ANCA-associated vasculitis: On clinical management and renal outcome

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THE DUTCH TRANSPLANTATION IN VASCULITIS (DUTRAVAS) STUDY: OUTCOME OF RENAL TRANSPLANTATION IN ANCA-ASSOCIATED GLOMERULONEPHRITIS

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ABSTRACT

Introduction: Data on the outcome of renal transplantation in anti-neutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (AAGN) patients are still limited. In particular, how disease recurrence in the renal allograft defines graft outcome is largely unknown. Therefore, we conducted a multi-center observational clinical and histopathological study to establish recurrence rate of AAGN in the allograft, and the impact of recurrence on allograft survival.

Methods: Using the nationwide Dutch Pathology Registry (PALGA), we retrospectively collected clinical and histopathological data of consecutive AAGN patients who had developed end-stage renal failure and received a kidney allograft in one of six Dutch university hospitals between 1984 and 2011. Transplant biopsies were scored using the Banff '09 classification. Renal disease recurrence was scored using the histopathological classification of AAGN.

Results: The post-transplantation recurrence rate of AAGN was 2.8% per patient year, accumulating to recurrence in a total of 11 out of 110 AAGN patients within the first 5 years after transplantation. Four of these 11 patients lost their graft, with one-year and 5-year graft survival rates of 94.5% and 82.8%, respectively. By multivariate analysis, AAGN recurrence was independently associated with subsequent graft loss.

Conclusions: In this study in 110 Dutch patients, the recurrence rate of AAGN within 5 years after kidney transplantation appeared slightly higher than in previous reports. Moreover, recurrence of AAGN contributed independently to kidney allograft loss, emphasizing the importance of clinical vigilance, since early treatment might be critical to rescuing the allograft.

INTRODUCTION

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) are the major subtypes of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Approximately 80% of patients with GPA and 90% of patients with MPA develop kidney involvement during the disease course.[1] ANCA-associated glomerulonephritis (AAGN) progresses to end-stage renal failure (ESRF) in approximately 20-40% of patients.[2-5] Data regarding the outcome of renal transplantation in AAGN patients are limited with only a few multi-center cohort studies. The studies vary widely with respect to graft survival and disease relapse rates; they reported 1-year graft survival rates of 86-100% and 5-year graft survival rates of 69-100%. In these studies, relapse rates ranged from 1.0-2.0% per patient year of follow-up.[6-10] National and international registry studies reported 1-, 3-, 5, and 10-year graft survival rates of 95%,[11] 70%,[12] 82-96%,[11, 13] and 80%,[14] respectively. These registry studies focused solely on graft survival and provided limited in-depth disease-specific data. Almost none of the published studies took into account histopathological findings. Only Little *et al.* commented briefly on vascular changes within the renal graft, but the complete renal transplant histopathology was not formally reviewed.[9]

In the current study, we investigated the outcome of renal transplantation in a Dutch cohort of AAGN patients, focusing on renal disease recurrence and graft survival rates within five years of transplantation. We formally reviewed the complete renal transplant histopathology and assessed the impact of disease recurrence within the allograft on graft survival, since it is largely unknown how disease recurrence in the renal allograft affects graft outcome.

METHODS

Patients

The study cohort included 113 patients who were retrospectively recruited from six academic hospitals in the Netherlands using a nationwide search for AAGN patients who received a renal transplant. We used the Dutch Pathology Registry (PALGA) (www.palga.nl), a nationwide network and registry of histo- and cytopathology in the Netherlands, encompassing all pathology laboratories.[15] Only patients with one of the following diagnoses were included: GPA (formerly Wegener's granulomatosis), MPA, proteinase 3 (PR3-)AAV, myeloperoxidase (MPO-)AAV, AAV not further specified, renal limited vasculitis (RLV), systemic vasculitis, or pauci-immune rapidly progressive glomerulonephritis. Experienced, academically based nephrologists and nephropathologists verified the diagnosis of each patient by reviewing the medical and histology reports. The diagnosis was based on a clinical presentation compatible

with AAGN and substantiated by a positive ANCA serology and/or histology (light microscopy and immunofluorescence and/or electron microscopy). Because the PALGA search was limited to patients with a renal transplant sample, we performed an additional, center-specific search for transplanted AAGN patients. Nephrologists at the participating centers searched their hospital's clinical database for additional patients. In summary, 113 patients with 34 native kidney biopsies and 136 renal transplant samples were included in this study. Diagnostic definitions were adapted from the 1994 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis.[16] All transplantations occurred between 1984 and 2011. We verified our search strategy using the Renine database (https://www.renine.nl/page?id=registry), which registers all patients in the Netherlands who receive chronic renal replacement therapy. This verification revealed no additional AAGN patients who were transplanted between 1984 and 2011. All patients were Caucasian and had ESRF secondary to AAGN. Only the first renal transplantation of each patient was analyzed.

We also performed a search for native renal biopsies of all included patients. This resulted in 34 native renal biopsies from 31 patients. This number was relatively low, because many of the native renal biopsies were taken in regional non-academic hospitals, where the tissue specimens were either not accessible or not preserved. In case of non-accessibility or non-availability, the histology reports were additionally reviewed centrally by an experienced, academically based nephropathologist (I.M.B.).

