Antiphospholipid antibodies in patients with autoimmune blistering disease

Antiphospholipid antibodies in patients with autoimmune blistering disease

Brief Report

Takeshi Echigo[1], MD, PhD; Minoru Hasegawa[1], MD, PhD; Makoto Inaoki[2], MD, PhD; Masahide Yamazaki[3], MD, PhD; Shinichi Sato[4], MD, PhD; Kazuhiko Takehara[1], MD, PhD

[1]Department of Dermatology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.

[2]Department of Dermatology, Kawasaki Medical School, Kurashiki, Japan.

[3]Department of Internal Medicine (III), Kanazawa University Graduate School of Medical Science, Kanazawa, Japan.

[4]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

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-β_2 glycoprotein I complex antibody (aCL/β_2GPI), 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 foliaceous, and bullous pemphigoid.

Results: The prevalence of IgG aCL, IgM aCL, aCL/β_2GPI, 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/β_2GPI: anti-cardiolipin-β_2 glycoprotein I complex antibody

aPS/PT: anti-phosphatidylserine-prothrombin complex antibody

PV: pemphigus vulgaris

PF: pemphigus foliaceous

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 [1]. Pemphigus, including pemphigus vulgaris (PV) and pemphigus foliaceous (PF), is an autoimmune intraepidermal blistering diseases that target desmoglein [2]. Bullous pemphigoid (BP) is an autoimmune subepidermal blistering disease that target BP180/NC16a [3].

The antiphospholipid syndrome (APS) is an acquired multisystem disorder characterized by hypercoagulation in which thrombosis and recurrent fetal loss develop [4]. The serological markers for this syndrome are antiphospholipid antibodies (aPLs), such as the lupus anticoagulant (LA), anti-cardiolipin antibody (aCL) [5, 6], antibody against anticardiolipin β_2-glycoprotein I complex antibody (CL/β_2GPI), and anti-phosphatidylserine-prothrombin complex antibody (aPS/PT) [7, 8].

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 [9-16], 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 ± 12 (mean ± SD) years), 21 patients with PF (12 males and 9 females; age, 62 ± 12 years), and 28 patients with BP (12 males and 16 females; age 69 ± 15 years). The patients were diagnosed according to their clinical, pathological, and immunological features typical for each ABD [17]. 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 ± 15 years), and 29 patients with SLE (3 males and 26 females; age, 37 ± 14 years), who fulfilled the criteria of the American College of Rheumatology [18] 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 /β_2GPI 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 [19].

Clinical assessment

Eighteen of the 71 ABD patients examined for aPLs and 2 with other autoimmune subepidermal blistering diseases (one cicatrial 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/β_2GPI 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/β_2GPI, 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/β_2GPI, 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 [20]. However, recent studies indicate that aPLs have also been detected in organ-specific autoimmune diseases, such as insulin dependent diabetes mellitus (34%) [9, 10], myasthenia gravis (22%) [11], autoimmune thyroid diseases (43~55%) [12, 13], inflammatory bowel disease (16~18%) [14, 15], and localized scleroderma (46%) [16]. Nevertheless, aPL positive patients with these diseases infrequently show clinical signs of thromboembolism [9, 11-16]. Only in patients with insulin dependent diabetes mellitus, the presence of aPLs is associated with complications throughout the pregnancy [10]. 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.

REFERENCES

1. Stanley JR. Cell adhesion molecules as targets of autoantibodies in pemphigus and pemphigoid, bullous diseases due to defective epidermal cell adhesion. Adv Immunol 1993;53:291-325.
2. Amagai M, Klaus-Kovtun V, Stanley JR. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. Cell 1991;67:869-77.
3. Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, et al. A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest 1993;92:2480-8.
4. Hughes GR. Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. Br Med J (Clin Res Ed) 1983;287:1088-9.
5. Gharavi AE, Harris EN, Asherson RA, Hughes GR. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. Ann Rheum Dis 1987;46:1-6.
6. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112:682-98.
7. Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. Blood 1988;72:512-9.
8. Atsumi T, Ieko M, Bertolaccini ML, Ichikawa K, Tsutsumi A, Matsuura E, et al. Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. Arthritis Rheum 2000;43:1982-93.
9. Lynch A, Marlar R, Murphy J, Davila G, Santos M, Rutledge J, et al. Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. Ann Intern Med 1994;120:470-5.

