Clinical presentations and vascular histopathology in autopsied patients with systemic lupus erythematosus and anticardiolipin antibodies

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Abstract

Objective
To examine histomorphological and immunohistological changes in an autopsy series of systemic lupus erythematosus (SLE) patients with or without anticardiolipin antibodies (aCL).

Methods
Fourteen SLE patients who died at our department from 1988 to 1996 were included. The patients’ medical files were reviewed for the clinical history and the presence of IgG and IgM aCL. Autopsy samples of various organs, including regularly the kidneys, heart, brain and skin, were studied by standard histological methods and the direct immunofluorescence technique.

Results
Thirteen of 14 (93%) autopsied SLE patients were persistently positive for IgG aCL and had common overt thrombotic complications and/or other clinical features related to the antiphospholipid syndrome. Their autopsy tissue samples showed frequent occlusive vascular changes such as bland thromboses, thrombotic microangiopathy (TMA) related changes and arterial intimal fibrous hyperplasia. The immune complex related vascular changes were mostly unremarkable and present mainly in low aCL positive patients, who also had more aggressive types of lupus glomerulonephritis (GN).

Conclusion
Increased IgG aCL were found in 13 out of 14 autopsied SLE patients who had predominant occlusive vascular histopathologic changes. The coincidence of bland thromboses with a characteristic TMA histopathology suggested two pathogenetic mechanisms associated with the presence of aCL, one related to abnormal coagulation and the other to endothelial cell injury. The extent of granular vascular immune deposits, typical of SLE, and the severity of lupus GN were inversely related to the aCL level.

Key words
Systemic lupus erythematosus, antiphospholipid syndrome, anticardiolipin antibodies, vascular histomorphology, thrombosis.

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Introduction

The antiphospholipid syndrome (APS) is a relatively new clinical entity characterized by multiple thromboembolic events, thrombocytopenia and recurrent fetal loss in the presence of a heterogeneous family of antiphospholipid antibodies (aPL) directed against phospholipid-binding plasma proteins such as 2-glycoprotein I (1, 2). The APS can be primary, i.e., not associated with another autoimmune disease, or secondary, most commonly associated with systemic lupus erythematosus (SLE). It is estimated that about a third of SLE patients harbor aPL and approximately 15% fulfill the diagnostic criteria for secondary APS (4-6).

A growing body of clinical and experimental data support a pathogenic role of aPL in the thrombotic tendency characteristic of APS. The thrombotic vasculopathy in APS may affect arteries and veins at any level of the vascular tree, resulting in diverse clinical presentations. Beside blood hypercoagulability, additional mechanisms may be involved in the development of some of the clinical features of APS (7, 8). The number of studies on the clinical and serologic presentations of APS far exceeds the number of reports on the histopathological changes in affected patients. Information on the morphology of vascular and organ lesions mainly derives from case reports and small patient series (reviewed in 9-11). The kidneys (12) and heart valves (11) appear to be the target organs most often examined in APS. Limited insight into the systemic involvement has been provided by the description of patients who died from catastrophic APS (13). However, no larger systematic studies, assessing multiorgan involvement in patients with APS secondary to SLE, have been presented yet.

In this study we present the histomorphological and immunohistological findings in various organs, including the kidneys, heart, brain and skin, obtained from 14 consecutive SLE patients who died in our department; 13 of them had been persistently positive for IgG aCL.

Patients and methods

Patients

Fourteen SLE patients (5 males, 9 females, mean age ± SD 40.1 ± 14.7 years, range 19-65 years) who died in our department over an 8-year period (1988-1996) and were tested for aCL at least twice more than 8 weeks apart were included. The diagnosis of SLE was based on the revised American College of Rheumatology classification criteria (14). The patients’ medical files were reviewed for the clinical history and laboratory data, with special emphasis on those features presumably related to the presence of aPL.