Patients transplanted after September 2000 received daclizumab as induction therapy (100 mg/day on the day of transplantation and 10 days after transplantation). The general maintenance regimen of the transplanted patients in this cohort was a triple therapy consisting of prednisolone, mycophenolate mofetil (MMF) and a calcineurin inhibitor (CNI). Fifty-one patients remained on this triple therapy regimen. In 22 patients, MMF or CNI was switched to azathioprine. Sixteen patients continued taking prednisolone and CNI, seven continued taking MMF and CNI, one continued taking prednisolone only, and four continued taking CNI only. In one patient, fingolimod was added to the standard regimen. In five patients, CNI was switched to a mammalian target of rapamycin (mTOR) inhibitor (three patients received sirolimus, and two received everolimus); in one patient, MMF was switched to everolimus, and a protein kinase C inhibitor was added. One patient continued on MMF, CNI and everolimus, and one patient continued on CNI and sirolimus.

Data collection

Patient data included clinical data, histology reports and —where available— native renal biopsies and/or renal transplant samples. Clinical data were collected using a questionnaire for review of the medical reports, which was completed by experienced nephrologists working in the participating academic hospitals. Native renal biopsies, renal transplant samples and histology reports were collected by experienced nephropathologists working at the participating academic centers. Data until January 2013 were collected. This study was conducted in accordance with the Declarations of Helsinki and Istanbul.

Clinical parameters

The following clinical data of the recipient between the time of diagnosis and the time of renal transplantation were collected: gender, date of birth, diagnosis (GPA/MPA), ANCA positivity during active disease (yes/no, PR3-ANCA/MPO-ANCA, cytoplasmic (c)ANCA/perinuclear (p)ANCA), time interval between diagnosis and transplantation, and (time on) dialysis. Clinical data at the time of transplantation included age, date of transplantation, donor type (deceased/living), and ANCA positivity. Clinical data collected after transplantation involved serum creatinine levels, date of last visit, disease relapse/renal disease recurrence, graft loss, death, ANCA positivity two and five years after transplantation, and transplantation-related immunosuppressive therapy. In case of disease relapse, the therapy regimen used to achieve disease remission was noted.

Patients were subdivided into two groups of GPA and MPA in accordance with established diagnostic definitions. RLV was regarded as a form of MPA. ANCA was categorized as PR3-ANCA in case of a positive PR3-ANCA ELISA or a positive cANCA pattern by indirect immunofluorescence (IIF) microscopy, and as MPO-ANCA in case of a positive MPO-ANCA ELISA or a pANCA pattern on IIF microscopy.

Parameters considered for analyses were: gender, age at transplantation, GPA/MPA, ANCA-type, time between diagnosis and transplantation, time on dialysis, donor type, ANCA status at transplantation, renal disease recurrence, and an acute rejection episode.

Histology

Native renal biopsies and renal disease recurrence in the graft with an available biopsy were scored according to the histopathological classification of AAGN.[17] All renal transplant samples were scored according to the Banff ’09 classification.[18-20]

The class was based on the first renal graft biopsy confirming the renal disease recurrence. All samples were scored centrally by an experienced, academically based nephropathologist (I.M.B.) who was blinded with respect to the clinical data.

Study outcomes

The primary clinical outcomes were renal disease recurrence and graft loss. The secondary outcome was disease relapse. Renal disease recurrence refers to the manifestation of the disease in the graft, independent of other organ involvement. Disease relapse refers to renal disease recurrence and/or extra-renal disease manifestations. Renal disease recurrence was defined as an increase in serum creatinine and new-onset hematuria or proteinuria (all attributable to active vasculitis) and/or histological confirmation. The appearance of cellular crescents and/or fibrinoid necrosis in the renal graft biopsy was considered evidence of renal disease recurrence. Extra-renal disease relapse was defined as new, worsened or recurred manifestations (all attributable to active vasculitis). Disease relapse was based on the expert opinion of the academically based nephrologists and nephropathologist (I.M.B.).

Statistical Analysis

Graft survival censored for death with a functioning graft was analyzed using the Kaplan-Meier survival method. For univariate analyses, log rank test and Cox regression analyses were used, with graft loss, renal disease recurrence and disease relapse as outcomes. In the univariate analyses, significant associations were found only with graft loss as outcome; therefore, multivariate Cox regression analyses were only performed with graft loss as dependent variable. All baseline parameters were considered to be fixed covariates. To assess the effect of renal disease recurrence and acute rejection on graft loss, both were considered to be time-dependent covariates. Due to the relatively low number of events, inclusion of all variables in the multivariate Cox regression analysis was not statistically feasible.[21] Therefore, we analyzed one multivariate Cox regression model based on the outcomes of the univariate analyses (all variables with $P<0.05$). For Cox regression analyses, hazard ratio (HR) and 95% confidence interval (CI) were calculated. A P-value <0.05 was considered statistically significant. All statistical calculations were performed using SPSS (v20.0; IBM Corp, Armonk, NY) and GraphPad Prism software (v6; GraphPad Software Inc, La Jolla, CA).

