Histopathologic Classification of ANCA-Associated Glomerulonephritis

Annelies E. Berden,* Franco Ferrario,† E. Christiaan Hagen,‡ David R. Jayne,§ J. Charles Jennette,¶ Kensuke Joh,∥ Irmgard Neumann,** Laure-Hélène Noël,†† Charles D. Pusey,‡‡ Rüdiger Waldherr,§§ Jan A. Bruijn,* and Ingeborg M. Bajema*

*Pathology, Leiden University Medical Center, Leiden, Netherlands; †Nephropathology Center, San Gerardo Hospital, Monza, Italy; ‡Department of Internal Medicine, Meander Medical Center, Amersfoort, Netherlands; §Renal Unit, Addenbrooke’s Hospital, Cambridge, United Kingdom; ¶Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina; ‡Division of Pathology, Sendai-Shaho Hospital, Sendai-city, Japan; **Department of Nephrology, Wilhelminenspital, Vienna, Austria; ††INSERM U 1016, Paris V University, Hôpital Cochin, Paris, France; ‡‡Imperial College Kidney and Transplant Institute, London, United Kingdom; and §§Department of Pathology, University of Heidelberg, Germany

ABSTRACT

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis is the most common cause of rapidly progressive glomerulonephritis worldwide, and the renal biopsy is the gold standard for establishing the diagnosis. Although the prognostic value of the renal biopsy in ANCA-associated glomerulonephritis is widely recognized, there is no consensus regarding its pathologic classification. We present here such a pathologic classification developed by an international working group of renal pathologists. Our classification proposes four general categories of lesions: Focal, crescentic, mixed, and sclerotic. To determine whether these lesions have predictive value for renal outcome, we performed a validation study on 100 biopsies from patients with clinically and histologically confirmed ANCA-associated glomerulonephritis. Two independent pathologists, blinded to patient data, scored all biopsies according to a standardized protocol. Results show that the proposed classification system is of prognostic value for 1- and 5-year renal outcomes. We believe this pathologic classification will aid in the prognostication of patients at the time of diagnosis and facilitate uniform reporting between centers. This classification at some point might also provide means to guide therapy.


Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, particularly Wegener’s granulomatosis and microscopic polyangiitis, often affect the kidneys, and renal involvement is an important factor with respect to patient morbidity and mortality. Although rapidly progressive renal failure in patients who are seropositive for ANCA by indirect immunofluorescence or ELISA is suggestive of ANCA-associated glomerulonephritis, the morphologic changes in the renal biopsy are still the gold standard for establishing a diagnosis. ANCA-associated glomerulonephritis is characterized on immunofluorescence microscopy by little or no glomerular staining for Igs or complement, the so-called pauci-immune staining pattern. By electron microscopy, subendothelial edema, microthrombosis, and degranulation of neutrophils are present, but immune deposits are absent. Light microscopy shows necrotizing and crescentic glomerulonephritis. Until now, there has been no histopathologic classification of ANCA-associated glomerulonephritis, although there is a clinical need to distinguish the levels of severity.

A large number of clinicopathologic studies investigating diagnostic and follow-up renal biopsies demonstrated that specific pathologic lesions—or the absence thereof—are important predictors for renal outcome in ANCA-associated vasculitis and that the combination of baseline GFR and renal histology is a better predictor of renal outcome than baseline GFR alone. One consistent finding in these studies is the relationship between a high percentage of normal glomeruli that are not affected by the disease process and favorable renal outcome. In fact, the percentage of normal glomeruli is a strong predictor, possibly the best his-
tologic predictor of short- or long-term renal outcome.\textsuperscript{14} In addition to the relation of normal glomeruli to outcome, a high percentage of globally sclerotic glomeruli has been associated with adverse renal outcome repeatedly.\textsuperscript{6,7,8} The percentage of active crescentic lesions, in particular cellular crescents, is related to recovery of renal function independent of baseline GFR.\textsuperscript{8} Conversely, the percentage of fibrous crescents adversely affects long-term renal outcome.\textsuperscript{14}

