The renal pathology of primary antiphospholipid syndrome: a distinctive form of endothelial injury

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Summary

Some features of the vascular and glomerular pathology of primary antiphospholipid syndrome (APS) are well recognized, but we describe novel glomerular ultrastructural changes that we consider to be pathognomonic of APS. Renal biopsies from eight patients with APS were examined by light and electron microscopy. All had anti-cardiolipin antibodies, and the clinical presentation ranged from fulminant multi-system disease to isolated proteinuria. By light microscopy, the hexamine silver stain showed a combination of glomerular basement membrane wrinkling and reduplication. By electron microscopy, redundant, wrinkled segments of basement membrane were accompanied by a ‘new’ straighter thin basement membrane adjacent to the endothelium. In two cases the presence of these antibodies was not suspected clinically, and there was no clinical history or evidence of a thrombotic microangiopathy. We describe a distinctive glomerular lesion that represents an unexplained form of endothelial injury in this syndrome.

Introduction

Antiphospholipid (anticardiolipin) antibodies can be detected either in patients with systemic autoimmune syndromes such as systemic lupus erythematosus or as isolated phenomena in the so-called primary antiphospholipid syndrome (APS). The laboratory hallmark of this syndrome is a positive test for lupus anticoagulant, the presence of anticoagulant antibodies or both. The presence of these antibodies is associated with pathological changes in the microvasculature of many organs and it is possible that the antibodies themselves are pathogenic, although this is open to some doubt. It is estimated that primary APS may account for 15–20% of all episodes of deep venous thrombosis, a third of new strokes occurring in patients under the age of 50, and 5–15% of cases of recurrent fetal loss. Data regarding the incidence of renal disease are only just emerging. Some of the histopathological changes associated with these antibodies have been described in case reports but have not previously been recognized as diagnostic of the condition. We report distinctive renal histology and ultrastructural pathology in eight patients with the primary antiphospholipid syndrome.

Methods

Patients

The patients were eight adults who presented with a variety of renal and systemic symptoms and were investigated by renal biopsy. All had anticoagulant antibodies but no other systemic autoimmune syndrome (Table 1).

Investigations

The clinical features of these patients and the results of their laboratory investigations were reviewed. The presence of lupus anticoagulant was
Table 1  Clinical features of 8 patients with primary antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex (years)</th>
<th>Clinical features</th>
<th>Livedo Raynaud’s BP (mmHg) [no of BP drugs]</th>
<th>Creatinine (µmol/l)</th>
<th>GFR (ml/min/1.73 m²)</th>
<th>Proteinuria (g/day)</th>
<th>Albumin (g/l)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>45/M</td>
<td>Multi-system involvement: heart, lung, kidney, liver</td>
<td>Yes Yes 150/80 [1]</td>
<td>400</td>
<td>10</td>
<td>2.0</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>69/F</td>
<td>Severe atherosclerosis, hyperlipidaemia</td>
<td>Yes Yes 160/70 [1]</td>
<td>140</td>
<td>43</td>
<td>0.4</td>
<td>41</td>
</tr>
<tr>
<td>3</td>
<td>44/F</td>
<td>Miscarriages × 4, arthralgia, migraine</td>
<td>Yes Yes 140/80 [1]</td>
<td>130</td>
<td>53</td>
<td>1.9</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>49/M</td>
<td>Migraine, cerebral infarctions, proliferative retinopathy</td>
<td>Yes Yes 120/80 [0]</td>
<td>200</td>
<td>40</td>
<td>4.8</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>41/M</td>
<td>Erectile dysfunction, proteinuria</td>
<td>No No 150/100 [0]</td>
<td>90</td>
<td>109</td>
<td>1.3</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>47/F</td>
<td>Microscopic haematuria</td>
<td>Yes No 150/90 [1]</td>
<td>83</td>
<td>100</td>
<td>0.2</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>54/M</td>
<td>Cerebral infarcts</td>
<td>Yes No 150/80 [1]</td>
<td>130</td>
<td>34</td>
<td>0.6</td>
<td>41</td>
</tr>
<tr>
<td>8</td>
<td>31/F</td>
<td>Miscarriages × 3, pre-eclampsia, gestational diabetes</td>
<td>No No 140/80 [0]</td>
<td>64</td>
<td>–</td>
<td>0.5</td>
<td>42</td>
</tr>
</tbody>
</table>

