A Clinicopathologic Study of Thrombotic Microangiopathy in IgA Nephropathy

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ABSTRACT

Thrombotic microangiopathy (TMA) occurs in IgA nephropathy, but its clinical significance is not well described. We retrospectively examined a series of 128 patients diagnosed with IgA nephropathy between 2002 and 2008 who had a mean follow-up of 44±27 months. In our series, 53% presented with lesions of TMA, acute or organized, in arteries and/or arterioles. Among patients with TMA, 4% were normotensive, 25% had controlled hypertension, and 71% had uncontrolled hypertension. Of those with uncontrolled hypertension, 26% had malignant hypertension. Histologically, the group with TMA had a significantly greater percentage of sclerotic glomeruli and worse tubulointerstitial fibrosis than those of the group without TMA. However, a significant minority of patients had near-normal histology, with minimal tubular atrophy (20%) and/or <20% interstitial fibrosis (24%). TMA rarely occurred in the absence of significant proteinuria. During follow-up, a doubling of serum creatinine or ESRD occurred in all patients with laboratory evidence of TMA, in 42% of those with morphologic evidence but no laboratory evidence of TMA, and in 11% of those without TMA. In summary, lesions of TMA are frequent in IgA nephropathy and may occur in normotensive patients with near-normal renal histology. Although the pathophysiologic mechanisms involved remain undetermined, the current study rules out severe hypertension or advanced renal disease as sole causes.


Thrombotic microangiopathy (TMA) is a heterogeneous disorder characterized by platelet thrombi in arteries and capillaries and on occasion in arteries.1,2 Renal histopathologic lesions in TMA tend to take one of two broad forms with considerable overlap: (1) predominant arteriolar, and lesser arterial, involvement, with thrombi and fibrinoid necrosis, particularly in thrombotic thrombocytopenic purpura, malignant hypertension (MHT), and scleroderma; or (2) glomerular involvement, with capillary thrombi, capillary loops with double contours due to mesangial interposition, and variable mesangiolysis, the latter most frequently seen in the hemolytic–uremic syndromes. These morphologic lesions occur in a number of other clinical settings as well, including anti-phospholipid antibody syndrome, or as a side effect of various pharmacologic agents, and are often associated with poor renal prognosis.1–3

In immunoglobulin A nephropathy (IgAN), the most common form of primary glomerular disease...
worldwide, it has long been recognized that intrarenal arterial and arteriolar lesions, such as arteriolar wall thickening and hyalin changes, may be a prominent feature. Further, TMA has been described in IgAN in a recent study and attributed by the authors to severe or malignant hypertension. However, a large-scale clinicopathologic analysis focused on TMA in IgAN has not been performed. This report describes the prevalence, associated clinical features, and outcome of histologic TMA lesions found in a retrospective survey of IgAN.

RESULTS

This study included 128 patients, with males predominating (69.5%). Among them, 118 (92.2%) were Caucasians, and 10 (7.8%) were Asians. Mean age was 38.7 years (range, 18–78 years). Mean proteinuria was 2.47 g/d (25th to 75th percentile: 0.8–3.00 g/d), and mean estimated GFR (eGFR) was 51.2 ml/min per 1.73 m² (25th to 75th percentile, 29–76 ml/min per 1.73 m²). All patients except one (who was pregnant and presented without TMA) received angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, or both, in case of hypertension or persistent proteinuria. Only one patient, in the non-TMA group, had received corticosteroid therapy prior to diagnosis, and none had steroid therapy subsequent to diagnosis. No patient had other immunosuppressive therapy, either prior to or subsequent to diagnosis.

Mean follow-up was 44 months (25th to 75th percentile, 23–60 months); for those who went to ESRD, mean time from diagnosis to dialysis was 15 months (25th to 75th percentile, 1–29 months).

TMA Is a Common Feature of IgAN

Among our patients, 68 (53.1%) presented with acute or organized TMA lesions. There were no significant differences in age or sex between the patients with and without TMA. Clinical and biological characteristics of TMA patients are summarized in Table 1. Hypertension was present in 71.0% and 23.3% of patients in the TMA and the non-TMA groups, respectively (P=0.00). MHT was noted in 26% of patients in the TMA group; no patient in the non-TMA group presented with MHT. Neurologic symptoms were absent (except in patients with MHT). Compared with patients in the non-TMA group, patients with TMA had significantly higher proteinuria, lower serum albumin, higher serum creatinine, and lower eGFR at the time of the biopsy (Table 1). No possible cause for TMA (such as radiotherapy, Shiga toxin–producing bacteria infection, or drug-induced TMA) was documented in any patient. Among the 52 patients tested, there was no difference between the groups for the presence of anticardiolipin antibody (26.5% versus 28.5%, P<0.10) or its titer when present. Only 1 of the 39 patients tested had lupus anticoagulant (although that patient indeed had TMA). Similarly, of the 47 patients tested, only 3 patients (2 with TMA and 1 without TMA) had anti-β2 glycoprotein (anti-β2GP1) antibody. It is evident, then, that none of these factors plays a major role in TMA. Eleven (8%) patients with TMA had complete complement assays and genetic screening for complement regulatory protein gene mutations; none presented such mutations.

Notably, 20 patients presented with TMA lesions (including acute lesions) either without associated hypertension or normotensive under treatment (Table 2). Of note, most (73.9%) patients from the TMA group did not have MHT at the time of biopsy or in their medical history.