10. Boddi M, Prisco D, Fedi S, Cellai AP, Liotta AA, Parretti E, et al. Antiphospholipid antibodies and pregnancy disorders in women with insulin dependent diabetes. Thromb Res 1996;82:207-16.
11. Colaco CB, Scadding GK, Lockhart S. Anti-cardiolipin antibodies in neurological disorders: cross-reaction with anti-single stranded DNA activity. Clin Exp Immunol 1987;68:313-9.
12. Paggi A, Caccavo D, Ferri GM, Di Prima MA, Amoroso A, Vaccaro F, et al. Anti-cardiolipin antibodies in autoimmune thyroid diseases. Clin Endocrinol (Oxf) 1994;40:329-33.
13. Nabriski D, Ellis M, Ness-Abramof R, Shapiro M, Shenkman L. Autoimmune thyroid disease and antiphospholipid antibodies. Am J Hematol 2000;64:73-5.
14. Koutroubakis IE, Petinaki E, Anagnostopoulou E, Kritikos H, Mouzas IA, Kouroumalis EA, et al. Anti-cardiolipin and anti-beta2-glycoprotein I antibodies in patients with inflammatory bowel disease. Dig Dis Sci 1998;43:2507-12.
15. Aichbichler BW, Petritsch W, Reicht GA, Wenzl HH, Eherer AJ, Hinterleitner TA, et al. Anti-cardiolipin antibodies in patients with inflammatory bowel disease. Dig Dis Sci 1999;44:852-6.
16. Sato S, Fujimoto M, Hasegawa M, Takehara K. Antiphospholipid antibody in localised scleroderma. Ann Rheum Dis 2003;62:771-4.
17. Wojnarowska F, Venning VA, Burge SM. Immunobullous diseases. In: Burns DA, Breathnach SM, Cox N, Griffiths CE, editors. Rook's Textbook of Dermatology. Massachusetts, VII: Blackwell Pub; 2004. p.41.1-59.
18. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.

19. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. Thromb Haemost 1995;74:1185-90.
20. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum 2002;46:1019-27.

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

Diag	Age	Sex	Thrombosis	LA	IgG aCL	IgM aCL	aCL/β_2GPI	aPS/PT
PV	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
PF	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.
BP	30	M	Brain	+	−	−	−	−
	62	F	−	−	21.4	−	−	−
	73	F	N.D.	−	−	N.D.	−	−
	85	F	N.D.	−	−	N.D.	−	−
CP	80	F	N.D.	−	−	N.D.	−	N.D.
LAD	61	F	−	−	17.9	N.D.	−	N.D.

N.D.: not done
CP: cicatrial 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/β_2GPI, and (D) IgG Abs against PS/PT in serum samples from patients with autoimmune blistering disease (ABD), pemphigus vulgaris (PV), pemphigus foliaceous (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.

