A Case of Antiphospholipid Syndrome Nephropathy related Disease Diagnosed by Assessing Phosphatidylserine-dependent Antiprothrombin Antibodies

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Abstract:
A 42-year-old Japanese woman was admitted for the evaluation of proteinuria. She had a history of four habitual abortions and valvular heart disease, including severe mitral regurgitation and moderate tricuspid regurgitation. A kidney biopsy showed fibrointimal thickening of interlobular arteries, fibrin thrombosis, and associated focal segmental sclerosis. Although the standard test for antiphospholipid (aPL) antibodies was negative, the patient was diagnosed with antiphospholipid syndrome (APS)-related disease by testing for phosphatidylserine dependent anti-prothrombin anticardiolipin antibody, a non-criterial aPL antibody. A kidney biopsy may lead to a diagnosis of APS in patients with negative laboratory test findings for APS.

Key words: antiphospholipid antibody syndrome nephropathy, antiphospholipid syndrome, systemic lupus erythematosus

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Introduction
According to the revised Sapporo classification criteria from 2006, the diagnosis of antiphospholipid syndrome (APS) is based on the presence of specific pregnancy-related morbidities and/or arterial or venous thrombosis in association with persistent antiphospholipid (aPL) antibodies: lupus anticoagulant (LAC) and anticardiolipin IgG/IgM or anti-beta 2-glycoprotein I IgG/IgM antibodies (1). One of the most frequent organ lesions in APS is renal disease, which is referred to as aPL antibody syndrome nephropathy (APSN). Intimal fibrous hyperplasia of interlobular arteries is considered the main chronic lesion of APSN (2-5).

We herein report a patient with a history of habitual abortions in whom a kidney biopsy showed typical findings of APSN. The laboratory tests needed to meet the APS criteria were negative, so APS could not be diagnosed by conventional criteria (1). However, APSN was diagnosed based on the novel marker of aPL antibodies. Therefore, measuring the new marker of aPL antibodies is expected to expand the diagnostic yield of APS.

Case report
A 42-year-old Japanese woman was admitted for the evaluation of proteinuria. She developed a fever and arthritis at 22 years old. At that time, the test for antinuclear anti-
bodies had been positive, but tests for anti-double-stranded deoxyribonucleic acid (ds-DNA) antibodies, anti-Sm antibodies, lupus anticoagulant (LAC), and antiphospholipid antibodies (ACA) were negative. An initial kidney biopsy did not show immune deposits on glomeruli. A diagnosis of systemic lupus erythematosus (SLE) was suspected, so treatment was started with prednisolone 40 mg/day, which was then reduced to 9 mg/day.

At 31 years old, the patient developed heart failure and hypertension, and severe mitral regurgitation was diagnosed. She was prescribed calcium channel blockers, and hypertension was controlled within the reference range (120-130/70-80 mm Hg). Subsequently, she had four habitual abortions.

At 40 years old, the patient had a seizure, and a stroke was suspected. She was hospitalized and started on treatment with aspirin (100 mg/day). During this hospitalization, proteinuria was noted, and lupus nephritis was suspected, so treatment was started with tacrolimus (2 mg/day) and mycophenolate mofetil (1000 mg daily). However, proteinuria persisted.

At the current admission, the patient was 162 cm tall and weighed 46 kg. Her blood pressure was 139/86 mm Hg, and her body temperature was 36.7 °C. A reflux systolic murmur was heard at the apex. Edema was prominent in the lower legs. The laboratory findings were as follows: hemoglobin, 12.4 g/dL; leucocytes, 10400/μL; thrombocytes, 20.6×10^11/μL; schizocytes, negative; serum albumin, 3.9 g/dL; serum creatinine, 1.18 mg/dL; estimated glomerular filtration rate, 40.9 ml/min/1.73 m^2; C-reactive protein, 0.0 mg/dL; prothrombin time 118.7 second (normal value, >75 seconds); and activated partial thromboplastin time 28.1 seconds (reference range, 27-40 seconds); D dimer 4.1 μg/mL (reference range, <0.5). Immunological studies were positive for speckled and homogenous antinuclear antibodies. Tests for anti-ds-DNA antibodies, anti-Smith antibodies, antiribonucleoprotein antibodies, LAC, and beta2-glycoprotein I-dependent ACA were negative. An initial kidney biopsy did not show electron-dense deposits. However, endothelial cell damage, including subendothelial edema in the glomerulus, was slight, and some foot process effacement was observed (Figure h), although in this case, endothelial cell proliferation and fibrosis in the renal interlobular artery were prominent.