Comparisons of Patients According to the Degree of Hypertension

Comparisons were made between completely normotensive patients, patients normotensive under treatment, hypertensive patients, and those with MHT; the clinical data and the morphologic parameters are presented in Table 2. Among the 63 normotensive patients, 44 (69.8%) were treated with one or more antihypertensive agents. MHT was found in 18 (14.1%) patients who, compared with patients with less severe hypertension, presented with much more advanced renal

Table 1. Comparison of clinical data between patients with and without TMA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TMA</th>
<th>No TMA</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>68</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.7 (29–49)</td>
<td>36.2 (26–44)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Male</td>
<td>49 of 68 = 72.1%</td>
<td>41 of 60 = 68.3%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Initial systolic BP (mmHg)</td>
<td>161 (135–177)</td>
<td>130 (120–140)</td>
<td>0.00</td>
</tr>
<tr>
<td>Initial diastolic BP (mmHg)</td>
<td>94 (80–107)</td>
<td>77 (67–85)</td>
<td>0.000002</td>
</tr>
<tr>
<td>Percentage hypertensive</td>
<td>48 of 68 = 70.6%</td>
<td>14 of 60 = 23.3%</td>
<td>0.00</td>
</tr>
<tr>
<td>Percentage MHT</td>
<td>18 of 68 = 26%</td>
<td>0 of 60 = 0</td>
<td>0.00</td>
</tr>
<tr>
<td>Final systolic BP (mmHg)</td>
<td>131 (120–144)</td>
<td>122 (110–130)</td>
<td>0.004</td>
</tr>
<tr>
<td>Final diastolic BP (mmHg)</td>
<td>81 (72–87)</td>
<td>74 (69–80)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Number of antihypertensive agents</td>
<td>2.8 (2–4)</td>
<td>1.4 (1–2)</td>
<td>0.00</td>
</tr>
<tr>
<td>Proteinuria Dx (g/day)</td>
<td>3.37 (1.2–4.0)</td>
<td>1.33 (0.40–1.85)</td>
<td>0.00</td>
</tr>
<tr>
<td>Macroscopic hematuria</td>
<td>11 of 59 = 18.6%</td>
<td>22 of 46 = 47.8%</td>
<td>0.0014</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>34.6 (30–39)</td>
<td>39.8 (36–43)</td>
<td>0.0001</td>
</tr>
<tr>
<td>eGFR (ml/min per 1.73 m²)</td>
<td>34 (14–50)</td>
<td>73 (51–90)</td>
<td>0.00</td>
</tr>
<tr>
<td>Final SCr (μmol/L)</td>
<td>340 (123–569)</td>
<td>156 (81–119)</td>
<td>0.00</td>
</tr>
<tr>
<td>Final eGFR (ml/min per 1.73 m²)</td>
<td>23 (0–49)</td>
<td>69 (50–96)</td>
<td>0.00</td>
</tr>
<tr>
<td>Bad outcomea</td>
<td>34 of 68 = 50.0%</td>
<td>6 of 53 = 11.3%</td>
<td>0.00</td>
</tr>
<tr>
<td>RRT</td>
<td>30 of 68 = 44.1%</td>
<td>5 of 53 = 9.4%</td>
<td>0.00</td>
</tr>
<tr>
<td>Family history</td>
<td>8 of 65 = 12.3%</td>
<td>0 of 55 = 0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>5 of 53 = 9.4%</td>
<td>7 of 39 = 17.9%</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

Values expressed as mean (25th to 75th percentile) or percentages. P values calculated by Mann–Whitney U test or Fisher’s exact test as appropriate. BP, blood pressure; Dx, diagnosis.

aValue of >0.10 after Holm–Bonferroni correction to minimize type 1 error (α=0.05).

