0%) of 88 patients showed cutaneous lesions in addition to mucosal lesions. Fifty-two and 46 of 87 patients had skin lesions on the trunk and extremities, respectively. Thirty-seven and 24 patients showed erythemas and blisters, respectively.

Thirteen, 9 and 4 patients showed erythema exsudativum multiform-like, pemphigus vulgaris-like and lichen planus-like skin lesions, respectively.

Associated neoplasms

Associated neoplasms are summarized in Table 2. Regarding hematological tumors, 43 patients had various types of malignant lymphoma, including follicular lymphoma. Fourteen patients had Castleman disease. Computed tomography suggested that 4 patients possibly had malignant lymphoma, although histological information was not available. Six patients had various types of leukemia. Three patients had chronic lymphocytic leukemia, while classification was not available in 3 patients. One patient had primary macrocryglobulinemia.

Twelve patients had various solid malignant tumors, including two different types of lung cancer, gastric cancer, uterine cancer, uterine cervix cancer, laryngeal cancer, hepatocarcinoma, renal cancer, colon cancer, ovarian cancer, breast cancer, thyroid cancer, esophageal carcinoma, gastrointestinal stromal tumor and basal cell carcinoma. Eight patients had thymoma. Five of the 12 patients with solid tumors had double cancers. Six patients had various types of sarcoma.

One patient each had fibrous histiocytoma and myofibroblastoma. However, 12

patients showed no detectable tumor.

Treatments

As treatments of associated tumors, 8 and 4 patients underwent tumor resection and radiotherapy, respectively. Regarding treatments for PNP, 49 and 21 patients underwent oral corticosteroids and steroid-pulse therapy, respectively. In addition to the steroid treatments, 4 and 2 patients were given immunosuppressive drugs and diaminodiphenylsulfone, respectively. In addition, 11, 6 and 3 patients underwent IVIG, plasmapheresis and rituximab treatment.

Prognosis

In this study, 40 patients died and 36 patients survived, while no information for prognosis was available for remaining 28 patients (Table S1). BO occurred in 20 patients. Sixteen of 40 patients with fatal outcome died of BO. Thus, 4 of the 20 patients with BO survived, at least from the available information. Other 24 patients died either of infection, mainly pneumonia or of associated tumors. The intervals between the first presentation of mucocutaneous lesions and death ranged from 40 days to 6 years.

Histopathological features

Histopathological findings were obtained from 61 patients. Thirty-two patients showed intraepidermal bulla and 9 patients showed subepidermal bulla. Additionally, 28 patients showed epidermal cells necrosis.

DIF

DIF was performed in 51 patients. Twently-two patients showed both keratinocyte cell surface and epidermal BMZ deposits of immunoglobulins and/or C3, while nineteen patients showed only keratinocyte cell surface deposits of immunoglobulins and/or C3 and ten patients showed only epidermal BMZ deposits of immunoglobulins and/or C3. Precisely, deposits of IgG, C3, IgA and IgM to epidermal keratinocyte were detected in 34, 18, 6 and 3 patients, whereas deposits of IgG, C3, IgA and IgM to epidermal BMZ were detected in 17, 26, 5 and 2 patients.

IIF

IIF of normal human skin and rat bladder was performed for all 104 patients (Table 3). In IIF of normal human skin, circulating IgG anti-cell surface autoantibodies were

found in 69 patients, IgG anti-BMZ autoantibodies were found in 4 patients, and concurrence of both antibodies were found in one patient. IIF of rat bladder showed positive reactivity in 83 (79.8%) of 104 patients.

IB

All 104 PNP patients reacted with a doublet of the 210 kDa envoplakin and the 190 kDa periplakin by IB of normal human epidermal extract (Table 3). In addition, 10 patients each reacted with the 230 kDa BP230 and the 130 kDa Dsg3, while 4 patients reacted with the 180 kDa BP180 and one patient each reacted with the 250 kDa desmoplakin I and 160 kDa Dsg1.

ELISAs for Dsg1 and Dsg3

ELISAs for Dsg1 and Dsg3 were performed for all 104 patients (Table 3). Thirty four (32.7%) and 82 (78.8%) patients had antibodies to Dsg1 and Dsg3, respectively. Twenty eight (26.9%) patients were positive for both Dsg1 and Dsg3, whereas 15 (14.4%) patients were negative for both Dsg1 and Dsg3.

ELISA for Dsc1, Dsc2 and Dsc3

We have previously reported the results of novel ELISAs for Dsc1, Dsc2 and Dsc3 for 79 PNP patients.[14] In the present study, the ELISAs were performed for 102 patients (Table 3). Nineteen (18.6%), 42 (41.2%) and 62 (60.8%) patients revealed positive reactivity with Dsc1, Dsc2 and Dsc3, respectively. Ten (9.8%) patients were positive for all Dscs, while twenty-nine (28.4%) patients were negative for all Dscs.

