Predicting Outcome in Patients with Anti-GBM Glomerulonephritis

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Abstract

Background and objectives Large studies on long-term kidney outcome in patients with anti-glomerular basement membrane (anti-GBM) GN are lacking. This study aimed to identify clinical and histopathologic parameters that predict kidney outcome in these patients.

Design, setting, participants, & measurements This retrospective analysis included a total of 123 patients with anti-GBM GN between 1986 and 2015 from six centers worldwide. Their kidney biopsy samples were classified according to the histopathologic classification for ANCA-associated GN. Clinical data such as details of treatment were retrieved from clinical records. The primary outcome parameter was the occurrence of ESRD. Kidney survival was analyzed using the log-rank test and Cox regression analyses.

Results The 5-year kidney survival rate was 34%, with an improved rate observed among patients diagnosed after 2007 ($P=0.01$). In patients with anti-GBM GN, histopathologic class and kidney survival were associated ($P<0.001$). Only one of 15 patients with a focal class biopsy sample ($\geq50\%$ normal glomeruli) developed ESRD. Patients with a sclerotic class biopsy sample ($\geq50\%$ globally sclerotic glomeruli) and patients with 100% cellular crescents did not recover from dialysis dependency at presentation. In multivariable analysis, dialysis dependency at presentation (hazard ratio [HR], 3.17; 95% confidence interval [95% CI], 1.59 to 6.32), percentage of normal glomeruli (HR, 0.97; 95% CI, 0.95 to 0.99), and extent of interstitial infiltrate (HR, 2.02; 95% CI, 1.17 to 3.50) were predictors of ESRD during follow-up.

Conclusions Dialysis dependency, low percentage of normal glomeruli, and large extent of interstitial infiltrate are associated with poor kidney outcome in anti-GBM GN. Kidney outcome has improved during recent years; the success rate doubled after 2007.

Introduction

Anti-glomerular basement membrane (anti-GBM) disease is an aggressive autoimmune disease (1), with an estimated incidence of 1–2 cases per million population per year (2). The disease affects glomerular capillaries, which usually leads to rapidly progressive GN, and pulmonary capillaries, possibly manifesting as alveolar hemorrhage. Kidney disease predominates in the majority of patients and is characterized by glomerular fibrinoid necrosis and crescent formation (3). To distinguish anti-GBM GN from other kidney diseases, immunofluorescence appearances are decisive, showing linear Ig deposits along the glomerular basement membrane (GBM) (4). In most cases, these deposits signify binding of circulating IgG autoantibodies with the NC1 domain of the α-3 chain of type IV collagen in the GBM (5,6). Anti-GBM autoantibodies can be detected in the serum of most patients, serving as a diagnostic tool. Besides anti-GBM antibody positivity, approximately one third of patients have ANCA (3,7).

The discovery of anti-GBM antibodies and their pathogenicity provide the rationale for treatment with plasma exchange and immunosuppressive agents, particularly cyclophosphamide and corticosteroids (8). This therapy combined with better supportive care and earlier diagnosis has improved the extremely poor outcome of patients with anti-GBM GN over the past decades (9–12). Despite these improvements, the rapid deterioration caused by the disease still causes ESRD, requiring dialysis at presentation in approximately 55% of patients (9). At 1-year follow-up, reported kidney survival rates range between 20% and 40% (10,12–15). Kidney outcome has been correlated with the severity of kidney failure at presentation and the percentage of crescents on kidney biopsy (9,11–16).

In a landmark paper from 2001, patients with anti-GBM GN who were dialysis dependent at presentation and had 100% crescents on their kidney biopsy did not recover kidney function (9). On the basis of this study, the Kidney Disease: Improving Global Outcomes guidelines currently recommend that patients who present

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with dialysis dependency and have 100% cellular crescents in an adequate biopsy sample, and who are without lung hemorrhage, can refrain from intensive treatment with plasma exchange, corticosteroids, and cyclophosphamide (17). However, a recent study suggested that all patients with anti-GBM GN should receive intensive therapy because the combination of plasma exchange, corticosteroids, and cyclophosphamide had a favorable effect even on patients who presented with clinical and/or histologic parameters indicative of a poor prognosis (11). In contrast, another recent study found that oligo-anuric patients who received intensive treatment and those who received minimal treatment had similarly poor patient and kidney survival (16). Because none of these patients recovered kidney function, a kidney biopsy may not be essential for predicting the prognosis of oligo-anuric patients (16).

Given the controversies regarding the predictive value of the kidney biopsy and the utility of intensive therapy in patients with anti-GBM GN, large studies on long-term kidney outcome are highly warranted to further improve patient-tailored therapy. Therefore, we investigated the clinical and histopathologic predictors of long-term kidney outcome in a large, international cohort of patients with anti-GBM GN.