Age, age at time of biopsy; no. of BP drugs, no. required to control blood pressure < 140/90; GFR, ⁵¹Cr-EDTA clearance; Albumin, plasma albumin (35–50 g/l).

Table 2  Immunological features of eight patients with primary antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lupus anti-coagulant</th>
<th>ACLA IgG/IgM (GPL/MPL)</th>
<th>Thrombocytopenia (&lt; 150 × 10⁹/l)</th>
<th>ESR (mm/h)</th>
<th>ANA/ds-DNA</th>
<th>ENA</th>
<th>Other auto-anti-bodies</th>
<th>Complement (C3,C4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>16/4.0</td>
<td>Yes</td>
<td>90</td>
<td>1:160/–ve</td>
<td>No</td>
<td>No</td>
<td>Low normal</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>25.2/0</td>
<td>Low normal</td>
<td>40</td>
<td>1:1280/–ve</td>
<td>No</td>
<td>Rh factor, thyroid, red cell</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>14/0</td>
<td>Low normal</td>
<td>60–100</td>
<td>1:40/–ve</td>
<td>No</td>
<td>Mitochondria</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>34.7/0</td>
<td>No</td>
<td>60</td>
<td>–/–ve</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>0.1/21.9</td>
<td>No</td>
<td>3</td>
<td>–/–ve</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>0.1/16.5</td>
<td>No</td>
<td>5</td>
<td>–/–ve</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>83/73</td>
<td>Yes</td>
<td>100</td>
<td>–/–ve</td>
<td>No</td>
<td>No</td>
<td>C3 low normal</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>16/0</td>
<td>No</td>
<td>5</td>
<td>–/–ve</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
</tr>
</tbody>
</table>

ACLA, anticardiolipin antibodies measured in IgG phospholipid units (GPL) normal <5 and IgM phospholipid units (MPL) normal <3; ESR, erythrocyte sedimentation rate; ANA/ds-DNA, antinuclear antibodies/double-stranded DNA antibodies; ENA, extractable nuclear antigens.
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Determined by coagulation-based assay. Anticardiolipin antibodies were measured by enzyme-linked immunosorbent assays (ELISAs) for IgG and IgM, using bovine heart cardiolipin (Sigma-Aldrich). Positive anticardiolipin values were taken for IgG as \( \geq 5 \) IgG antiphospholipid units (GPLU) and for IgM as \( \geq 3 \) IgM antiphospholipid units (MPLU).

The renal biopsies were examined after fixation in neutral buffered formalin and paraffin embedding. Sections for light microscopy were stained with haematoxylin and eosin (H&E), periodic acid Schiff (PAS), Jones’ hexamine silver and elastic van Gieson stains. Immunoperoxidase preparations for the detection of immunoglobulins IgG, IgM and IgA, complement components C3 and C1q, fibrin and MIB 1 were also examined. The tissue for electron microscopy was embedded in Araldite, thin sections from which were contrasted with uranyl acetate and lead citrate and examined in a Jeol (JEM 1200) electron microscope.

Results

Clinical features (Table 1)

The patients were four men and four women aged 31–69 (mean 47.5 years). Renal presentation ranged from asymptomatic proteinuria to acute renal failure. All patients had some proteinuria, but this varied from 0.2 g/day to 4.8 g/day. One patient (patient 1) went into acute renal failure while being investigated for respiratory symptoms that were shown at post mortem to be due to pulmonary veno-occlusive disease. All patients were normotensive or had mild hypertension easily controlled with one drug. Six of the eight patients had livedo reticularis and four had Raynaud’s phenomenon. Clinical syndromes ranged from fatal multi-system disease (1) to asymptomatic microscopic haematuria (1) and minimal proteinuria (1). Of the five other patients, two had typical primary APS with thrombosis and recurrent miscarriages, one presented in her late 60s with severe atherosclerosis and hyperlipidaemia, and two presented with multiple cerebral infarcts.