RESULTS

Patients and events

A total of 113 AAGN patients who received a renal transplant from 1984 through 2011 were included (Table 1). In three patients (3%) the graft did not gain proper function after transplantation and these were excluded from analyses. Of the remaining 110 patients, 88 (80%) retained the graft and did not die during the follow-up period. Fifty-seven of them (65%) completed the five years of follow-up. The remaining 31 patients (35%) had their last visit within five years of transplantation (median: 27.9 months, interquartile range (IQR): 21.0-41.8); two of them were lost to follow-up (reason unknown).

During follow-up, 79 patients (72%) experienced no event and six patients (5%) died with a functioning graft within five years of transplantation without experiencing a disease relapse (Figure 1). These six patients died due to infection (*n*=3), cancer (*n*=2), or a cardiovascular event (*n*=1).

Thirteen patients (12%) experienced 16 disease relapses (three patients experienced two relapses) within five years of transplantation (Figure 1). Of these 16 relapses, two involved extra-renal organs only, five involved the renal graft only, and nine involved both the renal graft and extra-renal organs (Table 2). The first disease relapse occurred within a median of 22.1 months (IQR: 10.3-46.3) following transplantation. The risk of experiencing a first disease relapse within 5 years of renal transplantation was 3.3% per patient year. The first renal disease recurrence occurred within a median of 18.0 months (IQR: 10.3-45.9). The risk of experiencing a first renal disease recurrence within five years was 2.8% per patient year. The relapses were equally distributed between 1986 and 2012.

Table 1 | Baseline characteristics of renal transplant recipients with end-stage renal failure due to ANCA-associated vasculitis

Characteristic	Value
Number of patients	113
Age at transplantation (years)	52.2 (14.7)[a]
Male	77 (68.1)
Diagnosis	
GPA	77 (68.1)
MPA	36 (31.9)
ANCA type	
PR3	37 (32.7)
MPO	52 (46.0)
ACPA[b]	6 (5.3)
Negative	5 (4.4)
Double positive	1 (0.9)
ANCA-positive not further specified	4 (3.5)
NR	8 (7.1)
Time between diagnosis and transplantation (months)	50.0 (26.6 – 95.2)[c]
Dialysis before transplantation[d]	
Yes	107 (94.7)
No (preemptive transplantation)	4 (3.5)
NR	2 (1.8)
Time on dialysis before transplantation[d] (months)	24.0 (16.7 – 40.3)[c]
Donor type	
Deceased	67 (59.3)
Living	46 (40.7)
ANCA status at time of transplantation	
Positive	27 (23.9)
Negative	25 (22.1)
NR	61 (54.0)
Era of transplantation	
Before 1990	8 (7)
1990 - 2000	37 (33)
After 2000	68 (60)

Data are presented as *n* (%) unless otherwise noted.
[a]Mean (SD).
[b]Former nomenclature for ANCA, and referred primarily to PR3-ANCA. These patients were tested before 1990, and no further specified test results were reported at a later time point.
[c]Median (25^{th} and 75^{th} percentile).
[d]Irreversible dialysis dependency.
Abbreviations: GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; ANCA, anti-neutrophil cytoplasmic antibody; PR3, proteinase 3; MPO, myeloperoxidase; ACPA, anti-cytoplasmic antibody; SD, standard deviation, NR, not reported/not performed.

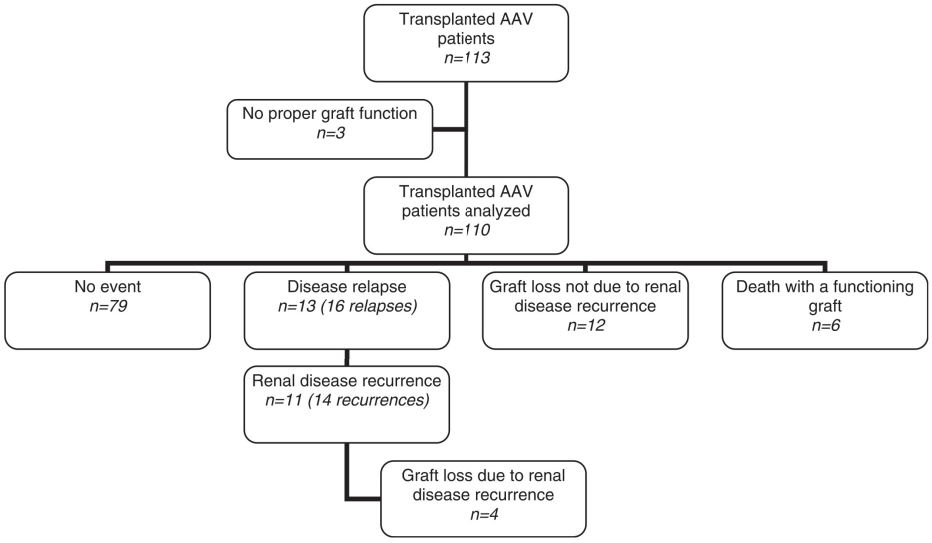


Figure 1 | Events.
Flowchart of the different events, which the 113 patients experienced within five years of renal transplantation.
Abbreviation: AAV, ANCA-associated vasculitis.