Materials and Methods
Study Cohort
Patients were eligible for this study if they had a clinical presentation compatible with anti-GBM GN in combination with positive (>1+) glomerular linear IgG staining on immunofluorescence and/or positive serum anti-GBM antibodies. Entry criterion for this study was the availability of a kidney biopsy sample, which was collected from a pathology department at one of the following centers: the University of North Carolina at Chapel Hill (n=34); Imperial College London (n=26); Addenbrooke's Hospital, Cambridge (n=25); LabPlus, Auckland (n=16); Leiden University Medical Center (n=15); and University Medical Center Utrecht (n=7). The cohort was divided according to year of diagnosis from which we created three groups of similar sizes in advance, which led to the following categorization: 1986–2000, 2001–2006, and 2007–2015. Patients with double positivity for anti-GBM antibodies and ANCA were included in the study, whereas patients with any other coexisting kidney disease were excluded. This study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki.

Biopsy Specimen Evaluation
A diagnostic kidney specimen was available for light microscopic evaluation in 118 patients. Biopsy specimens were evaluated independently by one of two experienced nephropathologists (I.M.B. or J.C.J.) who were blinded to the clinical data. The Berden classification for ANCA-associated GN was used to score the biopsy samples; focal class contained biopsy samples with ≥50% normal glomeruli, crescentic class contained biopsy samples with ≥50% glomeruli with cellular crescents, sclerotic class contained biopsy samples with ≥50% glomeruli with global sclerosis, and the remaining biopsy samples were designated as mixed class (18). Moreover, tubulointerstitial

parameters were evaluated; interstitial fibrosis and tubular atrophy and interstitial infiltrate were scored semiquantitatively (scale from 0 to 3), and tubulitis was scored as absent or present (0 or 1). Kidney biopsy samples from five patients could not be retrieved, but pathology reports had sufficient information to classify the biopsy samples according to the Berden classification, and medical files comprised complete clinical data. Results from immunofluorescence were assessed from the pathology report in all patients.

Clinical Data
Clinical data were retrieved from medical records at the participating centers. Kidney function at baseline (before start of dialysis) was expressed as serum creatinine (milligrams per deciliter) and was classified as serum creatinine <5.7 or ≥5.7 mg/dl as reported in previous studies (9,12,16). Dialysis dependency at presentation was defined as need for acute RRT during the first hospital admission. The outcome of ESRD was defined as dialysis dependency during follow-up or kidney transplantation. Kidney survival was expressed as time to ESRD, and patient survival as time to death. Standard therapy was similar between centers and consisted of plasma exchange (minimum of seven exchanges or until anti-GBM antibodies were negative), oral and/or intravenous cyclophosphamide for 3–6 months, and oral and/or intravenous corticosteroids. Missing data are reported in the legends of tables, and percentages of the total of nonmissing data are presented. Each analysis included patients with complete data on the variable of interest.

Statistical Analyses
Continuous variables are expressed as means±SD or medians (interquartile range), and were compared between groups using the t test, one-way ANOVA, Mann–Whitney U test, or Kruskal–Wallis H test, as appropriate. Categoric variables are expressed as numbers (%), and differences were assessed with the chi-squared test or chi-squared trend test. Kidney and patient survival was analyzed using the Kaplan–Meier method and log-rank test. Univariable and multivariable Cox regression analyses were performed to identify predictors of kidney survival. Results are expressed as hazard ratio (HR) with 95% confidence interval (95% CI). SPSS version 23 (IBM Corp., Armonk, NY) was used for all analyses, and P values below 0.05 were considered statistically significant.

Results
Patient Characteristics
The characteristics of the total 123 patients are depicted in Table 1. Males in the cohort (54%) were significantly younger than females (47±20 versus 55±18 years; P=0.02). Data on serology were available for 108 patients; 49% were positive for anti-GBM antibodies only ("single positive"); 32% were positive for anti-GBM antibodies and ANCA ("double positive"); 6% had detectable ANCA only; and 13% had no detectable anti-GBM antibodies or ANCA. Double-positive patients were older than single-positive patients (P=0.003; Table 2). Patients who had negative serology test results were significantly younger compared

Table 1.   Characteristics of the study population according to the year of diagnosis

Characteristic	Total (n=123)	1986–2000 (n=41)	2001–2006 (n=38)	2007–2015 (n=44)
Baseline (n=123)				
Age, yr	51±19	54±19	46±21	53±17
Males	66 (54)	22 (54)	24 (63)	20 (46)
Anti-GBM antibodies positive[a]	92 (82)	31 (87)	28 (78)	33 (81)
Anti-GBM antibodies, ANCA negative	53 (60)	12 (44)	20 (71)	21 (64)
Anti-GBM antibodies, ANCA positive	35 (40)	15 (56)	8 (27)	12 (36)
ANCA type[b]				
Anti-MPO	23 (85)	9 (90)	7 (100)	7 (70)
Anti-PR3	4 (15)	1 (10)	0 (0)	3 (30)
Lung involvement[c]	41 (35)	14 (37)	12 (33)	15 (35)
Serum creatinine, mg/dl[d]	7.0 (3.5–10.3)	9.0 (5.8–13.9)	7.3 (4.5–11.4)	4.6 (1.9–7.0)
Dialysis dependent[d]	69 (56)	28 (68)	27 (71)	14 (32)
Follow-up (n=123)				
Duration of follow-up, yr[d]	4.6±4.0	5.3±5.1	5.9±3.5	2.8±2.2
ESRD at end of follow-up[e]	82 (67)	31 (76)	29 (76)	22 (50)
Transplantation	32 (26)	9 (22)	15 (40)	8 (18)
Death[e]	34 (28)	17 (42)	11 (29)	6 (14)
Treatment (n=110)				
Plasma exchange	91 (83)	24 (71)	31 (89)	38 (88)
Median number of exchanges	7 (6–12)	6 (5–10)	10 (7–10)	7 (6–14)
Cyclophosphamide[e]	88 (80)	21 (62)	29 (88)	38 (88)
Oral[f]	43 (60)	13 (68)	14 (67)	16 (50)
Intravenous[f]	25 (35)	6 (32)	6 (28)	13 (41)
Both	4 (5)	0 (0)	1 (5)	3 (9)
Corticosteroids	106 (96)	33 (94)	32 (97)	42 (98)
Azathioprine	21 (19)	5 (15)	6 (18)	10 (23)
Mycophenolate mofetil	12 (11)	4 (12)	4 (12)	4 (9)
Rituximab[d]	10 (9)	0 (0)	0 (0)	10 (23)