bBad outcome defined as doubling of initial SCr or need for dialysis.
### Table 2. Clinical and morphologic differences between patients with normotension, moderate hypertension, and MHT at the time of diagnosis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive, No Antihypertensors</th>
<th>P Value</th>
<th>Normotensive on Antihypertensors</th>
<th>P Value</th>
<th>Hypertension</th>
<th>P Value</th>
<th>MHT</th>
<th>P Value (Versus Normotensive without Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>19</td>
<td>44</td>
<td>47</td>
<td>18</td>
<td>0.00</td>
<td>0.00001</td>
<td>0.00003</td>
<td>0.00004</td>
</tr>
<tr>
<td>Systolic BP Dx (mmHg)</td>
<td>119 (114–127)</td>
<td>&gt;0.10</td>
<td>126 (120–133)</td>
<td>0.00</td>
<td>162 (150–170)</td>
<td>0.001</td>
<td>193 (164–220)</td>
<td>0.00</td>
</tr>
<tr>
<td>Diastolic BP Dx (mmHg)</td>
<td>72 (62–80)</td>
<td>&gt;0.10</td>
<td>73 (66–80)</td>
<td>0.00</td>
<td>95 (88–100)</td>
<td>0.002</td>
<td>111 (97–125)</td>
<td>0.000001</td>
</tr>
<tr>
<td>SCr Dx (μmol/L)</td>
<td>113 (79–141)</td>
<td>&gt;0.10</td>
<td>173 (81–163)</td>
<td>0.001</td>
<td>215 (126–136)</td>
<td>0.00001</td>
<td>739 (248–1316)</td>
<td>0.000003</td>
</tr>
<tr>
<td>eGFR Dx (ml/min per 1.73 m²)</td>
<td>77 (68–89)</td>
<td>&gt;0.10</td>
<td>63 (44–89)</td>
<td>0.002</td>
<td>44.3 (26.4–55.6)</td>
<td>0.000001</td>
<td>15.8 (4.3–20.0)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Proteinuria Dx (g/day)</td>
<td>1.01 (0.11–1.90)</td>
<td>&gt;0.10</td>
<td>1.42 (0.66–2.00)</td>
<td>0.0004</td>
<td>3.34 (1.10–4.05)</td>
<td>&gt;0.10</td>
<td>4.21 (2.00–6.40)</td>
<td>0.00004</td>
</tr>
<tr>
<td>Laboratory evidence of TMA arterial acute, with fibrin</td>
<td>0 of 16 = 0</td>
<td>&gt;0.10</td>
<td>1 of 41 = 2.4%</td>
<td>0.01</td>
<td>2 of 45 = 4.5%</td>
<td>0.01</td>
<td>5 of 18 = 27.7%</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>organized arterial acute, with fibrin</td>
<td>0 of 19 = 0</td>
<td>&gt;0.10</td>
<td>2 of 44 = 4.5%</td>
<td>0.01</td>
<td>6 of 47 = 12.8%</td>
<td>&gt;0.10</td>
<td>6 of 18 = 33.3%</td>
</tr>
<tr>
<td></td>
<td>organized arteriolar acute, with fibrin</td>
<td>1 of 19 = 5.2%</td>
<td>&gt;0.10</td>
<td>5 of 44 = 11.3%</td>
<td>&gt;0.10</td>
<td>9 of 47 = 19.1%</td>
<td>&gt;0.10</td>
<td>4 of 18 = 22.2%</td>
</tr>
<tr>
<td></td>
<td>organized arteriolar</td>
<td>3 of 19 = 15.8%</td>
<td>0.07</td>
<td>3 of 44 = 6.8%</td>
<td>0.03</td>
<td>11 of 47 = 23.4%</td>
<td>&gt;0.10</td>
<td>6 of 18 = 33.3%</td>
</tr>
<tr>
<td>Any TMA (acute or organized, arterial or arteriolar)</td>
<td>3 of 19 = 15.8%</td>
<td>&gt;0.10</td>
<td>17 of 44 = 38.6%</td>
<td>0.01</td>
<td>31 of 47 = 65.9%</td>
<td>0.004</td>
<td>18 of 18 = 100%</td>
<td>0.00</td>
</tr>
<tr>
<td>Bad outcomea</td>
<td>1 of 19 = 5.2%</td>
<td>&gt;0.10</td>
<td>8 of 44 = 18.2%</td>
<td>0.04</td>
<td>17 of 45 = 37.8%</td>
<td>0.002</td>
<td>14 of 17 = 82.3%</td>
<td>0.00</td>
</tr>
<tr>
<td>RRT</td>
<td>0 of 19 = 0</td>
<td>0.05</td>
<td>8 of 44 = 18.2%</td>
<td>&gt;0.10</td>
<td>13 of 45 = 28.9%</td>
<td>0.0002</td>
<td>14 of 17 = 82.3%</td>
<td>0.00</td>
</tr>
<tr>
<td>Immediate RRTb</td>
<td>0 of 19 = 0</td>
<td>&gt;0.10</td>
<td>4 of 44 = 9.1%</td>
<td>&gt;0.10</td>
<td>3 of 45 = 6.7%</td>
<td>0.0002</td>
<td>9 of 17 = 57.9%</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Values expressed as mean (25th to 75th percentile) or percentages. P values calculated by Mann–Whitney U test or Fisher’s exact test as appropriate. BP, blood pressure; Dx, diagnosis.

*aBad outcome is defined as doubling of SCr or need for dialysis.
*bImmediate RRT is defined by RRT initiation <3 months after biopsy.
insufficiency and with much lower eGFR, 58% of them requiring renal replacement therapy from the outset compared with 7% with lesser hypertension ($P=0.00$; Table 2). They also had greater proteinuria (Table 2). Importantly, there was no difference in the frequency of anti-cardiolipin antibodies between the four groups of patients. As might be anticipated, MHT biopsies disclosed greater interstitial fibrosis, greater percentage of sclerotic glomeruli, worse glomerular extracapillary proliferation, and more frequent TMA than biopsies of hypertensive patients without MHT (Supplemental Table 1). All the MHT patients (100%) presented with TMA lesions versus 65.9% of hypertensive patients (without MHT) and 31.7% of normotensive patients overall (15.8% of entirely normotensive patients and 38.6% of patients normotensive on antihypertensive therapy) ($P=0.004$ and $P=0.0004$, respectively; Table 2).

**Histologic Findings in Patients with or without IgAN-Associated TMA**

TMA was nearly exclusively arterial and arteriolar in location (Figure 1). Only two cases (one in the original series and one in the supplemental cases stained for CD61 [see later]) had glomerular fibrin thrombi. There was no evidence in any case for glomerular capillary endothelial swelling, double contours, or mesangiolysis. The fresh fibrinous vascular thrombi (Figures 2–4) were characterized by the presence of fibrinous material (staining bright reddish on trichrome stain as performed in our laboratory using acetic acid–formol–absolute alcohol (AFA) fixative, as opposed to the blue staining of the hyalin deposits of hyalin arteriolosclerosis) and dilation with marked distension and smoothing out of the internal elastic lamina (Figures 1 and 2). Chronic lesions were basically organized thrombi with small recanalized vascular channels and reduction or obliteration of the lumen (Figures 1 and 5–7), sometimes having an “onion-skin” appearance. The organized fibrous tissue was generally oriented in the long axis of the lumen but lacked the lamellar quality of the fibroelastotic lesions of arteriosclerosis. Focal myocyte necrosis was seen, usually in association with thrombi but sometimes separately (Figure 8).

IgAN-associated TMA was associated with more severe other vascular lesions, both in terms of reduction of lumen (arterial intimal sclerosis and arteriolar lumen reduction) and smooth muscle hypertrophy (Supplemental Figures 1–6).
These differences between TMA and non-TMA cases were maintained when patients were divided into normotensive and hypertensive groups, although not all differences remained significant (Table 3) (patients with MHT were not included in this comparison because all had TMA). Consistent with these changes, hyperplasia of the juxtaglomerular apparatus was more frequent in patients with TMA ($P=0.04$).

In general, the biopsies with IgAN-associated TMA showed more extensive damage in terms of percentage of sclerotic glomeruli and tubulointerstitial damage (Supplemental Table 2). The ensemble of cases was also evaluated in terms of the Oxford Classification (Supplemental Table 2). As anticipated, all of the parameters were more frequent/worse among the patients with TMA than among those without.