Determination of anticardiolipin antibodies (aCL)

Serum aCL of IgG and IgM isotypes were detected by a standard enzyme-linked immunosorbent assay (ELISA), using fetal calf serum (FCS) as the blocking agent and sample diluent (15). Patients were considered aCL positive if their serum IgG or IgM aCL level was found to be increased (≥ 5 SD above the mean of 147 apparently healthy blood donors) at least twice, in tests conducted more than 8 weeks apart, in the last two years before death. Based on their serum aCL level, the patients were classified into 3 groups: negative, low, and medium to high positive. The cut-off points between these aCL categories were set at < 5 SD, 5-16 SD and > 16 SD, respectively. This was in agreement with the international aCL reference sera, both the GPL and MPL standards (16) and the Kingston Antiphospholipid Study Group Standards (17).

Histomorphological and immunohistological studies

Autopsy tissue samples of various organs, including regularly the kidneys, heart, brain and skin, obtained within 6-12 hours after death, were examined by standard light and immunofluorescent microscopy techniques. For light microscopy, standard staining methods with the addition of the acid picro-Mallory technique for fibrin were performed on 4 μm thick sections of
paraffin embedded tissue samples. For immunofluorescence microscopy, tissue samples were snap-frozen in liquid nitrogen and 6 mm sections were incubated with FITC-labelled antisera to human IgG, IgM, IgA, complement components C3, C1q and C4, and fibrin/fibrinogen (Dako, Denmark). The pattern of immune reactant deposits was defined either as granular or lumpy. The granular immunofluorescence pattern of predominant IgG, C1q and C3 was considered to represent immune complex deposition, while a lumpy pattern of IgM and/or C3 and C1q with or without fibrin/fibrinogen was assumed to result from passive insudation of plasma proteins into the vessel wall.

The following categories of histopathological changes were established:

i. Thrombotic changes including fibrin thrombi (recent, organized and recanalized), fibrous luminal obliteration and plexiform lesions.

ii. Thrombotic microangiopathy (TMA)-related changes (acute and chronic) including endothelial and subendothelial swelling, glomerular mesangiolysis and arterial/arteriolar intimal myxoid fibrosis.

iii. Suspected thrombotic/TMA sequela: excentric arterial intimal fibrosis, small vessel aneurysmatic changes, irregular proliferative arterioli-tis, focal nodular obliteratoral glomerular capillarosclerosis and double contours of the glomerular basement membrane without proliferation.

iv. Arterio- and arteriolosclerosis.

v. Lupus immune complex-related pathology: uncomplicated and hyalizing lupus microangiopathy or vasculitis - necrotizing, cell infiltrative (18), and glomerulonephritis (GN) categorized according to the revised WHO classification (19).

All histomorphological and immunohistological findings were semiquantitatively analyzed and graded from 0 to 3+.

### Results

**Demographic, clinical and laboratory data of patients**

Of the 14 autopsied SLE patients, 13 were found to be IgG aCL positive and 4 of these 13 patients were also low IgM positive, whereas only 1 patient proved to be aCL negative on repeated testing. The low IgM aCL levels, present simultaneously with increased IgG aCL, were not considered clinically significant and were therefore omitted from further analyses. The IgG aCL positive patients, repeatedly showing the same semiquantitative level of positivity, were classified as follows (Table I): 4 moderately to highly positive (patients 1–4), 9 low positive (patients 5–13) and 1 negative (patient 14). There were 8 females and 5 males in the aCL positive group (F/M ratio 1.6). The average age at death of the aCL positive patients was low (39.6 years) and 5 patients were under the age of 35 years. The average period from the diagnosis of SLE until death was 6.0 years. The patients’ medical files were further reviewed for data on the cause of death, the average level of anti-dsDNA antibodies, C3 complement component, average blood pressure and blood sugar (Table I). The 8 patients with arterial hypertension were receiving either calcium channel blockers or ACE inhibitors. Two patients exhibited mild hyperlipoproteinemia (one with elevated LDL cholesterol and the other with elevated triglycerides) which was not treated with hypolipemic drugs. All patients were receiving steroids and most

