Full Review

Twenty-five years of RENHIS: a history of histopathological studies within EUVAS

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ABSTRACT

In the early 1990s, an international working group of experienced renal pathologists, the Renal Histology group, set up a scoring system for biopsies with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis. This scoring system subdivided glomerular, interstitial and vascular lesions and served as a tool for the evaluation of all renal biopsies from studies of the European Vasculitis Study Group (EUVAS). Histopathological studies gave new insights into the prediction of renal outcome in patients with ANCA-associated glomerulonephritis. Percentage of normal glomeruli and a selected number of interstitial parameters were reliable predictors of long-term follow-up glomerular filtration rate in all studies. Out of these results, a histopathological classification distinguishing focal, crescentic, mixed and sclerotic classes of ANCA-associated glomerulonephritis was developed. Until today, 13 studies have validated this classification system. Future studies will try to determine if and how renal histology could be helpful in guiding treatment of ANCA-associated glomerulonephritis.

Keywords: ANCA-associated glomerulonephritis, classification, EUVAS, histopathology, RENHIS

EARLY BEGINNING

While the European Community/Bureau Centrale de Référence (EC/BCR) study was being conducted by Fokko van der Woude at Leiden University Medical Centre, a medical student with an interest in renal pathology was invited to assemble renal biopsies obtained from patients enrolled in this study. Ingeborg Bajema, supervised by Jan Anthonie Bruijn and Chris Hagen, took this task upon her in an era where there was no e-mail, just phones and faxes. She got in touch with the 12 European centres from which patients were enrolled and eventually collected 193 renal biopsy specimens. In the meantime, a group of pathologists among whom were Franco Ferrario, Laure-Hélène Noël, Rüdiger Waldherr and Jan Anthonie Bruijn [today known as the Renal Histology (RENHIS) group] came together in order to set up a scoring system for biopsies with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis (Figure 1). Anecdotally, it is often mentioned that the first issue of debate concerned the total number of glomeruli on which there was considerable disagreement. Should incomplete glomeruli be scored, and how incomplete could they be in order to be counted? Should multiple levels and stainings be taken into consideration to establish the total number of glomeruli, or should an encircled specimen be scored by all pathologists disregarding other levels? A form was devised subdividing glomerular, interstitial and vascular lesions, with the possibility to score separate lesions in a quantitative manner. This form, together with a study on the inter- and intraobserver variability among the pathologists, was published in 1996 [1]. Results showed that overall, interobserver agreement was good, but pathologists were doing better when they had to deal with quantitative data than when they had to score dichotomous data, i.e. deciding for a specific lesion whether it was present or absent. The explanation given for this difference is that for quantitative data, which usually referred to glomerular lesions that were scored per glomerulus, pathologists were forced to systematically evaluate each glomerulus in the biopsy. Obviously, this would bring their
scores closer together than in the case of dichotomous data (typically used for interstitial lesions), which were scored only once. The fact that definitions for lesions and how to score their extensiveness are important here is obvious, and over the past 25 years, the scoring form and the definitions for various renal lesions were fine-tuned many times. Still, it was decided that all renal biopsies enrolled in the various studies would have to be scored by two pathologists followed by consensus meetings in which the final score would have to be decided upon. In this way, all renal biopsies from the European Vasculitis Study Group (EUVAS) studies obtained a standardized evaluation based on the consensus of experienced renal pathologists.

The first study for which the results from the RENHIS group were used in a clinicopathological analysis was the EC/BCR study [2]. An important finding, which would later be reproduced in many other studies, was that the percentage of normal glomeruli in the renal biopsies correlated most significantly with renal function, both at the time of biopsy and during follow-up [3–5]. Moreover, the amount of glomerulosclerosis predicted for renal function at 1 year after the biopsy was taken [2]. These ends of the spectrum, representing the preserved and the irreversibly chronically affected parts of the kidney, turned out to be important parameters for patients’ prognosis in ANCA-associated glomerulonephritis, in part irrespective of the characteristic glomerular lesions such as crescents and fibrinoid necrosis. Furthermore, in a spin-off study from the main clinicopathological study, it was shown that the renal granuloma was a relatively infrequent finding in the renal biopsy (only present in 16 out of 157 patients) not related to renal function and very diverse in its histomorphology [6]. The aforementioned results lie at the basis of the later established classification of ANCA-associated glomerulonephritis, in which the focal and the sclerotic classes represent both ends of the spectrum [7]. Apart from glomerular lesions, diffuse interstitial infiltrates and tubular lesions (necrosis and atrophy) were also related to renal outcome [8, 9]. Today, it is debated if and how interstitial lesions should be incorporated into the histopathological classification system for ANCA-associated glomerulonephritis.