**Immunohistochemical Studies**
Staining using anti-CD61, an antiplatelet antibody, was performed for 12 recent cases of IgAN not included in the earlier main series reported here. All had evidence of either acute and/or organized TMA on routine Masson stain. Of these, 10 showed at least focal positivity on staining for CD61.

**Arteries and Arterioles**
In acute lesions, although sometimes platelet-rich thrombi completely filled the lumen (Figure 9A), typically platelets were present in fewer numbers, admixed in varying degrees with other elements (Figure 9B and Supplemental Figures 7 and 8), and might be present in one section of the lumen and absent in an adjacent one (Supplemental Figure 9). There frequently was staining for platelets in the media of arteries with...
acute lesions (Figure 9B and Supplemental Figure 8). Platelets progressively disappeared from the intima and media as lesions advanced (Figure 9C) and were generally entirely absent in organized TMA (Figure 9D).

**Glomeruli**

One of the 12 cases had glomerular thrombi, recognizable on CD61 staining (Figure 9E). Another case showed platelets at the site of a presumptive area of fibrinoid necrosis (Figure 9G). The corresponding glomerulus was not identifiable on the initial Masson stain, but another glomerulus from the same case showed clear fibrinoid necrosis (Figure 9H). In addition, several cases had isolated platelets or platelet aggregates in glomerular capillary lumens in a minority of glomeruli (Figure 9F), but these lesions were not recognizable by routine microscopy.

**Veins and Peritubular Capillaries**

CD61 staining permitted detection of rare capillary and venous lesions unapparent on routine Masson stain. Some of these represented definite venous thrombi (Supplemental Figure 10). Others may simply represent platelet aggregates (Supplemental Figures 11 and 12).

**TMA Associated with IgAN May Occur in Early and/or Mild Cases**

Because TMA in IgAN has previously been reported predominantly in patients with MHT,\(^7\) it is important to point out that TMA may occur in early/mild cases: 33% (23 of 69 cases) with systolic blood pressures \(\leq 140\) mmHg; 52% in patients with diastolic pressures \(\leq 90\) mmHg; 19% with serum creatinine (SCR) \(\leq 120\) \(\mu\text{mol/L}\); 16% with eGFR \(>60\) ml/min per 1.73 m\(^2\). In morphologic terms, 16 (23.2%) of the cases of TMA occurred in patients with minimal to mild interstitial fibrosis/tubular atrophy (Oxford class 0),\(^8\)\(^9\) with 4 (5.8%) cases showing only acute TMA, 5 (7.2%) cases only organized TMA, and 7 (10.1%) cases showing both. Conversely, however, IgAN-associated TMA rarely occurred in the absence of significant proteinuria, only 4.6% of cases having \(<0.5\) g/24 h versus 34.5% of cases without TMA (\(P=0.001\)). Similarly, TMA was tightly associated with the presence of glomerular lesions, only two (2.9%) cases having entirely normal glomeruli by light microscopy versus 14 (24.1%) cases among the non-TMA cases (\(P=0.0004\)). However, glomerular lesions, although present in 97% of TMA biopsies, were not necessarily severe in a given biopsy.

**TMA Is Associated with Bad Outcome**

Table 4 presents a univariate analysis of the various clinical and vascular parameters relatable to IgAN-associated TMA with bad outcome. Among the vascular parameters, all showed significant associations with bad outcome except hyalin arteriolar deposits. As anticipated, both fibrinoid and organized TMA were strongly associated with bad outcome.

### Table 3. Relationship between nonspecific lesions of arteries/arterioles and TMA in normotensive and hypertensive patients (MHT excluded)

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Normotensive Patients</th>
<th>Hypertensive Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMA Present</td>
<td>TMA Absent</td>
</tr>
<tr>
<td>arteriosclerosis (global)</td>
<td>2.21 (2–3)</td>
<td>1.14 (0–2)</td>
</tr>
<tr>
<td>arterial intimal sclerosis</td>
<td>1.20 (0–2)</td>
<td>0.54 (0–1)</td>
</tr>
<tr>
<td>arterial S/M hypertrophy</td>
<td>0.60 (0–1)</td>
<td>0.39 (0–1)</td>
</tr>
<tr>
<td>arterial S/M hyalin deposits</td>
<td>0.30 (0–1)</td>
<td>0.15 (0–0)</td>
</tr>
<tr>
<td>arteriolar lumen caliber</td>
<td>2.25 (2–3)</td>
<td>2.77 (3–3)</td>
</tr>
<tr>
<td>arteriolar S/M hypertrophy</td>
<td>0.1 (0–0)</td>
<td>0.01 (0–0)</td>
</tr>
<tr>
<td>arteriolar hyalin deposits</td>
<td>1.20 (0.5–2)</td>
<td>0.51 (0–1)</td>
</tr>
</tbody>
</table>

MHT cases could not be broken down in this fashion because all patients had some form of TMA. Values expressed as mean (25th to 75th percentile). \(P\) values calculated by Mann–Whitney \(U\) test. S/M, smooth muscle.
The contribution of TMA (compared with the Oxford criteria) to decline of eGFR and to bad outcome was analyzed by multiple linear regression and by Cox proportional hazards modeling, respectively (Table 5). Potential confounding factors such as mean arterial pressure, SCr, and proteinuria at the diagnosis were included. When this was done, the eGFR at diagnosis sorted as significant. Similarly, laboratory evidence for TMA sorted as significantly associated with decline of eGFR ($P=0.001$). However, the simple morphologic presence of TMA did not sort as significant.