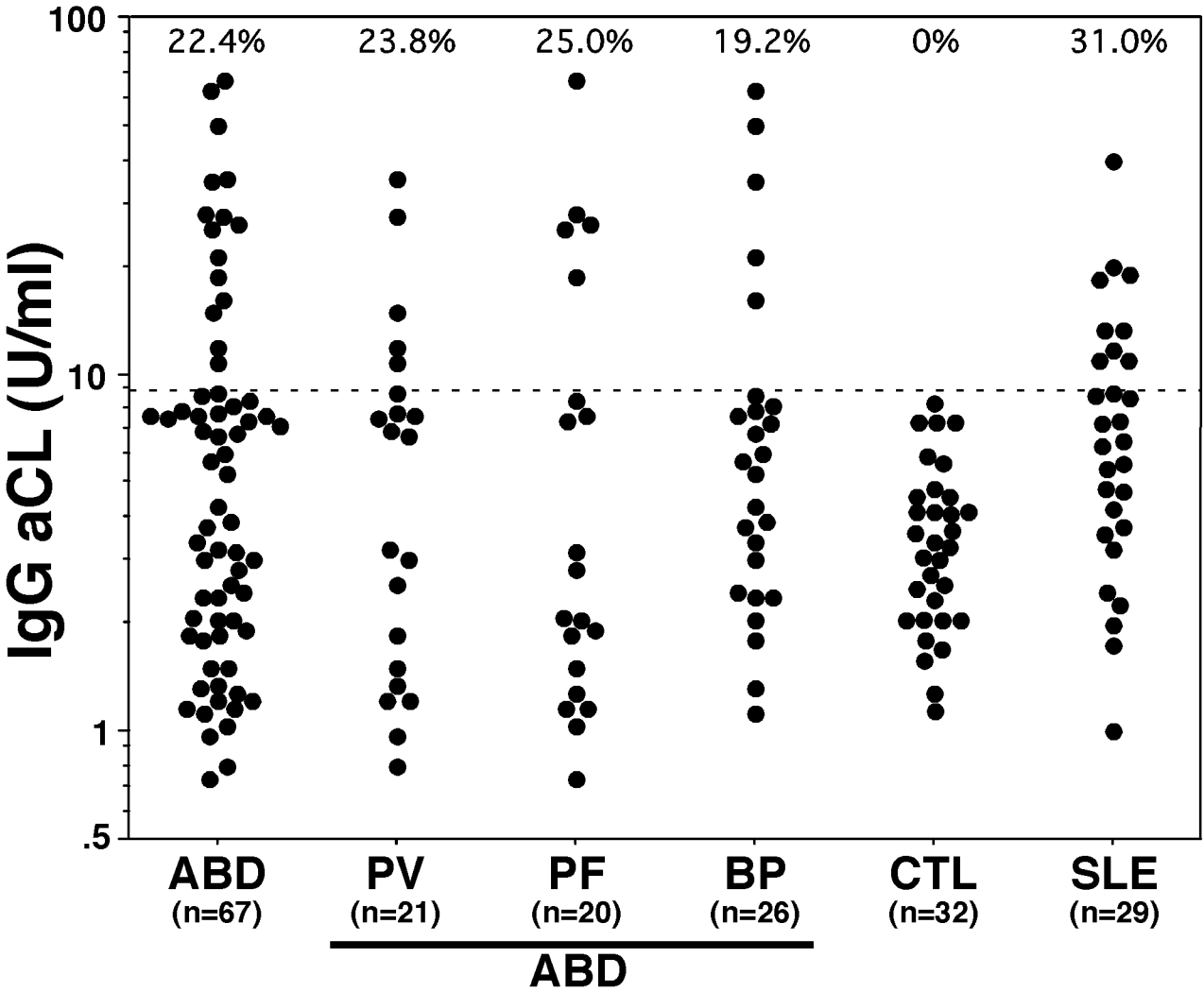

Figure 1A
Echigo T, et al.

