Serum Concentrations of Laminin-P1 in Thrombotic Microangiopathy: Usefulness as an Index of Activity and Prognostic Value

ALFONS SEGARRA,* RAFAEL SIMÓ,† LLUIS MASMIQUEL,‡ ROSA M. SEGURA,‡ VICENS FONOLLOSA,§ PERE HUGUET,‖ JOAQUIM MAJO,§ LLUIS PIERA,* and SIMÓ SCHWARTZ¶

Departments of *Nephrology, †Endocrinology, ‡Biochemistry, §Internal Medicine, and ‖Pathology, and ¶Biochemistry and Molecular Biology Research Center, Hospital Universitari Vall d’Hebron, Barcelona, Spain.

Abstract. Laminin is the main noncollagenous constituent of the basement membrane, and its serum levels could reflect the metabolic changes that occur in the basement membrane. Severe endothelial injury with thickening of basement membrane is a characteristic feature of thrombotic microangiopathy (TMA). With this background, the aim of the study was to investigate in a prospective way (1) the relationship among serum Lam-P1, the extent of renal histopathologic lesions, and the biochemical parameters commonly used as markers of TMA activity, and (2) the usefulness of serum Lam-P1 concentrations as a renal outcome prognostic index. To this end, 18 consecutive patients with active biopsy-proven TMA with renal involvement were studied. One hundred and twenty-one healthy control subjects, 20 patients with systemic sclerosis without renal involvement, and 35 patients with systemic lupus erythematosus (20 without nephropathy and 15 with diffuse proliferative type 4 lupus nephritis) were used as control groups. In addition, to analyze the influence of either renal failure or hemodialysis therapy on serum Lam-P1 levels, 91 patients on regular hemodialysis therapy and 81 patients with predialysis chronic renal failure of different etiologies were included in the study. Serum Lam-P1 was determined by RIA at admission, on days 10 and 30 of follow-up in all patients, and after 6 and 12 mo of follow-up in all surviving patients. Serum lactate dehydrogenase, haptoglobin, platelet count, hemoglobin, and serum creatinine were determined as markers of endothelial dysfunction and hemolysis. At admission, serum levels of Lam-P1 were significantly higher in patients with TMA than in healthy control subjects (3.39 ± 0.56 U/ml versus 1.40 ± 0.18 U/ml; P < 0.0001). In addition, patients with TMA had significantly higher serum Lam-P1 levels than the other groups included in the study. At the first control, Lam-P1 correlated with lactate dehydrogenase (P = 0.006) and hemoglobin (P = 0.002). During follow-up, platelet count and hemolysis indicators normalized in all patients, while serum Lam-P1 decreased only in patients with renal function recovery. In multivariate analysis, serum creatinine and Lam-P1 at day 10 were the only independent predictors of renal outcome (r² = 0.94; P < 0.0001) and also correlated with indices of histopathologic damage (P < 0.001). Serum Lam-P1 normalized in all patients with chronic renal failure in the samples obtained at 6 and 12 mo of regular hemodialysis after solving active TMA, thus suggesting that histopathologic lesions, but not renal function itself, would be mainly responsible for the high Lam-P1 serum concentrations detected in TMA. In conclusion, serum Lam-P1 concentrations are increased in patients with active TMA. Furthermore, patients with poor renal outcome show a prolonged increase of serum Lam-P1 that is related to the extent of renal histologic lesions. Unlike the biochemical markers of hemolysis commonly used to assess TMA activity, the sequential determination of serum Lam-P1 provides valuable information about long-term renal prognosis in patients with TMA.
membrane thickening, and accumulation of fluffy material in the subendothelium. These lesions suggest that important alterations of the metabolism and composition of capillary basement membranes are relevant in the pathogenesis of TMA (3,6–8).

Laminin is the main noncollagenous constituent of basement membranes. This glycoprotein is located in lamina rara, in close contact with endothelial cells, and is specific to basement membranes (9,10). It has been postulated that these characteristics confer to laminin a special usefulness to study basement membrane metabolism (11). Thus, serum concentrations of laminin have been studied in several diseases characterized by a large involvement of basement membranes, and increased levels have been reported in sera of patients with some neoplastic disorders, hepatic fibrosis, and diabetic microangiopathy (12–14). However, to date, there are no data about serum concentrations of laminin in TMA. Because serum levels of laminin or its fragments could reflect the changes observed in the basement membranes of patients with TMA, we measured, in a prospective way, the serum concentrations of laminin-P1, the largest pepsin-resistant fragment of laminin (Lam-P1), in a group of patients with TMA. Furthermore, we studied the relationship of Lam-P1 serum levels with other biochemical parameters commonly used as markers of disease activity. Finally, the usefulness of serum Lam-P1 concentrations as a renal outcome prognostic index, and the relation between serum Lam-P1 and the degree of histologic renal damage were also investigated.