Renal survival was 52.2% at 44 months among the TMA patients versus 93.5% among those without TMA ($P=0.00001$). However, a more telling separation comes from dividing the cases with morphologic lesions of TMA only compared with those TMA patients who had, in addition, laboratory evidence of TMA. All eight of the latter patients had a bad outcome within 6 months of presentation, with a highly significant difference between this group and those with morphologic lesions only ($P=0.0002$; Figure 10).

**DISCUSSION**

TMA was a frequently identified lesion in this study of IgAN in adults, being found in slightly more than one-half (53.1%) of our patients. This high frequency is in part attributable to the fact that our patients as a group had rather advanced disease.

A very high percentage of patients were either frankly hypertensive (48.4%) or normotensive on antihypertensive treatment (34.4%), with 18 (14%) patients presenting with MHT. This frequency of hypertension is substantially higher than that in other series$^{8,9}$ and is attributable to an active hypertension clinic in our institution from which many patients were drawn. This biased recruitment of patients accounts in large part for the much poorer survivals (80% of MHT patients went to ESRD). Because our data reveal that IgAN-associated TMA increases markedly in frequency with increasing hypertension (Table 2), this accounts in large part for the very high incidence of TMA in our series.

**Figure 9.** Immunohistochemical studies using anti-CD61 antibody. (A) CD61-positive Thrombi. These thrombi in an artery and arteriolar branch appear composed nearly entirely of platelets. Anti-CD61, original magnification $\times 400$. (B) Arterial and arteriolar thrombi. Platelets constitute roughly half of the thrombus in the artery (left) and are absent from the lumen of the arteriole on the right, but are present in the media (arrow). Anti-CD61, original magnification $\times 400$. (C) More advanced TMA. Rare CD61-positive platelets (arrows) remain in the intima of this advanced TMA, as well as in a glomerulus with near-total sclerosis. Anti-CD61, original magnification $\times 350$. (D) Organized TMA. This artery with advanced organized TMA is CD61 negative. Arrow indicates internal elastica for orientation. Anti-CD61, original magnification $\times 500$. (E) Glomerulus with capillary thrombus. A capillary thrombus is present, confined to the capillary lumen, along with isolated granules in other capillaries (arrows). Anti-CD61, original magnification $\times 400$. (F) Numerous CD61-positive glomerular capillary granules. These granules, representing isolated platelets or platelet aggregates (arrows), are located mostly in dilated capillaries filled with red blood cells. Such aggregates are not evident on routine microscopy. Anti-CD61, original magnification $\times 400$. (G) Probable glomerular fibrinoid necrosis. Numerous positive granules surround a central mass, and the glomerular basement membrane is not recognizable. Anti-CD61, original magnification $\times 500$. (H) Glomerular fibrinoid necrosis. Fibrinoid necrosis (arrow) in another glomerulus from the same case as in (G). Masson's trichrome, original magnification $\times 350$. 

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TMA: all vessels

TMA by vessel size

Malignant hypertension; S/M, smooth muscle.

Clinical parameters

Table 4. Univariate analysis of clinical and vascular factors associated with bad outcome

<table>
<thead>
<tr>
<th></th>
<th>Bad Outcomea</th>
<th>Preserved Renal Function</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>40</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Clinical parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic Dx (mmHg)</td>
<td>167 (140–197)</td>
<td>139 (120–150)</td>
<td>0.000002</td>
</tr>
<tr>
<td>diastolic Dx (mmHg)</td>
<td>97 (80–115)</td>
<td>82 (70–90)</td>
<td>0.0002</td>
</tr>
<tr>
<td>HBP Dx</td>
<td>37 of 40 = 92.5%</td>
<td>55 of 81 = 67.9%</td>
<td>0.003</td>
</tr>
<tr>
<td>systolic BP, end</td>
<td>136 (124–154)</td>
<td>123 (111–130)</td>
<td>0.000001</td>
</tr>
<tr>
<td>MHT</td>
<td>14 of 40 = 35%</td>
<td>3 of 81 = 3.7%</td>
<td>0.00</td>
</tr>
<tr>
<td>proteinuria Dx (g/day)</td>
<td>3.99 (1.81–5.30)</td>
<td>1.81 (0.49–2.52)</td>
<td>0.000001</td>
</tr>
<tr>
<td>proteinuria, end (g/day)</td>
<td>3.32 (1.80–4.92)</td>
<td>0.94 (0.10–1.07)</td>
<td>0.0001</td>
</tr>
<tr>
<td>serum albumin (g/L)</td>
<td>32.6±1.08</td>
<td>39.1±0.64</td>
<td>0.000004</td>
</tr>
<tr>
<td>SCr Dx (μmol/L)</td>
<td>537 (190–759)</td>
<td>125 (89–161)</td>
<td>0.00</td>
</tr>
<tr>
<td>eGFR Dx (ml/min per 1.73 m²)</td>
<td>22 (6–34)</td>
<td>66 (44–83)</td>
<td>0.00</td>
</tr>
<tr>
<td>laboratory evidence of TMA</td>
<td>8 of 38 = 21.1%</td>
<td>0 of 78 = 0</td>
<td>0.00</td>
</tr>
<tr>
<td>Vascular parameters (arbitrary units)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>arteriosclerosis, global</td>
<td>2.34 (2–3)</td>
<td>1.72 (1–2)</td>
<td>0.01</td>
</tr>
<tr>
<td>arterial intimal sclerosis</td>
<td>1.59 (1–3)</td>
<td>0.99 (0–2)</td>
<td>0.01</td>
</tr>
<tr>
<td>arterial S/M hypertrophy</td>
<td>0.99 (0–2)</td>
<td>0.52 (0–1)</td>
<td>0.002</td>
</tr>
<tr>
<td>arterial hyalin deposits</td>
<td>0.42 (0–1)</td>
<td>0.24 (0–0)</td>
<td>0.06</td>
</tr>
<tr>
<td>arteriolar lumen caliber</td>
<td>2.04 (1.5–2.0)</td>
<td>2.60 (2–3)</td>
<td>0.000001</td>
</tr>
<tr>
<td>arteriolar S/M hypertrophy</td>
<td>0.67 (0–1)</td>
<td>0.38 (0–1)</td>
<td>0.003</td>
</tr>
<tr>
<td>arteriolar hyalin deposits</td>
<td>0.82 (0–1)</td>
<td>0.81 (0–1.5)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>TMA by vessel size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>arterial fibrinoid TMA</td>
<td>11 of 40 = 27.5%</td>
<td>4 of 81 = 4.9%</td>
<td>0.0004</td>
</tr>
<tr>
<td>arterial organized TMA</td>
<td>10 of 40 = 25%</td>
<td>11 of 81 = 13.6%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>arteriolar fibrinoid TMA</td>
<td>10 of 40 = 25%</td>
<td>9 of 81 = 11.1%</td>
<td>0.05</td>
</tr>
<tr>
<td>arteriolar organized TMA</td>
<td>25 of 40 = 62.5%</td>
<td>26 of 81 = 32.1%</td>
<td>0.0014</td>
</tr>
<tr>
<td>TMA: all vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fibrinoid TMA</td>
<td>22 of 40 = 55%</td>
<td>19 of 81 = 23.5%</td>
<td>0.001</td>
</tr>
<tr>
<td>organized TMA</td>
<td>29 of 40 = 72.5%</td>
<td>28 of 81 = 34.6%</td>
<td>0.00001</td>
</tr>
<tr>
<td>any TMA</td>
<td>34 of 40 = 85%</td>
<td>34 of 81 = 42%</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Values expressed as mean (25th to 75th percentile) or percentages. P values calculated by Mann–Whitney U test or Fisher’s exact test as appropriate. Dx, diagnosis; HBP, high BP; MHT, malignant hypertension; S/M, smooth muscle.