The occurrence of (and time to first) relapse was not associated with any of the baseline characteristics listed in table 1, including type of immune-suppressive regimen. Four patients with renal disease recurrence in the graft, lost their grafts due to the disease recurrence within five years of transplantation. One of these patients experienced the first renal disease recurrence ten months after transplantation, which entered into remission following plasmapheresis, prednisolone and cyclophosphamide treatment. This patient's second renal disease recurrence occurred 13 months after the first event, and despite treatment with an increased dose of prednisolone with continuation of MMF and CNI, the patient lost the graft. In the other patients, the disease recurrences leading to graft loss were treated with prednisolone and cyclophosphamide (Table 2). These four grafts were lost before remission was achieved. All other patients were treated to remission without graft loss. At the time of relapse, 13 patients were ANCA-positive, two patients were ANCA-negative, and in one, the ANCA titer was not reported.

Table 2 | Characteristics of patients with disease relapse within five years of transplantation

Patient	Gender	Diagnosis	ANCA type	Age at Tx[a]	ANCA at Tx	Donor type	Year of Tx	Medication[b]	Disease relapse	Time after Tx[c]	Treatment relapse	ANCA status at relapse	Elevated ANCA titer before relapse	Graft loss ≤5 years of Tx[d]	Δ time[e]	Histopathological class
1	F	MPA	MPO	63	Positive	DD	1998	P, MMF, CNI	R	0.3	P, CYC	Positive	NR	Yes	1.0	Focal
2	M	GPA	PR3	51	Negative	DD	2000	P, MMF, CNI, AZA	RER	15.8	P, CYC	Negative	No	Yes	8.0	Mixed
3	F	GPA	PR3	58	Negative	LD	2001	P, MMF, CNI	RER	45.9	P, CYC	Positive	NR	Yes	3.5	Mixed
4	M	GPA	PR3	45	Positive	DD	2004	P, MMF, CNI, AZA	RER	10.3	Pph, P, CYC	Positive	Yes	No	N/A	Crescentic
4 (2nd relapse)									RER	23.0	P	Positive	Yes	Yes	8.0	N/A
5	M	GPA	PR3	51	NR	DD	2001	P, MMF, CNI	RER	18.0	MeP, P, CYC	Positive	NR	No	N/A	Focal
6	M	GPA	PR3	35	Positive	LD	2010	P, MMF, CNI, sirolimus	RER	10.4	P, RTX	Positive	Yes	No	N/A	Focal
7	M	GPA	ACPA	52	NR	DD	1984	P, CNI	RER	27.2	Cs	Positive	NR	No	N/A	Focal
7 (2nd relapse)									RER	46.1	P, CYC	Positive	NR	No	N/A	N/A
8	M	MPA	MPO	50	NR	LD	2010	P, MMF, CNI, AZA	R	8.1	P, CYC	Positive	NR	No	N/A	Focal
8 (2nd relapse)									R	26.6	P, RTX	Positive	Yes	No	N/A	N/A
9	M	MPA	MPO	40	Positive	DD	1990	P, CNI	R	59.2	P, AZA	Positive	No	No	N/A	Mixed
10	M	GPA	MPO	42	Positive	LD	1996	P, CNI	R	22.1	P, CYC	Positive	Yes	No	N/A	Mixed
11	M	GPA	MPO	62	NR	DD	1995	P, MMF, CNI	RER	55.7	NR	NR	NR	No	N/A	N/A
12	M	GPA	NR	50	NR	DD	1991	P, CNI	ER	36.0	NR	Positive	NR	No	N/A	N/A
13	F	GPA	PR3	18	Negative	DD	2008	P, MMF, CNI	ER	46.7	P	Negative	No	No	N/A	N/A

[a]Age in years.
[b]Transplantation-related immunosupressive medication.
[c]Time in months.
[d]Graft loss within five years of transplantation due to disease relapse with renal graft involvement (renal disease recurrence).
[e]Time difference in months between renal disease recurrence and graft loss.
Abbreviations: ANCA, anti-neutrophil cytoplasmic antibody; Tx, transplantation; F, female; M, male; MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; ACPA, anti-cytoplasmic antibody; DD, deceased donor; LD, living donor; P, prednisolone; MMF, mycophenolate mofetil; CNI calcineurin inhibitor; AZA, azathioprine; R, disease relapse with solely renal graft involvement; RER, disease relapse with renal graft and extra-renal involvement; ER, disease relapse with solely extra-renal involvement; CYC, cyclophosphamide; Pph, plasmapheresis; MeP, methylprednisolone; Cs, cyclosporine; RTX, rituximab; NR, not reported/not performed; N/A, no biopsy of the renal disease recurrence available or not applicable.

Twelve patients lost their grafts due to other causes than renal disease recurrence of AAGN: interstitial fibrosis and tubular atrophy (*n*=4), acute rejection (*n*=3), (uro)sepsis (*n*=2), post-transplant lymphoproliferative disorder (*n*=1), infarction (*n*=1) and acute cyclosporine toxicity (*n*=1). The 1-year graft survival rate was 94.5% (95% CI, 90.2%-98.8%), and the 5-year graft survival rate was 82.8% (95% CI, 75.0%-90.6%) (Figure 2). The era in which the transplantation was performed had no significant effect on graft survival (Figure 3).