Data are presented as numbers (%), means±SD, or medians (interquartile range). GBM, glomerular basement membrane; MPO, myeloperoxidase; PR3, proteinase 3.
[a]Results from anti-GBM antibody ELISA were available in 112 patients. Results from ANCA ELISA were available in 114 patients. Both tests were performed in 108 patients; four patients with positivity for anti-GBM antibodies had no available results from ANCA ELISA.
[b]Thirty-five patients were anti-GBM antibodies and ANCA positive. Of these, ANCA type (anti-MPO or anti-PR3) was known in 27 patients.
[c]Total, n=117 patients.
[d]Different between subgroups with P value <0.001.
[e]Different between subgroups with P value <0.05.
[f]Route of administration of cyclophosphamide was known for 72 patients. The mean oral cyclophosphamide dose at the start of therapy was 118±49 g (n=30), and the mean intravenous dose was 1113±460 g (n=8).

with patients with positive serology test results (*i.e.*, anti-GBM antibody and/or ANCA positivity) (P=0.02; Supplemental Table 1). Median duration of follow-up was 3.9 (1.3–6.1) years. Five patients were lost to follow-up within 1 year. The 5-year kidney survival rate was 34%, and the 5-year patient survival rate was 83%. Kidney survival at 5 years was 50% in patients who were diagnosed after 2007 (Figure 1).

Evaluation of Diagnostic Renal Biopsy Specimens

Immunofluorescence was performed successfully in 117 patients and was positive for linear IgG in all of them; six specimens for immunofluorescence did not contain assessable glomeruli. Ten biopsy samples were classified as sclerotic class, 15 biopsy samples as focal class, 72 biopsy samples as crescentic class, and the remaining 26 biopsy samples as mixed class (Table 3). The focal group had a younger mean age than the other three classes together

(41±19 versus 52±19 years; P=0.03). Serum anti-GBM antibodies were more frequently detected in patients from the crescentic class than in those from the other classes. All patients from the sclerotic class who were positive for anti-GBM antibodies and who were tested for ANCA showed double positivity for both antibodies. The amount of interstitial fibrosis and tubular atrophy differed significantly between classes, but the extent of interstitial infiltrate did not (Table 4). Double-positive patients tended to have a higher percentage of sclerotic glomeruli and a higher interstitial fibrosis and tubular atrophy score compared with single-positive patients (P=0.07 for both parameters; Table 2). The minimum number of glomeruli was set at six in this study, whereas the Berden classification set a minimum of ten glomeruli. The predictive value of glomerular lesions was greater in biopsy samples with ten or more glomeruli (n=101) compared with biopsy samples with 6–10 glomeruli (n=22). However, including biopsy

Table 2. Differences between single- and double-positive patients

Variable	Single Positive[a] (n=53)	Double Positive[a] (n=35)	P Value
Age, yr	47±19	59±17	0.003
Males	27 (51)	19 (54)	0.76
Lung involvement[b]	15 (28)	13 (39)	0.29
Serum creatinine at presentation, mg/dl	7.2 (3.5–10.0)	7.2 (3.9–10.1)	0.92
Dialysis dependent at presentation	32 (60)	20 (57)	0.76
Year of diagnosis			0.11
1986–2000	12 (23)	15 (43)	
2001–2006	20 (38)	8 (23)	
2007–2015	21 (39)	12 (34)	
ESRD at end of follow-up	36 (68)	25 (71)	0.73
Histopathologic class			0.03
Focal	7 (13)	4 (11)	
Crescentic	37 (70)	18 (51)	
Mixed	9 (17)	8 (23)	
Sclerotic	0 (0)	5 (14)	
Normal glomeruli, percentage[c]	0 (0–26)	0 (0–13)	0.40
Cellular crescents, percentage[c]	61±32	58±32	0.63
Sclerotic glomeruli, percentage[c]	2 (0–16)	13 (0–28)	0.07
Interstitial fibrosis and tubular atrophy[c]			0.07
0–1	36 (72)	18 (53)	
2–3	14 (28)	16 (47)	
Interstitial infiltrate[c]			0.10
0–1	22 (44)	9 (27)	
2–3	28 (56)	25 (73)	
Tubulitis[c]			0.20
0	18 (36)	17 (50)	
1	32 (64)	17 (50)	
Intensive treatment[d]	34 (71)	14 (45)	0.02
Maintenance therapy with azathioprine or mycophenolate mofetil[e]	7 (14)	13 (38)	0.01