**The diagnosis**

APS was suspected because of the patient’s history of habitual abortions and because the main features on a kidney biopsy-chronic microvascular lesions, intraglomerular thrombosis, and associated perihilar valiant of focal segmental glomerulosclerosis (FSGS)-were typical of APSN (1). Furthermore, there was no immunoglobulin deposition corresponding to lupus nephritis. However, according to the conventional diagnostic criteria, APS could not be diagnosed, as the conventional aPL antibody assay was negative (1).

**Diagnosing APS by assessing aPL antibodies**

Because the routine aPL antibody assay was negative, we arranged for a detailed aPL antibody assay to be performed. Tests for lupus anticoagulant, dilute Russell viper venom time, anti-cardiolipin IgG, anti-cardiolipin IgM, and phosphatidylserine-dependent anti-prothrombin (PS/PT) IgM ACA were all negative, but the test for PS/PT IgG ACA was positive (21.2 mg/dL; normal value, <1.20 mg/dL) (6). Therefore, the patient was diagnosed with APS-related disease. Although the conventional diagnostic criteria using a routine aPL antibody assay call for measurements to be performed at least twice, only one sample was measured with this antibody assay system.

**Clinical course**

The cause of proteinuria was considered to be FSGS related to APSN rather than lupus nephritis, so tacrolimus, and prednisolone were discontinued, and warfarin was started and maintained at a dose of 3 mg/day with a prothrombin time-international normalized ratio of 2.0. Leg edema improved after warfarin administration, but proteinuria did not decrease, and the renal function progressed slowly. There

**Kidney biopsy findings**

A light microscopic examination showed tubulointerstitial fibrosis and tubular atrophy in approximately 30% of the cortical area (Figure a). Cellular and fibroelastic intimal thickening (hyperplasia) of the interlobular arteries (Figure b) to the afferent arteriole and vascular pole (pre-glomerular arteriole) was a characteristic finding, as was glomerular capillary collapse (Figure c). Phosphotungstic acid-hematoxylin stain-positive fibrin thrombosis of the vascular pole (preglomerular arteriole) was observed (Figure d). Perihilar-type segmental sclerosis with increased mesangial matrix around the vascular pole was observed in three glomeruli (Figure e). In addition, global sclerosis with pseudotubulization was noted in 8 out of 20 glomeruli (Figure f), and extensive hyaline casts, known as a thyroid-like appearance, were seen (Figure g). These findings were concentrated in areas with advanced vascular and tubular lesions. Immunofluorescence microscopy showed no staining for IgG, IgA, IgM, C3, C4, or C1q. Electron microscopy of the preserved glomeruli did not show electron-dense deposits. However, endothelial cell damage, including subendothelial edema in the glomerulus, was slight, and some foot process effacement was observed (Figure h), although in this case, endothelial cell proliferation and fibrosis in the renal interlobular artery were prominent.

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Figure. a: A light microscopic examination showed strip tubulointerstitial fibrosis (arrow) and tubular atrophy. Masson Trichrome staining: original magnification ×40. b: The interlobular arteries showed cellular and fibroelastic intimal thickening (hyperplasia; arrow) within the internal elastic lamina (fibroelastosis). Masson Trichrome staining: original magnification ×200. c: Occlusion (arrow) or severe narrowing of the afferent arteriole led to glomerular capillary collapse. Masson Trichrome staining: original magnification ×200. d: Phosphotungstic acid Hematoxylin and Eosin staining showed fibrin thrombosis (arrow) of the vascular pole (preglomerular arteriole). Original magnification ×400. e: Glomeruli showed perihilar-type segmental sclerosis with increased mesangial matrix around the vascular pole. Periodic acid methenamine silver and Masson staining: original magnification ×200. f: Global sclerosis with pseudotubulization (arrow) was noted in 8 out of 20 glomeruli. Periodic acid–Schiff (PAS) staining: original magnification ×200. g: Extensive hyaline casts, known as a thyroid-like appearance, were noted. PAS staining: original magnification ×60. h: Electron microscopy of the preserved glomeruli did not show electron-dense deposits, and endothelial cell damage, including subendothelial edema in the glomerulus, was slight (arrow), with some foot process effacement observed.
were no clinical findings suggestive of SLE. At 47 years old, the patient experienced an acute myocardial infarction. However, she has been doing well since then and is satisfied that the moon face symptoms associated with long-term prednisolone use have resolved.