### Table I. Demographic, clinical and laboratory data of 14 autopsied SLE patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>SLE duration (years)</th>
<th>Cause of death</th>
<th>aCL (SD)</th>
<th>Anti-dsDNA* (% binding)</th>
<th>C3** (g/L)</th>
<th>BP</th>
<th>BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HF</td>
<td>M</td>
<td>33</td>
<td>1</td>
<td>Infection, respiratory failure</td>
<td>30</td>
<td>0.84</td>
<td>0.19</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2. KM</td>
<td>F</td>
<td>45</td>
<td>1</td>
<td>Cardiovascular failure</td>
<td>30</td>
<td>0.06</td>
<td>0.82</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>3. KS</td>
<td>F</td>
<td>43</td>
<td>14</td>
<td>Cardiovascular failure</td>
<td>19</td>
<td>0.70</td>
<td>0.15</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4. VA</td>
<td>M</td>
<td>47</td>
<td>15</td>
<td>Cardiovascular failure</td>
<td>30</td>
<td>0.86</td>
<td>0.49</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>5. KG</td>
<td>F</td>
<td>56</td>
<td>2</td>
<td>CNS vasculitis</td>
<td>9</td>
<td>0.47</td>
<td>0.85</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>6. KME</td>
<td>F</td>
<td>28</td>
<td>4</td>
<td>Respiratory failure</td>
<td>11</td>
<td>0.59</td>
<td>0.30</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7. MD</td>
<td>F</td>
<td>19</td>
<td>0.5</td>
<td>Respiratory and renal failure</td>
<td>10</td>
<td>0.88</td>
<td>0.16</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>8. MLM</td>
<td>M</td>
<td>32</td>
<td>8</td>
<td>Infection, cardiovascular failure</td>
<td>13</td>
<td>0.82</td>
<td>0.56</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9. OF</td>
<td>F</td>
<td>63</td>
<td>15</td>
<td>Cardiovascular failure</td>
<td>13</td>
<td>0.70</td>
<td>0.15</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>10. PB</td>
<td>F</td>
<td>33</td>
<td>6</td>
<td>Cardiovascular failure</td>
<td>10</td>
<td>0.40</td>
<td>0.52</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>11. PI</td>
<td>F</td>
<td>65</td>
<td>7</td>
<td>Infection</td>
<td>12</td>
<td>0.51</td>
<td>0.63</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12. SB</td>
<td>M</td>
<td>19</td>
<td>4</td>
<td>Infection</td>
<td>13</td>
<td>0.87</td>
<td>0.19</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13. SM</td>
<td>M</td>
<td>32</td>
<td>0.25</td>
<td>Pulmonary thrombembolism</td>
<td>10</td>
<td>0</td>
<td>0.74</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>14. DM</td>
<td>F</td>
<td>47</td>
<td>12</td>
<td>Gastrointestinal bleeding</td>
<td>&lt; 5</td>
<td>0.74</td>
<td>0.78</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

BP: blood pressure; BS: blood sugar.

*Anti-dsDNA antibodies were detected by the Farr radioimmunoassay; normal < 35%.

**Levels of C3 were measured by radial immunodiffusion technique; normal ≥ 0.526 g/L.

NOTE: In all tables different writing styles indicate the level of aCL positivity as follows: bold (moderate to high positive aCL), regular (low positive aCL), italic (negative aCL).
of them were receiving pulse cyclophosphamide for SLE; none was treated with cyclosporine A.

**Clinical features related to aPL**

There was a striking difference in the frequency and level of thrombocytopenia between the two categories of aCL positivity. While all the moderately or highly aCL positive patients exhibited significantly decreased platelet counts, mild thrombocytopenia was observed in only one low aCL positive patient (Table II).