**FiguRe 1:** One of the first meetings of the RENHIS group in Milan in the early 1990s. From left to right: Rüdiger Waldherr, Jan Anthonie Bruijn, Laure-Hélène Noël, Franco Ferrario, Ingeborg Bajema and Chris Hagen.

**RENAL HISTOLOGY STUDIES FROM CYCAZAREM, MEPEX AND RITUXVAS**

Treatment regimens in the EC/BCR study were not standardized and, therefore, an analysis of the effect of therapy on renal outcome in combination with the histopathological data was not performed. In subsequent studies from which renal biopsies were evaluated, standardized treatment, mostly in two treatment arms, was given. Two studies following the EC/BCR study in which treatment was standardized were the CYCAZAREM and the MEPEX studies [10, 11]. These studies were conducted in parallel. The inclusion criterion for the CYCAZAREM study was mainly based on a serum creatinine level of <500 µmol/L at entry, whereas the MEPEX study included patients with a serum creatinine level of >500 µmol/L. Consequently, the whole spectrum of renal involvement is represented in patients from these two studies, 292 in total, of whom 269 representative renal biopsies were collected. A number of clinical histopathological studies emerged from these two studies, with Hauer and De Lind van Wijngaarden in the lead.

The predictive value of clinical and renal histological features for renal outcome from 96 patients in CYCAZAREM, of whom renal biopsies were available, was reported first [3]. End points included renal function at 18 months and the occurrence of relapse or death. Results showed that renal function at the time of biopsy and predominantly chronic renal lesions (glomerulosclerosis and interstitial fibrosis and tubular atrophy) were potent predictors of renal function at 18 months after study entry. Parameters that most strongly correlated with estimated glomerular filtration rate (eGFR) at 18 months after correction for the baseline GFR were segmental and cellular crescents and fibrinoid necrosis. Interestingly, none of the clinical and histological features predicted the occurrence of relapse or death.

The predictive value of clinical and renal histological features for renal outcome from 100 patients in MEPEX, of whom renal biopsies were available, was reported secondly [8]. End points included renal function at the time of diagnosis, 12 months after diagnosis, dialysis at entry and 12 months after diagnosis, and death. Normal glomeruli were a positive predictor of dialysis independence and improved renal function after 12 months, indicating that the unaffected part of the kidney is vital in determining renal outcome. Both chronic and acute tubulointerstitial lesions predicted eGFR at 12 months. Fibrous crescents were predictive of dialysis at entry. No parameter predicted death. In a substudy investigating in more detail the chances of recovery for those patients who are dialysis dependent at diagnosis, it was found that even with ominous histologic findings, the chance of renal recovery exceeded the chance of therapy-related death when patients were treated with plasma exchange as adjunctive therapy [12].

In a spin-off study, data from the CYCAZAREM and MEPEX trials were used to gain more insight into the pathogenesis of vasculitis. Investigating histological differences between patients with MPO-ANCA and PR3-ANCA, Hauer et al. found that chronic lesions were more abundantly present
in MPO-ANCA-positive patients than in PR3-ANCA-positive patients, favouring the idea of different pathways in the pathogenesis of MPO-ANCA and PR3-ANCA vasculitides [13]. Besides gaining insights into the pathogenesis of ANCA-associated glomerulonephritis, MPO- and PR3-ANCA can be important outcome predictors. MPO-ANCA-positive patients present with worse renal function and have less recovery of renal function than PR3-ANCA-positive patients. Therefore, MPO-ANCA-positive patients more often reach end-stage renal disease (ESRD) [14].

More recently, the association between renal outcome and histopathological lesions was studied in 30 patients from the RITUXVAS trial [9]. In this trial, patients were treated in the induction phase with a rituximab-based regimen instead of the standard treatment with cyclophosphamide. In rituximab-treated patients, tubular atrophy and T cell tubulitis were the most important predictors of eGFR at 12 months.