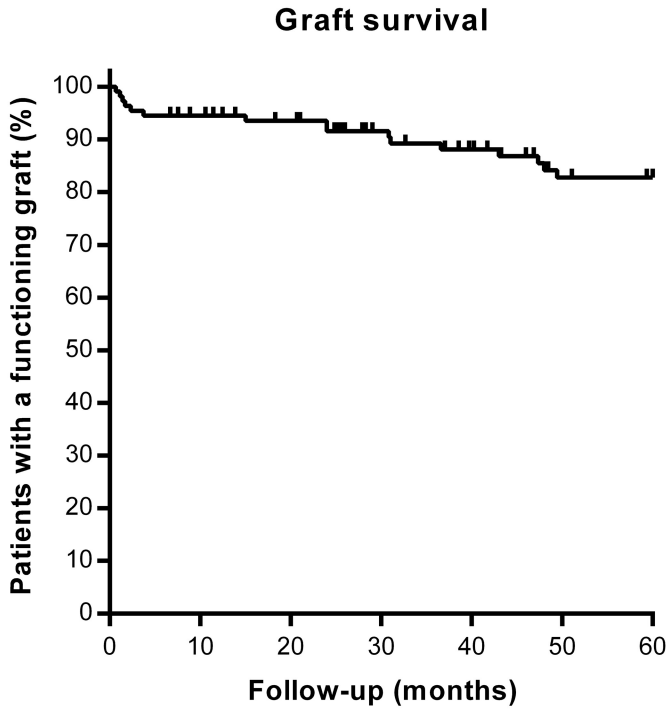
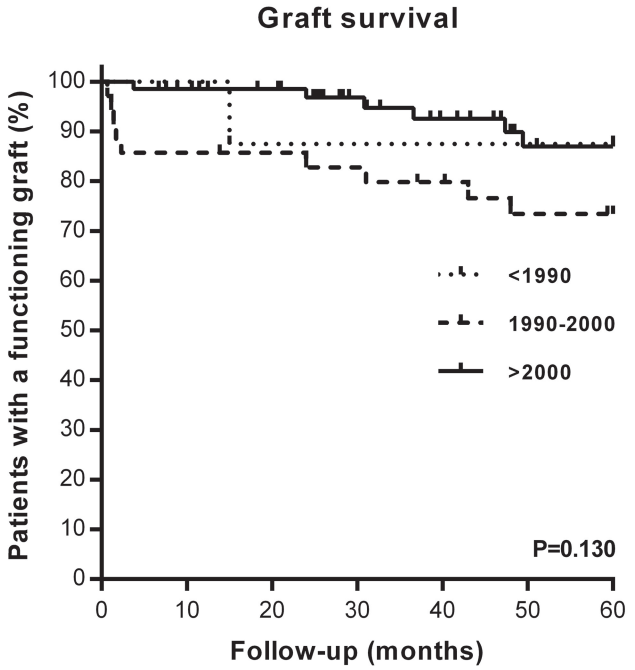


Figure 2 | Death-censored renal graft survival.
Kaplan-Meier curve of renal graft survival among 110 renal transplant recipients with ANCA-associated vasculitis. During follow-up 16 patients lost their graft within five years of transplantation. The one-year graft survival rate was 94.5% (95% CI, 90.2%-98.8%), and the 5-year graft survival rate was 82.8% (95% CI, 75.0%-90.6%). The data was censored for death with a functioning graft.



Patients at risk							
<1990	8	8	7	7	7	7	7
1990-2000	35	30	29	28	26	23	22
>2000	67	63	59	45	39	29	28

Figure 3 | Death-censored renal graft survival according to transplantation era.
Kaplan-Meier curves of renal graft survival according to the era's in which the 110 renal transplant recipients with ANCA-associated vasculitis were transplanted. The patients were divided into three era's: before 1990, 1990-2000 and after 2000. These era's were chosen in accordance with the study by Little *et al.*(9) There was no difference in graft survival between the groups (log-rank test: P=0.13).

Histopathology

Of the 136 renal transplant samples taken from these 110 patients within five years of transplantation, 108 fulfilled the criteria (≥7 glomeruli and ≥1 artery) for scoring using the Banff '09 classification (Table 3).[18-20] Of the 108 suitable samples, 24 were protocol biopsies (median: 4 months, IQR: 3 – 14), all the other biopsies were taken for cause. During follow-up, 23 patients experienced 28 biopsy-confirmed acute rejection episodes, three of which showed signs of humoral rejection. Six episodes were subclinical acute rejections detected by protocol biopsies.