Statistical analyses

Correlations between various clinical features and the results of all ELISAs were first statistically analyzed (Table S2). Correlations with statistically significant differences between clinical features and all ELISAs are shown. Nasal lesions were found only in PNP patients without anti-Dsg1 antibodies (p=0.043). Higher frequencies of genital lesions (p=0.015) and BO (P=0.049) were found in PNP patients with anti-Dsg3 antibodies. And a higher frequency of oral lesions was found in PNP patients without anti-Dsc1 antibodies (p=0.007). PNP patients without anti-Dsc2 antibodies showed a lower frequency of ocular lesions (p=0.003) and a higher frequency of solid tumors (p=0.011). PNP patients without anti-Dsc3 antibodies showed a lower frequency of skin lesions on the trunk (p=0.038), lower frequency of ocular lesion (p=0.012) and higher frequency of Castleman diseases (p=0.006). Figure 1 shows histograms of the

relevant results with statistically significant difference between clinical features and ELISA results.

Correlations with statistically significant differences among ELISAs are also shown. Anti-Dsg1 antibody-positive patients had anti-Dsc1 antibodies more frequently ($p=0.036$), whereas anti-Dsg3 antibody-positive patients had anti-Dsc3 antibodies more frequently ($p=0.025$). Anti-Dsc1 antibody-positive patients had anti-Dsg1 antibodies more frequently ($p=0.048$). Anti-Dsc2 antibody-positive patients had anti-Dsg3 antibodies ($p=0.032$) and anti-Dsc3 antibodies ($p<0.001$) more frequently. Anti-Dsc3 antibody positive patients had anti-Dsg3 antibodies ($p=0.008$) and anti-Dsc2 antibodies ($p<0.001$) more frequently.

Discussion

Clinical and histopathological findings in this study were generally similar to those in previous reports. Regarding the mucocutaneous lesions, 24 of 88 PNP patients had only mucosa lesions. Thus, PNP patients with only oral mucosal lesions should be carefully differentiated from mucosal dominant-type pemphigus vulgaris.

Regarding associated tumors, similar to previous reports, the most frequently associated neoplasms in our patients were malignant lymphomas, followed by

Castleman disease. Intriguingly, the third most frequent tumors in our study were solid tumors with diverse origins. Thus, although most PNP patients were considered to have hematologic tumors, association of solid tumors is not rare. Furthermore, no associated tumor was detected in 12 PNP patients in our study. The reason may be that limited scrutiny of diagnostic imaging overlooked small tumor lesions in early stages.

BO-like respiratory disease frequently causes death in PNP. Although the pathogenesis of PNP-related BO is still unclear, the original study detected acantholysis in bronchial epithelium, as well as IgG and C3 deposits to respiratory epithelia.[15] In addition, infiltrates almost exclusively of CD8+ T lymphocytes invaded the bronchial walls.[16] Furthermore, a recent PNP disease model study using Dsg3-transgenic mice demonstrated that pulmonary epithelia showed squamous metaplasia and ectopic expression of Dsg3, and that pulmonary injuries by naphthalene could recruit Dsg3-specific CD4+ T cells, indicating that Dsg3 was involved as a merger in PNP-related BO.[17] These previous results strongly suggested that BO in PNP caused by humoral and cellular autoimmunity against normal or ectopic expression of autoantigens. The autoantigens might be plakin proteins, as well as Dsg3 or other desmosomal cadherins.

Treatments of PNP are generally pursuant to the treatments of pemphigus vulgaris,

although PNP is usually very difficult to treat. Even when treatments are successful and skin lesions are improved, oral mucosal lesions are extremely intractable. In our study, although a number of combination therapies were used, no particular regimen had proved to have excellent effects.

Concerning various diagnostic studies in our study, IIF of rat bladder showed positive results in 79.8% of PNP patients, which was consistent to the previous studies showing positive reaction in 75% of PNP patients.[18] These finding indicate that IIF of rat bladder is useful for the diagnosis of PNP. However, because 20-25% of PNP sera were negative, this study may not be conclusive criterion.

Envoplakin and periplakin tether intermediate filaments to the membrane-bound adhesion junctions inside the cells. However, it is still unknown whether the antibodies to these two antigens are pathogenic in PNP, because all plakin family proteins are cytoplasmic molecules and autoantibodies cannot access them in intact cells. However, such antibodies may access cytoplasmic antigens, after cell membranes are injured by some other autoantibodies to cell membrane proteins. In support of this concept, in new born erythema multiforme model mice, injection of affinity purified autoantibodies against a carboxyl terminal domain of desmoplakin I and II caused the impairment of desmosome-keratin filament complexes.[19] Alternatively, these

autoantibodies may indirectly impair cell-cell adhesion by interfering with cell-cell signaling in some way. The previous study showed that ELISA titers of autoantibodies to envoplakin and periplakin decreased after resection of the tumors, suggesting pathogenic role of antibodies to envoplakin and periplakin.[7]

In this study, we also examined extensively autoantibodies to various desmosomal cadherins; i.e., Dsg1, Dsg3 and Dsc1-Dsc3. A prototype study of anti-Dsg antibodies in PNP reported that all PNP patients showed antibodies to Dsg3 and some patients showed anti-Dsg1 antibodies.[3] The study also confirmed that anti-Dsg3 antibodies could produce blister formation in neonatal mice. In contrast, another study reported that, although Dsg1 and Dsg3 are autoantigens in pemphigus foliaceus and pemphigus vulgaris, respectively, there was no clear association between clinical phenotype and anti-Dsg antibody profiles in PNP.[20] In addition, later studies showed that several PNP patients had only anti-Dsg1 antibodies,[21-25] and that neither anti-Dsg1 nor anti-Dsg3 antibodies were detected by ELISAs in some PNP patients.[26,27]

In this study, we also confirmed that 82 (78.8%) of 104 PNP patients showed anti-Dsg3 antibodies, and about one-third of these patients showed anti-Dsg1 antibodies in ELISAs. However, 15 (14.4%) of 104 PNP patients were negative for both anti-Dsg1 and anti-Dsg3 antibodies. Therefore, we speculated that, although anti-Dsg

antibodies are important antibodies in PNP, autoantibodies to non-Dsg antigens can produce PNP mucocutaneous lesions.