aBad outcome defined as doubling of SCr or need for dialysis.

Even taking the increased severity in our patients into account, however, it is evident that the incidence of TMA in IgAN generally is substantially higher than has previously been appreciated, as numerous examples occurred in patients who were either entirely normotensive or normotensive under therapy with normal/near-normal renal function. (In addition, we believe that our use of AFA fixative and the trichrome stain facilitates the search for these lesions, which may be inconspicuous on other stains.)

The only other study looking specifically at TMA in IgAN found MHT in 6 of 10 patients studied, with severe hypertension in another 3 patients, and favored the hypothesis that the TMA was the consequence of the MHT, the MHT itself being the consequence of advanced parenchymal lesions. This was a plausible theory for patient sample of that study, particularly given that MHT occurs in 7%–15% of IgAN. The association between MHT and TMA is well recognized, both in spontaneous and drug-induced MHT, the assumption being that the TMA is due to pressure-induced endothelial disruption. (In support of the pressure-induced mechanism for TMA, TMA has only been described in severe/malignant hypertension, not in mild to moderate essential hypertension.) However, our series essentially refutes the hypothesis that the TMA in IgAN is due to MHT. The frequency of TMA did indeed increase markedly in frequency with increasing blood pressure, leading to the conclusion that increasing blood pressure is a major aggravating factor. But it seems unlikely to be the sole cause of IgAN-associated TMA, as 20 of 69 (29%) cases occurred in patients with systolic pressures <140 mmHg at the time of biopsy, levels at which TMA has not been described in essential hypertension.

Nor did IgAN-associated TMA necessarily develop in a setting of advanced parenchymal lesions, 19% occurring in patients with an SCr <120 μmol/L and 23.9% occurring in patients with minimal to mild (Oxford class 0) interstitial fibrosis/tubular atrophy. It thus appears clear that TMA can precede the development of glomerulosclerosis and interstitial fibrosis rather than being a consequence of it, a sequence that has been suggested by others for vascular lesions in general in IgAN. Thus, neither hypertension nor advanced parenchymal lesions are necessary prerequisites to the development of TMA.

By contrast, the appearance of TMA in the biopsy did appear to be tightly linked to the presence of glomerular lesions and proteinuria. Only three (4.6%) TMA cases had proteinuria <0.5 g/24 h as opposed to 19 (34.5%) cases without TMA. Further, the frequency of TMA increased with increasing proteinuria, from 13.6% for cases <0.5 g/24 h to 80% for cases >3.0 g/24 h (P=0.00). Similar considerations held for the association of TMA with overt glomerular lesions. Only two (2.9%) TMA biopsies had normal glomeruli by light microscopy compared with 14 (24.1%) biopsies without TMA (P=0.0004). Any explanation of the mechanism(s) of TMA in IgAN must take its association with glomerular lesions and proteinuria into account.

Although CD61 staining revealed glomerular lesions to be slightly more extensive than appreciated on routine microscopy, IgAN-associated TMA remains a primarily arterial/arteriolar lesion. In this regard, it resembles scleroderma, and particularly, the kidney of malignant hypertension. A recent report of 21 patients with MHT-associated TMA found arterial or arteriolar lesions in all but glomerular thromboses in...
Table 5. Analysis of rate of decline of renal function and outcome