Apart from glomerular lesions, acute and chronic tubulointerstitial lesions have been associated with renal outcome, and tubular atrophy is an especially important risk factor for impaired renal function during follow-up.\textsuperscript{7,15} The relationship of vascular lesions to renal outcome has been reported less frequently, although arteriosclerosis in the initial biopsy is identified as a risk factor for long-term dialysis.\textsuperscript{7}

Although a standardized scoring protocol for renal biopsies of patients with ANCA-associated vasculitis, with good reproducibility, was developed previously,\textsuperscript{16} a histopathologic classification is still lacking. Considering the substantial diagnostic and prognostic value of the renal biopsy in ANCA-associated glomerulonephritis, we propose a histopathologic classification that is based on glomerular pathology. Most histologic classifications of renal diseases\textsuperscript{17–19} have been primarily based on expert experience; our proposed classification, however, is also validated with patient data.

### Classification Proposal for ANCA-Associated Glomerulonephritis

The proposed classification is based on glomerular pathology as assessed by light microscopy. For classification purposes, adequacy of tissue specimens and histopathologic techniques is essential. A minimum of 10 whole glomeruli are considered adequate.\textsuperscript{18} Hematoxylin and eosin, methenamine silver, and periodic acid-Schiff stainings are minimally required for examining renal histopathology. A Masson trichrome staining or one of its variants can be helpful to visualize fibrinoid necrosis, acute tubular necrosis, and interstitial fibrosis but is not necessary for our proposed classification schema.

The classification is built around four general categories: Focal, crescentic, sclerotic, and mixed. The categories labeled focal, crescentic, and sclerotic are based on the predominance of normal glomeruli, cellular crescents, and globally sclerotic glomeruli, respectively. The mixed category represents a heterogeneous glomerular phenotype wherein no glomerular feature predominates. Definitions of histologic variables used in our classification are reported in Table 1, and the classification schema is depicted in Table 2. The biopsies in the focal category contain $\geq 50\%$ normal glomeruli that are not affected by the disease process. The crescentic category contains biopsies with $\geq 50\%$ of glomeruli with cellular crescents. Biopsies from the sclerotic category contain $\geq 50\%$ of glomeruli with global sclerosis. All remaining biopsies (Figure 1) are, per definition, not characterized by one predominant glomerular phenotype and form the mixed category. These latter biopsies will have all aforementioned glomerular features to varying degrees.

The typical description of immunofluorescence findings in ANCA-associated glomerulonephritis is that of a so-called pauci-immune pattern, first described by Jennette et al.\textsuperscript{20} and defined as $<2+$ glomerular immunostaining for IgG. A coarse granular staining with positivity for mesangial IgA has been described in a small number of patients with ANCA-associated glomerulonephritis.\textsuperscript{21} This staining pattern is not an exclusion criterion for the

<table>
<thead>
<tr>
<th>Table 1. Definitions</th>
<th>Total number of glomeruli</th>
<th>Normal glomeruli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The maximum number of glomeruli in one of the sections excluding incomplete glomeruli on the edge</td>
<td>Glomeruli without vasculitic lesions or global sclerosis. Normal glomeruli may show subtle changes as a result of ischemia or a minimum number of inflammatory cells (fewer than four neutrophils, lymphocytes, or monocytes)</td>
</tr>
<tr>
<td>Exclusion criteria are</td>
<td>synchiae</td>
<td></td>
</tr>
<tr>
<td>local/segmental glomerulosclerosis</td>
<td>extensive ischemic changes (splitting of Bowman’s capsule, wrinkling of the GBM)</td>
<td></td>
</tr>
<tr>
<td>any other lesion unrelated to vasculitis (e.g., amyloid, träm tracking)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crescents</th>
<th>Purely cellular lesions or with cellular components</th>
</tr>
</thead>
<tbody>
<tr>
<td>fibrous</td>
<td>Fibrotic (sclerotic) lesion with fibroblasts filling Bowman’s space</td>
</tr>
</tbody>
</table>

| Global glomerulosclerosis | $>80\%$ of the glomerulus sclerosed |

| Table 2. Classification schema for ANCA-associated glomerulonephritis |
|-----------------------------|-------------------------------|
| Class                        | Inclusion Criteria*          |
| Focal                        | $\geq 50\%$ normal glomeruli  |
| Crescentic                   | $\geq 50\%$ glomeruli with cellular crescents |
| Mixed                        | $<50\%$ normal, $<50\%$ crescentic, $<50\%$ globally sclerotic glomeruli |
| Sclerotic                    | $\geq 50\%$ globally sclerotic glomeruli |