Autoantibodies to Dsc1-Dsc3 were occasionally identified in patients with atypical pemphigus by either cDNA transfection method using cultured COS-7 cells or by ELISAs using baculovirus recombinant proteins of Dsc1, Dsc2 and Dsc3.[28,29] However, the ELISAs using recombinant proteins of Dsc1, Dsc2 and Dsc3 showed very low sensitivity. [11] Therefore, we have recently developed sensitive ELISAs using mammal recombinant proteins of human Dsc1, Dsc2 and Dsc3, which were shown to be highly specific and sensitive.[14] The previous study showed that PNP patients frequently reacted with Dsc-Dsc3, particularly with Dsc2 and Dsc3. Intriguingly, in the studies for 102 PNP patients, positive rates of antibodies to Dsc2 and Dsc3 were higher than that of anti-Dsg1 antibodies.

The statistical analyses of the correlations between anti-Dsg antibodies and clinical parameters indicated that anti-Dsg1 antibodies were negatively related to nasal mucosal lesions, and that anti-Dsg3 antibodies may cause genital lesions and BO development. Particularly, the positive correlation between anti-Dsg3 antibodies and BO was important, because the recent mouse PNP model suggested involvement of Dsg3 in lung disease.[17]

The statistical analyses of the correlations between anti-Dsc antibodies and clinical parameters indicated that anti-Dsc1 antibodies were negatively related to oral mucosal lesions, and that without anti-Dsc2 antibodies were related negatively to ocular lesions and positively to solid tumors. In addition, without anti-Dsc3 antibodies were related negatively to skin lesions on the trunk and ocular lesions, and positively to Castleman diseases. Thus, these significant statistical correlations between antibodies to Dsg1, Dsg3 and Dscs and various clinical features may suggest that these antibodies were pathogenic in PNP.

In the final statistical analyses among the ELISA results, PNP patients with anti-Dsc2 antibodies had anti-Dsc3 antibodies more frequently, and vise versa. A previous report also found double antibodies to Dsc2 and Dsc3 in PNP patients with eosinophilic spongiosis.[30] Therefore, although anti-Dsc autoantibodies have not been widely examined, antibodies to Dscs, particularly Dsc2 and Dsc3, should be important diagnostic criterion for PNP.

After the original criteria for the diagnosis of PNP proposed by Anhalt et al, many patients who did not satisfy the criteria were reported as PNP. Accordance with the progress in pathophysiology in PNP, many new findings and diagnostic technologies have emerged. Based on the results in the present study, as well as accumulated

findings in previous studies, we would like to suggest the following criteria for the diagnosis of PNP. The two major inclusion criteria are the presence of mucosal lesions and the detection of envoplakin and periplakin by IB or ELISAs. IIF of rat bladder and ELISAs of Dsc1-Dsc3 frequently show positive results. Hematological malignancies are most frequently associated, although some patients have solid tumors or no apparent tumor.

Although PNP is a rare disease, clinical manifestations are serious and even fatal. Future studies should be performed to elucidate pathogenesis in PNP and to develop new therapies for PNP and PNP-relate BO.

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Figure legends

Fig 1. Histograms for the correlations with statistical significance between major clinical features and ELISAs of Dsgs and Dscs. (a) Negative association between anti-Dsg1 antibodies and nasal lesions. (b) Association between anti-Dsg3 antibodies and genital lesions. (c) Association between anti-Dsg3 antibodies and BO. (d) Negative association between anti-Dsc1 antibodies and oral mucosal lesions. (e) Negative association between anti-Dsc2 antibodies and solid tumor. (f) Negative association between anti-Dsc3 antibodies and Castleman disease.

Table 1 Summary of mucocuneous lesions and associated neoplasms in PNP patients

Parameter		**Number of positive patients / case with descriptions**
Mucocuneous lesions		
Site of cutaneouslesions	Trunk	52/87 (59.8%)
	Extremities	46/87 (52.9%)
Skin lesions	Erythemas	37/61 (60.7%)
	Blisters	24/59 (40.7%)
Sites of mucosal lesions	Oral	82/88 (93.2%)
	Ocular	33/81 (40.7%)
	Nasal	9/77 (11.7%)
	Genital	28/79 (35.4%)

PNP, paraneoplastic pemphigus.

