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ORIGINAL ARTICLE

High Titer of Anti–Phosphatidylserine-Prothrombin Complex Antibodies in Patients With Cutaneous Polyarteritis Nodosa

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Objective. **To investigate possible correlations between cutaneous polyarteritis nodosa (CPN) and antiphospholipid syndrome–associated antibodies.**

Methods. **Sixteen patients were referred with CPN features. To investigate the possible role of antiphospholipid antibodies (aPL) in CPN, we measured serum lupus anticoagulant (LAC), IgG and IgM anticardiolipin (aCL) and anti–phosphatidylserine-prothrombin complex (anti-PS/PT) antibodies, and anti–β_2-glycoprotein I–dependent cardiolipin (anti-β_2GPI/CL) antibodies in the 16 CPN patients, 8 microscopic polyangiitis (MPA) patients, 33 systemic lupus erythematosus (SLE) patients, and 23 healthy controls. LAC was determined according to the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibody guidelines. Anti-PS/PT, aCL, and anti-β_2GPI/CL antibodies were measured by enzyme-linked immunosorbent assay.**

Results. **Anti-PS/PT antibodies and/or LAC were detected in all CPN patients, but not in any controls. Serum IgM anti-PS/PT antibody was found in 13 (81.3%) CPN patients. The mean ± SD serum anti-PS/PT IgM level (19.9 ± 12.4 units/ml) in CPN patients was significantly elevated compared with SLE patients (5.7 ± 5.9 units/ml). IgG anti-PS/PT antibody was detected in 5 (31.3%) CPN patients, but not in any controls. The IgG PS/PT antibody titers were similar in CPN patients (12.3 ± 12.0 units/ml) and SLE patients (13.8 ± 14.3 units/ml). Three (18.8%) CPN patients were positive for IgG aCL antibody and 2 (12.5%) for IgM aCL antibody. No MPA patients had aPL. CPN skin manifestations included livedo reticularis (14 [87.5%]). Direct immunofluorescence (DIF) revealed C3 within the affected vessels in 7 (77.8%) of 9 CPN patients.**

Conclusion. **Our study demonstrated that presence of anti-PS/PT antibodies and/or LAC could serve as markers in CPN patients. CPN could be dependently associated with the presence of anti-PS/PT antibody. Clinicians need to recognize these titers to permit early accurate diagnosis and treatment. We believe that anti-PS/PT antibodies will become widely recognized as a new factor when diagnosing CPN.**

KEY WORDS. Cutaneous polyarteritis nodosa; Lupus anticoagulant; Anti–phosphatidylserine-prothrombin complex antibody; Anticardiolipin antibody.

INTRODUCTION

Polyarteritis nodosa (PAN) is a multisystem necrotizing vasculitis of small and medium-sized muscular arteries characterized by involvement of the renal and visceral arteries. In classic or systemic PAN, very high morbidity and mortality rates characterized by fulminant deterioration or by relentless progression are associated with intermittent acute exacerbations. There is a form of PAN that is restricted to the skin (cutaneous PAN [CPN]), which has a chronic relapsing benign course (1–3). The diagnosis is often problematic because the skin manifestations and their histologic findings in systemic PAN and CPN are identical. Several authors have described differing clinical presentations between patients with CPN and systemic PAN. However, the distinction remains controversial. Skin manifestations may not be helpful for differentiating between the 2 types, and extensive examinations are usually necessary for the final diagnosis (4). PAN and microscopic polyangiitis (MPA) are primary systemic necrotizing vasculitides. MPA is strongly associated with myeloperoxi-

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dase (MPO) antineutrophil cytoplasmic autoantibody (ANCA). In contrast, PAN is defined as medium-sized vasculitis without ANCA and is extremely rare (5). The underlying pathogenic mechanisms appear to be different, involving immune complexes in PAN and ANCA in MPA. Titers of MPO ANCA in MPA can reflect disease activity and play a pathogenic role.

