

## Seronegative Antiphospholipid Syndrome with Anti-phosphatidylethanolamine Antibody in a Boy

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Antiphospholipid syndrome (APS) is an autoimmune disease caused by antiphospholipid antibodies. At our institution, APS is diagnosed on the basis of the Sapporo criteria, which consist of thrombosis and recurrent pregnancy-related complications and the following laboratory findings: the presence of lupus anticoagulant, anticardiolipin antibody, or anti- $\beta_2$  glycoprotein 1 antibody. However, we sometimes treat patients we strongly suspect of having APS but who do not satisfy the laboratory criteria. To accommodate such suspected cases, a subtype of APS termed seronegative APS has been proposed. Here, we report on a man with chronic thrombocytopenic purpura since the age of 3 years and multiple cerebral infarctions since the age of 14 years who finally received a diagnosis of seronegative APS with positive antiphosphatidylethanolamine antibodies. (J Nippon Med Sch 2015; 82: 117–120)

**Key words:** antiphosphatidylethanolamine antibody, chronic thrombocytopenia, cerebral infarctions, seronegative antiphospholipid syndrome

### Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease caused by antiphospholipid antibodies; APS is associated with thrombosis and recurrent pregnancy-related complications<sup>1</sup>. At our institution, APS is diagnosed on the basis of the Sapporo criteria<sup>1</sup>, which consist of the above clinical manifestations and the following laboratory findings: the presence of lupus anticoagulant, anticardiolipin antibody, or anti- $\beta_2$  glycoprotein 1 antibody<sup>1</sup>. However, we sometimes treat patients we strongly suspect of having APS but who do not satisfy the laboratory criteria<sup>2,3</sup>. To accommodate such suspected cases of APS, Hughes et al have proposed a subtype of APS termed seronegative APS (SNAPS)<sup>3</sup>. The clinical course of SNAPS is reportedly similar to that of classic APS. In patients with SNAPS, the involvement of several other antigens, ranging from anionic phospholipids to phospholipid-protein complexes and plasma proteins, has been reported<sup>1</sup>. We describe herein a 23-year-old man with thrombocytopenic purpura since the age of 3 years and multiple cerebral infarctions since the age of 14 years

who finally received a diagnosis of SNAPS with antiphosphatidylethanolamine (anti-PE) antibodies when he was 21 years old. We obtained informed consent from the patient for publication.

### Case Presentation

A 21-year-old man was re-admitted to our hospital with difficulty speaking and numbness in both the hands and the lower limbs below the knee. At 3 years of age, he had exhibited purpura in the extremities after incidental nontraumatic contact, and immune thrombocytopenic purpura was diagnosed, although bone marrow examination revealed mild hypoplasticity without typical findings of megakaryocytes. He continued to show thrombocytopenia, despite treatment with steroids (prednisolone, 1 mg/kg, or pulse therapy with methylprednisolone) or intravenous gamma globulin or both.

At the age of 14 years, he had complained of numbness in the right hand. On admission at that time physical examination revealed: blood pressure, 152/84 mm Hg; height, 174.2 cm; and weight, 97.2 kg. Other than the

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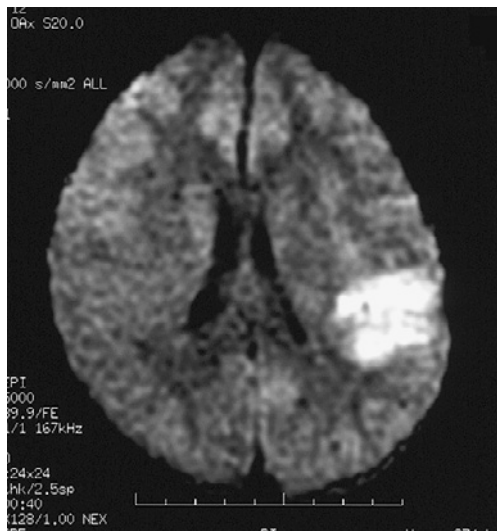


Fig. 1 Diffusion-Weighted MRI findings of first cerebral ischemia attack at 14 years of age. MRI examination at 2 days after cerebral ischemia attack showed high intensity signals in left parietal lobe on DWI, indicating acute cerebral ischemic changes.