Materials and Methods

Patients

Eighteen patients who presented with active biopsy-proven TMA and treated consecutively at our nephrology department between February 1990 and June 1994 were studied. TMA was defined by the presence of hemolytic microangiopathic Coombs-negative anemia, thrombocytopenia (<100 × 10^9 platelets/L), and acute or rapidly progressive renal failure with or without neurologic symptoms. At diagnosis, all patients entered in a study protocol to systematically investigate the etiology of TMA. The etiology of TMA was TTP (n = 4), HUS (n = 4), scleroderma renal crisis (n = 6), and systemic lupus erythematosus-associated TMA (n = 4). Diagnostic criteria used to define each primary form of TMA were as follows. (1) Idiopathic TMA: Presence of characteristic TMA signs in absence of a primary systemic disease, neoplasm, malignant arterial hypertension, infectious disease, pregnancy, or puerperium. Patients with neurologic involvement were diagnosed with TTP and the remaining patients were diagnosed with HUS (4,15). (2) Scleroderma renal crisis-associated TMA (SRC): Scleroderma was diagnosed according to the American Rheumatism Association (ARA) criteria (16). All patients suffered proximal sclerosis and Raynaud syndrome. According to the classification of LeRoy et al. (17), all patients had diffuse scleroderma. Scleroderma renal crisis was defined by the presence of acute onset renal failure, arterial hypertension, and TMA (18). (3) Systemic lupus erythematosus (SLE)-associated TMA: This form of TMA was considered in all patients with SLE diagnosed according the ARA criteria (19) who developed characteristic signs of TMA (4,15). One hundred and twenty-one healthy volunteers (mean age 36.6 ± 10.9 yr), 20 patients with systemic scleroderma without renal involvement, 35 patients with SLE (20 without nephropathy and 15 patients with diffuse proliferative type 4 lupus nephritis) were used as control groups. In addition, to analyze the influence of renal failure or hemodialysis therapy on serum Lam-P1 levels, 91 patients on regular hemodialysis therapy (30 chronic glomerulopathies, 22 nephroangiopathy, 18 polycystic renal disease, 11 chronic interstitial disease, and 10 unknown renal failure) and 81 patients with predialysis chronic renal failure (50 chronic glomerulopathies, 15 chronic interstitial disease, 10 vascular disease, and 6 polycystic renal disease) were included in the study.

Measurements

In all patients, platelet count, serum concentrations of lactate dehydrogenase (LDH), haptoglobin (HPT), creatinine, and hemoglobin (Hb) were monitored periodically (every 24 to 72 h). Serum levels of Lam-P1 were measured at admission and after 10 and 30 d of follow-up in all cases. Furthermore, in surviving patients Lam-P1 was determined again after 6 and 12 mo of follow-up. Biochemical determinations were performed using a Hitachi 917 autoanalyzer. Creatinine was measured by the colorimetric method (picric acid) of Jaffé. HPT was determined by immunonephelometry (Behring, Germany) and LDH by the optimized standard method (Boehringer Mannheim, Mannheim, Germany) in accordance with the recommendations of the Deutsche Gesellschaft für Klinische Chemie. Hb measurement and platelet and erythrocyte counts were performed with a Coulter Counter (Coulter Electronics, Hialeah, FL). Serum Lam-P1 concentrations were determined using a double antibody RIA (Behring Werke, Germany) according to the method described by Brocks et al. (11). This Lam-P1 assay is specific for laminin, and no cross reactions are detectable with several collagens or fibronectin. Our intrassay and interassay coefficients of variation were 3.6 and 5.8%, respectively. All samples were performed in duplicate.