Major thrombotic events were noted in 9 of the 13 (77%) aCL positive patients. The number of clinically overt thrombotic events per patient was similar in both groups of aCL positivity (4 events in 4 patients with medium to high positive aCL and 10 events in 9 patients with low positive aCL). By contrast, other aPL related features were more common in patients with moderately to highly increased IgG aCL (8 features in 4 patients vs. 3 features in 9 patients). The sole aCL negative patient experienced a non-transmural myocardial infarction and showed fibrous changes of the mitral valve (Table II).

**Histomorphological vascular changes**

Specific thrombotic vascular changes in various organs, either recent or chronic (Fig. 1), were found in 8 out of the 13 (75%) aCL positive patients, whose average age was just 32.5 years. Mainly the small vessels were affected and in only 3 patients were thromboses of the larger arteries and veins in different stages of organization and recanalization seen. Patient KM had extensive thrombotic changes in the intramyocardial arteries (Fig. 2), and patient HF in the intra-renal arteries, while patient SM had both the larger intra- and extrarenal veins affected in addition to the intra-renal arteries. The distribution of vascular changes (defined in Patients and Methods) in the examined organs is shown in Table III.
Fig. 1. An aneurysmatic arteriole at the glomerular hilus occluded by a recent, laminated thrombus in patient MD with WHO IVC lupus GN. Goldner’s trichrome x65.

Fig. 2. Multiple organizing thrombi occluding intramyocardial blood vessels in patient KM. Hematoxylin-eosin x32.

Fig. 3. Organized thrombosis of an arteriole at the glomerular hilus and reactive intimal arteritis in patient KM with WHO IIA lupus GN. Hematoxylin-eosin x65.
Fibrous endarteritis characterized by cellular and/or fibroelastic thickening of the intima (Fig. 2) was seen in 12 of the 13 aCL positive patients, involving arteries of various sizes. Irregular excentric intimal thickening was more frequent than concentric thickening. The internal elastic lamina was mainly preserved at the sites of fibrous endarteritis and no significant infiltration by inflammatory cells was noted.

Beside occlusive vascular lesions (thrombotic and fibrous endarteritis), the following, often multifocal, histological changes were common in the aCL positive patients: infarcts, fibrosis, dystrophic calcinations, atrophy of the parenchyma, and collapsed or sclerotic glomeruli. All of these changes most probably reflected non-specific tissue reactions to ischemia, suggesting the presence of additional vessel occlusions that were not seen directly.

In the kidneys, both specific vascular changes associated with thrombosis/TMA, as well as lesions indicating the sequelae of thrombosis/TMA and/or arterio-arteriolosclerotic changes, were more common in the moderately or highly aCL positive patients than in those who were low positive for aCL (Table III). By contrast, more aggressive forms of lupus GN, according to the WHO classification, were found in the low positive group of patients as compared with those moderately to highly positive for aCL. The GN WHO IV and V prevailed in the former, while in the latter GN II and III were most frequently observed (Table IV).

Overt extraglomerular vasculitis was not demonstrated in our series of SLE patients, although focal, mild, reactive endothelial cell proliferation and intimal leukocytic infiltration were noted in association with thrombotic lesions. The sole aCL negative patient had non-specific vascular changes in the kidneys and heart according to the categories defined in Patients and Methods (Table III).

**Immunohistological findings**

A remarkable difference between the two groups of aCL positive patients was noted with regard to the presence of immune reactants in the glomeruli and vessel walls (Table IV). Vascular granular immune deposits were generally more common in the patients low positive for aCL. They were particularly frequent in the kidneys (in 7 out of 9 patients), but were present in the other examined organs as well. In the moderately to highly aCL positive group, vascular granular immune deposits were found in 2 out of 4 patients. Lumpy deposits composed of C3 and/or C1q complement components, either alone or accompanied by IgM and fibrin/fibrinogen deposits, predominated in this group and were seen in all 4 patients, where they were also more intense than granular vascular immune deposits (Table IV).