Antiphospholipid syndrome (APS) is defined as the presence of lupus anticoagulant (LAC), anticardiolipin (aCL) antibodies, or anti–β_2-glycoprotein I–dependent cardiolipin (anti-β_2GPI/CL) antibodies and either vascular thrombosis or specific pregnancy complications (6). These antibodies constitute one of the criteria for classification of primary APS (7) and systemic lupus erythematosus (SLE) (8). LAC activity detected by a phospholipid-dependent coagulation assay is heterogeneous with respect to the specificities and functional capacities of the antibodies (9). The detection of LAC activity requires a careful, sequential series of steps. Despite internationally accepted guidelines and many efforts to improve the standardization of LAC assays, it is very difficult to standardize the laboratory diagnosis of LAC (10). Detection of antiphospholipid cofactor antibodies in addition to the classic aCL antibodies and LAC seems to be of considerable clinical importance. Prothrombin is another possible antigenic target of APS (11). Atsumi et al (12) and Amengual et al (13) suggested that anti–phosphatidylserine-prothrombin complex (anti-PS/PT) antibody rather than antiprothrombin antibody alone is associated with symptoms of APS and LAC activity.

In the present study, we examined the prevalence of LAC, aCL antibody, anti-PS/PT antibody, and anti-β_2GPI/CL antibody in 16 patients with CPN and 8 patients with MPA. Furthermore, we investigated whether these antibodies are strictly correlated with the clinical or serologic features of CPN, because early recognition of the serologic features of this disease may aid in diagnosis and treatment of CPN.

PATIENTS AND METHODS

Clinical investigation and tissue samples. Sixteen Japanese patients with CPN (6 men, 10 women; mean age 47 years [range 19–80 years]) and 8 patients with MPA (3 men, 5 women; mean age 48 years [range 27–80 years]) seen at the Department of Dermatology, St. Marianna University School of Medicine between 2003 and 2007 were examined. The patients were diagnosed with CPN following extensive examinations of internal organs, including arteriography, brain magnetic resonance imaging, and chest computed tomography. All tissue specimens were obtained by skin biopsy, fixed in 10% formalin, step-sectioned, and stained with hematoxylin and eosin and elastic–van Gieson stain. All biopsy samples were taken from the lower extremities. A diagnosis of vasculitis requires the presence of necrotizing vasculitis, such as fibrinoid degeneration, nuclear dust, neutrophilic infiltration, and erythrocyte extravasation. The skin biopsy samples in 9 CPN patients were also taken for direct immunofluorescence (DIF) staining according to standard practice. The tissue section was incubated with commercially prepared fluoresceinated antiserums specific to human IgG, IgM, or C3. None of the patients had received corticosteroids, immunosuppressants, or vasodilators at the time of serum sampling. Furthermore, none of the patients demonstrated any evidence of coexisting malignancy, other autoimmune diseases, or viral hepatitis, nor were any of the patients positive for mixed cryoglobulinemia. The CPN patients were compared with age- and sex-matched patients with SLE (n = 33) and healthy controls (n = 23). The experimental protocol was approved by the St. Marianna University, and informed consent was obtained from all patients.

Serologic studies. According to the guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibodies, LAC was screened by measuring dilute Russell's viper venom time and kaolin clotting time and confirmed by mixing studies and demonstration of phospholipid dependence (14). IgG and IgM anti-PS/PT antibodies were measured with a specific enzyme-linked immunosorbent assay (ELISA; Medical and Biological Laboratories, Nagoya, Japan), according to the manufacturer's protocol. Briefly, the serum samples diluted to 1:101 were added to 96-well plates coated with PS/PT and incubated for 1 hour at 20°C. After washing, bound antibodies were detected with peroxidase-conjugated anti-human IgG and IgM antibodies. Color was developed with 3,3′,5,5′-tetramethylbenzidine and H_2O_2 and the plates were read at 450 nm. IgG and IgM isotypes of aCL antibody were determined according to the standardized aCL antibody ELISA. Briefly, microtiter ELISA plates were coated with highly purified cardiolipin (Diagnostica Stago, Asnières, France). Polyclonal rabbit anti-human IgG and IgM antibodies labeled with horseradish peroxidase were used as conjugate solutions to recognize the 2 isotypes of aCL antibody. The plate was read in a spectrophotometer at 450 nm. Anti-β_2GPI/CL antibody was measured using an ELISA kit (Yamasa, Tokyo, Japan) (14).