Serum Lam-P1 concentrations obtained at admission in patients with TMA were compared with those obtained in control subjects. Moreover, differences in serum Lam-P1 concentrations were investigated among different groups of patients with TMA (TTP, HUS, SRC, and SLE). Finally, the relationship among serum Lam-P1 levels, the hematologic parameters commonly used as markers of TMA activity (platelet count, haptoglobin, LDH), and the renal outcome (renal function recovery versus irreversible renal failure) of the patients were also studied.
Histopathologic Study

A kidney biopsy was performed in all patients within the first week after admission. Renal tissue was fixed in 10% buffered formalin, embedded in paraffin, cut into 4- to 5-μm sections, and stained with hematoxylin-eosin, Masson’s trichrome, periodic acid-Schiff, and Jone’s methenamine. Tissue specimens for immunofluorescence studies were immediately frozen in liquid nitrogen, cut into 5-μm sections in a cryostat, and reacted with antibodies against immunoglobulins A, G, M, fibrinogen, and C3. In each biopsy specimen, we determined the glomerular sclerosis index, the severity of arteriolar lesions, and the extension of interstitial fibrosis and tubular atrophy as follows: (1) Glomerular sclerosis index was determined as the proportion of glomeruli with total or partial sclerosis. (2) The severity of vascular lesions was determined according to the method described by Bader and Meyer (20). With this method, the changes in the arteriolar walls are divided into four degrees of severity (1 to 4). In each biopsy, the number of arteriolar cross sections with the same grade of arteriolar sclerosis is multiplied by the grade number (1 to 4). The products for all grades are added together and the sum is divided by the total number of arteriolar sections on the biopsy. The resultant number is called the vascular index, with 1 and 4 the lowest and highest possible values, respectively. (3) The extent of tubular atrophy and interstitial fibrosis was determined measuring the proportion of interstitial area showing sclerotic lesions in trichromic stained sections using the following semiquantitative scoring system: 1, no interstitial fibrosis; 2, sclerosis involving <25% of the interstitial area; 3, sclerosis affecting 25% but <50% of the interstitial area; 4, sclerosis affecting 50% but <75% of the interstitial area; and 5, sclerosis affecting >75% of the interstitial area.

Immunohistochemical Study

Renal tissue was deparaffinized with xylene and alcohol solutions, placed in a 0.05% protease solution (Sigma type XIV, pH 7.6) at room temperature, and reacted with antibodies against immunoglobulins A, G, M, fibrinogen, and C3.

Table 1. Basic clinical, biochemical, and histopathologic characteristics of patients with TMA included in the study

| Characteristic                  | HUS/TTP (n = 8) | SRC (n = 6) | SLE-TMA (n = 4) |
|--------------------------------|----------------|------------|----------------|----------------|
| Age (yr)                       | 40 ± 20        | 54 ± 17    | 27 ± 8         |
| Creatinine (mg/dl)             | 5 ± 2          | 7 ± 1      | 6 ± 6          |
| Haptoglobin (g/L)              | 0.2 ± 0.2      | 0.1 ± 0.1  | 0.1 ± 0.1      |
| Hemoglobin (g/dl)              | 8.4 ± 1.5      | 9 ± 0.7    | 8.6 ± 1.9      |
| LDH (UI/L)                     | 1594 ± 622     | 2100 ± 936 | 1788 ± 940     |
| Platelet count (×10^9/L)       | 44 ± 21        | 63 ± 15    | 50 ± 36        |
| Lam-P1 (U/ml)                  | 3.4 ± 0.5      | 3.3 ± 0.5  | 3.4 ± 0.4      |
| Glomerular sclerosis index     | 25 ± 21        | 43 ± 38    | 27 ± 11        |
| Interstitial fibrosis score    | 2 ± 2          | 3 ± 4      | 2 ± 1          |
| Vascular index                 | 2 ± 3          | 3 ± 3      | 1 ± 2          |

Table 2. Evolution of the biochemical and hematologic parameters of patients with TMA during follow-up