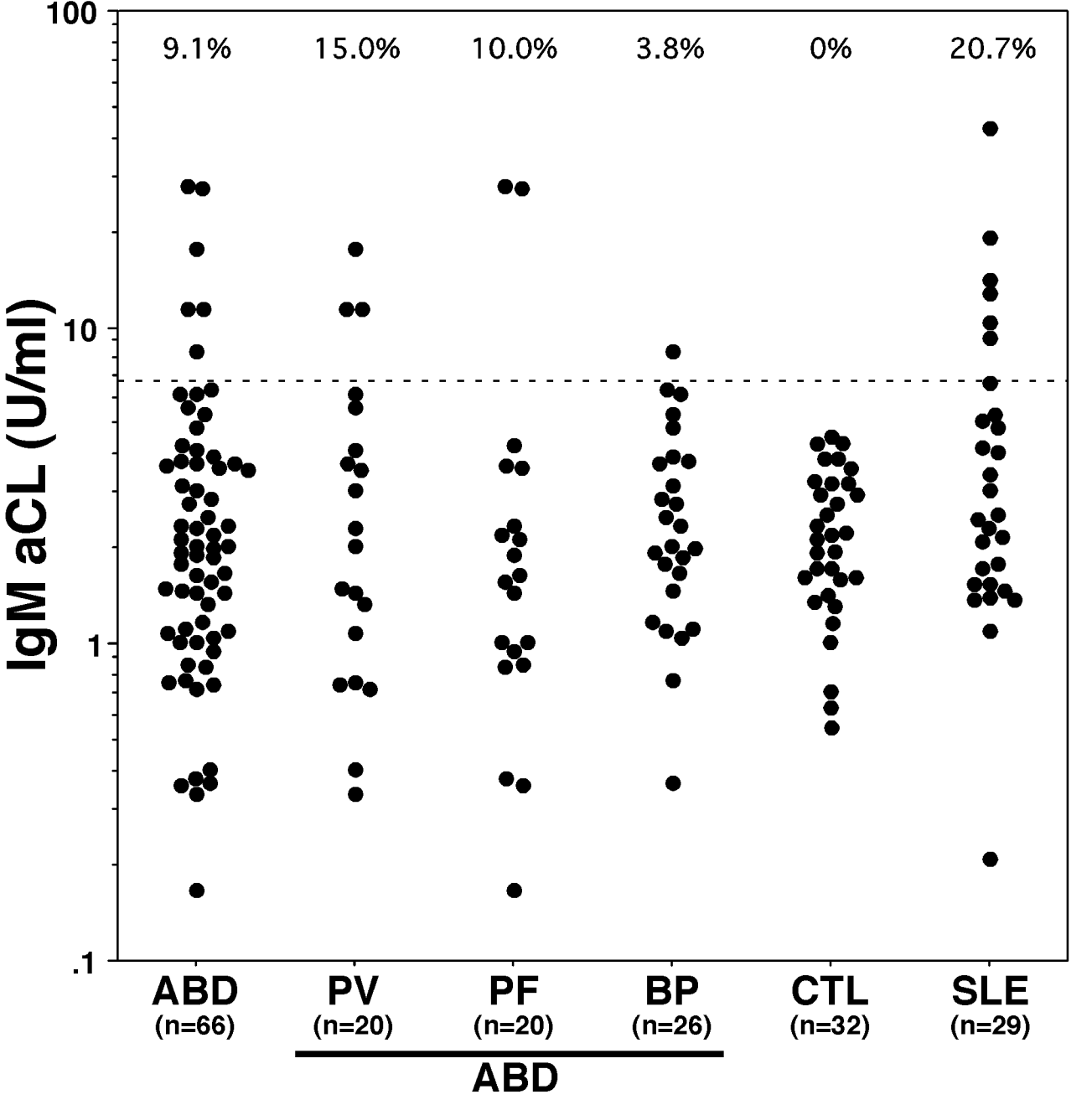

Figure 1B
Echigo T, et al.

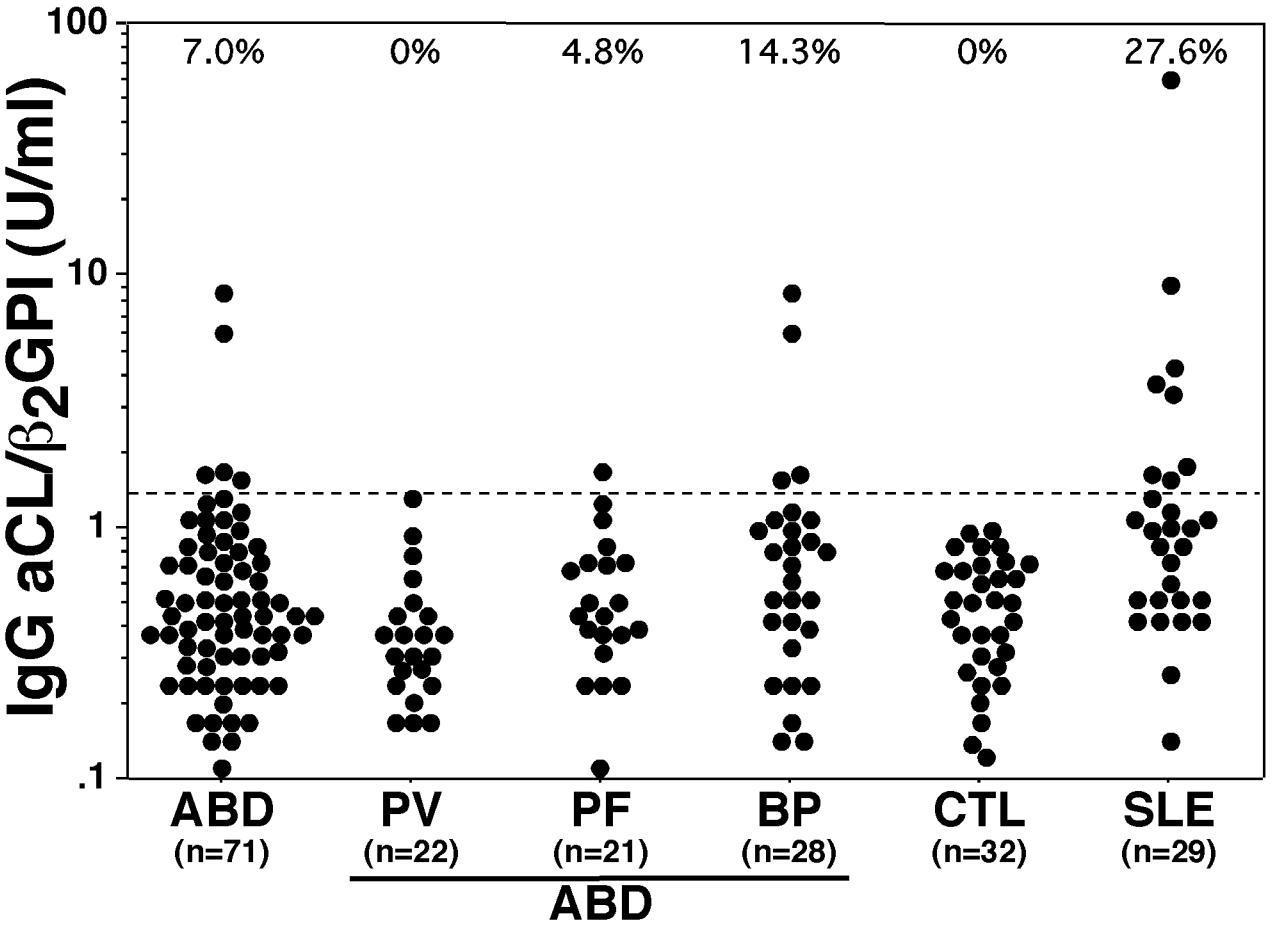

Figure 1C
Echigo T, et al.