Table 2 Summary of associated neoplasms in PNP patients

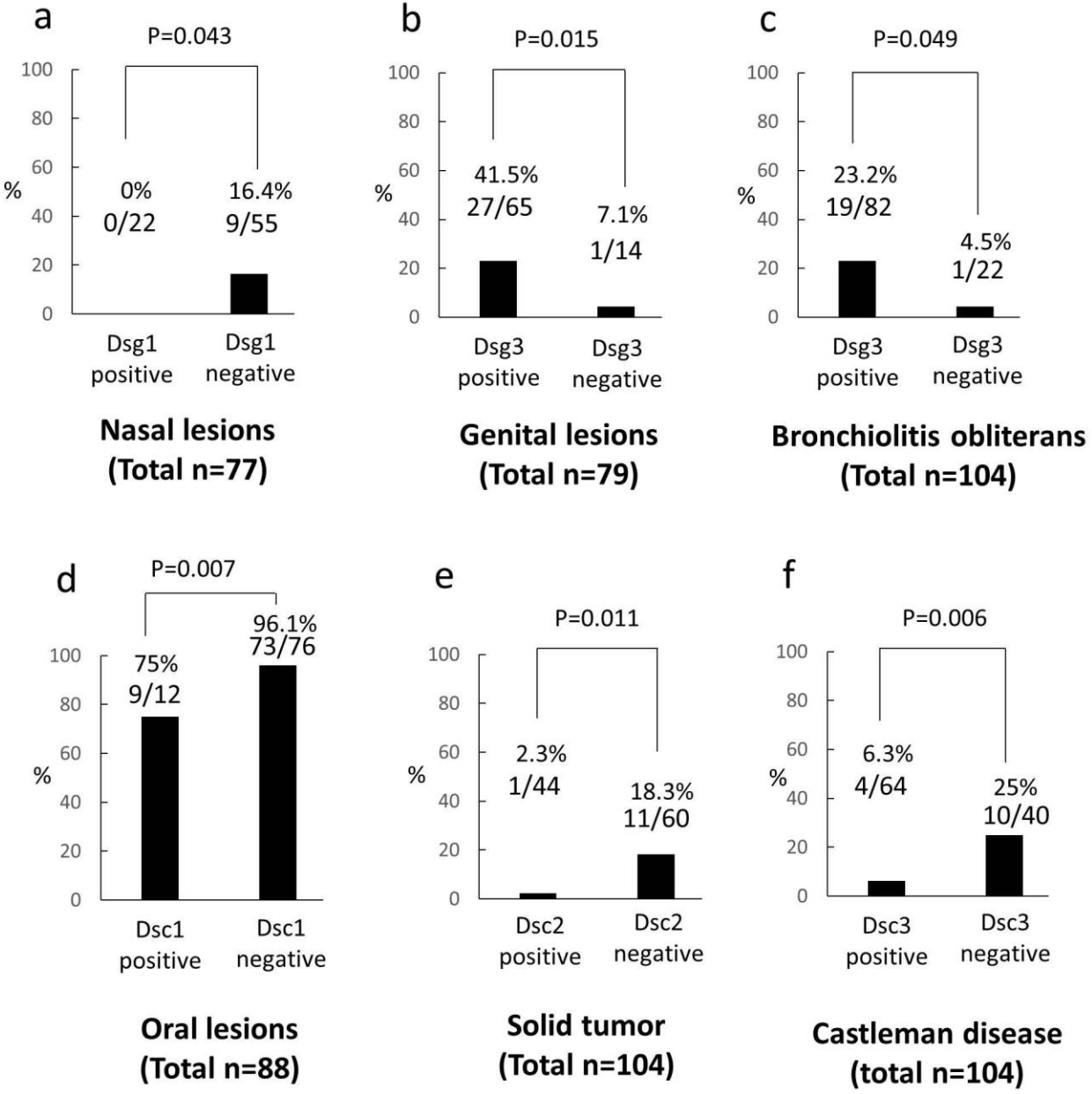
Disease	**Number of patients (total number)**
Malignant lymphomas	43
Castleman disease	14
Malignant solid tumor	12
Thymoma	8
Lukemia	6
Sarcoma	6
Benign tumors	2
Primary macroglobulinemia	1
Malignant lymphoma suspected	4
Undetected	12

PNP, paraneoplastic pemphigus.

Table 3 Summary of IIF, IB, ELISA in PNP patients

	Number of patients
IIF in PNP patients (n=104)	
Positive to keratinocyte cell surface (IgG)	69/104 (66.3%)
Positive to basement membrane zone (IgG)	4/104 (3.8%)
Positive to keratinocyte cell surface and basement membrane zone (IgG)	1/104 (0.9%)
Positive to rat bladder (IgG)	83/104 (79.8%)
IB in PNP patients (n=104)	
190kDa envoplakin	104 (100%)
210kDa periplakin	104 (100%)
230kDa BP230	10 (9.6%)
130kDa Dsg3	10 (9.6%)
180kDa BP180	4 (3.8%)
160kDa Dsg1	1 (0.9%)
250kDa Desmoplakin I	1 (0.9%)
ELISAs in PNP patients,Dsg1, Dsg3 (n=104)	
Positive to Dsg1	34/104 (32.7%)
Positive to Dsg3	82/104 (78.8%)
Positive to Dsg1 and Dsg3	28/104 (26.9%)
Negative to Dsg1 and Dsg3	15/104 (14.4%)
ELISAs in PNP patients, Dsc1, Dsc2 and Dsc3 (n=102)	
Positive to Dsc1	19/102 (18.6%)
Positive to Dsc2	42/102 (41.2%)
Positive to Dsc3	62/102 (60.8%)
Positive to all Dsc1-Dsc3	10/102 (9.8%)
Negative to all Dsc1-Dsc3	29/102 (28.4%)