Uncomplicated vascular immune deposits, the most typical lupus histomorphological vascular lesion with extensive granular vascular immune deposits, was present in 6 aCL positive patients. Five of them had low titer aCL.

**Discussion**

While the diverse clinical manifestations of APS have already been extensively documented, their histopathologic correlations are less well known.

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**Table IV. WHO classification of lupus glomerulonephritis (GN) and immunohistological findings in the renal, heart, brain and skin vessels from 14 autopsied SLE patients.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>GN WHO Class</th>
<th>Vascular deposits of immune reactants and fibrin/fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td>1. HF</td>
<td>IIB</td>
<td>0</td>
</tr>
<tr>
<td>2. KM</td>
<td>IIA</td>
<td>0</td>
</tr>
<tr>
<td>3. KS</td>
<td>IIIC</td>
<td>+++</td>
</tr>
<tr>
<td>4. VA</td>
<td>IVC + membranous component</td>
<td>++</td>
</tr>
<tr>
<td>5. KG</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>6. KME</td>
<td>VB</td>
<td>++</td>
</tr>
<tr>
<td>7. MD</td>
<td>IVC</td>
<td>+++</td>
</tr>
<tr>
<td>8. MLM</td>
<td>IVC</td>
<td>+++</td>
</tr>
<tr>
<td>9. OF</td>
<td>VB</td>
<td>+</td>
</tr>
<tr>
<td>10. PB</td>
<td>IVD</td>
<td>+</td>
</tr>
<tr>
<td>11. PI</td>
<td>IIA</td>
<td>0</td>
</tr>
<tr>
<td>12. SB</td>
<td>IVC</td>
<td>+++</td>
</tr>
<tr>
<td>13. SM</td>
<td>VA</td>
<td>++</td>
</tr>
<tr>
<td>14. DM</td>
<td>VA</td>
<td>+++</td>
</tr>
</tbody>
</table>

ICD: immune complex deposits (granular deposits of predominant IgG, C1q and C3); INS: insudation (lumpy deposits of IgM and/or C1q and C3); F: fibrin/fibrinogen deposits.

*: present (semiquantified); 0: absent; /: not performed.

NOTE. In the kidneys, glomerular capillary and extraglomerular vascular immune deposits were included, while glomerular mesangial immune deposits were not considered.
To gain insight into the vascular involvement in patients with SLE harboring aPL, we studied by classical histologic and immunofluorescent techniques autopsy tissue specimens of various organs (the kidneys, heart, brain and skin) obtained from 14 SLE patients who died in our department during the period from 1988 to 1996. All but one of the studied patients had been found to have persistently increased IgG aCL and 10 of them fulfilled the current diagnostic criteria for secondary APS (2). The lack of aCL negative SLE patients therefore precluded a comparison between the vascular lesions of SLE in the presence and absence of aCL. The high prevalence of aCL positivity in our cohort of autopsied SLE patients and their short longevity (the mean survival time from the diagnosis of SLE was 6.0 years) conform with the observation that patients with SLE and APS have a higher and earlier mortality rate than patients with SLE alone (6, 20, 21). Of interest is also the high proportion of men among our aCL positive patients (5 of 13) and their apparent predisposition to thrombosis.

There was no clear positive correlation between aCL levels and the frequency of thrombotic events per patient. However, other clinical and laboratory features believed to be associated with aCL occurred more often in the patients with moderately or highly increased antibody levels than in those who were low positive for aCL. Our results support the view that there is a rough correlation between aCL levels and the activity of thrombogenic/pathogenic processes, to which other pre-disposing factors may contribute (22, 23).

