Antiphosphatidylserine-Prothrombin Complex Antibodies in 3 Patients With Behçet Disease Involving Superficial Vein Thrombophlebitis

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Background: Superficial vein thrombophlebitis is the common vascular symptom in Behçet disease and is characterized as erythema nodosum-like eruptions. Some studies have reported the presence of antiphospholipid antibodies (Abs) in patients with Behçet disease.

Observations: We measured lupus anticoagulant, anticardiolipin, anti-β₂-glycoprotein I, and antiphosphatidylserine-prothrombin complex antibody (Ab) levels in 3 patients with Behçet disease involving superficial vein thrombophlebitis. High levels of IgM antiphosphatidylserine-prothrombin complex Abs were found (mean [SD], 50.3 [43.1] U/mL; normal, <10 U/mL). One of the patients with Behçet disease was positive for both IgM and IgG antiphosphatidylserine-prothrombin complex Abs, and 2 were positive for lupus anticoagulant. Two patients were also positive for IgM anticardiolipin Abs, but the titers were low. In contrast, none of the patients with Behçet disease was positive for IgG anticardiolipin Abs or IgG or IgM anti-β₂-glycoprotein I Abs.

Conclusions: A high titer of IgM antiphosphatidylserine-prothrombin complex Abs was found in our patients with Behçet disease involving superficial vein thrombophlebitis. We speculate that there is a relationship between the antiphospholipid Abs, especially IgM antiphosphatidylserine-prothrombin complex Abs, and superficial vein thrombophlebitis complications in Behçet disease. This study suggests that elevated serum antiphosphatidylserine-prothrombin complex Ab levels might play some role in the development of the vascular manifestations in Behçet disease.

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Behçet Disease (BD) is a multisystem, inflammatory disorder characterized by recurrent oral aphthous ulcers and at least 2 of the following features: recurrent genital ulcers, eye lesions, skin lesions, and a positive pathergy test result. Some authors suggest that the common histopathological lesion in all organ systems of the patient with BD is vasculitis.1,2 It is believed that much of the pathologic process in BD may be secondary to vasculitis.3 Vessel lesions of various sizes, such as small-vessel vasculitis and large venous or arterial lesions, can be involved, and venous lesions are a characteristic manifestation of the disease.4,5 The common cutaneous manifestations were characterized as erythema nodosum-like eruptions, a purpuric pathergic tissue reaction to needle trauma, oral and genital ulceration, and acneform folliculitis. Microscopic examination of the erythema nodosum-like lesions in the lower extremities reveals superficial vein thrombophlebitis (SVT) in the deep dermis to subcutaneous fat and small-vessel vasculitis involving, in particular, the venules, with perivascular neutrophilic and lymphocytic infiltrations in the vessel walls. The cutaneous vasculitis in BD is predominantly venulitis or thrombophlebitis with relative sparing of the arterial compartment.3 Superficial vein thrombophlebitis tends to be the common cutaneous vascular symptom in BD, although the mechanism is unknown.

Lupus anticoagulant (LAC) activity detected by a phospholipid-dependent coagulation assay is heterogeneous with respect to the specificities and functional capacities of the antibodies (Abs).6 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.7 Detection of antiphospholipid cofactor Abs in addition to the classic anticardiolipin (aCL) Abs and LAC seems to be of considerable clinical importance. Atsumi et al8 and Amengual et al9 suggested that antiphosphatidylserine-prothrombin com-
plex (aPS/PT) Abs, rather than antiprothrombin Abs alone, are associated with symptoms of LAC activity. Some studies have reported the presence of antiphospholipid (aPL) Abs in patients with BD.10,11 Other reports have indicated that erythema nodosum–like lesions with BD in the lower extremities resemble cutaneous polyarteritis nodosa.12,13 We previously reported a high titer of aPS/PT Abs in patients with cutaneous polyarteritis nodosa.14

In this study, we investigated LAC and levels of aCL Abs, aPS/PT Abs, and anti–β2-glycoprotein I (aβ2GPI) Abs in the serum of the 3 patients with BD involving SVT. To our knowledge, serum levels of aPS/PT Abs in patients with BD have not been previously investigated.