*Pauci-immune staining pattern on immunofluorescence microscopy (IM) and $\geq 1$ glomerulus with necrotizing or crescentic glomerulonephritis on light microscopy (LM) are required for inclusion in all four classes. See Figure 1 for hierarchical structure.
The proposed classification system hinges on the recognition of normal glomeruli, glomeruli with cellular crescent formation, and glomeruli with global glomerulosclerosis. Straightforward as this may seem, interobserver disagreement may arise for recognition of these features in individual glomeruli. We refer to Figure 2 for typical examples of glomeruli that belong or do not belong to the various categories of classification. Furthermore, we now offer explicit guidelines to distinguish these features in more detail.

**Normal Glomeruli**

According to the definition provided in Table 1, a normal glomerulus does not exhibit features of vasculitic lesions or global glomerulosclerosis. It also should not show intracapillary proliferation, meaning no extensive endothelial swelling or proliferation in more than one capillary loop or more than four intracapillary inflammatory cells (neutrophils, lymphocytes, or monocytes) in all of the glomerular capillary bed. Normal glomeruli should not have synechiae or any local or segmental glomerulosclerosis. Normal glomeruli may show subtle signs of ischemia: Slight collapse of the tuft, focal splitting of Bowman’s capsule, or focal wrinkling of the GBM. Ischemia may lead to a more prominent appearance of the parietal epithelium of Bowman’s capsule. As long as the epithelium remains as a monolayer and does not show signs of atypia or influx of inflammatory cells, these changes could be accepted within the scope of ischemia and not be regarded as extracapillary proliferation. We refer to Figure 2 showing examples of subtle versus overt changes as a result of ischemia, giving guidance as to which are still acceptable in the context of a normal glomerulus.

**Crescents**

Cellular crescents are defined as either purely or partially cellular crescents in which fibrous components are allowed. They are distinct from fibrous crescents, which are defined as purely fibrotic lesions in which a cellular component is virtually absent. If >90% of a crescent consists of extracellular matrix, then the term fibrous crescent is used. As long as

---

Figure 1. Classification flowchart. Biopsies should be scored for glomerular lesions in the following order: Globally sclerotic glomeruli, normal glomeruli, and cellular crescent glomeruli. Any biopsies that do not fit into one of the categories on the basis of a predominant glomerular phenotype will automatically be included in the mixed category.

Figure 2. Typical examples of glomerular lesions in each of the four categories. (A through C) Normal glomeruli, allowing for fewer than four leukocytes in the capillary tuft (B) or mild ischemic changes such as wrinkling of the GBM (C). Cellular crescents contain >10% of cellular components. Whether crescents are segmental or circumferential is irrelevant for the classification schema. (D through G) Examples of cellular crescents. The amount of fibrinoid necrosis is irrelevant. (H through J) If >90% of a crescent consists of extracellular matrix, then the term fibrous crescent is used. (K) Global glomerulosclerosis refers to sclerotic changes in the glomerulus composing >80% of the tuft. Global glomerulosclerosis excludes the designation of any other glomerular lesion.
the crescent contains cellular components >10%, it is regarded as a cellular crescent, irrespective of whether it is segmental or circumferential or whether it contains other components such as fibrin or is accompanied by a periglomerular granulomatous reaction or by breaks in Bowman’s capsule. Whether the glomerulus has a fibrinoid necrotic lesion is not regarded as relevant for classification purposes. Segmental crescents show extracapillary proliferation in <50% of the circumference of Bowman’s space, whereas circumferential crescents show extracapillary proliferation in >50% of Bowman’s space.

