

# Antiphospholipid antibodies in patients with autoimmune blistering disease

著者	Echigo Takeshi, Hasegawa Minoru, Inaoki Makoto, Yamazaki Masahide, Sato Shinichi, Takehara Kazuhiko
journal or publication title	Journal of the American Academy of Dermatology
volume	57
number	3
page range	397-400
year	2007-09-01
URL	<a href="http://hdl.handle.net/2297/9508">http://hdl.handle.net/2297/9508</a>

doi: 10.1016/j.jaad.2006.07.004

## **Antiphospholipid antibodies in patients with autoimmune blistering disease**

### Brief Report

Takeshi Echigo<sup>1</sup>, MD, PhD; Minoru Hasegawa<sup>1</sup>, MD, PhD; Makoto Inaoki<sup>2</sup>, MD, PhD;  
Masahide Yamazaki<sup>3</sup>, MD, PhD; Shinichi Sato<sup>4</sup>, MD, PhD; Kazuhiko Takehara<sup>1</sup>, MD, PhD

<sup>1</sup>Department of Dermatology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.

<sup>2</sup>Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan.

<sup>3</sup>Department of Internal Medicine (III), Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.

<sup>4</sup>Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Address correspondence to: Dr. Minoru Hasegawa, Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan.

Phone: 81-76-265-2342

Fax: 81-76-234-4270

E-mail: [minoruha@derma.m.kanazawa-u.ac.jp](mailto:minoruha@derma.m.kanazawa-u.ac.jp)

Funding Sources: None

Conflict of Interest Disclosure: None declared.

Word count 1052

Number of references 20

Number of tables 1

Number of figures 1

## ABSTRACT

**Objective:** To determine the serum levels and frequency of antiphospholipid antibodies (aPLs) and confirm the clinical importance of this antibodies in patients with autoimmune blistering disease (ABD).

**Methods:** IgG and IgM anti-cardiolipin antibodies (aCL), IgG anti-cardiolipin- $\beta_2$  glycoprotein I complex antibody (aCL/ $\beta_2$ GPI), and IgG anti-phosphatidylserine-prothrombin complex antibody (aPS/PT) were examined with an enzyme-linked immunosorbent assay in 71 patients with ABD, including pemphigus vulgaris, pemphigus foliaceus, and bullous pemphigoid.

**Results:** The prevalence of IgG aCL, IgM aCL, aCL/ $\beta_2$ GPI, and IgG aPS/PT were positive for 22.4%, 9.1%, 9.9%, and 25.4% of the ABD patients, respectively, while they were not detected in any of the normal controls. Ten of 20 (50%) patients with ABD who were attending our hospital in 2004 were positive for aPLs, and thromboembolism was detected in 7/10 (70%) patients with aPLs.

**Conclusion:** aPLs are frequently detected in ABD patients. Careful examination and follow up for thromboembolism may be necessary in ABD patients with aPLs.

*Abbreviations used*

aPL: antiphospholipid antibody

Ab: antibody

ABD: autoimmune blistering disease

aCL: anti-cardiolipin antibody

aCL/ $\beta_2$ GPI: anti-cardiolipin- $\beta_2$  glycoprotein I complex antibody

aPS/PT: anti-phosphatidylserine-prothrombin complex antibody

PV: pemphigus vulgaris

PF: pemphigus foliaceus

BP: bullous pemphigoid

SLE: systemic lupus erythematosus

## INTRODUCTION

Autoimmune blistering disease (ABD) is a typical acquired organ-specific autoimmune disease forming blisters on the skin and/or mucous membranes <sup>1</sup>. Pemphigus, including pemphigus vulgaris (PV) and pemphigus foliaceus (PF), is an autoimmune intraepidermal blistering diseases that target desmoglein <sup>2</sup>. Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease that target BP180/NC16a <sup>3</sup>.