Data are presented as numbers (%), means±SD, or medians (interquartile range).
[a]Single-positive patients were positive for serum anti-glomerular basement membrane (anti-GBM) antibodies and negative for ANCA. Double-positive patients were positive for anti-GBM antibodies and positive for ANCA.
[b]Total, n=86 patients.
[c]Total, n=84 patients.
[d]Intensive treatment consisted of at least seven plasma exchanges, corticosteroids, and cyclophosphamide, mycophenolate mofetil, or rituximab. Excluding patients without intensive treatment and relatively preserved renal function at presentation. Total, n=79 patients.
[e]The minimal duration of maintenance therapy was 3 months. Total, n=84 patients.

samples with 6–10 glomeruli did not change the predictive value of the glomerular lesions (Supplemental Table 2); therefore, they were included. No differences in treatment were observed between histopathologic classes, except for cyclophosphamide; patients from the focal and mixed class received cyclophosphamide more frequently than patients from the crescentic and sclerotic class (Table 5).

Kidney Function According to Histopathologic Class

Kidney function at baseline in terms of serum creatinine was significantly better in the focal class compared with the other three classes (Table 3). No patient from the focal class required dialysis at presentation, and only one developed ESRD during follow-up (Figure 2). Most patients (90%) from the sclerotic class developed ESRD at some time point, whereas the outcome in crescentic and mixed classes was more variable (Figures 2 and 3A). When patients were categorized according to the percentage of normal glomeruli, kidney survival decreased with each lower percentage category of normal glomeruli (Figure 3B). Patient survival did not differ between histopathologic classes (Figure 3C).

Predictors of ESRD

In Kaplan–Meier analysis, double-positive patients had a similar kidney survival to single-positive patients (P=0.75; Supplemental Figure 1A). Kidney survival was also similar in patients with negative and positive serology (P=0.69; Supplemental Figure 1B). Age, serum creatinine level, dialysis dependency at presentation, histopathologic classification, percentage of normal glomeruli, percentage of cellular crescents, extent of interstitial fibrosis and tubular atrophy, and extent of interstitial infiltrate were associated with ESRD in univariable Cox regression analyses (Table 6). Dialysis dependency at presentation was a discontinuous variable that predicted ESRD most significantly. Therefore, each parameter that was significant in univariable analysis was evaluated separately in a multivariable Cox regression analysis that included dialysis dependency at presentation.
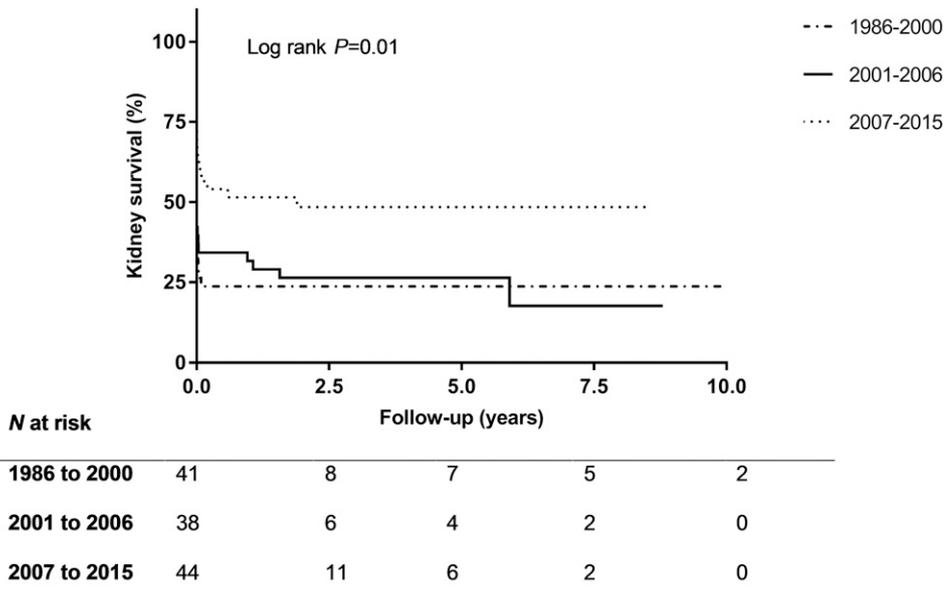
Figure 1. | The kidney survival is highest in patients who were diagnosed between 2007 and 2015.

The percentage of normal glomeruli and the extent of interstitial infiltrate remained significant predictors of ESRD in multivariable analysis. A multivariable analysis, including dialysis dependency, percentage of normal glomeruli, and extent of interstitial infiltrate, showed that these parameters predicted ESRD independently (Table 6).