REPORT OF CASES

CASE 1

A 32-year-old Japanese man presented with recurrent fever, erythema nodosum–like lesions, and orogenital ulcerations. He had a 6-year history of recurrent oral aphthous ulcers and acneiform lesions. The patient complained of recent arthralgia of his ankles and knees, as well as myalgias on both his legs. A physical examination revealed erythematous macules on his lower extremities, several ulcers in the oral cavity, and ulcers on the right scrotum (Figure 1A). The results of a pathergy test were positive. A biopsy specimen of the macules showed medium-sized vessel vasculitis and thrombi with a mild to moderate inflammatory cell infiltrate in the septa and lobules of the subcutaneous adipose tissue (Figure 1B).

CASE 2

A 28-year-old Japanese man with a 4-week history of fever was admitted to our hospital. The patient reported having skin eruptions, myalgias, and arthralgias on his lower extremities for approximately 3 weeks. The results of a pathergy test were positive. Physical examination revealed erythematous macules scattered over his legs (Figure 2A). Histopathological analysis revealed a medium-sized, thrombosed blood vessel with moderate cell infiltration and panniculitis in the dermis to subcutaneous fat (Figure 2B). Elastic–van Gieson staining revealed local irregular internal elastic lamina in the thick-walled vessels (Figure 2C).

CASE 3

A 53-year-old Japanese woman with a 1-month history of recurrent, tender nodules on her lower extremities and of recurrent aphthous ulcers of her oral mucosa, and genital ulcers was referred to our clinic. She has been thrombocytopenic for several years and had had a late miscarriage. Physical examination revealed multiple erythema nodosum–like lesions and livedo reticularis over both thighs and legs (Figure 3A). In addition, she had be-
gun to experience arthralgias and myalgias, with cutaneous eruptions on her legs and feet. Pathergy test results were positive. A skin biopsy specimen obtained from the erythema of her right lower extremity demonstrated SVT in the subcutaneous fat (Figure 3B). There was an inner elastic lamina confirming that the blood vessel was a vein (Figure 3C).

LABORATORY FINDINGS

The following blood tests in all 3 patients were within the normal range or negative: thyroid function; liver function; cryoglobulins; complement fractions C3/C4/CH50; antinuclear Abs; anti-DNA, anti-SSA, anti-SSB, anti-RNP, and anti-Sm antineutrophil cytoplasmic Abs; serological tests for hepatitis B and C, human cytomegalovirus, human immunodeficiency virus, and Epstein-Barr virus; prostatic-specific antigen; and carcinoembryonic antigen. None of the patients demonstrated any abnormal evidence of prothrombin time, partial thromboplastin time, protein S and protein C activity, antithrombin III, factor V Leiden (resistance to activated factor V), prothrombin gene mutation (G20210A), and homocysteine.

METHODS

The diagnoses in the 3 patients with BD were made according to criteria proposed by the International Study Group for BD.15
Each patient presented with erythema nodosum–like lesions on their lower extremities. We found SVT in all 3 patients, based on the presence of cutaneous lesions in their skin biopsy specimens. The 3 patients did not have any other vascular involvement including arterial and venous thrombosis. All plasma and serum samples were collected and immediately centrifuged at 3000 rpm for 30 minutes at 4°C. After filtration, aliquots of platelet-free plasma were stored at −70°C until used for the LAC clotting tests. According to guidelines recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibody, LAC was screened by measuring diluted Russell viper venom time and kaolin clotting time and was confirmed by mixing studies and demonstration of phospholipid dependence. IgG and IgM (IgG/IgM) aPS/PT Abs and IgG/IgM aCL Abs were measured using a specific enzyme-linked immunosorbent assay (ELISA) (Medical & Biological Laboratories, Nagoya, Japan), according to the manufacturer’s protocols. IgG and IgM aβ2,GPI Abs were determined according to a standardized aβ2,GPI Ab ELISA (Diagnostica Stago, Asnières, France). The cutoff values for IgG/IgM aPS/PT Abs, IgG/IgM aCL Abs, and IgG/IgM aβ2,GPI/CL Abs were set at 12 U/mL and 10 U/mL, 10 U/mL and 1 U/mL, and 10 U/mL, respectively. All data are expressed as means and standard deviations.

The experimental protocol was approved by the St Marianna University ethics committee, and informed consent was obtained from all patients.