<table>
<thead>
<tr>
<th>Follow-Up Day and Parameter</th>
<th>HUS/TTP</th>
<th>SRC</th>
<th>SLE-TMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>creatinine (mg/dl)</td>
<td>6 ± 4</td>
<td>8 ± 2</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>haptoglobin (g/L)</td>
<td>0.4 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>hemoglobin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (UI/L)</td>
<td>1048 ± 150</td>
<td>1750 ± 423</td>
<td>1535 ± 878</td>
</tr>
<tr>
<td>platelet count (×10^9/L)</td>
<td>49 ± 20</td>
<td>82 ± 6</td>
<td>60 ± 17</td>
</tr>
<tr>
<td>Lam-P1 (U/ml)</td>
<td>2.5 ± 0.9</td>
<td>3.8 ± 0.3</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>Day 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>creatinine (mg/dl)</td>
<td>6.7 ± 6.9</td>
<td>9.8 ± 4.3</td>
<td>4.8 ± 6.7</td>
</tr>
<tr>
<td>haptoglobin (g/L)</td>
<td>2.4 ± 1.1</td>
<td>2 ± 0.7</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>hemoglobin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH (UI/L)</td>
<td>385 ± 106</td>
<td>367 ± 88</td>
<td>440 ± 142</td>
</tr>
<tr>
<td>platelet count (×10^9/L)</td>
<td>191 ± 68</td>
<td>173 ± 48</td>
<td>165 ± 49</td>
</tr>
<tr>
<td>Lam-P1 (U/ml)</td>
<td>2.3 ± 1</td>
<td>3.8 ± 0.3</td>
<td>2.3 ± 1.1</td>
</tr>
</tbody>
</table>

a Abbreviations as in Table 1.
b Blood transfusions required by most patients with irreversible renal failure prevented consideration of hemoglobin concentrations obtained during follow-up in the analysis of the results.
temperature for 10 min, and put into 3% H₂O₂ to block endogenous peroxidase. The sections were washed with phosphate-buffered saline, reacted with 5% normal rabbit serum at room temperature for 30 min, and reacted with specific antilaminin antibodies (Biogenex, San Ramón, CA) in a moist chamber at 4°C overnight. The sections were stained with biotinylated anti-rabbit IgG antibody at room temperature for 30 min, followed by staining with horseradish peroxidase-conjugated streptavidin (Biogenex) at room temperature for 60 min. After the final washing with phosphate-buffered saline, a dianinobenzidine-H₂O₂ substrate was used to visualize immunoreactivity. In each biopsy, we analyzed the distribution of laminin staining and the concordance between laminin staining and the histopathologic indices mentioned above.

**Statistical Analyses**

Results are given as the mean ± 1SD for normally distributed variables or the median and the interquartile range for non-normal variables. Qualitative variables were compared using the χ² test. Correlation analyses among quantitative variables were done using the Pearson correlation test. A P value <0.05 was considered statistically significant. To analyze the biochemical and histologic variables associated with renal function at 30 d, all variables with P values <0.1 in univariate analyses were entered into stepwise multiple regression analysis considering the logarithm of serum creatinine at day 30 as the dependent variable. We determined the most parsimonious model by removing single variables. Statistical analyses were performed with the Statistical Package for the Social Sciences for Windows 6.1.2.

**Results**

**Lam-P1 Concentrations at Diagnosis of TMA**

Baseline characteristics of patients with TMA are shown in Table 1. At diagnosis, all patients with TMA had increased serum Lam-P1 concentrations when compared with healthy control subjects (3.39 ± 0.56 U/ml *versus* 1.40 ± 0.18 U/ml; *P* < 0.0001). Patients with TMA also showed higher Lam-P1 concentrations than patients with predialysis renal failure (3.39 ± 0.56 U/ml *versus* 1.98 ± 1.6 U/ml; *P* < 0.001) and patients on chronic hemodialysis therapy (3.39 ± 0.56 U/ml *versus* 2.06 ± 0.85 U/ml; *P* < 0.01). We did not observe significant differences in Lam-P1 levels between patients with predialysis renal failure and patients on chronic hemodialysis.

On the other hand, serum Lam-P1 levels were not different between patients with chronic renal failure and healthy control subjects (1.98 ± 1.6 U/ml *versus* 1.40 ± 0.18 U/ml).

Patients with SRC had significantly higher serum Lam-P1 levels than patients with scleroderma without renal involvement (3.31 ± 0.56 U/ml *versus* 1.9 ± 0.31 U/ml; *P* < 0.001). In addition, patients with SLE-associated TMA showed higher Lam-P1 levels than SLE patients without nephropathy (3.41 ± 0.46 U/ml *versus* 1.9 ± 0.28 U/ml; *P* < 0.001) and than patients with lupus nephritis (3.41 ± 0.46 U/ml *versus* 2.2 ± 0.36 U/ml; *P* < 0.01). No differences were observed when serum Lam-P1 levels were compared among the four groups of TMA patients (TTP: 3.28 ± 0.48 U/ml *versus* HUS: 3.62 ± 0.56 U/ml *versus* SRC: 3.31 ± 0.50 U/ml *versus* SLE: 3.41 ± 0.46 U/ml; *P* = NS).