The cutoff values were 12 units/ml and 10 units/ml for IgG and IgM anti-PS/PT antibody, respectively; 10 units/ml for both IgG and IgM aCL antibody; and 3.5 units/ml for anti-β_2GPI/CL antibody. When ANCA was detected, specificity was characterized by ELISA for reactivity with MPO or proteinase 3 using the technique recommended by the European ANCA-assay Standardization Group (15).

Statistical analysis. Statistical analysis was performed using the Mann-Whitney U test for the comparison of antibody levels and Fisher's exact probability test for the comparison of frequencies, and Bonferroni test for multiple comparisons was also used. A P value less than 0.05 was considered to be statistically significant. The correlation between serum C-reactive protein (CRP) level and variation of the APS-associated antibodies was assessed by Pearson's correlation coefficient. All data are expressed as the mean ± SD.

Table 1. Clinical, serologic, and DIF features in 16 cutaneous polyarteritis nodosa patients*

Patient no.	Age	Sex	PS/PT IgG (units/ml)	PS/PT IgM (units/ml)	aCL IgG (units/ml)	aCL IgM (units/ml)	β_2GPI/CL (units/ml)	LAC	CRP (mg/dl)	Arthralgia	Myalgia	Skin ulcer	Nodule	LR	Purpura	DIF
1	19	F	6	20	–	6	–	+	0.06	–	–	–	+	+	–	IgM, C3
2	27	F	–	20	–	6	–	–	0.73	–	–	–	+	+	–	ND
3	36	M	11	–	10	–	–	+	3.11	–	–	+	+	–	+	C3
4	51	F	25	18	–	–	–	–	0.6	+	–	–	+	+	–	ND
5	42	F	5	13	–	–	–	+	0.59	+	+	–	+	+	–	ND
6	64	M	–	45	–	–	–	+	11.5	+	+	–	+	+	–	–
7	80	F	–	40	–	–	–	–	3.25	+	–	+	+	+	+	IgG, IgM, C3
8	32	M	28	12	–	13	–	–	0.91	+	–	+	+	+	+	C3
9	60	F	25	9	12	–	–	–	5.92	+	+	+	+	+	+	C3
10	56	F	25	30	–	6	–	–	0.07	–	–	+	+	+	–	ND
11	68	F	–	15	13	–	–	+	0.77	+	–	–	+	+	–	ND
12	23	F	–	15	–	–	–	–	0.05	–	–	–	+	+	–	IgM, C3
13	48	F	6	40	–	28	–	–	0.04	+	+	–	+	+	–	ND
14	40	M	–	8	–	–	–	+	0.84	+	+	+	+	+	–	ND
15	70	M	40	15	–	–	–	+	2.7	+	–	+	+	+	+	–
16	48	M	8	15	–	–	–	–	0.1	+	–	+	+	–	–	C3

* PS/PT = anti–phosphatidylserine-prothrombin complex; aCL = anticardiolipin antibodies; β_2GPI/CL = anti–β_2-glycoprotein I–dependent cardiolipin; LAC = lupus anticoagulant; CRP = C-reactive protein; LR = livedo reticularis; DIF = direct immunofluorescence; ND = not done.