Global Glomerulosclerosis
We define global glomerulosclerosis as sclerotic changes in the glomerulus that compose >80% of the tuft. It is irrelevant whether the global glomerulosclerosis is attributable to ANCA-associated glomerulonephritis. In our classification system, global glomerulosclerosis excludes the designation of any other glomerular lesion.

RECOMMENDATIONS FOR REPORTING OF TUBULOINTERSTITIAL AND VASCULAR LESIONS
The current proposal for ANCA-associated glomerulonephritis is based purely on the presence of glomerular lesions; however, tubulointerstitial lesions may also be of prognostic value in ANCA-associated vasculitis.7,13 For guidelines on how to report systematically on tubulointerstitial and vascular lesions, we refer to the scoring form that was devised previously for the standardized evaluation of biopsies with ANCA-associated glomerulonephritis.16 Unless the findings are remarkable, tubulointerstitial and vascular lesions need not be mentioned in the final diagnosis. Examples of remarkable findings are a dominance of any cell type in the infiltrate (plasma cells or eosinophils), a high number of interstitial granulomas, or extensive arteriolar sclerosis. Some of these findings may have clinical consequences or be of importance in the differential diagnosis of other diseases, such as drug hypersensitivity, infection, or cardiovascular disease.

VALIDATION STUDY FOR THE CLASSIFICATION

Patients and Data
Following the stringent inclusion criteria described in the Concise Methods section, a total of 100 patients with at least 1 year of follow-up and adequate renal histology were included in a validation study. These patients came from 32 centers in nine European countries. Median age at baseline was 62.6 years (range 20.4 to 80.7 years). The male-to-female ratio was 54:46. All 100 patients had a clinicopathologic diagnosis of Wegener’s granulomatosis (n = 39) or microscopic polyangiitis (n = 61) with pauci-immune crescentic glomerulonephritis. ANCA test results by indirect immunofluorescence or ELISA were available for 97% of patients (PR3-ANCA n = 45, MPO-ANCA n = 47, negative ANCA test n = 2, missing n = 3). Thirty-five patients reached ESRD, and mean time to reach ESRD was just over 1 year from baseline. The median number of glomeruli per biopsy was 14.8 (range 10.0 to 49.0).

Classifying 100 Renal Biopsies
Following the proposed classification and flow chart (Table 2, Figure 1), 13 biopsies were classified as sclerotic ANCA-associated glomerulonephritis (≥50% globally sclerotic glomeruli). Of the 87 biopsies left, 16 were classified as focal (≥50% normal glomeruli). After taking out the biopsies that were classified as sclerotic or focal, 71 were left for study. Fifty-five of these biopsies demonstrated ≥50% of glomeruli with cellular crescents, and these biopsies were classified as crescentic. No biopsy showed >50% purely fibrous crescents. The remaining 16 biopsies could not be classified into a predominantly sclerotic, focal, or crescentic phenotype and were classified as a mixed phenotype. None of the biopsies in this cohort exhibited 50% globally sclerotic glomeruli together with 50% normal glomeruli or glomeruli with cellular crescents. Likewise, no biopsies exhibited 50% normal glomeruli and 50% crescentic glomeruli.

The 16 biopsies that were classified as demonstrating a mixed phenotype had on average approximately 27% globally sclerotic glomeruli, approximately 21% normal glomeruli, and approximately 32% glomeruli with cellular crescents. Approximately 15% of glomeruli in these biopsies exhibited purely fibrous crescents, and the remaining 5% of glomeruli exhibited either local/segmental glomerulosclerosis or ischemia.

Categories in Relation to Clinical Presentation and Renal Outcome
As depicted in Table 3 and Figure 3, the renal biopsy categories were correlated to the degree of renal function at presentation and at 1- and 5-year follow-up (all P ≤ 0.001), with the sequence of category (focal, crescentic, mixed, and sclerotic) corresponding to the order of severity of renal function loss. In multiple linear regression analyses investigating independent predictors for estimated GFR (eGFR) at 1 and 5 years, the focal and crescentic categories were the only variables that remained statistically significant.