The antiphospholipid syndrome (APS) is an acquired multisystem disorder characterized by hypercoagulation in which thrombosis and recurrent fetal loss develop <sup>4</sup>. The serological markers for this syndrome are antiphospholipid antibodies (aPLs), such as the lupus anticoagulant (LA), anti-cardiolipin antibody (aCL) <sup>5,6</sup>, antibody against anticardiolipin  $\beta_2$ -glycoprotein I complex antibody (CL/ $\beta_2$ GPI), and anti-phosphatidylserine-prothrombin complex antibody (aPS/PT) <sup>7,8</sup>.

aPLs are frequently found in patients with systemic autoimmune diseases, especially in systemic lupus erythematosus (SLE). They are also found in patients with organ-specific autoimmune diseases <sup>9-16</sup>, however the frequency of aPL in ABD remains unknown. In this study, we investigated whether aPLs were detected in patients with ABD, and whether these Abs correlated with the clinical symptoms of APS.

## METHODS

### Patients and serum samples

Serum samples were obtained from 22 patients with PV (6 males and 16 females; age,  $55 \pm 12$  (mean  $\pm$  SD) years), 21 patients with PF (12 males and 9 females; age,  $62 \pm 12$  years), and 28 patients with BP (12 males and 16 females; age  $69 \pm 15$  years). The patients were diagnosed according to their clinical, pathological, and immunological features typical for each ABD <sup>17</sup>. None of the ABD patients were treated with systemic corticosteroids or immunosuppressive agents when the serum samples were collected. Age-matched 32 healthy Japanese people (12 males and 20 females; age,  $60 \pm 15$  years), and 29 patients with SLE (3 males and 26 females; age,  $37 \pm 14$  years), who fulfilled the criteria of the American College of Rheumatology <sup>18</sup> were used as controls.

### Detection of aPLs

The aCL of the IgG and IgM isotypes, and IgG aPS/PT were measured with specific ELISAs (Medical & Biological Laboratories, Nagoya, Japan), and the IgG aCL/ $\beta_2$ GPI was also measured with specific ELISAs (Yamasa, Tokyo, Japan), according to the manufacturer's protocol. LA was determined according to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibodies <sup>19</sup>.

### Clinical assessment

Eighteen of the 71 ABD patients examined for aPLs and 2 with other autoimmune subepidermal blistering diseases (one cicatrical pemphigoid and one linear IgA bullous dermatosis) attended our hospital in 2004. Eighteen of the 20 ABD patients were treated with systemic corticosteroids or immunosuppressive agents when the serum and plasma samples were collected. Only one patient (61-year-old male patient with PV) has symptomatic of

thromboembolism (skin ulcers of the legs and mononeuropathy multiplex). We examined aPLs and clinical signs of APS with magnetic resonance imaging scans of the brain, ventilation/perfusion pulmonary scintigraphy, electrocardiography, echocardiography, and phlebography, and we enquired as to the history of the intrauterine fetal loss.

The protocol was approved by the Kanazawa University Graduate School of Medical Science, and informed consent was obtained from all patients.

### **Statistical analysis**

Statistical analysis was performed using Fisher's exact probability test for the comparison of frequencies. A p value less than 0.05 was considered statistically significant.

## RESULTS

### Prevalence of aPLs in patients with ABD, SLE, and normal controls

Values higher than the mean + 2SD of the normal control serum samples were considered positive in this study. IgG aCL was detected more frequently in the patients with ABD (22.4%), PV (23.8%), PF (25.0%), BP (19.2%), and SLE (31.0%) than in the normal controls (0%,  $p < 0.01$ , respectively, **Figure 1A**). The detection rate of IgM aCL was higher in the patients with PV (15.0%,  $p < 0.05$ ) and SLE (20.7%,  $p < 0.01$ ) than in normal controls (0%, **Figure 1B**). IgG aCL/ $\beta_2$ GPI was detected more frequently in the patients with BP (14.3%,  $p < 0.05$ ) and SLE (27.6%,  $p < 0.01$ ) than in the normal controls (0%, **Figure 1C**). IgG aPS/PT was detected significantly higher in the patients with ABD (25.4%,  $p < 0.01$ ), PV (18.2%,  $p < 0.05$ ), PF (23.8%,  $p < 0.05$ ), BP (32.1%,  $p < 0.01$ ), and SLE (55.2%,  $p < 0.01$ ) than in the normal controls (0%, **Figure 1D**).