Table 3. Characteristics stratified by histopathologic class: Baseline and follow-up characteristics

Characteristic	Focal (n=15)	Crescentic (n=72)	Mixed (n=26)	Sclerotic (n=10)	P Value
Age, yr	41±19	53±19	53±16	50±25	0.17
Males	9 (60)	41 (57)	10 (39)	6 (60)	0.37
Anti-GBM antibodies positive[a]	11 (79)	58 (91)	17 (71)	6 (60)	0.02
Anti-GBM antibodies, ANCA negative	7 (64)	37 (67)	9 (53)	0 (0)	0.03
Anti-GBM antibodies, ANCA positive	4 (36)	18 (33)	8 (47)	5 (100)	
ANCA type[b]					0.001
Anti-MPO	0 (0)	14 (100)	7 (87)	2 (100)	
Anti-PR3	3 (100)	0 (0)	1 (13)	0 (0)	
Lung involvement[c]	7 (50)	26 (37)	4 (16)	4 (57)	0.06
Serum creatinine at presentation, mg/dl	1.4 (1.0–1.7)	7.8 (6.4–12.6)	4.2 (2.6–7.2)	6.6 (4.5–15.2)	<0.001
Dialysis dependent at presentation	0 (0)	51 (71)	10 (39)	8 (80)	<0.001
Year of diagnosis					
1986–2000	3 (20)	28 (39)	4 (15)	6 (60)	<0.01
2001–2006	2 (13)	26 (36)	8 (31)	2 (20)	
2007–2015	10 (67)	18 (25)	14 (54)	2 (20)	
Duration of follow-up, yr	4.7±3.7	4.9±4.2	4.3±3.8	3.4±3.6	0.72
ESRD at end of follow-up	1 (7)	57 (79)	15 (58)	9 (90)	<0.001
Transplantation	0 (0)	25 (35)	4 (15)	3 (30)	0.01
Death	2 (13)	24 (33)	5 (19)	3 (30)	0.32

Data are number (%), mean±SD, or median (interquartile range). GBM, glomerular basement membrane; MPO, myeloperoxidase; PR3, proteinase 3.
[a]Results from anti-GBM antibody ELISA were available in 112 patients. Results from ANCA ELISA were available in 114 patients. Both tests were performed in 108 patients.
[b]Thirty-five patients were anti-GBM antibodies and ANCA positive. Of these, ANCA type (anti-MPO or anti-PR3) was known in 27 patients.
[c]Total, n=117 patients.

Table 4. Characteristics stratified by histopathologic class: Biopsy specimen characteristics

Characteristic	Focal (n=15)	Crescentic (n=68)	Mixed (n=25)	Sclerotic (n=10)	P Value
Total number of glomeruli	22±12	17±9	19±9	19±9	0.35
Normal glomeruli, percentage	71 (62–90)	0 (0–8)	9 (0–31)	0 (0–0)	<0.001
Cellular crescents, percentage	12±10	81±16	32±15	19±16	<0.001
Sclerotic glomeruli, percentage	0 (0–5)	0 (0–14)	17 (8–30)	63 (58–70)	<0.001
Interstitial fibrosis and tubular atrophy					<0.001
0	8 (53)	16 (24)	1 (4)	0 (0)	
1	7 (47)	30 (44)	11 (44)	1 (10)	
2	0 (0)	19 (28)	10 (40)	3 (30)	
3	0 (0)	3 (4)	3 (12)	6 (60)	
Interstitial infiltrate					0.16
0	5 (33)	5 (8)	1 (4)	1 (10)	
1	9 (60)	17 (25)	12 (48)	2 (20)	
2	1 (7)	22 (32)	6 (24)	7 (70)	
3	0 (0)	24 (35)	6 (24)	0 (0)	
Tubulitis					0.02
0	12 (80)	27 (40)	15 (60)	6 (60)	
1	3 (20)	41 (60)	10 (40)	4 (40)	

Data are number (%), mean±SD, or median (interquartile range). Excluding five patients without kidney biopsy samples available for re-evaluation. Total, n=118 patients.

Treatment

Data on therapy were complete for 110 patients. Intensive treatment consisting of at least seven plasma exchanges (19), corticosteroids, and cyclophosphamide, mycophenolate mofetil, or rituximab was given to 61 patients. Of the 49 remaining patients, 25 patients were treated with plasma exchange but not with cyclophosphamide and 24 with cyclophosphamide but no or less than seven plasma exchanges. Five of these 49 patients had relatively preserved kidney function at presentation (serum creatinine ≤1.4 mg/dl); therefore, they were not included in the analysis comparing intensive versus mild treatment (Supplemental Table 3). Older patients, double-positive patients, and patients with severe kidney failure at presentation were less likely to receive intensive treatment. Moreover, patients with a lower percentage of normal glomeruli and a higher percentage of crescentic glomeruli were treated less intensively. Over time, there was a significant trend toward increased use of an intensive treatment regimen (Supplemental Table 3). Twenty-one patients received azathioprine, and 12 patients received mycophenolate mofetil for induction and/or remission therapy. Double-positive patients received maintenance therapy with azathioprine or mycophenolate mofetil more frequently than single-positive patients (Table 2). Ten patients were treated with rituximab; nine of these patients also received cyclophosphamide concurrently or before treatment with rituximab.