Investigations (Table 2)

A lupus anticoagulant was found in four patients. Anticardiolipin antibodies were present in all eight patients, predominantly IgG in six and predominantly IgM in two. Erythrocyte sedimentation rate was substantially elevated in five patients (range 40–100 mm/h). Antinuclear antibodies were found in three patients, but antibodies to double-stranded DNA and extractable nuclear antigens (ENA) were not found in any patient. No other autoantibodies were found, apart from rheumatoid factor, thyroid...
and red cell antibodies in patient 2 and antimitochondrial antibodies in patient 3.

Pathology (Table 3)

Glomeruli—light microscopy

The glomeruli were not all affected equally, but they were generally enlarged, and although the cellularity was approximately normal, the number of capillary loop outlines per glomerulus was increased (Figures 1 and 2) and the capillary walls were diffusely thickened. The appearance with H&E resembled that seen in membranous glomerulonephritis (Figure 1). Appearances with PAS were less strikingly abnormal. With a silver stain, the glomerular basement membranes in thickened capillary loops were seen to have double contours, but unlike the double contours seen in the thrombotic microangiopathy associated with the haemolytic uraemic syndrome (HUS), which are generally parallel, the outer contour often had a wrinkled outline with redundant folds and the inner contour had a more direct course (Figure 3). Between the basement membranes, cellular interposition was sometimes seen. In some patients there were three or even four layers of basement membrane which had a complex, intertwining appearance (Figures 4 and 5). In two patients (2 and 5), all the glomeruli which were not obsolete had reduplicated basement membranes. In three patients (1, 4 and 7) these changes were present in some but not all glomeruli. Other glomeruli in the same biopsies showed simple ischaemic basement membrane wrinkling and collapse without double or multiple contours (Figure 6). In the remaining patients (3, 6 and 8), double contours were not detected by light microscopy but were seen with electron microscopy. A single hyaline thrombus was found within a capillary loop in one patient in our series (patient 4), but in no case was there glomerular necrosis. In one patient (patient 1), central mesangiolysis was seen in several glomeruli, as is seen in the thrombotic microangiopathy associated with HUS, but the peripheral capillary loops showed the changes seen in the other cases of APS (Figure 7).

Immunoperoxidase staining for immunoglobulins IgA, IgG and IgM and complement components C3 and C1q was negative. Fibrin was demonstrated in one case only (patient 1) where a positive reaction was found in mesangial areas and in capillary loops in some glomeruli. MIB 1, the antibody which detects the proliferation marker Ki-67, was negative in the glomeruli of the seven cases in which it could be examined. Some tubular epithelial cells were positive in some cases, indicating that they were ‘in cycle’ and acting as a positive control.

Glomeruli—electron microscopy

The ultrastructure shared many features with the milder changes of thrombotic microangiopathy, as seen with the haemolytic uraemic syndrome (HUS). There was patchy lucency in the mesangial matrix (early mesangiolysis). In some capillary loops, separation had occurred between the endothelium and the glomerular basement membrane, the subendothelial space being filled with moderately lucent flocculent material (Figure 8). However in other capillary loops distinctive changes were found. The glomerular basement membrane was wrinkled and thickened and the wrinkled segments were separated from the capillary lumen by a ‘new’, straighter, thin basement membrane closely related to the endothelium (Figures 8 and 9). In some glomeruli, the new and old basement membranes were parallel as is seen in other forms of thrombotic microangiopathy. The new and old basement membranes in some loops were separated by cellular interposition (Figures 8 and 9). The interposed cells were probably mesangial cells but they appeared in places to be continuous, through gaps in the basement membrane, with endothelial cells (Figure 9). As the nuclei of mesangial cells are indistinguishable in appearance from endothelial cell nuclei, it is possible that the interposed cells were in fact trapped endothelial cells. In two patients, segments of basement membrane which were not wrinkled or reduplicated were abnormally thin, measuring from 110 to 240 nm, but in the other four patients no basement membrane thinning was found.