**Classic TMA Activity Markers, Serum Lam-P1 Concentrations, and Patient Outcome**

The evolution of the biochemical and hematologic parameters of patients with TMA is summarized in Table 2. A stable complete remission was obtained in five (two TTP and three HUS) of the eight patients with idiopathic TMA. The three remaining patients presented an irreversible renal failure and required permanent hemodialysis treatment. The hematologic parameters practically normalized in all these patients after the second week of treatment, but Lam-P1 remained elevated >3 U/ml at days 10 and 30 only in patients with irreversible renal failure. Renal function recovered in all but one patient with SLE-related TMA. As in the former group, the hematologic indices of microangiopathic hemolysis nearly normalized in SLE patients after the second week of follow-up. In contrast, serum Lam-P1 remained elevated at days 10 and 30 in the single case that required hemodialysis treatment. In the four patients (2 TTP, 1 HUS, 1 SLE) with renal insufficiency, Lam-P1 concentrations showed a decrease in samples obtained after 6 mo of regular hemodialysis treatment (Lam-P1 at day 30 *versus* Lam-P1 at 6 mo: 3.62 ± 0.53 U/ml *versus* 1.85 ± 0.08 U/ml; *P* < 0.01) and remained low at 12 mo of follow-up (1.75 ± 0.12 U/ml). No patients suffered further relapses during the first year of follow-up. In addition, no apparent

---

**Table 3. Correlations between Lam-P1 and the other laboratory variables considered in TMA patients during the study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Creatinine (mg/dl)</th>
<th>Hemoglobin (g/dl)</th>
<th>LDH (U/L)</th>
<th>Haptoglobin (g/L)</th>
<th>Platelets (×10¹²/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lam-P1 (U/ml) at diagnosis</td>
<td><em>r</em> = −0.317</td>
<td><em>r</em> = −0.616</td>
<td><em>r</em> = 0.616</td>
<td><em>r</em> = −0.374</td>
<td><em>r</em> = −0.377</td>
</tr>
<tr>
<td><em>P</em> = 0.200</td>
<td><em>P</em> = 0.002</td>
<td><em>P</em> = 0.006</td>
<td><em>P</em> = 0.126</td>
<td><em>P</em> = 0.123</td>
<td></td>
</tr>
<tr>
<td>Lam-P1 (U/ml) day 10</td>
<td><em>r</em> = 0.884</td>
<td><em>r</em> = 0.604</td>
<td><em>r</em> = −0.201</td>
<td><em>r</em> = −0.200</td>
<td></td>
</tr>
<tr>
<td><em>P</em> = 0.000</td>
<td><em>P</em> = 0.008</td>
<td><em>P</em> = 0.42</td>
<td><em>P</em> = 0.425</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lam-P1 (U/ml) day 30</td>
<td><em>r</em> = 0.954</td>
<td><em>r</em> = 0.304</td>
<td><em>r</em> = 0.255</td>
<td><em>r</em> = 0.108</td>
<td></td>
</tr>
<tr>
<td><em>P</em> = 0.000</td>
<td><em>P</em> = 0.891</td>
<td><em>P</em> = 0.306</td>
<td><em>P</em> = 0.670</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations as in Table 1.

* Blood transfusions required by most patients with irreversible renal failure prevented consideration of hemoglobin concentrations obtained during follow-up in the analysis of the results.
Changes were observed in serum Lam-P1 concentrations obtained in two of the former patients after 6 and 12 mo of successful kidney transplant (1.68 and 1.73 U/ml and 1.82 and 1.79 U/ml, respectively). SRC patients developed a rapidly progressive renal failure and required permanent hemodialysis since diagnosis. In this group, the microangiopathic hemolysis also tended to remit during treatment, and platelet counts as well as the indicators of hemolysis were in the normal range in most of the patients at the 30th day of follow-up. All of these patients died within the 6-mo period following diagnosis (mean 3.1 mo; range, 1 to 6 mo). Serum Lam-P1 remained >3 U/ml in all of these patients not only in the control obtained at days 10 and 30, but also at 3 mo (3.53 [3 to 6] U/ml) and at 6 mo (4.02 [3.5 to 4] U/ml) of follow-up. All non-SRC patients who required hemodialysis therapy at the end of the first month were still on dialysis after 12 mo of follow-up.