<table>
<thead>
<tr>
<th>Class</th>
<th>eGFR Entry Mean ± SD</th>
<th>eGFR 12 Months Mean ± SD</th>
<th>eGFR 12 Months* Mean ± SD</th>
<th>eGFR 60 Months Mean ± SD</th>
<th>eGFR 60 Months* Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Focal</td>
<td>15.4 ± 9.5</td>
<td>13</td>
<td>16.6 ± 15.9</td>
<td>8</td>
<td>12.8 ± 12.4</td>
</tr>
<tr>
<td>Distal</td>
<td>8.3 ± 2.1</td>
<td>11</td>
<td>9.5 ± 2.5</td>
<td>3</td>
<td>9.5 ± 2.5</td>
</tr>
<tr>
<td>Proximal</td>
<td>10.8 ± 9.5</td>
<td>13</td>
<td>16.6 ± 15.9</td>
<td>8</td>
<td>12.8 ± 12.4</td>
</tr>
<tr>
<td>Sclerotic</td>
<td>10.8 ± 9.5</td>
<td>13</td>
<td>16.6 ± 15.9</td>
<td>8</td>
<td>12.8 ± 12.4</td>
</tr>
</tbody>
</table>

*Corrected for entry eGFR.
years and taking into account patient age, treatment limb, baseline eGFR, and the classification system, baseline eGFR and renal biopsy category were the only independent predictors for eGFR at both follow-up events, as depicted in Table 4. Adjusted $R^2$ values for the models at 1 and 5 years are 0.61 and 0.49, respectively, indicating that these models account for considerable percentages of the variance in eGFR at these time points.

Regarding the hard end point of development of ESRD, as depicted in Figure 4, the absolute number of events was limited. Renal survival data were available for 82 of 100 patients. A total of 25 patients developed ESRD during the follow-up period. ESRD developed in one of 14 patients with focal, in 11 of 45 patients with crescentic, in six of 13 patients with mixed, and in seven of 10 patients with sclerotic ANCA-associated vasculitis. The data show that the percentage of patients who developed ESRD increases with ascending category ($P = 0.003$). A multiple Cox regression analysis, including patient age, treatment, baseline eGFR, and the classification, demonstrates that patients who present with crescentic ANCA-associated glomerulonephritis are at decreased risk for developing ESRD compared with patients who present with sclerotic ANCA-associated glomerulonephritis (hazard ratio 0.176; 95% confidence interval 0.057 to 0.574; $P = 0.003$).

Investigating renal outcome by looking at renal function during follow-up does not take into account patients who have died, and in survival analyses, these patients are censored. We have taken the Kidney Disease Outcomes Quality Initiative (KDOQI)$^{28}$ classification of chronic kidney disease stages as an example to describe different categories of renal outcome at 1 year, primarily on the basis of renal function (ml/min per 1.73 m$^2$). The following four classes are considered: eGFR $\geq$ 60, eGFR 15 to 59, eGFR <15 or on dialysis, and death within the first year. Results of this exercise are depicted in Table 5 and illustrate that patients with sclerotic ANCA-associated glomerulonephritis not only have decreased chances of renal survival but also are at a higher risk for death.

Adding Tubulointerstitial Parameters to the Classification System

To assess the contribution of tubulointerstitial parameters to the classification system, we investigated the influence on the classification of either a combined score of fibrosis and tubular atrophy or individual scores of fibrosis, tubular atrophy, and intraparenchymal infiltrates. Although, in general, a slight dichotomy could be seen within the four glomerular classes, wherein patients with more extensive tubulointerstitial damage had worse renal outcome, the data were not convincing enough to adjust the classification system accordingly for any of the tubulointerstitial parameters. Particularly, adjusted $R^2$ values obtained for the models taking into account the glomerular classification system as well as tubulointerstitial parameters and renal function at 1 and 5 years, did not differ from the

\[
\text{Table 4. Independent predictors of renal outcome} \]

<table>
<thead>
<tr>
<th>Model</th>
<th>eGFR at 1 Year</th>
<th>eGFR at 5 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>$P$</td>
</tr>
<tr>
<td>eGFR at entry</td>
<td>0.554</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Classification</td>
<td>$-0.256$</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Adjusted $R^2$ model eGFR 1 year = 0.61; adjusted $R^2$ model eGFR at 5 years = 0.49.
increases its complexity.