Table 5. Characteristics stratified by histopathologic class: Treatment

Treatment	Focal (n=14)	Crescentic (n=65)	Mixed (n=23)	Sclerotic (n=8)	P Value
Intensive treatment[a]	8 (57)	33 (51)	16 (70)	4 (50)	0.46
Plasma exchange	12 (86)	53 (82)	21 (91)	5 (63)	0.32
Cyclophosphamide	13 (93)	46 (71)	23 (100)	6 (75)	<0.01
Corticosteroids	14 (100)	62 (95)	23 (100)	7 (88)	0.33
Azathioprine	4 (29)	8 (12)	6 (26)	3 (38)	0.11
Mycophenolate mofetil	2 (14)	7 (11)	2 (9)	1 (13)	0.92
Rituximab	3 (21)	3 (5)	4 (17)	0 (0)	0.06

Data are number (%), mean±SD, or median (interquartile range). Total, n=110 patients.
[a]Intensive treatment was defined as treatment consisting of at least seven plasma exchanges, corticosteroids, and cyclophosphamide, mycophenolate mofetil or rituximab.
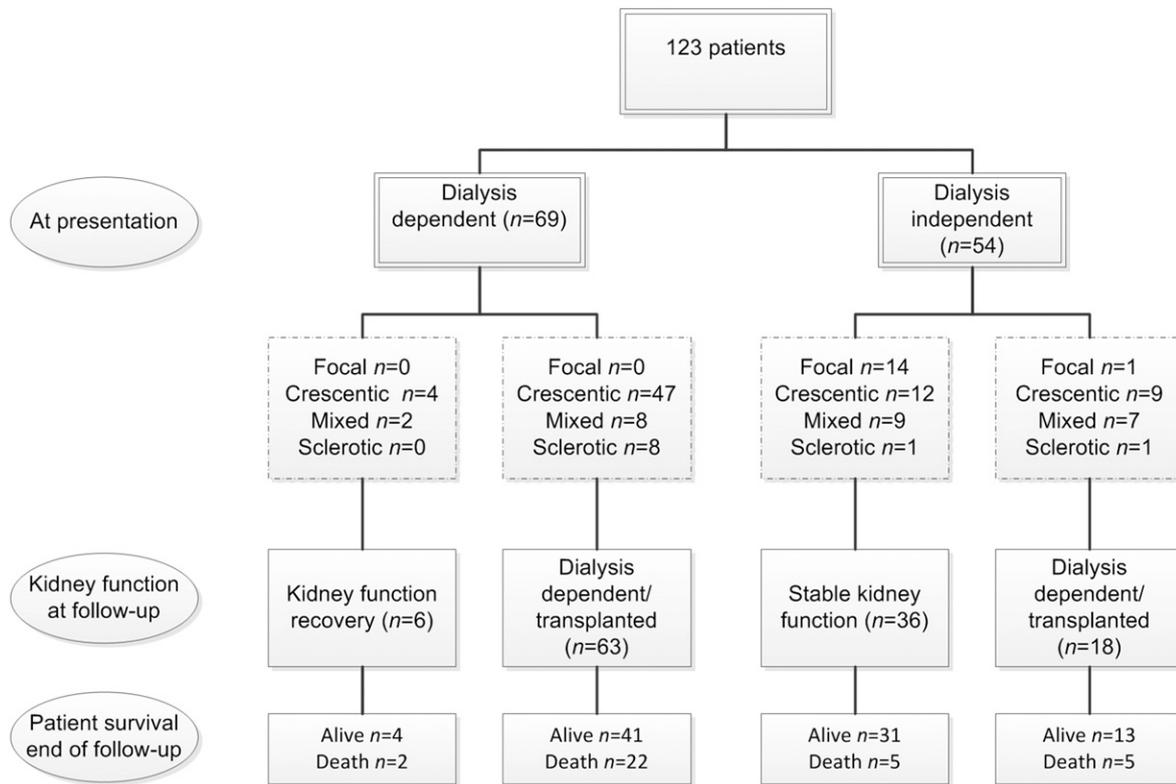
Figure 2. | **The flowchart shows a favorable outcome in the focal class, a poor outcome in the sclerotic class, and variable outcomes in the mixed and crescentic class.**

Discussion

In one of the largest studies to date, we investigated the long-term outcome of 123 patients with anti-GBM GN from six centers worldwide. We analyzed the predictive value of the kidney biopsy in anti-GBM GN by applying the histopathologic classification for ANCA-associated GN (18). The histopathologic classification was a significant predictor of kidney survival in univariable analysis, but not in multivariable analysis including dialysis dependency at presentation. However, the percentage of normal glomeruli and the extent of interstitial infiltrate remained significant predictors in multivariable analysis. We also found that patients with ≥50% globally sclerotic glomeruli did not recover from the need for acute dialysis. The crescentic and mixed classes, as defined for ANCA-associated GN, seemed less important in predicting the outcome of anti-GBM GN because their kidney outcome was variable. In line with the study by Levy et al. (9), we found that patients who were dialysis dependent at presentation and had 100% cellular crescents at biopsy did not recover kidney function.

Age seemed to be an important denominator in determining the therapeutic strategy and in outcome. Younger patients more often had a focal class biopsy specimen, were more likely to stay dialysis independent, and received intensive treatment more frequently. However, when we performed multivariable analyses, age was no longer significantly associated with ESRD. From our data, we can conclude that histopathologic parameters, i.e., percentage of normal glomeruli and extent of interstitial infiltrate, are more predictive of outcome. Nevertheless, we feel that it is important to take age into account

when defining the optimal individual treatment strategy. Interestingly, older age and double positivity were significantly associated, but double positivity was not associated with a worse outcome compared with single positivity.