The correlation between serum Lam-P1 and the other variables considered in TMA patients is presented in Table 3. At the first control, Lam-P1 correlated with hemolysis parameters including Hb and LDH, but not with serum creatinine. At day 10, Lam-P1 correlated only with serum creatinine.

Figures 1 and 2 show the evolution of LDH, platelet count, and serum Lam-P1 concentrations during follow-up of TMA patients according to the renal outcome. At diagnosis, statistically significant differences were not detected between patients with and without renal function recovery in any of the variables studied. However, at day 10 of follow-up, patients with irre-

---

**Figure 1.** Lactate dehydrogenase (LDH) (A), and platelet count (B) during follow-up in thrombotic microangiopathy (TMA) patients with irreversible renal failure (●) and TMA patients with renal recovery (○). Observe the trend to normalize all variables regardless the renal outcome of the patients.

**Figure 2.** Serum Lam-P1 concentrations during follow-up in patients with TMA. At day 10, patients with irreversible renal failure (●) showed significantly higher serum concentrations of Lam-P1 than patients with reversible renal failure (○). This difference remained significant for control performed at day 30.

**Figure 3.** Linear regression between serum Lam-P1 concentrations obtained after 10 d of follow-up and final serum creatinine (day 30). ■, values in patients with irreversible renal failure; ▲, values in patients with renal recovery.
versatile renal failure showed significantly higher serum concentrations of Lam-P1 and LDH than patients with reversible renal failure (Lam-P1: 3.73 ± 0.34 U/ml versus 1.92 ± 0.13 U/ml; P < 0.0001, and LDH: 1677 ± 588 U/I versus 1067 ± 182 U/I; P < 0.05) These differences only remained significant for Lam-P1 (3.72 ± 0.38 U/ml versus 1.68 ± 0.12 U/ml; P < 0.0001) at control performed after 30 d of follow-up.

The relationship between Lam-P1 measures at day 10 and final values of serum creatinine obtained in patients with TMA is represented in Figure 3. It must be emphasized that no overlap exists between the values from patients with irreversible renal failure and those from patients with renal function improvement. So, serum Lam-P1 concentrations at day 10 were higher than 3.07 U/ml in all patients needing renal replacement therapy and lower than 2.11 in all patients with final acceptable renal function.

### Histologic Parameters, Renal Outcome, and Serum Lam-P1 Concentrations

Microscopically, analyses of kidney biopsies revealed four types of lesions coexisting in different degrees: glomerular ischemic retraction, glomerular thrombi associated with thickening of the glomerular basement membrane and sclerosis, concentrically myointimal hyperplasia of medium vessels, and interstitial sclerosis. The results of histopathologic indices considered in kidney biopsy are shown in Table 1. It must be emphasized that we found significant correlation among sclerosis index, interstitial fibrosis score, and vascular index (Table 4).

When compared with patients with reversible renal failure, those with irreversible renal failure showed higher vascular and glomerular sclerosis indices (3.10 ± 0.46 versus 1.25 ± 0.87; P < 0.0001, and 51.10 ± 13.60 versus 22.50 ± 8.01; P < 0.0001, respectively), as well as higher interstitial fibrosis score (2.50 ± 0.52 versus 1.12 ± 0.83; P < 0.001). When analyzed by stepwise multiple regression analysis, the main determinants of renal outcome were vascular index and interstitial fibrosis score, both accounting for 76% of variability in serum creatinine levels at day 10 (P < 0.001) and 90% of variability in serum creatinine at day 30 (P < 0.0001), respectively. The final model included either vascular index or glomerular sclerosis index, but not both, and fitted significantly better when vascular index was introduced instead of glomerular sclerosis index. When vascular index was introduced into multiple regression analysis together with serum Lam-P1 and creatinine at day 10, we detected multicolinearity between vascular index and both serum Lam-P1 and creatinine. These data indicate that vascular index is providing the same information about renal outcome as Lam-P1 and serum creatinine at day 10.