### Association of aPL with clinical features

LA, IgG aCL, IgM aCL, aCL/ $\beta_2$ GPI, and aPS/PT were detected in 6/20 (30%), 7/20 (35%), 3/14 (21%), 0/20 (0%), and 3/17 (18%) patients with ABD attending our hospital in 2004, respectively (**Table I**). Ten of 20 (50%) patients with ABD were positive for any one of aPLs, and five of ten patients had plural aPLs. Thromboembolism was detected in 7/10 (70%) patients with aPLs, while a history of intrauterine fetal loss was not found in any of the patients. Interestingly, all patients with plural aPLs had clinical signs of thromboembolism.

## COMMENT

In the present study, IgG aCL, IgM aCL, IgG aCL/ $\beta_2$ GPI, and IgG aPS/PT were positive for 22.4%, 9.1%, 9.9%, and 25.4% of ABD patients, respectively (**Figure 1**). Thromboembolism was detected in high frequency (7/10 cases, 70%) in ABD patients with aPLs (**Table I**).

It is well known that aPLs are frequently detected in SLE and some systemic autoimmune diseases, and SLE comprised 36% of the cases among those with secondary APS<sup>20</sup>. However, recent studies indicate that aPLs have also been detected in organ-specific autoimmune diseases, such as insulin dependent diabetes mellitus (34%)<sup>9,10</sup>, myasthenia gravis (22%)<sup>11</sup>, autoimmune thyroid diseases (43~55%)<sup>12,13</sup>, inflammatory bowel disease (16~18%)<sup>14, 15</sup>, and localized scleroderma (46%)<sup>16</sup>. Nevertheless, aPL positive patients with these diseases infrequently show clinical signs of thromboembolism<sup>9, 11-16</sup>. Only in patients with insulin dependent diabetes mellitus, the presence of aPLs is associated with complications throughout the pregnancy<sup>10</sup>. Our findings suggest that ABD is another organ-specific autoimmune disease that can be accompanied by APS.

Since most of thromboembolism was asymptomatic in ABD patients (**Table I**), hypercoagulative states due to aPLs may be modest in ABD patients compared with patients with primary APS or SLE. Nonetheless, we propose that patients with ABD should be examined for the presence of aPLs, since the frequency of some aPLs was significantly higher than that in normal controls. While systemic corticosteroid is often used for treatment of ABD, this may enhance the hypercoagulative states. Therefore, careful observation and follow up may be required to prevent symptomatic thrombotic events in patients with aPLs. Further follow-up studies, especially with a large patient group, will be needed to clarify the clinical relevance of aPLs in ABD.

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**Table I. The profiles of antiphospholipid antibodies in patients with autoimmune blistering disease, and clinical involvements of thrombosis**

<b>Diag</b>	<b>Age</b>	<b>Sex</b>	<b>Thrombosis</b>	<b>LA</b>	<b>IgG aCL</b>	<b>IgM aCL</b>	<b>aCL/<math>\beta_2</math>GPI</b>	<b>aPS/PT</b>
<b>PV</b>	37	M	Femoral head	+	-	-	-	-
	46	M	N.D.	-	-	-	-	-
	61	M	Skin ulcer, Nerve, Brain	+	11.7	N.D.	-	18.6
	63	F	Lung, Brain	+	27.7	17.8	-	-
	64	F	Popliteal vein	+	10.8	-	-	-
	71	M	Brain	-	32.2	11.5	-	33.4
<b>PF</b>	47	F	N.D.	-	-	-	-	-
	48	F	-	-	-	27.8	-	-
	54	M	N.D.	-	-	-	-	-
	56	M	N.D.	-	-	-	-	-
	60	M	Lung	+	27.5	-	-	26.9
	67	M	N.D.	-	-	-	-	-
	70	M	N.D.	-	-	-	-	-
73	M	N.D.	-	-	N.D.	-	N.D.	
<b>BP</b>	30	M	Brain	+	-	-	-	-
	62	F	-	-	21.4	-	-	-
	73	F	N.D.	-	-	N.D.	-	-
	85	F	N.D.	-	-	N.D.	-	-
<b>CP</b>	80	F	N.D.	-	-	N.D.	-	N.D.
<b>LAD</b>	61	F	-	-	17.9	N.D.	-	N.D.