Dsc, desmocollin; Dsg, desmoglein; ELISA, enzyme-linked immunosorbent assays; IB, immunoblotting; IIF, indirect immunofluorescence; PNP, paraneoplastic pemphigus.


a
P=0.043
100
80
60
40
20
0
%
0%
0/22
16.4%
9/55
Dsg1
positive
Dsg1
negative
Nasal lesions
(Total n=77)
b
P=0.015
41.5%
27/65
7.1%
1/14
Dsg3
positive
Dsg3
negative
Genital lesions
(Total n=79)
c
P=0.049
23.2%
19/82
4.5%
1/22
Dsg3
positive
Dsg3
negative
Bronchiolitis obliterans
(Total n=104)
d
P=0.007
75%
9/12
96.1%
73/76
Dsc1
positive
Dsc1
negative
Oral lesions
(Total n=88)
e
P=0.011
2.3%
1/44
18.3%
11/60
Dsc2
positive
Dsc2
negative
Solid tumor
(Total n=104)
f
P=0.006
6.3%
4/64
25%
10/40
Dsc3
positive
Dsc3
negative
Castleman disease
(total n=104)

SUPPLEMENTARY DATA

Table S1 Summary of the results of clinical and immunological features in all 104 PNP patients

No.	Ages	Genders	Normal IIF	Rat IIF	Epidermal IB	ELISA (INDEX)		ELISA (OD)			Bronchiolitis obliterns	Clinical course
						Dsg1	Dsg3	Dsc1	Dsc2	Dsc3		
1	39	M	CS+	+	190kd, 210kd	6.16	135.85	0.047	1.694	1.775	-	dead
2	58	M	CS+	+	190kd, 210kd	-2.63	114.10	0.100	1.749	1.939	-	dead
3	63	M	CS+	+	190kd, 210kd, 230kd, 250kd	-0.50	47.00	0.024	0.411	0.569	-	dead
4	62	F	CS+	+	190kd, 210kd	-0.10	0.20	0.026	0.011	0.019	-	dead
5	unknown	unknown	CS+	+	190kd, 210kd	0	0.30	0.216	0.076	0.463	-	unknown
6	80	F	CS+	+	190kd, 210kd, 230kd	0	-0.40	0.157	0.054	0.130	-	unknown
7	35	F	CS+	+	190kd, 210kd	0	2.90	-	-	-	-	dead
8	46	F	CS+	+	190kd, 210kd	0	16.60	0.034	0.012	0.124	-	alive
9	66	M	-	+	190kd, 210kd	0	13.90	0.013	0.009	0.026	-	dead
10	72	F	CS+	-	190kd, 210kd	0.09	3.60	0.046	0.016	0.050	-	alive
11	55	F	CS+	+	190kd, 210kd	0.20	75.76	0.197	0.072	0.063	-	alive
12	53	F	CS+	+	190kd, 210kd	0.41	161.90	0.032	0.155	1.639	-	dead
13	60	unknown	CS+	+	190kd, 210kd	0.48	88.30	0.012	1.160	0.720	-	unknown
14	47	M	CS+	+	190kd, 210kd	0.49	143.10	0.048	0.013	0.152	-	dead
15	unknown	unknown	CS+	+	190kd, 210kd	0.63	0.80	2.147	0.023	0.941	-	dead
16	60	F	CS+	+	190kd, 210kd	0.85	8.00	0.112	0.09	0.694	+	dead
17	57	F	CS+	+	190kd, 210kd	0.95	108.36	0.020	0.010	0.538	-	dead
18	48	F	CS+	+	180kd, 190kd, 210kd	0.95	129.26	0.205	1.358	1.353	-	unknown
19	58	F	CS+	+	190kd, 210kd	0.95	47.40	0.056	0.090	0.735	+	dead
20	11	M	CS+	+	190kd, 210kd, 230kd	1.00	93.38	1.756	0.039	0.284	-	unknown
21	unknown	unknown	-	+	190kd, 210kd	1.03	125.05	0.047	0.004	0.035	-	unknown
22	64	F	-	+	190kd, 210kd	1.11	29.60	0.158	0.178	0.350	-	dead
23	51	M	CS+	+	190kd, 210kd	1.14	153.40	0.037	0.012	0.055	-	alive
24	unknown	unknown	CS+	+	190kd, 210kd	1.35	168.85	0.564	2.193	2.036	-	unknown
25	49	M	CS+	+	130kd, 190kd, 210kd	1.57	190.43	0.046	1.093	1.739	-	dead
26	35	F	CS+	+	190kd, 210kd	1.69	268.10	0.029	0.012	0.018	-	alive
27	57	F	-	+	190kd, 210kd	2.08	145.08	0.048	0.043	0.664	-	dead
28	unknown	F	-	+	190kd, 210kd	2.14	39.71	0.373	0.065	0.095	-	unknown
29	64	F	CS+	+	190kd, 210kd	2.15	9.15	0.166	0.024	0.030	+	dead
30	58	F	CS+	+	190kd, 210kd	2.24	40.03	0.032	0.012	0.211	+	dead
31	59	M	CS+	-	130kd, 190kd, 210kd, 230kd	2.50	255.37	0.050	0.025	0.562	-	dead
32	50	M	CS+	+	190kd, 210kd	2.67	67.46	0.059	0.183	1.545	+	unknown
33	42	M	CS+	+	190kd, 210kd	2.95	85.22	0.040	0.008	0.717	+	dead
34	69	F	-	-	190kd, 210kd	2.97	115.20	0.061	0.032	0.033	-	unknown
35	70	M	-	+	190kd, 210kd	3.01	121.37	0.073	0.041	0.221	-	alive
36	67	F	-	+	190kd, 210kd, 230kd	3.10	36.17	0.014	0.004	0.058	+	dead
37	33	F	-	+	190kd, 210kd	3.28	15.00	0.097	0.026	0.024	-	alive
38	49	F	CS+	+	190kd, 210kd	3.48	33.55	0.044	0.041	0.170	+	alive
39	74	F	CS+	+	190kd, 210kd	3.70	126.86	0.136	0.135	0.397	+	dead
40	63	F	CS+	+	190kd, 210kd	3.73	15.36	0.043	0.021	0.051	-	dead