The classification system is composed of four categories. The focal category contains biopsies wherein ≥50% of glomeruli are not yet affected by the disease. In the crescentic category, more than half of the glomeruli have cellular crescents. The mixed category involves biopsies in which a combination of normal, crescentic, and sclerotic glomeruli are present, all occurring in <50% of glomeruli. Forming the sclerotic category are biopsies characterized by ≥50% globally sclerotic glomeruli.

Our validation study shows that the phenotypical order of the classes corresponds to the order of severity of renal dysfunction during follow-up. Patients with focal ANCA-associated glomerulonephritis present with relatively preserved renal function and have a relatively favorable renal outcome. Patients with crescentic ANCA-associated glomerulonephritis present with highly active renal disease and severely reduced renal function but stand a good chance for renal function recovery. Patients with a mixed phenotype have an intermediate outcome profile. Patients with sclerotic ANCA-associated glomerulonephritis at the time of biopsy run the highest risk for not recovering renal function and also have a higher risk for death within the first year after diagnosis.

This classification proved practical during an initial validation exercise. None of the 100 biopsies exhibited 50% globally sclerotic glomeruli together with 50% normal glomeruli or glomeruli with cellular crescents. Likewise, no biopsies that exhibited 50% normal glomeruli and 50% crescentic glomeruli were encountered. This indicates, in this cohort, that each biopsy clearly has one predominant glomerular feature or demonstrates a mixed phenotype. No biopsy had an overlap between categories. Additional validation cohorts will be required to confirm these conclusions. In cases in which exactly 50% of glomeruli are consistent with one feature and 50% with another feature, the flow chart (Figure 1) will be helpful in making the final decision.

The limitations of this study reflect problems encountered when studying relatively rare diseases. Material for the validation study came from various centers where it was processed in comparable but not exactly identical ways. Although this was an international study, all patients were seen in European centers only. The interobserver variation for the histopathologic parameters on which the classification was based was previously established, and consensus was

**DISCUSSION OF THIS CLASSIFICATION**

ANCA-associated vasculitis is the most frequent cause of rapidly progressive glomerulonephritis worldwide, and the renal biopsy is the gold standard for establishing the diagnosis of ANCA-associated glomerulonephritis.\textsuperscript{29,30} The diagnostic and prognostic value of the renal biopsy in ANCA-associated glomerulonephritis is widely known. We present here a proposal for a pathologic classification for ANCA-associated glomerulonephritis. The proposed classification schema has been developed by an international working group of renal pathologists, and we report its validation on a set of 100 renal biopsies that were scored in a standardized manner.

The classification system is composed of four categories. The focal category contains biopsies wherein ≥50% of glomeruli are not yet affected by the disease. In the crescentic category, more than half of the glomeruli have cellular crescents. The mixed category involves biopsies in which a combination of normal, crescentic, and sclerotic glomeruli are present, all occurring in <50% of glomeruli. Forming the sclerotic category are biopsies characterized by ≥50% globally sclerotic glomeruli.

Table 5. Classification and outcome at 1 year

<table>
<thead>
<tr>
<th>Class</th>
<th>eGFR ≥60 (n [%])</th>
<th>eGFR 15 to 59 (n [%])</th>
<th>eGFR&lt;15 or on Dialysis (n [%])</th>
<th>Death (n [%])</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal</td>
<td>8 (50)</td>
<td>7 (44)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Crescentic</td>
<td>3 (6)</td>
<td>29 (53)</td>
<td>8 (15)</td>
<td>15 (27)</td>
<td>55 (100)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1 (6)</td>
<td>7 (44)</td>
<td>4 (25)</td>
<td>4 (25)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Sclerotic</td>
<td>0 (0)</td>
<td>4 (31)</td>
<td>4 (31)</td>
<td>5 (39)</td>
<td>13 (100)</td>
</tr>
</tbody>
</table>
reached for each parameter during various meetings\textsuperscript{16}; however, the division into the four categories now devised is based on the initial scores of individual parameters. No interobserver variation study was undertaken purely for classifying biopsies according to our proposal.