As expected, in the presence of aCL (9-12) specific thrombotic and TMA related vascular changes were common in our series of SLE patients. Similarly to previous studies, the small vessels were predominantly or (occasionally) exclusively affected, even though in the setting of SLE there were no signs of underlying overt vasculitis (9-12). As our patients had unusually short longevity, they were probably not representative of ordinary SLE patients with aCL. The data, however, point to a chronic progressive occlusive microangiopathy involving several organs in the presence of aCL.

The most common histomorphologic vascular lesion in the aCL positive patients was fibrous endarteritis, characterized by either concentric or excentric intimal thickening. The lesion was present in different tissue specimens from 12 of the 13 aCL positive patients, involving arteries of various sizes, mainly with irregular, and occasionally only a focal, distribution pattern. Fibrous endarteritis, also designated endarteritis obliterans, is a well known form of APS vasculopathy. It has been documented in the kidneys, peripheral arteries, skin, myocardium, lungs, brain and leptomeninges of aCL positive patients (reviewed in 9-11). Recurrent thrombosis, resulting in chronic hypoxic stress which induces reactive cellular proliferation, was suggested to be the initial pathophysiologic event in the development of fibrous endarteritis (24-26).

Alternatively, it has been speculated that in some instances myointimal cell proliferation might be the primary process (27). Primary myointimal hyperplasia could be mediated by the interaction of aPL with endothelial cells, resulting in endothelial activation and the consequent secretion of proinflammatory cytokines, chemokines and growth factors, which can promote recruitment and proliferation of other cells in the vessel walls (28, 29).

In our study, excentric intimal thickening was more common than concentric lesions. The excentric fibrous intimal thickening could be a consequence of former thrombosis and, in the larger elastic type arteries, could also be related to atherosclerosis. In contrast, the concentric intimal thickening in the smaller arteries bore a close resemblance to hypertensive vascular changes and accelerated occlusive vasculopathy in transplanted organs (30). The latter resemblance supports the immunologic hypothesis of the development of fibrointimal hyperplasia in the smaller arteries in APS and may also imply a role for cellular immunity in its pathogenesis. Furthermore, by analogy with other conditions associated with fibrous intimal hyperplasia (30), it may be speculated that vasodilators possessing anti-proliferative properties, such as calcium channel blockers and ACE inhibitors, could slow the progression of fibrous endarteritis also in APS.

As already established for fibrous endarteritis in primary APS, there were no signs of overt vasculitis in our series of SLE patients. It also appeared that the extent of vascular immune complex deposits was inversely related to the aCL level. By contrast, lumpy deposits of IgM and/or complement components C3 and C1q predominated in moderately to highly aCL positive patients. Representing passive plasma protein insudation into the vessel wall, the abundant lumpy deposits suggested endothelial cell injury associated with high aCL. These immunofluorescence findings were in concordance with the findings of thrombosis/TMA related changes or at least suspected thrombotic/TMA sequelae, additionally indicating that endothelial cells injury was implicated in both plasma protein insudation and thrombotic tendency.

More aggressive types of lupus GN WHO classes IV and V predominated in the low aCL positive patients, whereas less severe GN WHO classes II and III prevailed in those with moderate to high aCL levels. This observation differs from the results of previous studies, which found no relationship between the presence of aPL and the type of lupus GN or the renal histologic pattern (12, 31). In contrast to lupus GN, which appeared to be milder at higher aCL levels, specific thrombosis/TMA related and thrombosis/TMA suspected vascular changes, as well as arterio-and arteriolosclerosis in the kidneys, were more frequent in the medium to high aCL positive patients than in those low positive for aCL.

A link between aPL and atherosclerosis has recently been proposed (21,32, 33). However, in our series of aCL positive SLE patients prominent atherosclerotic lesions of the larger arteries were not observed. The only common vascular lesion, resembling atherosclerotic changes to some extent, was excentric intimal hyperplasia, but even this lesion is not specific for atherosclerosis and could be attributed to former thrombo-
Vascular histomorphology in SLE patients with aCL / A. Sipek-Dolnicar et al.

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