**Discussion**

We presented a case in which APS was diagnosed by performing a detailed assessment of aPL antibodies. Below, we compare the kidney biopsy findings in this patient with those in other reports.

Saracino et al. reviewed the renal histology of five patients with primary APS that was positive for both LAC and anti-β2 glycoprotein I ACA. Intimal fibrous hyperplasia or intimal edema of interlobular arteries was noted in four patients, and FSGS was noted also in four patients. All patients had tubulointerstitial lesions (3). Fakhuri et al. evaluated 29 patients with primary APS that was positive for both LAC and anti-β2 glycoprotein I ACA. Twenty patients showed only typical vascular lesions of APSN, including fibrous intimal hyperplasia, intraglomerular thrombi, and arteriolar thrombi, but the other nine also showed membranous nephropathy, minimal change disease/focal segmental glomerulosclerosis, mesangial C3 nephropathy, and pauci-immune crescentic glomerulonephritis (4). Nochy et al. assessed the renal histopathology of 16 patients with APSN and reported that characteristic findings were intimal fibrous hyperplasia of the interlobular arteries, arteriolar occlusions, fibrin thrombosis of arterioles, and thyroidization in the interstitium. LAC and ACA were present in all cases (5). Daugas et al. examined the renal histopathology of 114 cases of SLE and reported that renal findings of APSN were present in 32%, both in addition to and independently of lupus nephritis. Proliferation of myofibroblasts in the intima with reduction of the lumen of small-caliber arteries was identified as renal histology specific to APSN. Tests for aPL antibodies with LAC and/or ACA were positive in all patients (6).

Taken together, these reports indicate that the presence of fibrous intimal hyperplasia of renal small arteries is characteristic of APS and that tests for LAC and ACA are positive in most patients.

In patients with clinical and kidney biopsy findings specific for APS who did not meet the APS criteria because of negative standard laboratory tests for aPL antibodies, Mishima et al. and Heikal et al. reported detecting IgG and IgM antibodies against the phosphatidylserine/prothrombin complex, which led to a diagnosis of APS (7, 8). Similar to our report, the case reported by Mishima et al. was a woman with a history of two habitual abortions and valvular heart disease, and the kidney biopsy findings correspond to APSN in whom conventional tests for aPL antibodies were negative; the patient was ultimately diagnosed with APS because the PS/PT IgG antibody assay was positive. A PS/PT IgG antibody assay was suggested as another basis for diagnosing APS when clinical findings correspond to APS but the conventional aPL antibody assay was negative (9).

The mechanism by which aPL antibodies are related to vascular lesions in APSN is unknown, but it has been hypothesized to be related to hemodynamics because of the similarity of APSN to malignant hypertension. The vascular lesions of APSN differ from those of typical malignant nephrosclerosis: First, in APSN, the thickened arterial intima tends to be cellular, in contrast to the relatively acellular, densely collagenous intima in malignant nephrosclerosis; second, there tends to be less inner elastic laminating in APSN than in malignant nephrosclerosis. The differential diagnosis between APS and malignant nephrosclerosis must be made on the basis of clinical findings, as malignant nephrosclerosis also occurs in patients with untreated high blood pressure (4, 10).

In conclusion, our patient had a history of four habitual abortions and valvular heart disease, and the kidney biopsy findings were typical of APSN; however, the conventional aPL antibody assay was negative, so the patient could not be diagnosed with APS according to the conventional criteria. However, we described a patient in whom APS was diagnosed by a PS/PT IgG antibody assay, one of the most well characterized non-criterial aPL antibodies. This case indicates that if kidney biopsy findings are similar to those presented here, physicians should consider the possibility of APSN.

**The authors state that they have no Conflict of Interest (COI).**

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**Statement of Ethics**

The present report was produced in conformity with the Declaration of Helsinki, and the patient gave her written consent for this case report to be published.

**Disclosures**

The authors have no competing financial interests or conflicts of interest to declare.

**Abbreviations**

ACA, anticardiolipin antibodies; ds-DNA antibodies, anti-double stranded deoxyribonucleic acid antibodies; APS, antiphospholipid syndrome; aPL antibodies, antiphospholipid antibodies; APSN, antiphospholipid antibody syndrome nephropathy; FSGS, focal segmental glomerulosclerosis; LAC, lupus anticoagulant; LN lupus nephritis; PTAH, phosphotungstic acid-hematoxylin; PS/PT ACA, phosphatidylserine-dependent anti-prothrombin anticardiolipin antibodies; SLE, systemic lupus erythematosus

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