numbness of the right hand, no neurological abnormalities were noted. The results of laboratory examination were as follows: C-reactive protein, 0.23 mg/dL; white blood cells, 3,430 / $\mu$ L; red blood cells,  $379 \times 10^4$  / $\mu$ L; hemoglobin, 12.0 g/dL; hematocrit, 38.5%; platelets,  $4.3 \times 10^4$  / $\mu$ L; aspartate aminotransferase, 51 IU/L; alanine aminotransferase, 47 IU/L; lactate dehydrogenase, 515 IU/L; alkaline phosphatase, 518 U/l; creatine phosphokinase, 229 U/l; antinuclear antibody, 80x; anti-DNA antibody, <2 IU/mL; lactate dehydrogenase test, negative; Rheumatoid Arthritis test, negative; rheumatoid factor, 16 X; C3, 154 mg/dL; C4, 48.6 mg/dL; CH50, 49 U/mL; perinuclear antineutrophilic cytoplasmic antibody, <13 U/mL; and cytoplasmic antineutrophilic cytoplasmic antibody, <13 U/mL. With regard to coagulation,  $\beta$ -thromboglobulin was 46.0 ng/mL (<50 ng/mL), platelet factor 4 was 15.5 ng/mL (<20 ng/mL), and coagulation factors VII, VIII, IX, X, XI and XII, and von Willebrand factor showed normal ranges. Anticardiolipin antibody immunoglobulin (Ig) M was 4 U/mL (normal: <8), anticardiolipin antibody IgG was 12 U/mL (normal, <10 U/mL), lupus anticoagulant test was 1.5 (normal, <1.3), and anti- $\beta$ -2-glycoprotein I antibodies were <1.3 U/mL. Diffusion-weighted magnetic resonance imaging (MRI) 2 days after admission showed high-intensity signals in the left parietal lobe, indicating an acute cerebral ischemic change (Fig. 1).

We diagnosed cerebral infarction and treated the pa-

tient with concentrated glycerin and edaravone without heparin and discontinued danazol. Transesophageal echocardiography showed no intracardiac thrombosis. By 1 week after admission, the patient's condition had improved, and we discontinued concentrated glycerin and edaravone. However, 2 days later, he had generalized tonic-clonic convulsions for 5 minutes. We therefore re-administered concentrated glycerin and edaravone with an anticonvulsant. An electroencephalogram was normal. Multiple, old cerebral infarctions were found with MRI 2 weeks after the convulsions. On discharge, he had no complaint of left hand numbness or speech difficulties but reported needing more time to convert from hiragana (Japanese phonetic symbols) words to Roman letters when typing E-mail on his cellular phone for input.

At the age of 15 years, he again had a cerebral infarction. We strongly suspected APS; however, the Sapporo criteria were not fulfilled because of a lack of persistent positivity for lupus anticoagulant, anticardiolipin, or anti- $\beta$ -2-glycoprotein I antibodies.

At the age of 21 years, he had a fifth cerebral ischemia attack and was again admitted to our hospital. Diffusion-weighted MRI revealed high-intensity signals in the left lobe of the cerebellum, the right subcortical area of the frontal lobe, the right occipital lobe, and the subcortical area of the parietal lobe (Fig. 2a, b, c, d). However, the concentration of anticardiolipin IgG antibodies was 6 U/mL (normal: <106 U/mL) and that of anti- $\beta$ -2-glycoprotein I antibodies were <1.3 U/mL (negative). Because we noted looseness of the facial skin, we performed a skin biopsy and chromosomal examination. The skin biopsy showed a lack of elastin, and chromosomal analysis revealed 46, XY, i (7) (q10). Although the body-mass index was high (32.1 kg/m<sup>2</sup>; body weight, 98.4 kg; body height, 175 cm), the findings of low total cholesterol (119 mg/dL), high-density lipoprotein cholesterol (28 mg/dL), the absence of atherosclerosis on cervical MRI, and a normal echocardiogram indicated that the cause of the stroke was not atherosclerosis.

Because we suspected SNAPS, we measured levels of anti-PE IgG (normal range <0.36 U/mL) and IgM antibodies (normal range <0.456 U/mL) with an enzyme-linked immunosorbent assay with or without kininogens using a method previously described<sup>45</sup>. The PE was purchased from Avanti Polar Lipids (Birmingham, AL, USA), and phosphatidylserine and alkaline phosphatase-conjugated monoclonal antibodies to human IgG and IgM were purchased Sigma Chemical Co). Anti-PE-IgG titer was 0.189 with addition of kininogen for making the