Table 3 | Overview of the histological findings in 108 renal transplant samples of 110 patients

Histological finding[a]	Number of samples[b]	Number of patients[c]	Time after Tx[d]
Normal	13	11	0.7 (0.3-3.0)
Acute rejection	28[e]	23	5.2 (0.5-16.8)
Borderline changes	9[e]	8	1.3 (0.8-5.5)
IFTA	25	21	12.0 (1.3 – 28.3)
CNI toxicity	8	8	2.1 (0.9-10.5)
ATN	4	4	0.3 (0.2-0.3)
Renal disease recurrence	10[e]	10	19.0 (9.7-31.8)
BK-nephropathy	1	1	22.3
TMA	1	1	0.4
Pyelonephritis	1	1	0.4
Infarction	2	2	0.1 and 1.7
Extensive vasculopathy	1	1	0.9
Slight hyalinosis	1	1	1.1

[a]Adapted from the Banff '09 classification.
[b]The total number of samples/episodes differs from 108, since categories may coincide in the same sample and some numbers represent number of episodes rather than number of samples (see [e]).
[c]The total number of patients in the table differs from the total number of the cohort (*n*=110), since histological lesions may coincide in the same patient and not all patients were biopsied after transplantation.
[d]Time in months: median (IQR).
[e]These numbers represent episodes: in some cases more than one biopsy has been performed on the same episode.
Abbreviations: Tx, transplantation; IFTA, interstitial fibrosis and tubular atrophy; CNI, calcineurin inhibitor; ATN, acute tubular necrosis; TMA, thrombotic microangiopathy; IQR, interquartile range.

Renal biopsies were available for 10 of the 14 renal disease recurrences. All ten biopsy-confirmed recurrences were first-time renal disease recurrences. The histopathological classes of these ten recurrences were focal (5), crescentic (1), and mixed class (4) (Table 2). The biopsies contained a median of 16 glomeruli (IQR: 12-22). In the three patients who had lost their graft within five years of transplantation as a result of their first renal disease recurrence, one recurrence was focal, and two were mixed class. In one of the patients with a mixed class recurrence, the recurrence changed to crescentic class before graft loss (S Table 1 in the supplementary material).

Outcome statistical analyses

Four baseline parameters were associated with graft loss in a univariate Cox regression analysis (S Table 2 in the supplementary material). Male gender of the recipient (HR 0.33, 95% CI 0.12-0.88, P=0.03) and a living donor (HR 0.22, 95% CI 0.05-0.95, P=0.04) were associated with better graft survival. Experiencing renal disease recurrence (HR 12.43, 95% CI 3.61-42.89, P<0.001) and experiencing an acute rejection (HR 3.18, 95% CI 1.08-9.36, P=0.04) were significantly associated with worse graft survival. No baseline parameter was associated with either renal disease recurrence or disease relapse (S Table 3 in the supplementary material).

In the multivariate analysis with graft loss as outcome and including all baseline parameters that were statistically significant in the univariate analysis, only the associations with gender of the recipient (HR, 0.27; 95% CI, 0.09-0.81; *P*=0.02) and experiencing renal disease recurrence persisted (HR, 18.48; 95% CI, 4.96-68.89; *P*<0.001) (Table 4).

Table 4 | Outcome of the multivariate analysis regarding graft loss

	Parameter	P-value	HR (95% CI)
Multivariate model	Male gender recipient	0.02	0.27 (0.09-0.81)
	Living donor	0.08	0.26 (0.06-1.18)
	Renal disease recurrence	<0.001	18.48 (4.96-68.89)
	Acute rejection episode	0.25	1.94 (0.62-6.03)

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

DISCUSSION

We investigated the outcome of renal transplantation within the first five years of transplantation in a Dutch cohort of 113 AAGN patients transplanted between 1984 and 2011. In this cohort, the 1-year and 5-year graft survival rates were 94.5% and 82.8%, respectively, which is similar to graft survival rates reported by registry data of the general transplantation population in Europe and North America.[22-24] Compared to the Dutch general renal transplantation population transplanted in the period 1984-2011, the graft survival in our AAV cohort seemed to be better: 1-year graft survival 94.5% vs. 86.0%, 5-year graft survival 82.8% vs. 70.0% (Dutch Transplantation Foundation (NTS) database; registers all transplantations in the Netherlands, accessed 12-06-2015). This discrepancy is most likely due to the larger proportion of transplantations in the earlier years in the general population compared to our cohort. In addition, compared to the European and North American registry data, transplantations from an earlier period have been included in the Dutch registry data. The risk of experiencing a first disease relapse or renal disease recurrence within 5 years of transplantation was 3.3% and 2.8% per patient year, respectively. The principal finding in this cohort was that renal disease recurrence was an important cause of graft loss within the first 5 years of transplantation.

Our graft survival rates are similar to those reported in previous studies on AAGN, although some studies reported a slightly better 1- or 5-year graft survival rate.[7-9,13] Most of these studies included transplantations performed in a later time period compared to our study; from the late 90's on. In our cohort the era in which the transplantation was performed had no significant effect on graft survival. These slight differences can also be a consequence of the relative small cohorts in the different studies. The largest cohort with 919 recipients transplanted between 1997-2007 showed similar graft survival rates as our study.[11] There were no noteworthy differences in treatment protocols between the studies that could explain the differences.