N.D.: not done

CP: cicatricial pemphigoid

LAD: linear IgA bullous dermatosis

Femoral head: avascular necrosis of the femoral head

Nerve: mononeuropathy multiplex

Brain: cerebral thrombosis

Lung: pulmonary embolism

Popliteal vein: stenosis of bilateral popliteal vein

**FIGURE LEGENDS**

**Figure 1. (A) IgG Abs against cardiolipin (CL), (B) IgM Abs against CL, (C) IgG Abs against CL/ $\beta_2$ GPI, and (D) IgG Abs against PS/PT in serum samples from patients with autoimmune blistering disease (ABD), pemphigus vulgaris (PV), pemphigus foliaceus (PF), bullous pemphigoid (BP), systemic lupus erythematosus (SLE), and normal controls (CTL). Each Ab levels were determined with specific ELISA. A broken line represents the cut-off value. Percentage above the broken line indicates the frequency of antibody-positive patients in each subgroup.**

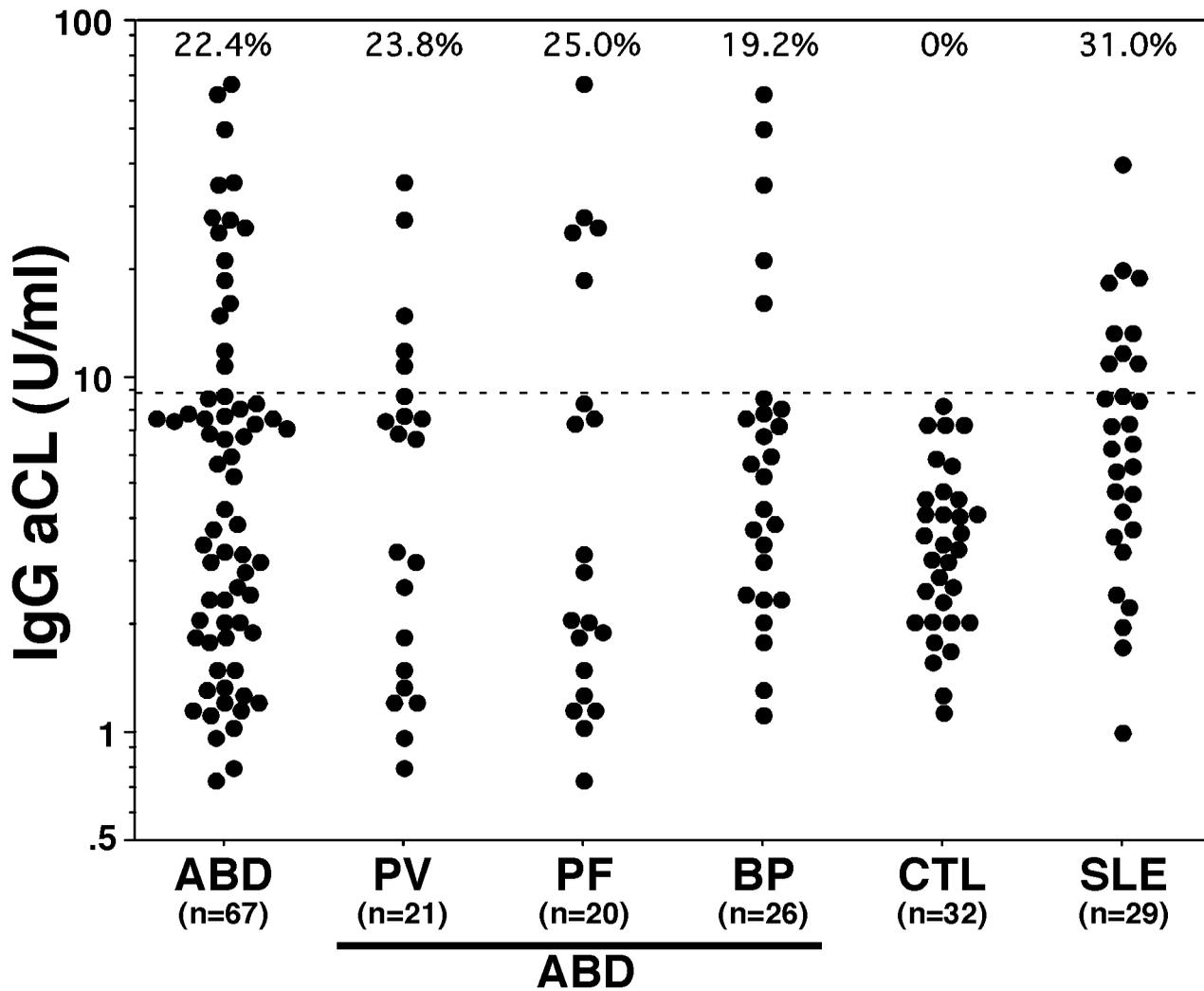
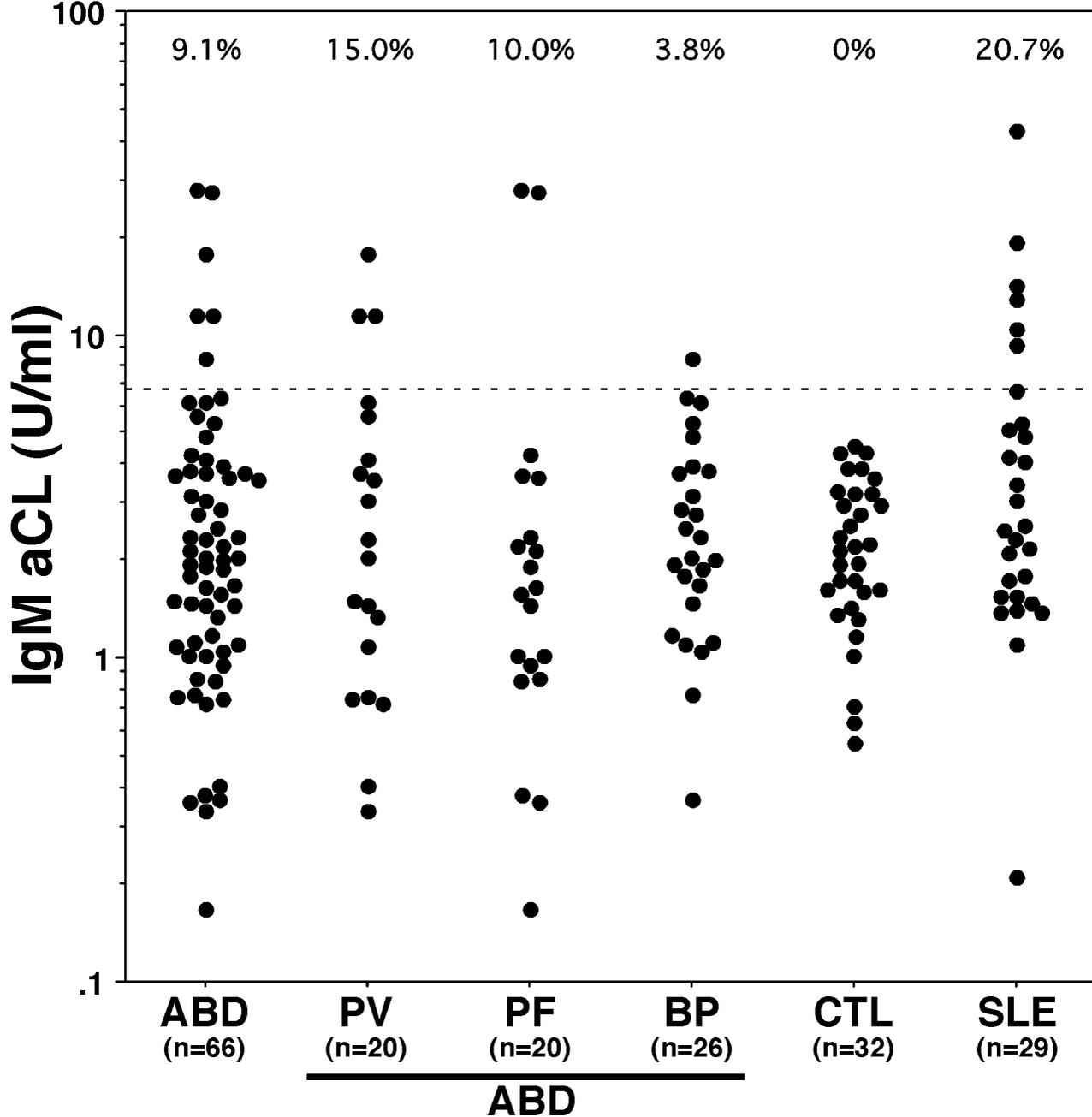