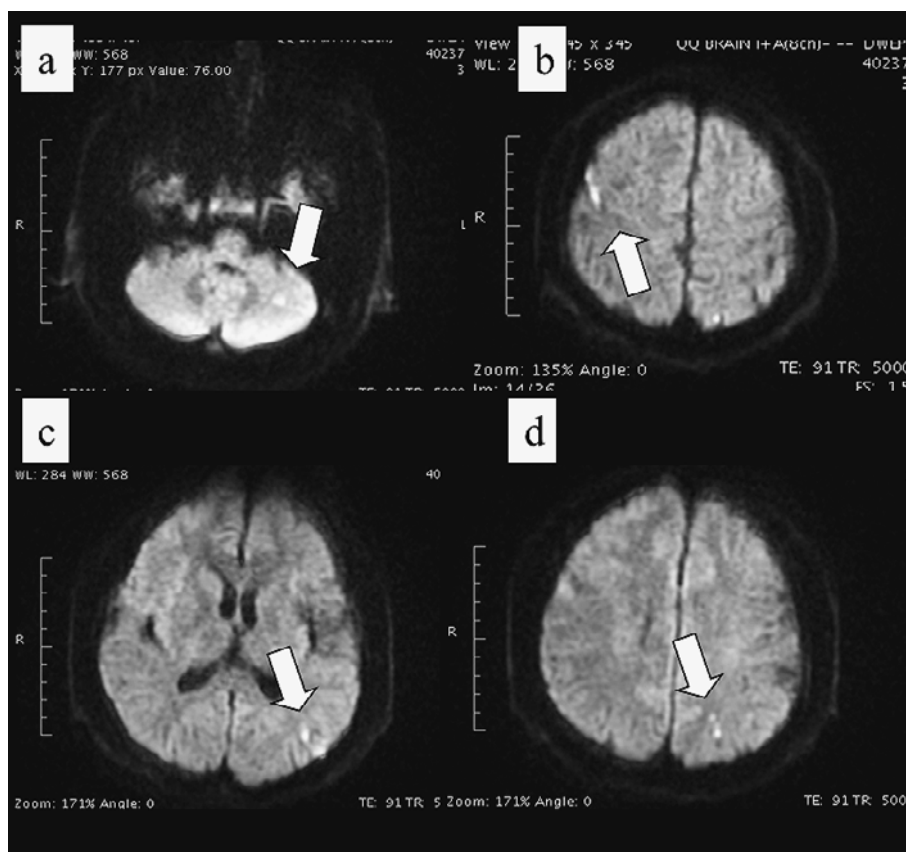


Fig. 2 a. Diffusion-Weighted MRI findings for fifth cerebral ischemia attack at 21 years of age. High intensity in the left lobe of the cerebellum was noted.  
 b. Diffusion-Weighted MRI findings for fifth cerebral ischemia attack at 21 years of age. High intensity in the right subcortical area of the frontal lobe was noted.  
 c. Diffusion-Weighted MRI findings for fifth cerebral ischemia attack at 21 years of age. High intensity in the right occipital lobe was noted.  
 d. Diffusion-Weighted MRI findings for fifth cerebral ischemia attack at 21 years of age. High intensity in the subcortical area of the parietal lobe was noted.

kininogen-platelet complexes, 0.255 without kininogen, and anti-PE-IgM titer was 0.811 with addition of kininogen and 0.459 without kininogen<sup>5</sup>. We therefore diagnosed serum negative antiphospholipid syndrome (SNAPS) with positivity for anti-phosphatidylethanolamine antibody.

### Discussion

APS is characterized by diffuse thrombosis, recurrent fetal loss, and the persistent presence of anti-phospholipid antibodies<sup>1</sup>. On the basis of the Sapporo criteria, however, patients with clinical features highly suggestive of APS but who test negative for anti-phospholipid antibodies cannot be classified as having APS; Hughes et al proposed classifying such patients as having seronegative APS (SNAPS)<sup>3</sup>. Several studies have examined the relationship between anti-PE antibody and thrombosis, which is a major clinical feature of APS<sup>6</sup>. Other studies

have focused on anti-PE antibodies as a potential alternative laboratory criterion for diagnosing APS<sup>7</sup>. In one study, positivity for anti-phospholipid antibodies was confirmed in 58.3% of patients with SNAPS, with anti-PE antibodies being detected in 30.5% of these patients<sup>8</sup>. Thus, the value of positivity for anti-PE antibodies in patients with SNAPS remains a controversial topic. We treated a male patient who had had chronic thrombocytopenic purpura from the age of 3 years and multiple cerebral infarctions from the age of 14 years, and in whom SNAPS with anti-PE antibodies was finally diagnosed when he was 21 years old. As mentioned above, several reports have indicated that the clinical manifestations of APS are caused by anti-PE antibodies<sup>5</sup>. PE is found mainly in the inner leaflets of mammalian plasma membranes, and it accounts for 20% to 50% of total phospholipids. PE works as an anticoagulant, enhancing activated protein C (APC) activity in blood coagulation

reactions, downregulating procoagulant function<sup>9</sup>, and inhibiting factor Xa-prothrombin (PT)<sup>10</sup>. Cell surface-exposed PE plays a major role in translocating protein C inhibitor proteins across the plasma membrane<sup>10</sup>.

In our patient, anti-PE-IgM was positive only when kininogen was added, so we considered the anti-PE-IgM to be an anti-Kininogen-PE complex-specific antibody. Kininogen-dependent anti-PE antibodies augment thrombin-induced platelet aggregation in vitro, and kininogens bind to platelets and inhibit thrombin-induced platelet aggregation<sup>5</sup>. Therefore, kininogen-dependent anti-PE antibodies may cause thrombosis in vivo by disrupting the normal antithrombotic effects of kininogen<sup>5</sup>.

Cerebral ischemia is a major APS-related event. The biological links between the presence of PE antibodies and the occurrence of thrombosis are unclear. However, the role of PE antibodies in thrombotic events may be due to the prothrombotic activity of a subpopulation of anti-phospholipid antibodies via selective inhibition of the protein C anticoagulant pathway<sup>9</sup>. This hypothesis is supported by the fact that the presence of PE enhances the anticoagulant function of APC, and by the ability of anti-phospholipids to inhibit APC activity in the presence of PE<sup>9</sup>.

In conclusion, our study indicates that anti-PE antibody is a marker of SNAPS in patients, including children, with unexplained thrombosis in the absence of anti-anionic phospholipid antibodies and early diagnosis of SNAP using anti-PE antibody might be benefit for the patients with SNAP in terms of prompt treatment.

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