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rate of Decline of eGFR (ml/min per 1.73 m² per year)</th>
<th>Factors Associated with Bad Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple Linear Regression</td>
<td>Cox Proportional Hazards Modeling</td>
</tr>
<tr>
<td></td>
<td>$F(9,82)=5.2877, P&lt;0.00001, R^2=0.3672$</td>
<td>$\chi^2=111.34, df=9, P=0.00000$</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>SEM  $\beta$</td>
</tr>
<tr>
<td>Mesangial hypercellularity</td>
<td>0.0668</td>
<td>0.1082</td>
</tr>
<tr>
<td>Segmental glomerulosclerosis</td>
<td>0.2154</td>
<td>0.1006</td>
</tr>
<tr>
<td>Endocapillary proliferation</td>
<td>0.1502</td>
<td>0.1130</td>
</tr>
<tr>
<td>Percentage tubular atrophy/interstitial fibrosis</td>
<td>0.2926</td>
<td>0.1484</td>
</tr>
<tr>
<td>Proteinuria Dx</td>
<td>0.0957</td>
<td>0.1124</td>
</tr>
<tr>
<td>eGFR Dx</td>
<td>0.4052</td>
<td>0.1514</td>
</tr>
<tr>
<td>Mean arterial pressure Dx</td>
<td>0.0108</td>
<td>0.1119</td>
</tr>
<tr>
<td>Morphologic TMA</td>
<td>0.0817</td>
<td>0.1175</td>
</tr>
<tr>
<td>Laboratory evidence of TMA</td>
<td>0.3367</td>
<td>0.0959</td>
</tr>
</tbody>
</table>

Bold designates $P<0.05$. Dx, diagnosis.

*aBad outcome defined as doubling serum creatinine or ESRD.

none, with only 6 patients showing laboratory evidence for TMA.17

The question remained whether some of the lesions thought to represent TMA on routine microscopy might instead represent simply banal hyalin arteriolosclerosis rather than brinoid material, despite the marked differences in staining on Masson stain as performed in our laboratory—blue for the former, bright red for the latter. Staining for anti-CD61, an antiplatelet antibody, largely put this question to rest, revealing that the acute lesions were extensively positive for platelets, although staining varied from artery to artery (Figure 9, A and B and Supplemental Figures 7–9). The platelet staining here corresponds in large part with that seen in other situations.18

Other arterial and arteriolar lesions of IgAN have been reported as being associated with other clinical and histologic poor prognostic factors5,19,20 and even potentially independently associated with the degradation of renal function.21 In our study, both fibroinoid and organized TMA, as well as other vascular lesions, particularly arteriolar lumen size, were significant on univariate analysis (Table 4).

However, multiple linear regression of rate of decline of eGFR and Cox proportional hazards modeling of outcome both show similar results (Table 5). Laboratory evidence of TMA sorts as a significant factor in eGFR decline and bad outcome in both models, but simple morphologic TMA does not. We have in effect a “tip of the iceberg” effect, with all eight patients with thrombotic tendencies severe enough to lead to laboratory manifestations going on to bad outcome. Those with only morphologic TMA, the “underwater” part, nonetheless had a substantially greater frequency of bad outcome (42.1% versus 11.3%, $P=0.0004$). The only other study looking at IgAN-associated TMA found that all of the patients for whom follow-up data were available evolved to terminal renal insufficiency within a year of diagnosis of TMA.7

Notably, in our study, TMA appears to be associated with worse lesions of arteriolosclerosis, particularly striking in the normotensive patients, where possible confounding effects of hypertension can be excluded from consideration (Table 3). Although the evaluation of the arterial/arteriolar lesions was simply a semiquantitative estimate, the differences in Table 3 are sufficiently great that they seem likely to reflect a real link between TMA and vascular sclerosis. Obviously, however, extensive morphometric studies will be required to confirm this result.

The causes of TMA in IgAN are uncertain. Certain statements can be made from our analysis. First, although TMA clearly increases markedly in frequency with increasing blood pressure (Table 2), it may appear early, in situations ruling out both severe hypertension or advanced parenchymal damage and renal insufficiency as necessary to its development. However, glomerular lesions and proteinuria are integral elements of the setting in which TMA develops.

Anti-phospholipid syndrome antibodies (anti-cardiolipin, anti-β2GP1 antibodies, or lupus anticoagulant) have also been described in IgAN.22,23 However, in our series, these antibodies were present in a minority of cases, and there was no significant difference in frequency of TMA between cases with and without these antibodies. Thus, they clearly do not play a role in causation in the majority of cases.

Mutations of complement factor H (CFH) and complement factor I (CFI) and membrane cofactor protein genes have been associated with TMA and kidney involvement.2 No genetic abnormalities were identified in 11 patients from our series chosen for their severe TMA. This mitigates against the possible influence of the regulation of alternative pathway in this disease. In addition, recently, Edey et al.24 have reported the absence of mutations of CFH in a large series of patients with IgAN.

Other possible mechanisms for TMA exist that our study cannot address. First is possible alteration and/or diminution of function of vascular endothelial growth factor (VEGF).25,26 Inhibitors of VEGF, are known to lead to proteinuria regularly and less frequently to TMA.27–29 It is known that aberrantly glycosylated IgA downregulates synthesis of VEGF in mesangial cells,30 and there is diminution of podocyte staining for VEGF in IgAN.31 Anti-endothelial cell antibodies are
another possibility to be considered. A study from the 1980s found a 32% incidence of anti–endothelial cell antibodies in IgAN compared with 4% in controls and 9% in other glomerular diseases. Little had been done since in this area until a recent study32 found anti–endothelial cell antibodies in 34 of 75 (45.3%) patients with IgAN (24 of the 34 having MHT) compared with 3 of 19 patients with primary MHT (P=0.02).

This study has several limitations. It is retrospective and observational and will need to be validated with a prospective cohort of patients. Further, the evaluation of prognosis was rendered difficult by the variable nature of the treatment received.

In conclusion, we have shown in this study that the lesions of TMA are frequent and severe in IgAN and have a poor prognosis. They increase in frequency with both increasing blood pressure and proteinuria. Lesions of TMA are particularly associated with MHT, but their frequent presence in patients who are normotensive either naturally or under antihypertensive therapy indicates that they are not the result of the MHT. The causative factors responsible for this TMA remain to be determined. We believe that these lesions should be systematically sought on renal biopsy, so that the TMA may be addressed therapeutically, with the future goal being to optimize treatment for this lesion when it occurs in IgAN.