We encourage further validation of this classification for ANCA-associated glomerulonephritis in different cohorts throughout the world, and hopefully this will lead to classification refinements. This classification will be of aid in the prognostication of patients at the time of diagnosis and will facilitate uniform reporting between centers.

CONCISE METHODS

Data
Renal histology data from patients who were entered into two randomized controlled trials (Cyclophosphamide or Azathioprine As a Re-Mission therapy for vasculitis [CYCAZAREM] and Methylprednisolone versus Plasma Exchange as additional therapy for severe, ANCA-associated glomerulonephritis [MEPEX]\textsuperscript{1,3,32}) conducted by the European Vasculitis Study Group between March 1995 and September 2002 were pooled. Trial outcomes and clinicopathologic studies from these trials were previously published.\textsuperscript{7,8,31,32} For this study, patients who had been followed-up for at least 12 months (including patients who died within the first 12 months but excluding patients who were lost to follow-up) were included. Five-year follow-up was available for a subset of patients and reported on. Adequacy of tissue specimens and histopathologic techniques are mandatory for a reliable classification. For this validation exercise, we included biopsies with a minimum of 10 whole glomeruli.\textsuperscript{18} Hematoxylin and eosin, methenamine silver, periodic acid-Schiff, and Masson trichrome stainings were available for evaluation. All biopsies were scored independently by two pathologists, who were blinded to patient data, from a group of five pathologists (F.F., I.M.B., J.A.B., L.H.N., and R.W.), according to a previously standardized protocol; discrepancies were resolved during consensus meetings.\textsuperscript{16} Patients with Churg-Strauss syndrome were not included in this study, and this classification proposal is not validated for these patients. GFRs were estimated using the four-variable Modification of Diet in Renal Disease (MDRD) equation.\textsuperscript{33,34} To evaluate an independent predictive effect of the classification on eGFR at 1 and 5 years of follow-up, we corrected for the eGFR at baseline. The corrected eGFR at a time point was defined as the difference between the observed eGFR at that time point and its linear prediction on the basis of baseline eGFR.\textsuperscript{33,35} In addition to renal function at different follow-up times, renal survival, defined as time to end stage renal failure, was assessed.

Statistical Analysis
\(\chi^2\), one-way ANOVA, and multiple linear regression analyses were performed as appropriate. Renal survival was assessed using the Kaplan-Meier method. Differences between categories were assessed using the log-rank test. Hazard ratios were acquired using Cox proportional hazards regression. \(P < 0.05\) was considered significant.

ACKNOWLEDGMENTS
The CYCAZAREM trial was supported by contracts (BMH1-CT93-1078, CIPD-CT94-0307, BMH4-CT97-2328, and IC20-CT97-0019) with the European Union; the MEPEX trial was designed and launched as part of the European Community Systemic Vasculitis Trial project (BMH1-CT93-1078 and CIPD-CT94-0307) and finished as part of the ANCA Associated Vasculitis European Randomized Trial project (BMH4-CT97-2328 and IC20-CT97-0019) funded by the European Union.