**Towards a Histopathological Classification for ANCA-Associated Glomerulonephritis**

Combining the results from previous EUVAS studies, a first attempt was made towards a formula representing the most robust parameters to predict for renal outcome 1 year after disease onset. Vergunst et al. incorporated data of 160 patients from MEPEX and CYCAZAREM and established the following formula to calculate GFR at 1 year after the biopsy: 36.96 + 0.65 × (GFR at 0) + 10.52 (if normal glomeruli present) + 7.72 (if fibrinoid necrosis present) − 0.42 × age [15]. This formula was based on data showing that renal function at the time of biopsy was the best predictor for renal function 1 year later, but combining renal function with percentage of normal glomeruli, the amount of fibrinoid necrosis and patient’s age could effectively explain for 60% of the variation in renal function 1 year after disease onset. ANCA subset was no independent predictor for renal outcome.

To the purpose of summarizing the most important data from the renal biopsy more succinctly, in order to give an indication about long-term renal outcome, a histopathological classification for ANCA-associated glomerulonephritis was launched in 2010 [7]. Four classes were distinguished, named the focal, crescentic, mixed and sclerotic classes. Renal survival during long-term follow-up could be predictive on the basis of these four categories, since the order of categories (focal, crescentic, mixed and sclerotic) corresponded to the severity of renal function loss [7]. Since 2010, 13 studies have validated the classification system. These studies came from Japan, China, Australia, the United States, the Netherlands, Turkey, Canada, the United Kingdom and India [16–28] and confirmed that the classification system is of predictive value for renal outcome [29]. Slightly conflicting outcomes are noticed with regard to crescentic and mixed class biopsies. In some studies [16–20, 22], the outcome of patients with a crescentic class renal biopsy is worse or similar to that of patients with mixed class renal biopsies, which is in contrast to the original validation study [7]. An overview of the differences between the validation studies and the primary study is provided in Table 1. Although further studies are needed to address these differences in more detail, one suggestion is that interobserver variability in the evaluation of the crescentic lesion may

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Outcome difference with primary study</th>
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<tbody>
<tr>
<td>Iwakiri et al. [16]</td>
<td>102 Japanese patients, mostly MPO-ANCA positive</td>
<td>No significant differences in eGFR at 1 year among crescentic, mixed and sclerotic classes. No significant difference in probability of progressing to ESRD between crescentic and mixed classes.</td>
</tr>
<tr>
<td>Togashi et al. [17]</td>
<td>54 Japanese patients, all MPO-ANCA positive</td>
<td>No significant difference in eGFR at entry and follow-up between crescentic and mixed classes. Higher probability of progressing to ESRD in crescentic than mixed class.</td>
</tr>
<tr>
<td>Muso et al. [18]</td>
<td>87 Japanese patients, mostly MPO-ANCA positive</td>
<td>Slightly better renal survival in mixed than crescentic class.</td>
</tr>
<tr>
<td>Chang et al. [19]</td>
<td>121 Chinese patients, mostly MPO-ANCA positive</td>
<td>Higher probability of progressing to ESRD in crescentic than mixed class.</td>
</tr>
<tr>
<td>Hilhorst et al. [20]</td>
<td>164 Dutch patients, only 1 biopsy classified as sclerotic</td>
<td>No difference in eGFR at follow-up and 5-year renal survival between crescentic and mixed classes. Subdividing these classes on the basis of % normal glomeruli showed that patients with &gt;25% normal glomeruli had a significantly better renal survival.</td>
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<tr>
<td>Ford et al. [21]</td>
<td>120 Australian patients</td>
<td>No significant difference in eGFR at 1 year and probability of progressing to ESRD among focal, crescentic and mixed classes.</td>
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<tr>
<td>Ellis et al. [22]</td>
<td>76 American patients</td>
<td>No significant difference in eGFR at 1 year between crescentic and mixed classes. No significant difference in renal survival at 1 year between classes.</td>
</tr>
<tr>
<td>Uńúa et al. [23]</td>
<td>141 Turkish patients</td>
<td>Classification predicted dialysis requirement in the log-rank test but not in the Cox regression model.</td>
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<tr>
<td>Nohr et al. [24]</td>
<td>67 Canadian patients</td>
<td>No difference with primary study.</td>
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<tr>
<td>Quintana et al. [25]</td>
<td>136 Spanish and British patients</td>
<td>No significant difference in ESRD between crescentic and mixed classes.</td>
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<tr>
<td>Tanna et al. [26]</td>
<td>104 British patients</td>
<td>No significant difference in outcome between mixed and crescentic classes. No significant differences in renal function at follow-up among classes in multivariate analysis.</td>
</tr>
<tr>
<td>Naidu et al. [27]</td>
<td>73 Indian patients, much lower mean age</td>
<td>No significant differences in improvement rates and probability of progressing to ESRD among classes.</td>
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<tr>
<td>Noone et al. [28]</td>
<td>40 Canadian children, mixed and crescentic classes were combined for analysis</td>
<td>No difference with primary study.</td>
</tr>
</tbody>
</table>
account in part for this discrepancy. Crescentic class ANCA-associated glomerulonephritis is defined by a majority of glomeruli with cellular crescents, leaving out those crescentic lesions that are fibrocellular or fibrous. Given the relatively low interobserver agreement amongst pathologists for evaluating ANCA-associated glomerulonephritis as recently reported by Ford et al. [20], it is possible that the difficulty to distinguish between cellular crescents (characteristic of a crescentic class) and fibrocellular and fibrous crescents (which would more likely be found in the mixed class) has led to these discrepant results.