Blood vessel changes

The changes in the blood vessels were graded 1 to 3 as follows: Grade 1, endothelial swelling, mild fibrous arterial intimal thickening and/or mild patchy arteriolar hyalinosis; Grade 2, moderate fibrous intimal thickening or moderate arteriolar hyalinosis; Grade 3, severe occlusive intimal thickening, thrombosis or fibrinoid vascular necrosis.

Arterioles

In some the arteriolar lumen was narrowed by endothelial swelling (Figure 10). In others the entire arteriolar wall was replaced by nodular hyaline material (Grade 2). In the patient who presented with acute renal failure (patient 1) there was arteriolar thrombosis and fibrinoid necrosis (Grade 3) (Figure 11). With electron microscopy, the arteriolar endothelial basal lamina was reduplicated.

Arteries

The intima in interlobular arteries was thickened by eccentric intimal oedema (Figure 12) or there was medial thickening of varying severity (Figure 13), but
Figure 1. With haematoxylin and eosin staining the glomerular capillary walls appear diffusely thickened, not unlike the H&E appearance of membranous glomerulonephritis. Patient 5. H&E × 400.

Figure 2. Jones’s hexamine silver stain accentuates an apparent increase in the number of capillary loops in the glomerulus. Patient 5. Hexamine silver × 400.

the changes in the arterioles were usually more severe (Figures 12 and 13).

Tubules and the interstitium
Tubular atrophy reflected the extent of any glomerular obsolescence. Interstitial inflammation was not a feature. Large scars, presumed to be vascular in origin, ran through the biopsies from patients 3, 7 and 8.

Clinicopathological correlation
There was little correlation between the pathology and the level of proteinuria in these 8 patients but the severity of the vascular pathology appeared to correlate with the renal function. Patient 1, who was in acute renal failure, had severe vascular pathology including fibrinoid vascular necrosis (Grade 3). Patients 2, 4 and 7, who had glomerular filtration
rates of 43, 40 and 34 ml/min/1.73 m$^2$, respectively, all had Grade 2 vascular pathology.

No association could be found between the level of antiphospholipid antibodies or the antibody class and either the severity of the clinical features or the grade of pathology. This is perhaps not surprising, as antibody levels vary over time in any one patient.

**Discussion**

The reduplication of the glomerular basement membrane shares some features with other causes of glomerular endothelial injury such as HUS and transplant glomerulopathy (chronic vascular rejection). The similarities have been noted by other authors, and some have illustrated glomerular pathology similar to the pathology we describe, but they have not identified it as a particular form of microangiopathy, morphologically identifiable as APS. The outer folded or wrinkled basement membrane and inner straighter membrane beneath the endothelium are characteristic. This ultrastructure can be seen in transplant glomerulopathy, which is hardly likely to be a source of clinical confusion, but the light microscopical appearance of an excess of glomerular capillaries is not seen in transplant nephropathy. We believe that this pathology is
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Figure 5. Higher power of figure 4. Hexamine silver ×1000.

Figure 6. In some glomeruli, simple ischaemic collapse and basement membrane wrinkling occur, presumably due to occlusion of a more proximal vessel. Patient 1. Hexamine silver × 400.

sufficiently notable in a native kidney biopsy to suggest the diagnosis of APS on morphological grounds alone.