Table S1 Summary of the results of clinical and immunological features in all 104 PNP patients (Continued)

No.	Ages	Genders	Normal IIF	Rat IIF	Epidermal IB	ELISA (INDEX)		ELISA (OD)			Bronchiolitis obliterns	Clinical course
						Dsg1	Dsg3	Dsc1	Dsc2	Dsc3		
41	34	F	-	-	190kd, 210kd	3.75	1.86	0.072	0.017	0.025	+	dead
42	75	F	CS+	-	190kd, 210kd	3.90	159.31	0.015	2.045	0.365	-	dead
43	45	F	CS+	-	190kd, 210kd	4.01	221.22	0.045	0.076	1.118	-	dead
44	68	M	CS+	+	190kd, 210kd	4.49	105.59	0.126	0.047	0.535	-	dead
45	52	F	-	+	190kd, 210kd	4.66	8.59	0.138	0.289	0.088	-	alive
46	70	F	-	+	130kd, 190kd, 210kd	4.68	119.43	0.161	0.398	0.865	-	dead
47	74	F	CS+	+	190kd, 210kd	4.70	160.40	0.223	1.138	2.272	-	unknown
48	53	F	CS+	+	190kd, 210kd	4.80	139.93	0.263	0.285	2.039	-	unknown
49	64	M	CS+	-	190kd, 210kd	4.80	127.13	0.080	0.135	0.683	-	unknown
50	70	unknown	CS+	-	190kd, 210kd	4.85	177.13	0.122	2.036	2.358	-	unknown
51	64	F	CS+	+	190kd, 210kd	4.90	9.19	1.284	0.015	0.018	-	alive
52	67	M	CS+	-	190kd, 210kd	5.00	5.26	0.013	0.011	0.011	-	dead
53	unknown	unknown	CS+	-	190kd, 210kd	5.20	198.89	0.023	0.986	2.06	-	unknown
54	40	F	CS+	-	190kd, 210kd	5.47	109.43	0.215	0.065	1.018	-	alive
55	59	F	CS+	+	190kd, 210kd	5.59	194.30	0.163	1.691	2.448	+	dead
56	39	M	-	-	190kd, 210kd	5.64	94.43	0.159	0.057	0.088	-	alive
57	72	M	-	-	180kd, 190kd, 210kd	6.48	17.57	0.013	0.208	1.000	-	dead
58	74	F	BMZ+	-	180kd, 190kd, 210kd, 230kd	6.80	28.91	0.103	0.017	0.043	-	unknown
59	44	F	CS+	-	190kd, 210kd	7.01	1.22	—	—	—	-	dead
60	57	F	CS+, BMZ+	+	190kd, 210kd	7.25	13.90	0.029	0.015	0.048	-	dead
61	75	F	-	+	190kd, 210kd	7.40	97.28	0.324	0.387	1.033	-	alive
62	37	M	-	+	190kd, 210kd	7.47	218.00	0.179	0.050	0.464	-	alive
63	44	F	CS+	+	190kd, 210kd	7.49	12.00	0.001	0.003	0.000	+	dead
64	51	M	CS+	+	190kd, 210kd	7.80	10.00	0.030	0.026	0.048	-	unknown
65	52	F	CS+	+	190kd, 210kd	8.10	-0.50	1.245	0.037	0.019	-	alive
66	unknown	unknown	-	+	190kd, 210kd	8.23	6.50	0.105	0.034	0.049	-	alive
67	47	F	CS+	+	190kd, 210kd	9.29	139.25	0.459	1.102	2.217	+	dead
68	unknown	unknown	CS+	+	190kd, 210kd	9.86	158.10	0.170	0.066	0.980	+	alive
69	54	F	CS+	+	190kd, 210kd	11.40	55.50	0.029	0.007	0.007	+	alive
70	68	M	CS+	+	190kd, 210kd	12.48	14.21	0.048	0.007	0.470	-	unknown
71	57	M	CS+	+	190kd, 210kd	15.16	112.00	0.118	0.135	2.030	+	dead
72	30	F	CS+	+	190kd, 210kd, 230kd	15.19	122.16	0.050	0.070	0.428	-	alive
73	24	M	-	-	130kd, 190kd, 210kd	15.84	173.77	0.209	0.026	0.102	-	alive
74	63	F	CS+	+	190kd, 210kd	15.96	17.00	0.020	0.012	0.214	-	unknown
75	39	F	-	+	190kd, 210kd	17.51	28.48	0.057	0.019	0.104	-	alive
76	unknown	M	CS+	+	130kd, 190kd, 210kd	18.33	149.24	0.290	0.421	2.285	-	alive
77	30	M	CS+	-	190kd, 210kd	19.05	-1.60	0.097	0.019	0.020	-	alive
78	37	F	-	+	190kd, 210kd	21.20	125.77	0.098	0.096	0.096	+	dead
79	58	F	-	+	190kd, 210kd	22.84	210.00	0.063	0.054	0.225	-	alive
80	67	F	CS+	+	190kd, 210kd	24.61	56.58	0.073	0.044	0.054	+	dead
81	63	M	-	+	130kd, 160kd, 190kd, 210kd	25.00	19.98	0.034	0.031	0.036	-	alive