**CONTEMPLATIONS**

In the majority of our studies, we investigated renal outcome of ANCA-associated vasculitis with respect to histopathologic findings of the renal biopsy at disease onset, mostly in relation to clinical parameters at onset. We would like to conclude with a number of considerations on the merits of our studies and make recommendations for future investigations. With respect to the data derived from the renal biopsies, it can be stated that these were in the vast majority of cases not influenced by therapy initiated before the biopsy was taken and therefore reflected the actual state of the disease at study entry. To what extent duration of disease influenced the amount of acute and chronic lesions in the biopsy remains an interesting point of discussion and one that cannot be solved easily by human studies. Especially, in the case of ANCA-associated vasculitis, it is practically impossible to determine the duration of disease before a diagnosis is made because the early symptoms can be non-specific and regarded both by the patient and by the physician as relatively benign. Consequently, in many patients, no further diagnostic measurements are taken until the moment at which more serious symptoms present themselves such as hearing loss, recurrent otitis media, persistent (bloody) rhinorrhea, dyspnoea, haemoptysis and haematuria [30]. Although it is likely that duration of disease before diagnosis is highly variable among patients, and therefore, an important contributor to the findings in the renal biopsy, it cannot be ruled out that other factors may be of equal or even greater importance here. To illustrate this point, we refer to recent findings of Xiao et al., showing that the genetic makeup in mice greatly determines the percentage of glomeruli with crescents in a model of ANCA-associated glomerulonephritis [31].

Over the years, it was discussed many times how many glomeruli a biopsy should contain in order to be diagnostic or to be of prognostic relevance. According to a statistical analysis into the predictive value of the renal biopsy [32], only biopsies with 20 glomeruli or more start to predict with moderate accuracy for the state of the entire organ. But 20 glomeruli is fairly high, and for practical purposes also decisions have to be made on biopsies that are less generous in their material. For some of our studies, we set the minimum at 7 [33], for others at 10 [7].

In most of the validation studies on the ANCA-associated glomerulonephritis classification, the question of whether to include tubulointerstitial parameters or not has been raised [18–21, 25]. Tubular atrophy and tubulitis predict eGFR at 12 months in ANCA-associated glomerulonephritis patients treated with a rituximab regimen [9]. However, adding these parameters to the classification had no significant effect on the prognostic value and were therefore not included in the current classification [7]. The validation study performed by Quintana et al. suggested that tubulointerstitial fibrosis enhances the prognostic value of the classification, but this was not analysed in a multivariate regression analysis [25]. The variability in histologic features that have been found to be of significant importance in ANCA-associated glomerulonephritis may be due to differences in patient demographics or different treatment regimens [24], and therefore, a worldwide study is needed to solve this issue.

As for prognosis, we ponder upon a number of fundamental questions. First, it is uncertain over which period of time findings from the renal biopsy may be expected to have clinical significance. In our studies, we took various time points for renal function: 1 year, 2 years and for the classification of histopathological lesions even 5 years and more. A graph in which histological class is set out against renal survival [7] seems to show that even during long-term follow-up, renal histology remains to play a role in the prediction of outcome. It has been argued that because of the numerous events that may occur in the meantime, e.g. disease relapses and toxicity of treatment, the predictive values of renal biopsy findings should not be overestimated and confined to 1 or 2 years, but our results seem to be in favour of the counterargument. Second, it may be questioned for which clinical outcome we actually want to predict. Most studies focus on renal outcome in terms of renal function, but some new studies are currently being conducted investigating histology in relation to renal relapse, development of proteinuria and cardiovascular events. For each of these outcome parameters, we should always question the likeness and then the weight of the renal biopsy parameters at entry playing a role.