An explanation for the appearance of the glomerular basement membrane in APS is not immediately obvious, but it is possible that it represents recanalization of previously occluded and collapsed glomerular capillaries. Thrombi are occasionally found in capillary loops, although they are not common, but recanalization would probably occur fairly rapidly. The presence of three and four layers of basement membrane could be explained by recurrent episodes of thrombosis and recanalization. The interposed cells could be endothelial cells trapped in the thrombosis/recanalization process. These cells might on the other hand be mesangial cells. Mesangial cells are seen to extend around capillary loops in other conditions, apparently in response to the presence of subendothelial immune complex deposits. There is no evidence either by immunohistochemistry or electron microscopy of any glomerular immune complex deposits in primary APS, so if the interposed cells are in fact mesangial cells, their presence at this site is not a response to the presence of immune complexes. Some mesangial expansion into the subendothelial space can also be seen in HUS. An explanation for the increase in the number of glomerular capillary loops is also speculative. Perhaps when recanalization of a thrombosed capillary lumen occurs, several new lumens are formed, as is seen in recanalization of larger vessels. Each of these new endothelial ‘tubes’ could become invested with its own basement membrane and, ultimately, by its own epithelium, thus creating an increased number of smaller loops. We were unable to detect any evidence of glomerular endothelial proliferation using the proliferation marker MIB 1, but this technique might not be relevant to the timescale over which these changes occur. It is however difficult to believe that glomerular thrombosis had been occurring in two of our patients with normal function, minimal proteinuria and yet diffuse glomerular basement
Figure 7. This glomerulus shows central mesangiolysis indistinguishable from that seen in HUS but the peripheral capillary loops show changes characteristic of APS. Patient 1. Hexamine silver × 400.

Figure 8. Electron micrograph. Three capillary loops display separation of the endothelium from the glomerular basement membrane, the resulting space containing flocculent material and dense particles. Cellular interposition is present in the two lower loops and there is widespread flattening of the epithelial foot processes. × 6200. The lower left insert is an enlargement of area A showing what appears to be two layers of endothelium with pinocytic vesicles (p). The lower right insert, an enlargement of area B, shows continuity between the endothelium and the interposed cell. × 10 900.

membrane changes. Some other as yet unidentified form of endothelial injury may be involved. Changes in afferent arterioles and interlobular arteries are no different in APS from those in other forms of thrombotic microangiopathy such as HUS and thrombotic thrombocytopenic purpura or in
hypertensive vascular disease. It is only the changes in the glomeruli which are distinctive.

It is not known whether patients with APS who do not have clinical evidence of renal disease have morphological changes in there kidneys. We have only biopsied patients with primary APS who have clinical evidence of renal disease. In patients with systemic lupus erythematosus who have antiphospholipid antibodies, some patients have only pathology which is attributable to immune complexes, and none of the changes we have associated with antiphospholipid antibodies, while in others a mixed picture is observed. Kant et al. found thrombosis without necrosis in lupus patients with a circulating lupus anticoagulant, whereas thrombosis with necrosis correlated more closely with immune-complex-mediated pathology.

The pathogenetic mechanism of these antibodies is not understood, but their presence in the blood is
strongly linked with widespread thrombotic lesions in many organs. Whether these antibodies directly stimulate prothrombotic activities of endothelial cells and monocytes or interfere with the kinetics of coagulation reactions is a subject for debate; alternatively, they may be a secondary phenomenon. There is in vitro evidence that these antibodies interfere in the protein-C-dependent pathway, but this fails to explain the range of thrombotic manifestations of APS. Also although platelet-reactive antibodies are also often present, there is little evidence that in vivo platelet activation is a direct result of autoantibody binding. It has been hypothesized that as yet unidentified antibodies are present with antiendothelial properties that might cause cell damage or apoptosis. During apoptosis, phosphatidyl serine is exposed on the cell surface, which can interact with circulating proteins such as $\beta_2$-glycoprotein-1 and prothrombin, and other epitopes are revealed to which antiphospholipid antibodies may form. In this model antiphospholipid antibodies are a secondary phenomenon in a disorder of vascular endothelial autoimmune damage.

In summary, we describe distinctive renal glomerular histology and ultrastructure associated with the presence of antiphospholipid antibodies, which probably represents a novel and unexplained form of endothelial injury.

### References

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