Table S1 Summary of the results of clinical and immunological features in all 104 PNP patients (Continued)

82	73	M	-	+	130kd, 190kd, 210kd	26.71	167.86	0.079	0.012	0.103	-	alive

Table S1 Summary of the results of clinical and immunological features in all 104 PNP patients (Continued)

No.	Ages	Genders	Normal IIF	Rat IIF	Epidermal IB	ELISA (INDEX)		ELISA (OD)			Bronchiolitis obliterns	Clinical course
						Dsg1	Dsg3	Dsc1	Dsc2	Dsc3		
83	72	F	CS+	+	190kd, 210kd	34.14	156.10	0.098	0.169	0.158	+	dead
84	unknown	unknown	CS+	+	130kd, 190kd, 210kd	36.40	170.22	0.044	1.401	1.757	-	alive
85	46	F	-	+	190kd, 210kd	38.30	20.00	0.048	0.155	0.472	-	alive
86	83	F	CS+	+	190kd, 210kd	39.34	0	0	0.179	0.840	-	alive
87	unknown	unknown	CS+	+	130kd, 190kd, 210kd	47.00	172.44	1.207	0.661	1.829	-	alive
88	80	M	CS+	+	190kd, 210kd	48.58	99.23	0.067	0.583	2.229	-	dead
89	61	F	CS+	+	190kd, 210kd	58.32	2.14	0.067	0.560	1.938	-	alive
90	61	M	-	+	190kd, 210kd	58.96	1.39	0.220	0.309	0.147	-	alive
91	46	M	CS+	±	190kd, 210kd	64.72	91.85	0.034	0.027	0.068	+	dead
92	28	M	CS+	+	190kd, 210kd	64.90	2.76	1.175	0.021	0.147	-	alive
93	72	F	CS+	+	190kd, 210kd	69.03	102.50	0.025	0.486	2.254	-	unknown
94	unknown	M	-	+	190kd, 210kd, 230kd	78.70	0.340	0.059	0.008	0.013	-	unknown
95	69	M	-	+	190kd, 210kd, 230kd	79.20	4.50	0.168	0.054	0.092	-	unknown
96	79	F	CS+	-	190kd, 210kd	80.11	110.78	0.076	0.034	0.049	-	alive
97	54	F	BMZ±	-	190kd, 210kd	85.30	0.42	0.039	0.007	0.010	-	unknown
98	72	F	BMZ+	±	180kd, 190kd, 210kd	86.45	1.41	0.248	0.002	0.011	-	unknown
99	60	F	-	+	190kd, 210kd	89.46	65.00	0.045	0.218	2.218	-	unknown
100	66	F	BMZ+	-	190kd, 210kd	92.10	0.76	0.042	0.006	0.077	-	unknown
101	53	M	-	+	190kd, 210kd	108.5	36.55	0.075	0.067	0.177	-	alive
102	66	F	CS+	+	190kd, 210kd	150.38	2.74	0.004	0.019	0.009	-	unknown
103	69	M	CS+	-	190kd, 210kd	173.30	152.40	0.155	0.024	0.124	-	alive
104	64	F	-	+	190kd, 210kd	183.11	105.58	0.155	0.057	2.315	-	unknown
positive total			74	83		34	82	19	42	62		

BMZ, basement membrane zone; CS, cell surface; Dsc, desmocollin; Dsg, desmoglein; Epi, epithelia; IB, immunoblotting; IIF, indirect immunofluorescence.
+, positive; ±, weakly positive; -, negative.
130kDa Dsg3, 160kDa Dsg1, 180kDa BP180, 190kDa periplakin, 210kDa envoplakin, 230kDa BP230, 250kDa desmoplakin I.