Immunohistochemical studies showed that laminin was present in both glomerular lesions and intimal hyperplasia of arteries and arterioles, but not in the interstitium. This pattern was strongly coincident with the glomerular and vascular lesions observed in trichromed stained sections (Figure 4). The histologic abnormalities observed in a representative patient with irreversible renal failure compared to a patient with renal recovery are shown in Figure 5.

The relationship among serum Lam-P1, creatinine, LDH, and the morphologic lesions is presented in Table 5. At first control, no relationship was observed between the indices of histopathologic lesion and serum Lam-P1 concentrations. However, during follow-up, serum Lam-P1 levels significantly correlated with the histopathologic parameters considered in the study, especially the glomerular sclerosis index.

### Predictors of Renal Outcome in TMA Patients

Because we wanted to analyze the prognostic value of the different biochemical markers, we had to define an end point for the renal outcome. We decided to consider serum creatinine at day 30 for two reasons. First, all patients who suffered irreversible renal failure required permanent hemodialysis therapy within the first month of follow-up and remained on dialysis afterward. On the other hand, patients with reversible renal failure did not suffer further relapses during the first year of follow-up. So, serum creatinine at the end of the first month seemed to be an adequate end point reflecting the final state of renal function in a given patient.

In univariate analysis, the variables associated with renal function (logarithm of serum creatinine) at day 30 were age (P = 0.04), LDH at day 10 (P = 0.03), serum creatinine at day 10 (P = 0.002), serum Lam-P1 at day 10 (P = 0.0001), vascular index (P = 0.001), and percentage of interstitial sclerosis in kidney biopsies (P = 0.045). In single regression analysis, creatinine levels at day 10 and serum levels of Lam-P1 at day 10 accounted for 65% (r² = 0.65; P < 0.001) and 45% (r² = 0.45; P < 0.001) of the variability of serum creatinine at day 30, respectively (P < 0.05). When analyzed by stepwise multiple regression analysis, the final model included only serum creatinine at day 10 and serum levels of Lam-P1 as independent predictors of renal outcome (r² = 0.94; P < 0.0001). Standardized β coefficients of the regression model were 0.48 for serum creatinine and 0.52 for Lam-P1, indicating that the two variables contributed in a similar degree to explain the variability of serum creatinine at day 30.

### Discussion

The term thrombotic microangiopathy refers to a unique pathologic lesion that accompanies several clinical conditions characterized by microangiopathic hemolytic anemia and some.
degree of kidney dysfunction (2–4,15). This lesion consists of intravascular thrombi in small vessels, thickened basement membranes, and detachment of endothelial cells with accumulation of fluffy material in the subendothelium. At later stages, glomerular and arterial fibroproliferative lesions can be observed (3,6–8). The origin of these lesions is unknown, but both endothelial dysfunction and disturbed metabolism and composition of capillary basement membranes seem to play an important role in its pathogenesis (2,3,21).

Laminin, unlike other adhesive glycoproteins, is specific to basement membranes and is located mainly in lamina rara in close contact with endothelial cells (9,10,22). Thus, serum levels of this protein or its fragments could reflect the changes observed in the basement membranes of TMA patients. In the present study, we describe two main findings. First, we clearly demonstrate that serum Lam-P1 concentrations are strongly increased in patients with TMA regardless of the primary etiology. Second, we found that serum Lam-P1 levels obtained at day 10 of follow-up were an independent predictor of renal outcome in patients with TMA.

Figure 4. Morphologic pattern in a case of irreversible renal function stained with Masson’s trichrome (A) showing important interstitial fibrosis lacking laminin expression in the immunostaining (B), where deposition of this protein can be seen in the vessel wall, being coincidental to the arteriolar fibrosis with severe intimal hyalinization noticed in the Masson’s trichrome stain. Magnification, ×100.
To our knowledge, higher serum Lam-P1 concentrations than those observed in our patients with active TMA have only been reported in a case of giant retroperitoneal schwannoma (23). This finding could be explained by the severity of endothelial lesion that occurs in TMA. However, another factor that must be taken into account is the important role of the kidney in the pathologic scene of these patients. This organ consists of an important capillary bed in which, unlike other organs, there are basement membranes of a double layer of lamina rara (lamina rara interna and externa) that can be affected by the endothelial injury (24).