Figure 1A  
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**Figure 1B**  
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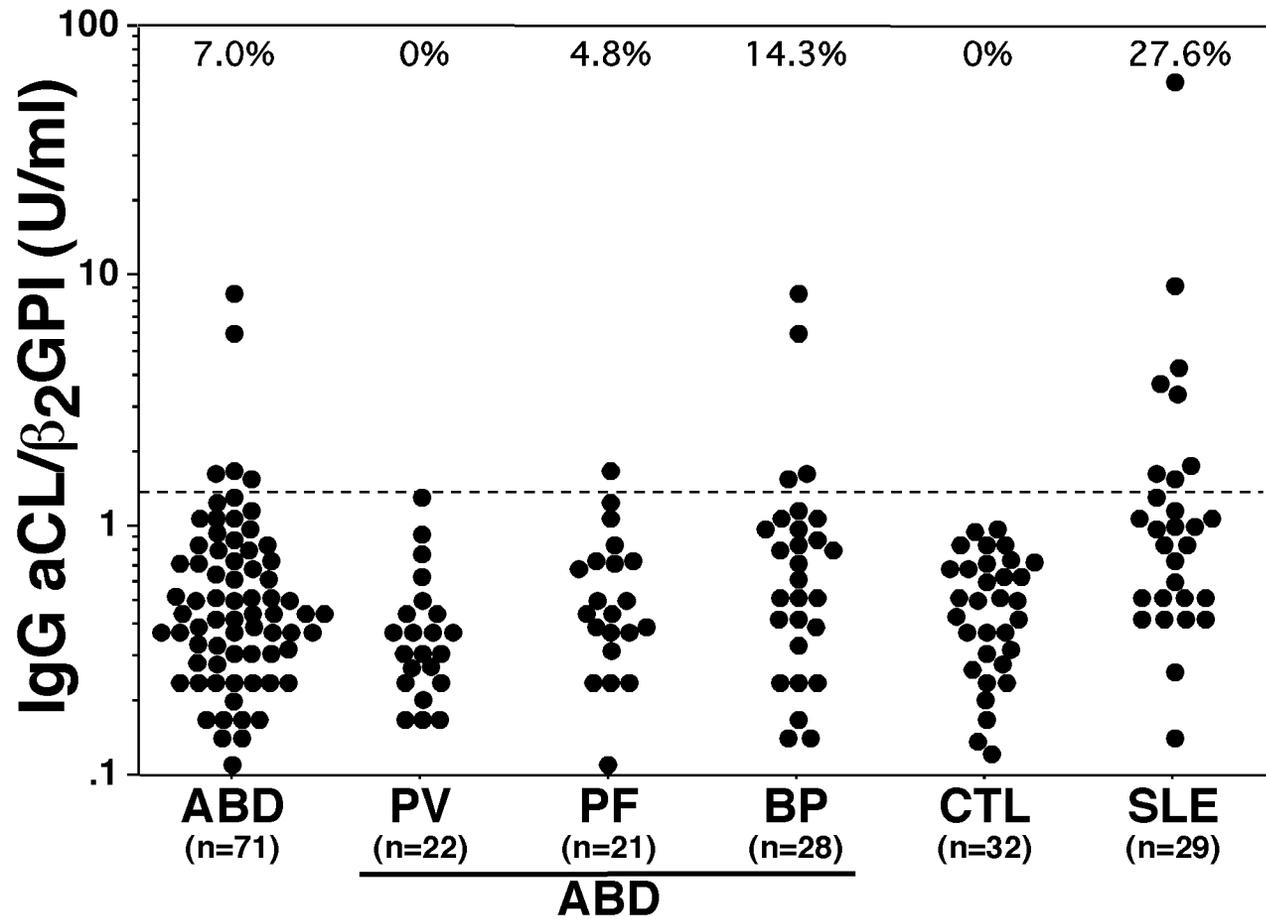