It is evident that ultimately, we do not only want to be able to predict renal outcome to inform our patients about the future of their health status but also to adjust therapy according to these findings. Given the severe and life-threatening complications of immunosuppressive therapy, it would, for instance, be helpful if in some cases where chronic lesions dominate the histological picture, we could advise that a milder therapy regime could suffice because of the relatively low chances of renal recovery in relation to the risk of complications. This has not been investigated thus far. In fact, our study on histopathological determinants of renal outcome in patients who had severely disturbed renal function at entry showed that age, normal glomeruli, tubular atrophy, intraepithelial infiltrate and GFR at baseline are predictive of eGFR at 12 months [8]. To our knowledge, there are no studies yet that investigate how histology-guided treatment in human native renal diseases could be approached. This is opposed to the setting of renal transplantation, where renal transplant biopsy findings seem to have more direct consequences of the therapeutic regime than in native renal diseases. The relatively low threshold of taking multiple biopsies in renal transplant patients over time probably plays an important role in this issue. It should be realized that because of the abundance of protocolized and for cause biopsies in renal transplantation patients, we have gained a tremendous amount of knowledge on the development of
lesions in renal grafts, and we know far more about, e.g. the reversibility of lesions and of their grumbling nature than in many native diseases. Only few studies have reported on repeat biopsies in ANCA-associated vasculitis; in a recent study, it was shown that protocolized biopsies taken \( \sim 1 \) year after disease onset overall showed an increase of chronic lesions while some acute lesions were still present, resulting in 11 out of 17 patients in a higher class of the histopathological classification of ANCA-associated glomerulonephritis in the repeat biopsy in comparison to the biopsy at disease onset [34]. Another study by Hauer et al. emphasized the stability of the amount of unaffected glomeruli in repeat biopsies taken for cause [35]. With reference to the genetic study by Xiao et al. [31], it is interesting to speculate that also in humans, genetic background may influence the severity of disease in ANCA-associated glomerulonephritis. However, many clinicians are reluctant to take repeat biopsies in ANCA-associated glomerulonephritis, which is an obstacle for our further understanding of the development of lesions and the possibility of histology-guided treatment in these patients.

**THE PRESENT AND THE FUTURE**

Currently, the RENHIS group is evaluating renal biopsies from the recently conducted MYCYC trial and from the ongoing PEXIVAS trial. A website was launched this year that makes it possible for the renal pathologists to score scanned slides and

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Music written in 1898 by: Eduardo di Capua (Napels, 1865-1917), lyrics by Giovanni Capurro (Napels, 1859-1920)

**First four lines:**

_Che bella cosa è na jurnata 'e sole, n’aria serena dopo na tempesta!_  
_Pe’ ll’aria fresca para già na festa..._  
_Che bella cosa na jurnata 'e sole._

**Music:**

[Music notation image]

**Famous recordings:**

1915, Charles W. Harrison records the first English translation of "O sole mio"

1920, Summer Olympics in Antwerp, "O sole mio" was played when the music to the national anthem of Italy could not be found

1931, song sung with harp accompaniment in the Marx Brothers movie Monkey Business

1959, Elvis Presley comes out with the English version It’s Now or Never, a rewrite by Aaron Schroeder and Wally Gold

2012, Alfie Boe performs part of the song, as well as a portion of Elvis Presley’s "It’s Now or Never", at the Diamond Jubilee Concert in celebration of the Diamond Jubilee of Elizabeth II

1997-2014, Franco Ferrario performs the song at EUVAS meetings and ANCA workshops

**FIGURE 2:** O sole mio.
transmit data electronically. These new logistics also facilitate the scoring of biopsies in parallel, glass slides no longer need to be distributed by regular mail and the biopsies will be returned to the participating centres much more rapidly than in the past. We could not have established many of our goals without Franco Ferrario singing O sole mio for us during EUVAS meetings and workshops. In Figure 2, we give an overview of what is behind the song and how the sun has given us so much energy in discussing moonlight crescents. The RENHIS group remains to have consensus meetings at regular intervals. Moreover, it has coined a new name for itself to celebrate 25 years of fruitful and joyful collaboration: we are now The Dream Team!

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


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