The demonstration that serum Lam-P1 is an independent predictor of renal outcome in TMA patients, and its relationship with the classic TMA activity markers deserve further comment. At diagnosis, the close correlation found between hemolysis markers and Lam-P1 and the lack of correlation between Lam-P1 and renal function suggested that Lam-P1 would behave as a hemolysis marker. However, during follow-
up, hemolysis markers normalized in all patients whatever the renal outcome, a condition that has been reported previously by other authors (3,25,26). Conversely, serum Lam-P1 normalized only in patients with reversible renal failure, and at control performed at day 10 of follow-up, serum Lam-P1 predicted the long-term renal function in all TMA patients. The lack of correlation between Lam-P1 and hemolysis was particularly evident at day 30 when Lam-P1 only correlated with serum creatinine. This finding raises the question of whether the additional value of serum Lam-P1 is a marker in clinical practice, over that of serum creatinine now in use. In this regard, it should be noted that in multivariate analysis, serum creatinine and serum Lam-P1 at day 10 were the only independent predictors of renal outcome, both contributing to an equal degree to explain renal function variability at the end of the first month of follow-up. In addition, we found a significant correlation between Lam-P1 measures at days 10 and 30 of follow-up and both glomerular and vascular scores of renal biopsies. Moreover, the pattern of laminin staining was coincident with the glomerular and vascular lesions observed in trichromic stained kidney sections. This finding indicates that laminin is a relevant component of glomerular thickening of basement membranes, sclerotic glomerular lesions, and concentric myointimal hyperplasia of medium-sized vessels. Curiously, we found a significant correlation between Lam-P1 at day 10 and interstitial fibrosis score, but we did not find laminin deposits in the interstitium of kidney biopsies. This apparent paradox could be explained by the close correlation found between vascular index and interstitial fibrosis, which is in agreement with the concept that in TMA, irreversible renal failure results mainly from occlusive arteriolopathy and tubulointerstitial ischemia (27). Taken as a whole, our data show that follow-up of serum Lam-P1 levels can give additional information on renal outcome that obtained from renal monitoring and provides a rational basis to consider serum Lam-P1 as a marker of renal lesions in TMA. Interestingly, Lam-P1 remained increased in all SRC patients who eventually died within the first 6 mo of follow-up. These data suggest that serum Lam-P1 would also be a marker of patient outcome, but this hypothesis could not be evaluated in our study because mortality was restricted to patients with SCR.

Although during follow-up Lam-P1 correlated with serum creatinine, several reasons suggest that renal failure itself cannot be considered as a significant factor accounting for the high Lam-P1 serum concentrations detected in TMA. First, the liver but not the kidney is the main organ responsible for the clearance of plasma laminin (28,29). Second, in the present study, serum Lam-P1 normalized in all patients who remained in hemodialysis treatment after solving the acute TMA. Third, patients on chronic renal failure without TMA but with serum creatinine values comparable to TMA cases showed significant lower serum Lam-P1 concentrations. Finally, in agreement with other authors (30), we have found previously that serum Lam-P1 concentration is mainly increased in renal diseases that involve the glomerular basement membrane independently of serum creatinine levels (31,32). On the other hand, it seems that the hemodialysis procedure does not influence serum Lam-P1 concentrations because we did not observe differences between predialysis patients and patients on regular hemodialysis.

A few years ago, Bergstein et al. (33) reported that duration of elevated plasminogen-activator inhibitor type 1 activity in patients with HUS correlated with the outcome of the disease. This observation together with our results favor the concept that serum markers of endothelial and/or basement membrane damage could be used as a prognostic index in patients with TMA.

In conclusion, serum Lam-P1 is increased in patients with active TMA. Furthermore, contrary to patients with renal function recovery, those patients with irreversible renal failure showed a prolonged increase of serum Lam-P1 concentrations. This fact could probably be related to the extent of renal lesions, since during follow-up Lam-P1 levels clearly correlated with the indices of histopathologic damage. Finally, unlike other biochemical parameters commonly used to assess TMA activity, the sequential determination of serum Lam-P1 provided useful information about long-term renal prognosis in TMA patients.

Acknowledgment

This work was supported by a grant from Fondo de Investigaciones Sanitarias (F.I.S. 98/1246), Ministerio de Sanidad y Consumo, Spain.
References