RESULTS

Anti-PS/PT antibody levels in CPN. We detected the IgM isotype of anti-PS/PT antibody in 13 (81.3%) of 16 CPN patients (Table 1). Of the 3 patients who were negative for IgM anti-PS/PT antibody, 2 men were positive for LAC and 1 woman was positive for IgG anti-PS/PT antibody. IgM anti-PS/PT antibody was not detected in any of the healthy controls. The mean IgM anti-PS/PT antibody level in patients with CPN was significantly higher than in patients with SLE (19.9 ± 12.4 units/ml versus 5.7 ± 5.9 units/ml; $P < 0.01$) (Figure 1). IgG anti-PS/PT antibody was detected in 5 (31.3%) of the 16 CPN patients, whereas it was not detected in any of the normal controls. There was no significant difference in the elevation of serum IgG levels of anti-PS/PT antibody between patients with CPN and those with SLE (12.3 ± 12.0 units/ml versus 13.8 ± 14.3 units/ml) (Figure 2). By contrast, IgG and IgM anti-PS/PT antibody levels were both within normal limits in all patients with MPA.

Prevalence of aCL and anti-PS/PT antibody. Three (18.8%) of the 16 CPN patients were positive for IgG aCL antibody and 2 (12.5%) were positive for IgM aCL antibody. Serum IgG aCL levels were significantly higher in SLE patients compared with CPN patients (32.7 ± 38.3 units/ml versus 4.6 ± 3.5 units/ml; $P < 0.01$) (Figure 3). None of the normal controls or MPA patients was positive for this isoform of antibody. Similar trends were seen with respect to IgM aCL antibody levels. Serum IgM aCL antibody levels differed significantly between SLE patients and CPN patients (11.8 ± 13.4 units/ml versus 5.8 ± 6.5 units/ml; $P < 0.05$) (Figure 4).

Clinical, serologic, and DIF findings in CPN patients. All CPN patients were positive for anti-PS/PT antibody and/or LAC (Table 1). Of the 16 CPN patients, LAC activity was observed in 7 patients (43.8%). Serum anti-β_2GPI/CL antibody was not detected in any of the CPN patients. There was a significant positive correlation between serum IgM anti-PS/PT antibody and CRP levels in LAC-positive CPN patients ($r_s = 0.83$, $P = 0.021$) (Figure 5). Subcutaneous nodule on the lower extremities was the most common skin manifestation and was observed in all CPN patients. Fourteen (87.5%) of the 16 patients had livedo reticularis. Eight (50%) of these patients had skin ulcers, 5 (31.3%) had purpuric lesions ranging from petechiae to extensive ecchymosis. Eleven (68.8%) of the CPN patients had arthralgia and 5 (31.2%) had myalgia. However, the clinical severity of the cutaneous findings did not always seem to correlate with other indicators, such as arthralgia and myalgia. There was no evidence of MPO ANCA or proteinase 3 ANCA in any of the CPN patients. A DIF study performed on skin biopsy samples in 7 (77.8%) of 9 CPN patients showed deposits of C3 within necrotizing vasculitis. In addition, 3 CPN patients (patients 1, 7, and 12) showed IgM deposits within vessels in the lesions. No deposits were detected at the dermoepidermal junction.