Figure 1C  
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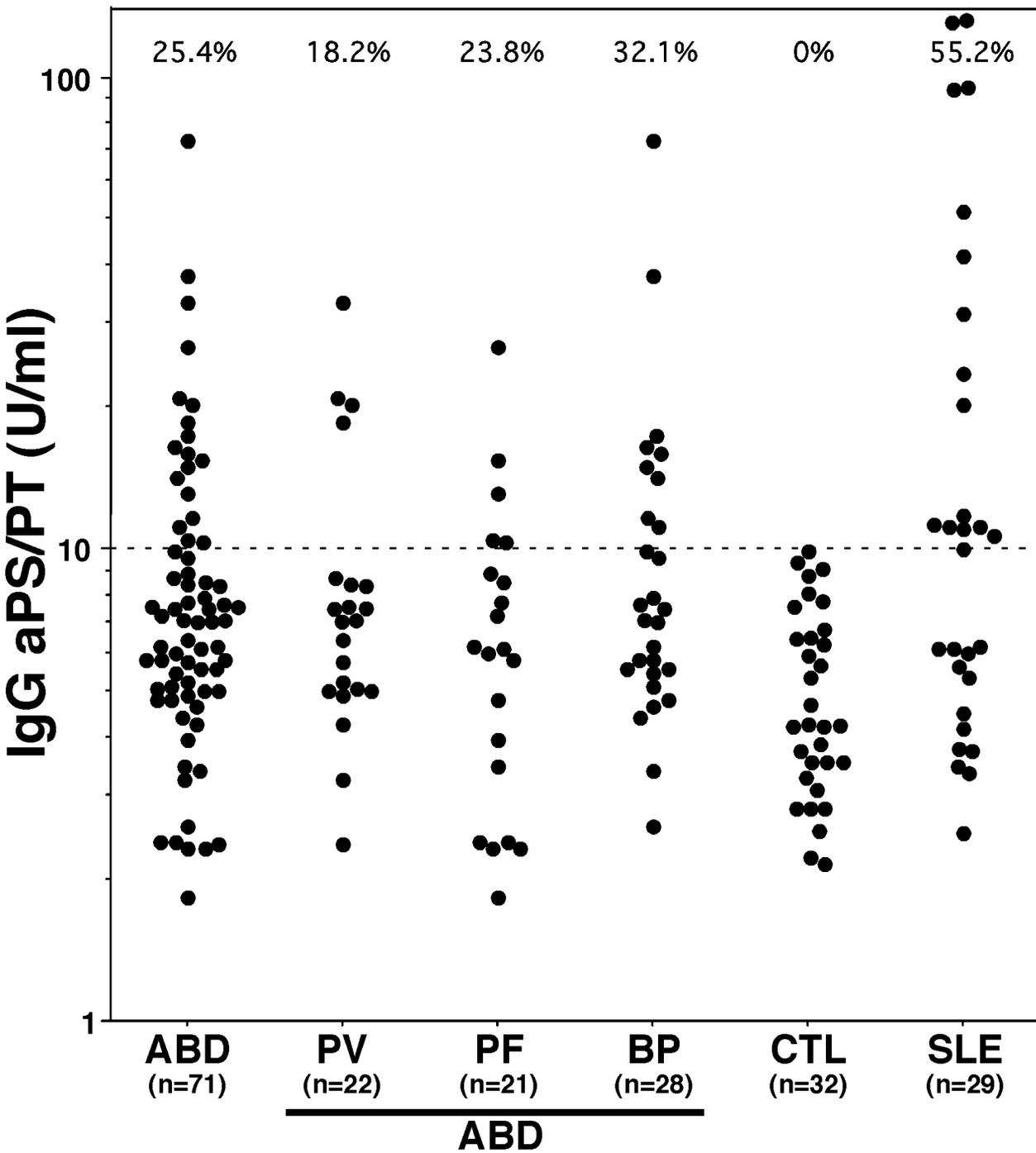


Figure 1D  
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