Table S2 Summary of the relationship between various parameters and results of ELISAs

Parameter (n= case with description)		Dsg1 by ELISA		Dsg3 by ELISA		Dsc1 by ELISA		Dsc2 by ELISA		Dsc3 by ELISA	
		positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
Genders(n=93)	Females	20/28 (71.4%)	39/65 (60%)	46/74 (62.2%)	13/19 (68.4%)	12/17 (70.6%)	47/76 (61.8%)	26/37 (70.2%)	33/56 (58.9%)	33/55 (60%)	26/38 (68.4%)
	Males	8/28 (28.6%)	26/65 (40%)	28/74 (37.8%)	6/19 (31.6%)	5/17 (29.4%)	29/76 (38.2%)	11/37 (29.7%)	23/56 (41.1%)	22/55 (40%)	12/38 (31.6%)
Cutaneous lesions (n=87)	Trunk	17/25 (68%)	35/62 (56.5%)	40/69 (58.0%)	12/18 (66.7%)	6/13 (46.2%)	46/74 (62.2%)	23/33 (69.7%)	29/54 (53.7%)	34/49 (69.4%)	18/38 (47.4%)
	Extremities	14/25 (56%)	32/62 (51.6%)	34/69 (49.3%)	12/18 (66.7%)	7/13 (53.8%)	39/74 (52.7%)	20/33 (60.6%)	26/54 (48.1%)	29/49 (59.2%)	17/38 (44.7%)
Clinical manifestations	Erythema (n=61)	12/18 (66.7%)	25/43 (51.8%)	28/49 (57.1%)	9/12 (75%)	4/8 (50%)	33/53 (62.3%)	14/23 (60.9%)	23/38 (60.5%)	22/36 (61.1%)	15/25 (60%)
	Bulla (n=59)	6/16 (37.5%)	18/43 (41.9%)	21/49 (42.9%)	3/10 (30%)	1/8 (12.5%)	23/51 (45.1%)	10/21 (47.6%)	14/38 (36.8%)	15/34 (44.1%)	9/25 (36%)
Mucosal lesions	Oral (n=88)	25/26 (96.2%)	57/62 (91.9%)	67/71 (94.4%)	15/17 (88.2%)	9/12 (75%)	73/76 (96.1%)	32/34 (94.1%)	50/54 (92.6%)	48/51 (94.1%)	34/37 (91.9%)
	Ocular (n=81)	7/25 (28%)	26/56 (46.4%)	30/66 (45.5%)	3/15 (20%)	2/11 (18.1%)	31/70 (44.3%)	20/33 (60.6%)	13/48 (27.1%)	25/48 (52.1%)	8/33 (24.2%)
	Nasal (n=77)	0/22 (0%)	9/55 (16.4%)	9/63 (14.3%)	0/14 (0%)	0/9 (0%)	9/68 (13.2%)	5/29 (17.2%)	4/48 (8.3%)	7/45 (15.6%)	2/32 (6.3%)
	Genital (n=79)	8/24 (33.3%)	20/55 (36.4%)	27/65 (41.5%)	1/14 (7.1%)	2/10 (20%)	26/69 (37.7%)	12/30 (40%)	16/49 (32.6%)	20/45 (44.4%)	8/34 (23.5%)
Rat bladder indirect IF (n=104)		28/34 (82.4%)	55/70 (78.6%)	68/82 (82.9%)	15/22 (68.2%)	18/21 (85.7%)	65/83 (78.3%)	37/44 (84.1%)	46/60 (76.7%)	54/64 (84.4%)	29/40 (72.5%)
Dsg1 indeces (n=104)		-	-	23/82 (28.0%)	11/22 (50%)	11/19 (57.9%)	23/85 (27.1%)	15/44 (34.1%)	19/60 (31.7%)	24/64 (37.5%)	10/40 (25%)
Dsg3 indeces (n=104)		27/34 (79.4%)	55/70 (78.6%)	-	-	13/21 (61.9%)	69/83 (83.1%)	38/44 (86.4%)	44/60 (73.7%)	55/64 (85.9%)	27/40 (67.5%)
Dsc1 OD value (n=102)		10/34 (29.4%)	9/68 (13.2%)	13/82 (15.9%)	6/20 (30%)	-	-	10/42 (23.8%)	9/60 (15%)	14/62 (22.6%)	5/40 (12.5%)
Dsc2 OD value (n=102)		14/34 (41.2%)	28/68 (41.2%)	38/82 (46.3%)	4/20 (20%)	10/19 (52.6%)	32/83 (38.6%)	-	-	39/62 (62.9%)	3/40 (7.5%)
Dsc3 OD value (n=102)		23/34 (67.6%)	39/68 (57.4%)	55/82 (67.1%)	7/20 (35%)	14/19 (73.7%)	48/83 (57.8%)	39/42 (92.9%)	23/60 (38.3%)	-	-
Broncholitis obliterans (n=104)		8/34 (23.5%)	12/70 (17.1%)	19/82 (23.2%)	1/22 (4.5%)	1/21 (4.8%)	19/83 (22.9%)	9/44 (20.5%)	11/60 (18.3%)	12/64 (18.8%)	8/40 (20%)

Table S2 Summary of the relationship between various parameters and results of ELISAs

Dsc, desmocollin; Dsg, desmoglein; ELISA, enzyme-linked immunosorbent assays.