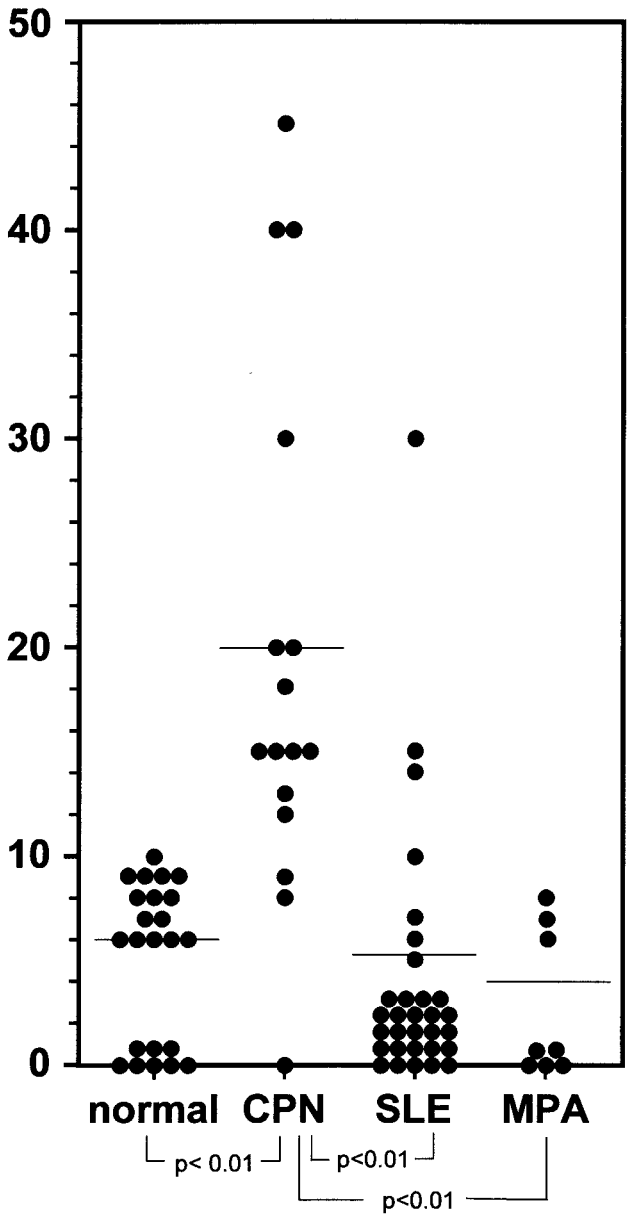

Figure 1. IgM antibodies against phosphatidylserine-prothrombin (PS/PT) in serum samples from normal controls and patients with cutaneous polyarteritis nodosa (CPN), systemic lupus erythematosus (SLE), and microscopic polyangiitis (MPA). Anti-PS/PT antibody levels were determined by enzyme-linked immunosorbent assay. The detection limit was 5 units/ml and the cutoff value was 10 units/ml. The horizontal lines indicate the mean value in each group.

DISCUSSION

The etiology and pathogenic mechanisms responsible for CPN development have yet to be determined. In the present study, anti-PS/PT antibody and/or LAC were detected in all of our CPN patients, whereas they were not detected in any of the normal controls. There was a significantly higher presence of IgM anti-PS/PT antibody in CPN patients compared with SLE patients ($P < 0.01$) (Figure 1). Furthermore, we found a significant correlation between serum IgM anti-PS/PT antibody and CRP level in the 7 LAC-positive CPN patients (Figure 5). CRP is an inflammatory marker and an elevated CRP titer likely contributes to the aggressive clinical condition. IgM anti-PS/PT antibody in patients with CPN may reflect disease activity and play a pathogenic role.

Anionic phospholipids such as cardiolipin and phosphatidylserine, which are not normally expressed on the surface of viable cells, are translocated to the surface of the plasma membrane of cells during apoptosis (16). Recent studies have demonstrated that β_2GPI or prothrombin binds specifically to the surface of apoptotic cells (17,18).