Our relapse and recurrence rates are slightly higher compared to those reported in other studies. Most studies on the outcome of renal transplantation in AAV described rates of 1.0-2.0% per patient year.[6-9] Three studies reported higher rates of 7.6%, 6.0% and 10.0% per patient year.[25-27] Several of these studies described patients with renal disease recurrence with consequent graft loss, sometimes in >50% of the patients with recurrence.[7, 9-11, 13] Our study is the first study which specifically assesses the impact of renal disease recurrence on graft survival, showing that renal disease recurrence is associated with subsequent graft loss.

The patients who lost their graft after renal disease recurrence were not treated more or less aggressively than the patients who did not lose their graft. In view of recent evidence from the RAVE trial (NCT00104299) showing that rituximab treatment has an advantage over cyclophosphamide treatment in case of relapse with severe disease manifestations,[28, 29] we suggest that also in the transplantation setting there may be a benefit of treating recurrent disease with rituximab. In fact, two patients in our cohort who were treated with rituximab for a renal disease recurrence did not lose their graft. Another study also described remission induction with rituximab in patients with renal disease recurrence in the graft.[30] However, we were unable to compare the rituximab-treated patients with standard cyclophosphamide-treated patients due to the small patient numbers. Our results show that the recurrence of AAGN is an important cause of graft loss, and therefore, continuously monitoring these transplanted patients for relapse is highly important.

Currently no distinction is made in the clinical setting based on the clinical defined diseases or ANCA-specifity. They are treated the same way and have the same procedure regarding renal transplantation in AAGN. Our study showed that there is no difference in graft loss, renal disease recurrence or disease relapse when comparing disease subtypes or ANCA-specificity. This is also described in other studies on renal transplantation in AAGN.[7, 9, 13] Further studies are needed to determine whether distinction in ANCA specificity or disease subtype is needed for optimal clinical management.

Our study and other studies revealed that patients can relapse without a positive ANCA titer before the relapse. This finding is consistent with other renal transplantation studies.[26, 31-34] A meta-analysis found that a rise in—or persistence of—ANCA has modest predictive value for future disease relapse in AAV patients without ESRF.[35] Therefore, it may be concluded that the isolated use of serial ANCA measurements is not reliable for management decision-making in transplanted AAGN patients.

This is the first study that formally reviewed the complete renal transplant histopathology and classified renal disease recurrence in the graft according to the histopathological classification of AAGN. With regards to the histopathological presentation of the renal disease recurrence in the grafts, we found that the disease can recur as a different class in the graft and that histopathological class can change over time in the renal graft after disease recurrence. These findings, although based on small numbers (S Table 1 and S Table 4 in the supplementary material), may suggest that the histopathological classes represent different phases of the disease. This is

in line with recent evidence from a study by Hruskova *et al*. describing a class change over native renal biopsies in 86% of the patients in protocolized repeat biopsies after one year.[36]

In the last 30 years, important developments were made in the field of post-transplantation immunosuppressive therapy. In 1983, cyclosporine was introduced after the 'azathioprine era'. This resulted in improved graft survival rates.[37] After increased use of cyclosporine over time, in 1992 new trends developed including waning of cyclosporine and the rise of induction therapy. The use of induction therapy gradually increased resulting in 59% of all recipients receiving induction therapy in 2001. During this period, corticosteroid therapy was an important component of maintenance therapy. The waning of cyclosporine was accompanied with a rise of tacrolimus use. In 1992, azathioprine was predominantly used as antimetabolite, but by 2001 most centers used MMF. In 1996, rapamycine was introduced as an alternative to spare other immunosuppressive drugs, in particular the nephrotoxic CNI. These developments resulted in the currently most used regimen of induction therapy followed by a triple therapy consisting of prednisolone, MMF and tacrolimus.[38] This probably has decreased the relapse rates of AAV after renal transplantation when comparing the different studies over time, but none of them could demonstrate this, probably due to a lack of power.[6-9, 25-27]

AAV is diagnosed on the clinical manifestation compatible with AAV and substantiated by a positive ANCA serology and/or histology. In the 90s, solid phase assays (ELISAs) for PR3-ANCA and MPO-ANCA detection were developed and standardized.[39] In addition, a disease specific activity index was introduced, which was revised two times since then; Birmingham Vasculitis Activity Score.[40-42] The gold standard for establishing AAGN is a renal biopsy. These diagnostic tools are also used in the setting of transplantation for detecting disease relapse. When detecting disease relapse in general the patient receives induction therapy based on a cyclophosphamide regimen as is the case with newly diagnosed AAV. As discussed above, rituximab is now also more frequently used in case of renal disease recurrence after transplantation.

The retrospective design is a limitation of our study. Although complete data were available for most patients, some cases had missing data or material was not available. This made it impossible to determine the duration of remission prior to transplantation in relation to post-transplant outcome. Moreover, numbers were too low to detect an impact of maintenance immunosuppression or therapy of recurrent disease on graft loss. Nevertheless, given our data we do not expect that a specific regimen was

associated with a negative outcome, as the majority of patients who experienced disease relapse, renal disease recurrence and/or graft loss received conventional therapy.