Participating physicians include D. Abramowicz, J. Sennesael, Free University of Brussels, Brussels, Belgium; M. Wissing, P. Madhoun, Edith Cavell Medical Institute, Brussels, Belgium; M. Dhaene, Clinique Louis Caty, Baudour, Belgium; D. Blockmans, University Hospital, Leuven, Belgium; J. Stolear, IMC de Tournai, Tournai, Belgium; V. Chabova, I. Rychlik, V. Tesar, Charles University Hospital, Prague, Czech Republic; N. Rasmussen, Rigshospitalet, Copenhagen, Denmark; A. Wiik, Statens Seruminstitutet, Copenhagen, Denmark; C. Grönhagen-Riska, E. Ekstrand, University of Helsinki, Helsinki, Finland; P. Lesavre, Hôpital Necker, Paris, France; L. Guillevin, Hôpital Cochin, Paris, France; P. Vanhille, Centre Hospitalier, Valenciennes, France; K. Andrassy, O. Hergesell, Heidelberg University Hospital, Heidelberg, Germany; F. van der Woude, R. Nowack, University of Mannheim, Mannheim, Germany; K. de Groot, University Hospital, Hannover, Germany; H. Ruppprecht, P. Weber, S. Weidner, Klinikum Nürnberg, Nürnberg, Germany; W. Schmitt, W. Gross, University of Luebeck and Rheumaklinik Bad Bramstedt, Luebeck, Germany; M. Schneider, C. Specker, Heinrich Heine Universität, Düsseldorf, Germany; M. Vischedyk, St. Vinzenz-Hospital Paderborn, Paderborn, Germany; C. Feighery, St. James Hospital, Dublin, Ireland; G. Gregorini, Spedali Civili, Brescia, Italy; R. Sinico, F. Ferrario, Ospedale San Carlo Borrromeo, Milan, Italy; R. Confaloni, Ospedale Niguarda, Niguarda, Italy; J. Dadoniené, University of Vilnius, Vilnius, Lithuania; C. Kallenberg, C. Stegeman, University Hospital Groningen, Netherlands; E. van Gurp, E. Hagen, Meander Medical Center, Amersfoort, Netherlands; C. Stegert, C. Verburch, R. de Lind van Wijngaarden, Leiden University Medical Center, Leiden, Netherlands; J.W. Cohen Tervaert, Maastricht University Medical Center, Maastricht, Netherlands; A. Serra, Hospital Germans Trias i Pujol, Badalona, Spain; E. Mirapeix, Hospital Clinic i Provincial, Barcelona, Spain; M. Valles, Hospital Doctor Josep Trueta, Girona, Spain; R. Poveda, Hospital Bellvitge, Barcelona, Spain; J. Ballarin, Fundació Puigvert, Barcelona, Spain; J. Ballarin, F. Calero, Fundación Puigvert, Barcelona, Spain; A. Bruchfeld, E. Pettersson, M. Heimbürger, Huddinge University Hospital, Stockholm, Sweden; G. Gemnias, Danderyds Sjukhus, Danderyds, Sweden; D. Selga, K. Westman, University of Lund, Lund, Sweden; Z. Heigl, I. Lundberg, E. Svenungssen, Karolinska Sjukhuset, Stockholm, Sweden; M. Segelmark, G. Sterner, University Hospital of Malmö, Malmö, Sweden; M. Tidman, Nephrology University Hospital, Orebro, Sweden; P. Mathiesen, C. Tomson, Southmead Hospital, Bristol, United Kingdom; R. Watts, Ipswich Hospital, Ipswich, United Kingdom; J. Feehally, University Hospital, Leicester, United Kingdom; A. Burns, Royal Free Hospital, London, United Kingdom; R. Luqmani, N. Turner, Royal Infirmary, Edinburgh, United Kingdom; D. Adu, L. Harper, C. Savage, P. Bacon, University of...
Birmingham, Birmingham, United Kingdom; G. Gaskin, Imperial College, London, United Kingdom; P. Mason, Churchill Hospital, Oxford, United Kingdom; D. Oliveira, St. George’s Hospital, London, United Kingdom; J. Stevens, Southampton Hospital, Southampton, United Kingdom; A. Williams, Morriston Hospital, Swansea, United Kingdom.

We thank Oliver Flossmann (Cambridge), Herbert Hauer (Leiden), and Rob de Lind van Wijngaarden (Leiden) for data management.

DISCLOSURES
None.

REFERENCES

29. Jayne DR, Marshall PD, Jones SJ, Lockwood CM: Autoantibodies to GBM and