The 5-year kidney survival rate in this study was 34%, which is higher than reported by Cui et al. (11), but in line with results from Levy et al. (9). Patients who were diagnosed after 2007 had a two-fold higher kidney survival rate compared with patients who were diagnosed before 2007. Interestingly, most patients with a focal class biopsy sample were diagnosed recently (2007–2015), suggesting earlier detection of the disease or increased awareness of the heterogeneity of the disease (20,21). The favorable outcome of this focal group can partially explain the observation of improved kidney outcome since 2007. The other contributing factor to this improvement is possibly the significant trend toward increased use of intensive therapy. From 1986 to 2000, 36% of the patients received intensive therapy, whereas 64% of the patients diagnosed after 2007 were treated intensively.

The heterogeneity of anti-GBM disease has recently been described by Nasr et al. (21) They described an atypic variant of anti-GBM disease, characterized by linear GBM staining for Igs, negative serum anti-GBM antibodies, absence of a crescentic phenotype, mild renal insufficiency, and absence of pulmonary hemorrhage. In our study, 20 patients had linear IgG deposits, but no detectable serum anti-GBM antibodies; three of them fulfilled the description of atypic anti-GBM disease. Therefore, the group of patients without detectable serum anti-GBM antibodies, but with linear IgG deposits, is rather heterogeneous.
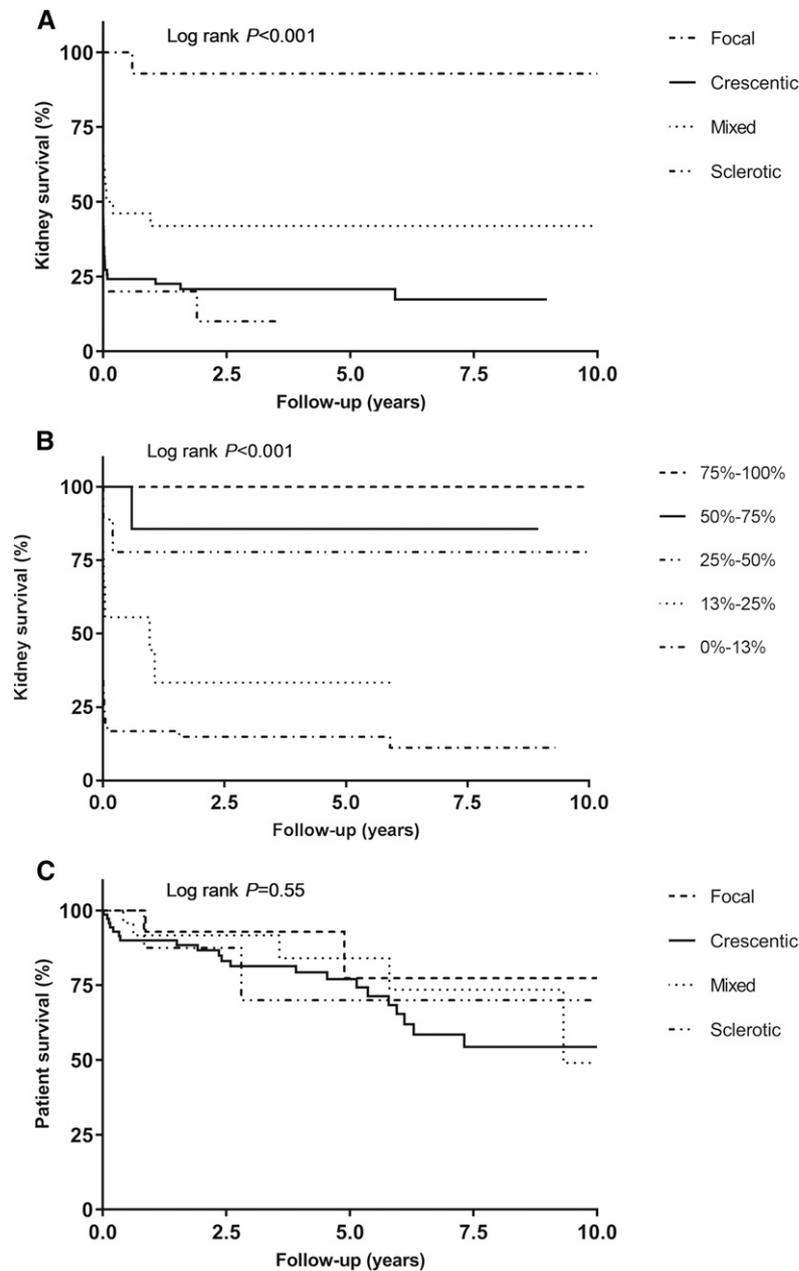
Figure 3. | Kidney survival is highest in patients with a focal class biopsy, and patient survival is similar in all histopathological classes. (A) Kidney survival according to histopathologic class. (B) Kidney survival according to percentage of normal glomeruli. (C) Patient survival according to histopathologic class.