### RESULTS

High titers of serum IgM aPS/PT in the patients with BD (50.3 [43.1] U/mL) were detected (Table). One of the patients with BD (case 1) was positive for both IgM and IgG aPS/PT Abs. Two patients (cases 2 and 3) were positive for LAC. Two of the 3 patients with BD were positive for IgG aCL Abs, but the titers (13 U/mL and 10 U/mL) were relatively low. None of the patients was positive for IgG aCL Abs or IgG/IgM aβ2,GPI Abs.

### COMMENT

The 3 patients with BD in our study showed skin biopsy–proven SVT in their erythema nodosum–like lesions, and we detected high titers of IgM aPS/PT Abs in their serum samples. In contrast, while we detected IgG aCL Abs in 2 of our patients, the titer was not high. Zouboulis et al. reported high IgM aCL Ab titers in patients with both BD and erythema nodosum. We detected LAC in the 2 patients who had erythema nodosum–like eruptions. Mader et al. reported that their patients with BD were not positive for LAC. Ethnic and geographical differences in BD related to the clinical manifestations are well known, and LAC and aCL Abs might be influenced by those factors. In the present study, we speculate on the relationships between the presence of aPL Abs, especially IgM aPS/PT Abs, and the SVT complication in BD. We propose that serum aPS/PT Abs could have an important role as biomarkers of disease and should become more widely available. It is important for clinicians to determine these titers to permit early vascular complication and treatment.

Behçet disease is a multisystem inflammatory disorder of unknown etiology that is sometimes associated with thrombosis and vasculitis. Some studies have suggested that BD should be accepted as a hypercoagulable or prothrombotic state. Recently, factor V Leiden mutation, prothrombin gene mutation, and homocysteine have been described in the pathogenesis of thrombosis in BD. In general, aPL Abs are regarded as activating endothelial cells, thus creating a hypercoagulable state. We have previously suggested an association between microvascular occlusions and cutaneous vessel vasculitis in the presence of a high level of aPS/PT Abs. Phosphatidylserine is a regular constituent of the inner leaflet of the cell membrane, which is only exposed on the outside of the cell membrane during apoptosis or by damaged endothelial cells. Some studies have shown that prothrombin binds specifically to the surface of apoptotic cells. We believe that prothrombin binds to apoptotic endothelial cells and combines phosphatidylserine in the SVT. The complexes may cause IgM aPS/PT Ab production in SVT, which would probably be locally produced. These conditions, comprising SVT and elevated aPS/PT Ab level, may be closely related to the pathogenic factors that trigger the development of vasculitis and thrombosis. This study reflects pilot data on a limited number of patients. More experimental work in combination with clinical, histopathological and serological observations is required to further elucidate the role of aPL Abs in BD.

**Table. Lupus Anticoagulant (LAC) and Antiphospholipid Antibodies in 3 Patients With Behçet Disease Involving Superficial Vein Thrombophlebitis**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>LAC</th>
<th>IgG aPS/PT</th>
<th>IgM aPS/PT</th>
<th>IgG aCL</th>
<th>IgM aCL</th>
<th>IgG aβ2,GPI</th>
<th>IgM aβ2,GPI</th>
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<tr>
<td>1/M/32</td>
<td>−</td>
<td>20</td>
<td>28</td>
<td>−</td>
<td>13</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2/M/28</td>
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<td>23</td>
<td></td>
<td>−</td>
<td>11</td>
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<td>100</td>
<td></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</table>

Abbreviations: aβ2,GPI, anti-β2-glycoprotein I; aCL, anticardiolipin; aPS/PT, antiphosphatidylserine-prothrombin complex; +, positive; −, negative.

a The numbers in the Table indicate serum titer levels (units per milliliter) measured by enzyme-linked immunosorbent assay. The minus sign indicates that the titer was within normal levels or undetectable.

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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 analy-
sis. Study concept and design: Kawakami, Mizoguchi, and Soma. Acquisition of data: Kawakami and Yamazaki. Analysis and interpretation of data: Kawakami and Yamazaki. Drafting of the manuscript: Kawakami and Mizoguchi. Critical revision of the manuscript for important intellectual content: Kawakami and Yamazaki. Statistical analysis: Kawakami and Yamazaki. Administrative, technical, and material support: Kawakami and Soma. Study supervision: Kawakami, Mizoguchi, and Soma.

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