CONCISE METHODS

Patients
All the adult (>18 years) patients diagnosed with IgAN from January 2002 to January 2008 at the Pathology Department of the Hôpital Européen Georges Pompidou (Paris, France) were enrolled in this study. These biopsies came from four different medical centers. The diagnosis was based on the presence of predominant IgA and C3 deposits in the mesangium. Patients with SLE, Henoch–Schönlein purpura, chronic liver disease, or HIV infection were excluded, as well as patients whose renal biopsy specimen contained less than eight glomeruli. Clinical and laboratory data including age, gender, blood pressure, number of antihypertensive agents used, immunosuppressive therapy, proteinuria, hematuria, familial history of IgAN, SCr, and presence of anti-cardiolipin antibody and lupus anticoagulant (defined by spontaneously prolonged activated partial thromboplastin time and abnormal specific lupus anticoagulant test). Anti-β2GP1 antibodies were collected at the time of renal biopsy and at the end of follow-up (or institution of renal replacement therapy). Seven patients were lost to follow-up shortly after biopsy and were not included in the outcome analysis. The following definitions were used. (1) Normotension: Systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg. (2) Hypertension: Systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or the need for antihypertensive medication to maintain pressures below these levels (this latter group considered separately in some analyses). (3) MHT: Marked elevation of blood pressure (mean in this study, 193/111 mmHg), obligatorily associated with central nervous system symptoms, such as blurred vision, headaches, nausea, vomiting, or papilledema. (4) Laboratory evidence of TMA: Association of anemia and/or thrombocytopenia, low haptoglobin, presence of schizocytes, elevated lactate dehydrogenase. (5) Bad outcome: Persistent doubling of SCr or requirement for renal replacement therapy (RRT). The glomerular filtration rate was estimated (eGFR) with the simplified modification of diet in renal disease formula.33

Renal Histopathology

The renal biopsies were processed for light microscopy and direct immunofluorescence. Tissue for histology was fixed in AFA and processed and stained by standard methods. Six-micrometer sections were stained for immunofluorescence study with FITC-conjugated antibodies specific for human IgG, IgM, IgA, C1q, C3, κ, and λ light chains, and fibrinogen (DAKO, Carpinteria, CA). All biopsy slides were re-reviewed by two senior pathologists (D. Nochy and G.S. Hill) without knowledge of clinical outcomes. The biopsies were graded according to the Oxford classification of IgAN.8,9 TMA lesions were described as (1) “acute,” with fibrin deposits, or (2) as “organized,” with evident fibrosis and recanalization and narrowing of the lumen at the arterial and arteriolar levels. TMA lesions were also classified according to location: arterial, arteriolar, or glomerular. The severity of interstitial cell infiltration and tubular atrophy was semiquantitatively scored on a scale of 0–4+. Interstitial fibrosis was also estimated as a percentage of

Figure 10. Survival from renal bad outcome. Three groups are compared: those with no TMA versus those with only morphologic TMA and those having morphologic TMA with recognizable laboratory manifestations. Significance of differences in survival between groups was calculated by log-rank test.
the renal parenchyma involved. In a separate analysis performed by one pathologist (G. S. Hill), arteries and arterioles were evaluated semiquantitatively for global estimation of arteriosclerosis on a scale of 0–4+ (0, no lesions; 1+, minimal recognizable intimal sclerosis with or without mild recognizable medial fibrosis; 2+, intimal sclerosis with <25% luminal occlusion with or without mild medial fibrosis; 3+, intimal fibrosis with <50% lumenal occlusion with definite medial fibrosis and smooth muscle atrophy; 4+, advanced lesions with >50% luminal occlusion-marked medial lesions); arterial intimal sclerosis on a scale of 0–4+ (0, none; 1+, recognizable intimal sclerosis but no luminal compromise; 2+, intimal sclerosis with <25% luminal occlusion; 3+, 25%–50% occlusion; 4+, >50% occlusion); smooth muscle hypertrophy on a scale of 0–2+ (0, absent; 1, recognizable, minimal to mild; 2, moderate to severe); size of arteriolar lumen on a scale of 0–4+ (0, total occlusion; 1, marked narrowing; 2, definite narrowing; 3, normal diameter; 4, dilated); and hyaline deposits in arteries and arterioles on a scale of 0–2+ (0, absent; 1, present, small, nonocclusive of lumen; 2, present, extensive, and/or impinging on lumen).

Immunohistochemical Studies
Twelve recent cases of IgAN, not included in the original series, all having either acute fibrinoid and/or organized TMA were stained with anti-CD61, an anti-platelet antibody (Y2/51; DAKO). Three cases of TMA of other causes (cocaine-induced, hemolytic–uremic syndrome) and five cases of IgAN without TMA were used as confirmatory positive and negative controls, respectively.

Complement Assays and Genetic Screening
Analyses were performed using EDTA plasma samples at the immunology laboratory of the Hôpital Européen Georges Pompidou. Plasma concentrations of CFH and CFI were measured by ELISA, and all CFH, membrane cofactor protein, and CFI exons were sequenced as previously described. Analyses were performed using EDTA plasma samples at the immunology laboratory of the Hôpital Européen Georges Pompidou.

Statistical Analyses
Results were expressed as numerical values and percentages for categorical variables. Continuous variables are expressed as mean (25th to 75th percentiles) because the majority had non-Gaussian distribution. Comparisons were based on Fisher’s exact test for categorical data and the t test for normally distributed continuous data. For non-Gaussian–distributed parameters, we used the nonparametric Mann–Whitney U test to compare continuous variables and the Wilcoxon test to compare two paired groups. The associations of the Oxford criteria with decline in eGFR were evaluated by standard multiple linear regression analysis and with outcome by Cox proportional hazards modeling. P<0.05 was regarded as statistically significant.

DISCLOSURES
None.

REFERENCES


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