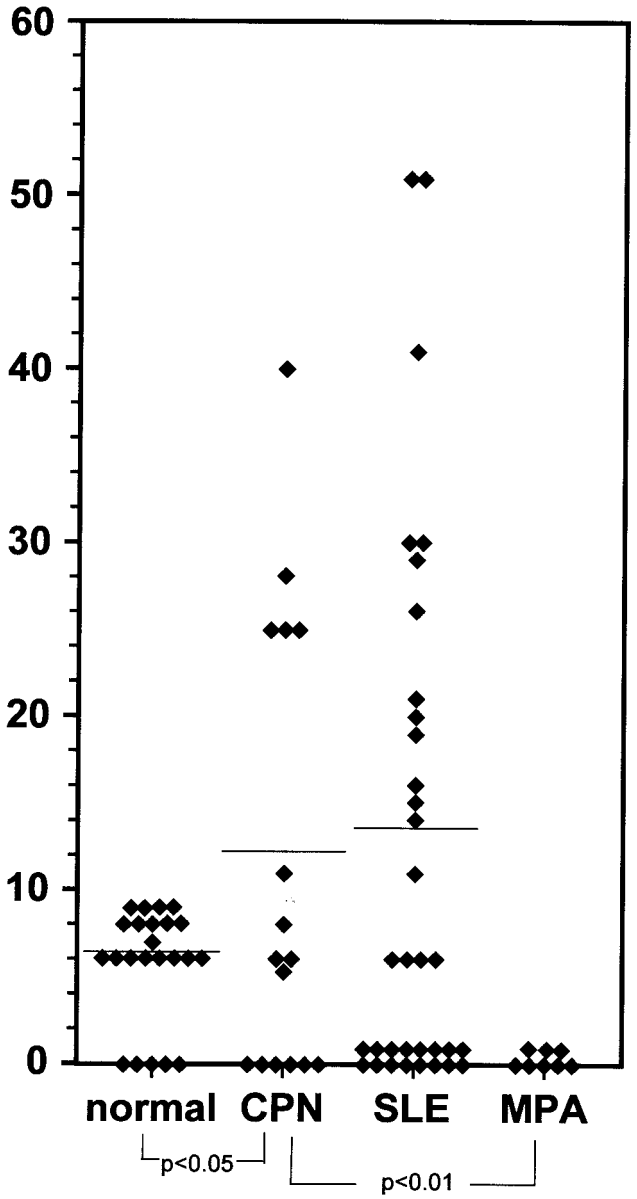

Figure 2. IgG anti–phosphatidylserine-prothrombin antibody levels expressed as relative optical density were measured by enzyme-linked immunosorbent assay in serum samples from normal controls and patients with cutaneous polyarteritis nodosa (CPN), systemic lupus erythematosus (SLE), and microscopic polyangiitis (MPA). The detection limit was 5 units/ml and the cutoff value was 12 units/ml. The horizontal lines indicate the mean value in each group.