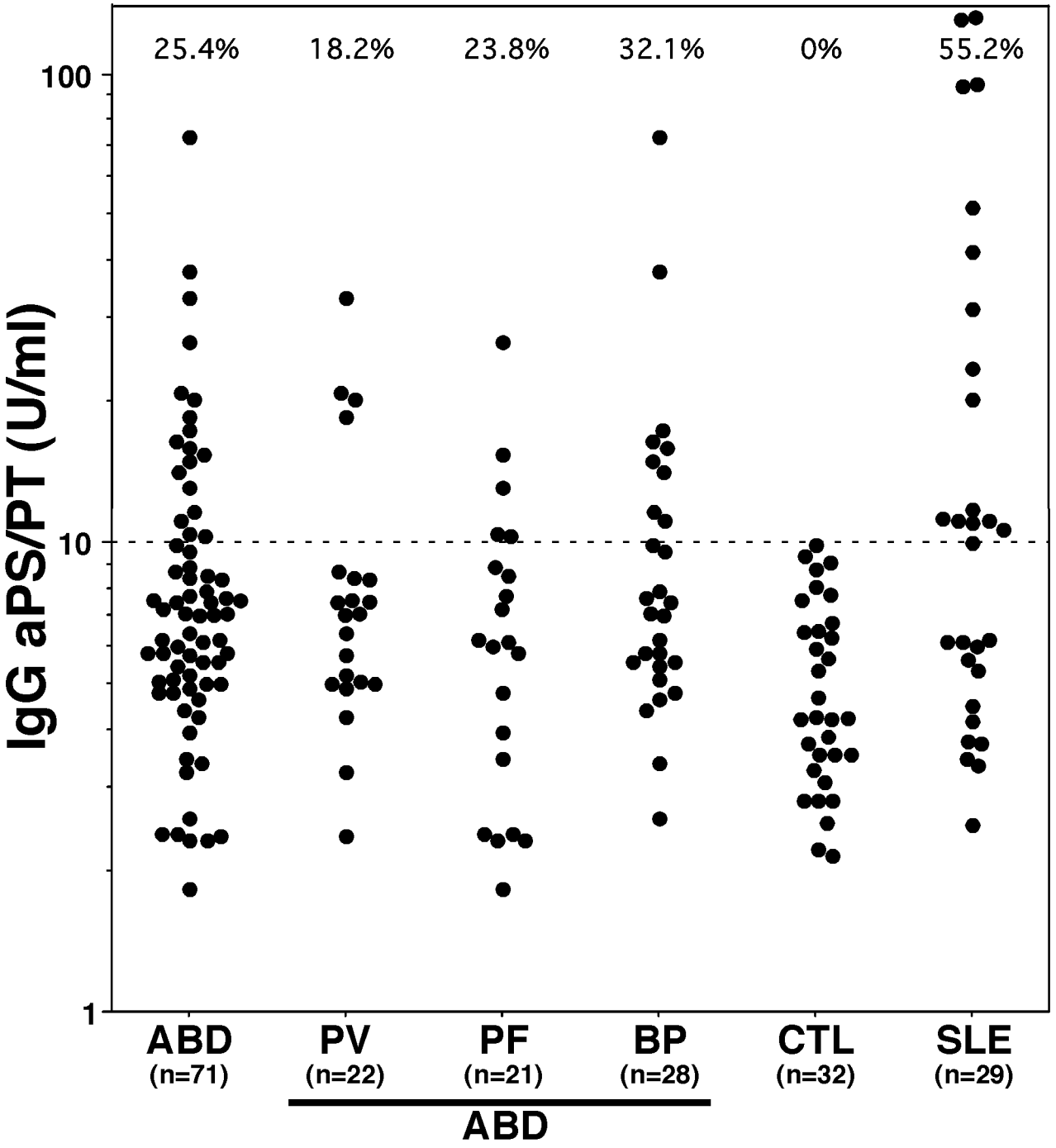

Figure 1D
Echigo T, et al.