We conclude that in a substantial proportion (36%) of AAGN patients with disease recurrence in the renal graft, the recurrence led to graft loss within five years of transplantation. In a multivariate analysis, renal disease recurrence was independently associated with subsequent graft loss. So, although the risk of renal disease recurrence is rather low, once a recurrence of the disease occurs in the graft, the risk of graft loss is considerable. This study confirms that renal transplantation is a viable treatment option for AAGN patients with ESRF, but it also serves as a warning that clinicians must remain cognizant of the risk of graft loss when the disease has recurred in the renal graft.

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DISCLOSURES

None to declare.

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SUPPLEMENTARY MATERIAL

The Dutch transplantation in vasculitis (DUTRAVAS) study: outcome of renal transplantation in ANCA-associated glomerulonephritis

S Table 1 | Overview of the patients with follow-up biopsies of their biopsy-confirmed renal disease recurrence

Patient[a]	Class recurrence - first biopsy[b]	Class recurrence - follow-up biopsy 1[b]	Class recurrence - follow-up biopsy 2[b]	Second renal disease recurrence ≤5 years of Tx	Graft loss ≤5 years of Tx[c]
1	Focal (0.3)	Focal (0.6)	Focal (1.1)	No	Yes
2	Mixed (15.8)	Crescentic (17.1)	-	No	Yes
7	Focal (27.2)	Mixed (32.8)	-	Yes	No
8	Focal (8.1)	Focal (10.0)	-	Yes	No

[a]The patient numbers correspond with Table 2.
[b]Class (months after transplantation).
[c]Graft loss within five years of transplantation due to disease relapse with renal graft involvement (renal disease recurrence).
Abbreviations: Tx, transplantation.

S Table 2 | Outcomes of the univariate analyses regarding graft loss

Parameter	Graft loss	
	P-value	HR (95% CI)
Gender recipient[a]	0.03	0.33 (0.12-0.88)
Age at transplantation (years)	0.36	0.99 (0.96-1.02)
Diagnosis[b]	0.20	1.92 (0.72-5.17)
PR3-ANCA[c]	0.60	0.75 (0.25-2.24)
MPO-ANCA[c]	0.81	1.14 (0.40-3.29)
Time between diagnosis and transplantation (months)	0.85	1.00 (0.99-1.01)
Time on dialysis (months)	0.27	1.01 (0.99-1.04)
Donor type[d]	0.04	0.22 (0.05-0.95)
ANCA status at transplantation[e]	0.31	0.49 (0.12-1.96)
Renal disease recurrence[f]	<0.001	12.43 (3.61-42.89)
Acute rejection episode[g]	0.04	3.18 (1.08-9.36)

[a]Ref: female.
[b]Ref: granulomatosis with polyangiitis.
[c]Ref: negative.
[d]Ref: deceased donor.
[e]Ref: ANCA-negative.
[f]Ref: patients experiencing no renal disease recurrence (time-dependent covariate).
[g]Ref: patients experiencing no acute rejection episode (time-dependent covariate).
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibody; MPO, myeloperoxidase.

S Table 3 | Outcomes of the univariate analyses regarding renal disease recurrence and disease relapse

Parameter	Renal disease recurrence		Disease relapse	
	P-value	HR (95% CI)	P-value	HR (95% CI)
Gender recipient[a]	0.42	1.89 (0.41-8.74)	0.59	1.43 (0.39-5.19)
Age at transplantation (years)	0.64	0.99 (0.95-1.03)	0.27	0.98 (0.95-1.01)
Diagnosis[b]	0.52	1.50 (0.44-5.12)	0.81	1.15 (0.36-3.74)
PR3-ANCA[c]	0.64	1.35 (0.39-4.67)	0.43	1.62 (0.49-5.31)
MPO-ANCA[c]	0.81	0.86 (0.25-2.97)	0.58	0.72 (0.22-2.36)
Time between diagnosis and transplantation (months)	0.64	1.00 (0.99-1.02)	0.74	1.00 (0.99-1.02)
Time on dialysis (months)	0.20	1.02 (0.99-1.05)	0.10	1.02 (1.00-1.05)
Donor type[d]	0.78	0.84 (0.25-2.88)	0.51	0.67 (0.21-2.18)
ANCA status at transplantation[e]	0.27	2.54 (0.49-13.12)	0.47	1.69 (0.40-7.08)

[a]Ref: female.
[b]Ref: granulomatosis with polyangiitis.
[c]Ref: negative.
[d]Ref: deceased donor.
[e]Ref: ANCA-negative.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; PR3, proteinase 3; ANCA, anti-neutrophil cytoplasmic antibody; MPO, myeloperoxidase.

S Table 4 | Overview of the patients with a native renal biopsy and a biopsy-confirmed renal disease recurrence after transplantation

Patient[a]	Class diagnostic native biopsy	Class follow-up native biopsy, if available	Class renal disease recurrence[b]
1	Focal	Mixed	Focal (10.4)
2	Crescentic	-	Mixed (45.9)
3	Mixed*	-	Mixed (59.2)
4	Sclerotic	-	Mixed (142.4)

[a]Patient 1, 2 and 3 correspond with patient 6, 3 and 9, respectively, in Table 2.
[b]Class (months after transplantation).
*One week after cyclophosphamide treatment was started.