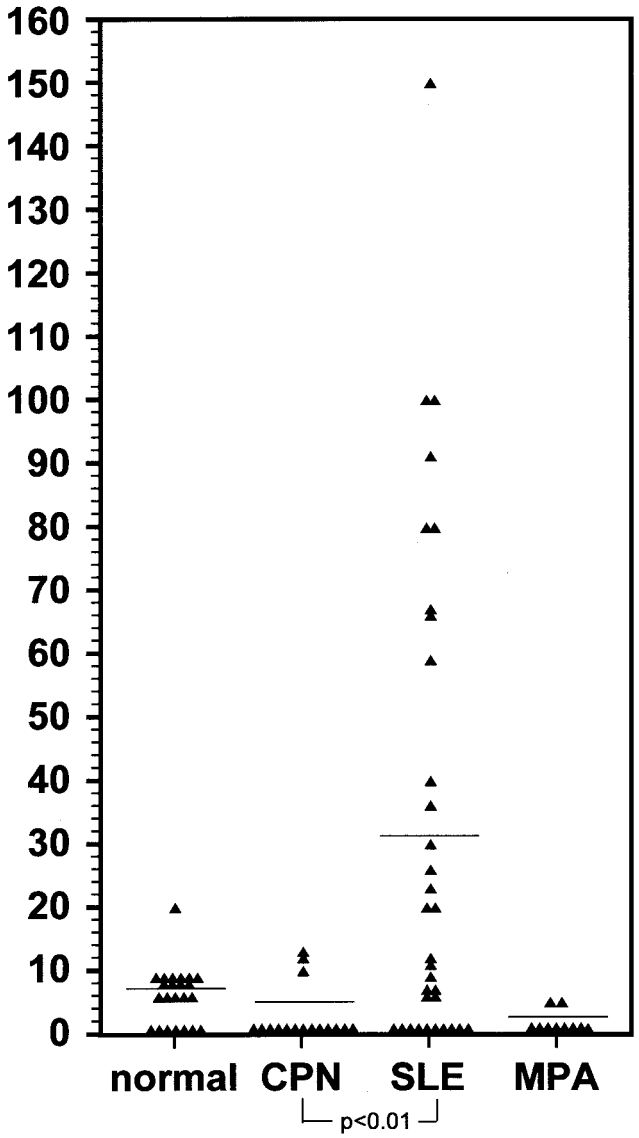

Figure 3. IgG anticardiolipin antibody levels were measured by enzyme-linked immunosorbent assay in serum samples from normal controls and patients with cutaneous polyarteritis nodosa (CPN), systemic lupus erythematosus (SLE), and microscopic polyangiitis (MPA). The detection limit was 5 units/ml and the cutoff value was 10 units/ml. The horizontal lines indicate the mean value in each group.

Although antibodies against β_2GPI/CL and/or PS/PT are major components of LAC, the main pathogenesis of CPN may be dependent on anti-PS/PT antibody but not β_2GPI/CL. Because β_2GPI that combines in apoptotic cells is immunogenic and can induce anti-β_2GPI/CL antibody (19), we believe that prothrombin bound to apoptotic endothelial cells is also immunogenic and causes anti-PS/PT antibody production in patients with CPN. DIF studies demonstrated elevated complement and/or immunoglobulin expression on damaged endothelial cells within necrotizing vasculitis lesions in 7 (77.8%) of 9 patients with CPN. Based on the DIF findings, we believe that a localized Arthus phenomenon may be involved in CPN. These findings indicate that a complement system has been activated via the classical pathway in patients with CPN. Immunoglobulins may represent extravasation of serum components trapped in fibrinoid deposits around the injured vessels. We suggest that complement activation might be due to immunoglobulin aggregates, which would probably be locally produced. In patients with CPN, anti-PS/PT antibodies bind to prothrombin complexes on the membranes of activated platelets and influence platelet activation and aggregation. We speculated that the presence of immunoglobulins in vasculitis lesions of patients with CPN may be a phenomenon that is somehow related to the pathogenesis of the disease process. ANCA titer closely correlates with MPA disease activity and patients who are