Forty-five percent of our patients did not receive intensive treatment and the reason for refraining from intensive therapy was not always reported. However, some medical reports stated that treatment was withdrawn because of the results of kidney biopsy (*i.e.*, a high percentage of affected glomeruli), therapy-related complications such as serious infections, or lack of improvement under therapy. Five patients who did not receive intensive treatment presented with relatively preserved kidney function, which was probably the reason for not initiating the full treatment regimen. Our data suggest that patients who are dialysis dependent at presentation and have either ≥50% globally sclerotic glomeruli or 100% cellular crescents do not benefit

from intensive therapy and could therefore avoid the risk of immunosuppression and follow a conservative regimen. Other patients, including those with alveolar hemorrhage, should be treated intensively.

In our cohort, ten patients were treated with rituximab. In one center, rituximab along with cyclophosphamide has become standard therapy in patients who are double positive for anti-GBM antibodies and ANCA. Other indications for rituximab were found in three patients. In one patient, the kidney biopsy specimen showed a large infiltrate of CD20 positive B cells, providing a rationale to start anti-CD20 therapy (*i.e.*, rituximab). Another patient did not tolerate azathioprine; therefore, rituximab was given instead. A third patient received

Table 6. Prognostic significance of clinical and histopathologic parameters on ESRD

Variable	Univariable Analysis		Multivariable Analysis[a]		Multivariable Analysis[b]	
	HR (95% CI)	P Value	HR (95% CI)	P Value	HR (95% CI)	P Value
Age, yr	1.02 (1.01 to 1.03)	<0.01	1.01 (1.00 to 1.03)	0.06		
Males	1.19 (0.77 to 1.84)	0.44				
Lung involvement	1.19 (0.75 to 1.88)	0.47				
Double positivity	1.07 (0.64 to 1.78)	0.80				
Serum creatinine at presentation ≥5.7 mg/dl	4.83 (2.75 to 8.47)	<0.001	1.98 (0.96 to 4.10)	0.07		
Dialysis dependency at presentation	6.50 (3.68 to 11.47)	<0.001	a	a	3.2 (1.6 to 6.3)	0.001
Histopathologic classification		<0.01		0.15		
Focal (reference)						
Crescentic	20.29 (2.80 to 147.26)		8.38 (1.10 to 63.87)			
Mixed	11.67 (1.54 to 88.47)		6.77 (0.87 to 52.65)			
Sclerotic	25.72 (3.24 to 204.51)		11.17 (1.34 to 3.13)			
Percentage normal glomeruli	0.95 (0.93 to 0.98)	<0.001	0.97 (0.95 to 0.99)	<0.01	0.97 (0.95 to 0.99)	0.01
Percentage cellular crescents	1.02 (1.01 to 1.02)	<0.001	1.02 (1.00 to 1.05)	0.09		
Percentage globally Sclerotic glomeruli	1.01 (1.00 to 1.02)	0.20				
Interstitial fibrosis and tubular atrophy, score 2–3	1.88 (1.20 to 2.95)	<0.01	1.38 (0.87 to 2.18)	0.17		
Interstitial infiltrate, score 2–3	2.35 (1.44 to 3.83)	0.001	1.90 (1.16 to 3.13)	0.01	2.02 (1.2 to 3.5)	0.01
Tubulitis, present	1.51 (0.96 to 2.35)	0.07				

HR, hazard ratio; 95% CI, 95% confidence interval.
[a]Each parameter that was significant in univariable analysis, was analyzed in a multivariable analysis including dialysis dependency at presentation. Dialysis dependency at presentation remained significant in each multivariable analysis with P values ≤0.002.
[b]Parameters that were significant in the multivariable analysis[a] were included in this multivariate analysis.

rituximab, because the side-effects of cyclophosphamide were likely to cause problems. Because only nine patients were treated with rituximab and cyclophosphamide, conclusions on the response to rituximab could not be drawn.

This study included 123 patients with anti-GBM GN and is therefore the largest study evaluating kidney biopsy samples in relation to clinical outcome in this rare disease. Another strength of the study is the relatively long duration of follow-up (median 3.9 years). We consider the possibility of the development of ESRD after the last date of follow-up unlikely, because progression to ESRD usually occurs in the initial phase of this disease (9). Because of the rapid onset of anti-GBM disease, patient or referral delay have probably not influenced our findings. A limitation of the study is the possibility of selection bias; we only included patients with a diagnostic kidney biopsy performed, thereby excluding patients with a contraindication for a kidney biopsy (*e.g.*, frail patients). Moreover, treatment was a potential confounder in our study; therefore, we have investigated treatment in detail. Future studies on histopathology in anti-GBM GN with patients who are treated similarly are highly warranted.

In summary, dialysis independency at presentation, a high percentage of normal glomeruli, and a lower extent of interstitial infiltrate are associated with a favorable outcome in patients with anti-GBM GN. Dialysis-dependent patients with 100% cellular crescents or ≥50% sclerotic glomeruli are very unlikely to recover, and may be refrained from intensive therapy. Kidney survival has improved during recent years, doubling the success rate after 2007. This is possibly the result of earlier detection accompanied by the increased use of intensive therapy in anti-GBM GN.

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