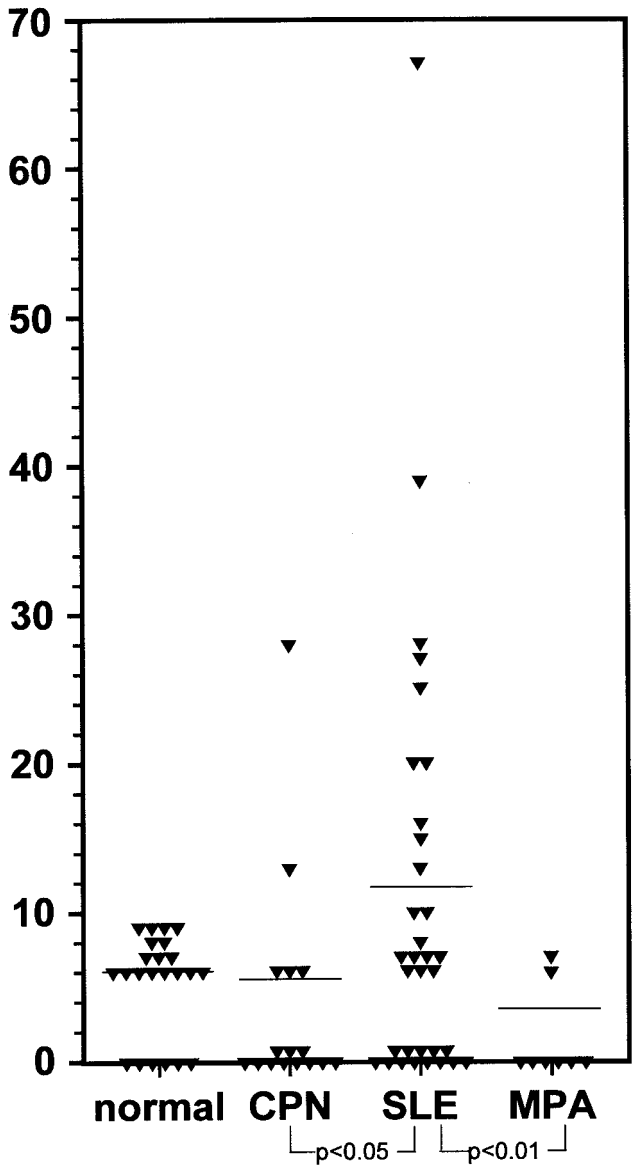

Figure 4. IgM anticardiolipin antibody levels were measured by enzyme-linked immunosorbent assay in serum samples from normal controls and patients with cutaneous polyarteritis nodosa (CPN), systemic lupus erythematosus (SLE), and microscopic polyangiitis (MPA). The detection limit was 5 units/ml and the cutoff value was 10 units/ml. The horizontal lines indicate the mean value in each group.

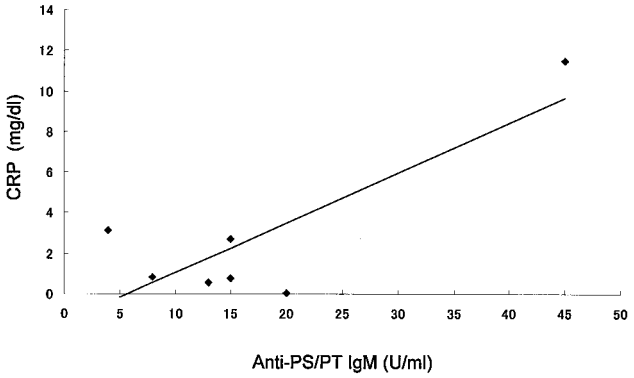

Figure 5. Positive correlation between serum IgM anti–phosphatidylserine-prothrombin (anti-PS/PT) antibody and C-reactive protein (CRP) levels in lupus anticoagulant–positive patients with cutaneous polyarteritis nodosa. Serum IgM anti-PS/PT antibody levels are shown on the ordinate, and serum CRP levels are shown on the abscissa ($r_s = 0.83$, $P = 0.021$).

persistently ANCA positive during remission are prone to relapses (20). In the present study, anti-PS/PT antibody was not detected in any of our MPA patients. Based on these data, we speculate that anti-PS/PT antibodies will become widely recognized as a new factor when diagnosing CPN. The treatment for anti-PS/PT antibodies such as warfarin therapy could have an effect in patients with CPN.

Livedo reticularis is a well-known, relatively common physical finding consisting of macular, violaceous, connecting rings that form a net-like pattern. This physical sign is a marker for vascular disease, both inflammatory vasculitis and thrombotic vascular disease (21). There are many potential causes, which can make the evaluation of a patient presenting with this finding very difficult (22). The Eleventh International Congress on antiphospholipid antibodies (aPL) defined aPL-associated livedo reticularis and advised subclassification of livedo reticularis variants for clinical studies (23). In the present series, livedo reticularis was detected in 14 (87.5%) of the 16 patients with CPN. The clinical appearance of livedo reticularis is caused by the anatomy and physiology of the cutaneous microvascular system. Anything that increases the visibility of the venous plexus can result in a livedo appearance. Our clinical data suggest that the association between microvascular occlusions and cutaneous vessel vasculitis has a predictive value for livedo reticularis pathogenesis in patients with CPN. Furthermore, the 2 pathologies, thrombosis and vasculitis, might be closely associated with each other in CPN disease activities. The thrombotic process may be the trigger for the development of cutaneous necrotizing vasculitis to a certain extent.

AUTHOR CONTRIBUTIONS

Dr. Kawakami had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Kawakami.
Acquisition of data. Kawakami, Yamazaki.
Analysis and interpretation of data. Kawakami, Yamazaki.
Manuscript preparation. Kawakami, Mizoguchi, Soma.
Statistical analysis. Kawakami.

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