ORIGINAL ARTICLE

Predictors of graft survival at diagnosis of antibody-mediated renal allograft rejection: a retrospective single-center cohort study

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SUMMARY

Antibody-mediated rejection (ABMR) is a major cause of graft loss in renal transplantation. We assessed the predictive value of clinical, pathological, and immunological parameters at diagnosis for graft survival. We investigated 54 consecutive patients with biopsy-proven ABMR. Patients were treated according to our current standard regimen followed by triple maintenance immunosuppression. Patient characteristics, renal function, and HLA antibody status at diagnosis, baseline biopsy results, and immunosuppressive treatment were recorded. The risk of graft loss at 24 months after diagnosis and the eGFR slope were assessed. Multivariate analysis showed that eGFR at diagnosis and chronic glomerulopathy independently predict graft loss (HR 0.94; $P = 0.018$ and HR 1.57; $P = 0.045$) and eGFR slope (beta 0.46; $P < 0.001$ and beta −5.47; $P < 0.001$). Cyclophosphamide treatment (6× 15 mg/m^2) plus high-dose intravenous immunoglobulins (IVIG) (1.5 g/kg) was superior compared with single-dose rituximab (1× 500 mg) plus low-dose IVIG (30 g) (HR 0.10; $P = 0.008$ and beta 10.70; $P = 0.017$) and one cycle of bortezomib (4× 1.3 mg/m^2) plus low-dose IVIG (HR 0.16; $P = 0.049$ and beta 11.21; $P = 0.010$) regarding the risk of graft loss and the eGFR slope. In conclusion, renal function at diagnosis and histopathological signs of chronic ABMR seem to predict graft survival independent of the applied treatment regimen. Stepwise modifications of the treatment regimen may help to improve outcome.

Transplant International 2020; 33: 149–160

Key words
antibody-mediated rejection, graft survival, immunosuppression, renal function, renal transplantation

Received: 13 April 2019; Revision requested: 8 May 2019; Accepted: 10 September 2019;
Published online: 15 October 2019

Introduction

Despite some progress during the past years, antibody-mediated rejection (ABMR) following renal transplantation remains a significant obstacle associated with an unfavorable prognosis. Currently, much effort is undertaken to evaluate the available treatment options in order to improve overall long-term graft survival. However, despite these efforts the existing evidence is anything but satisfactory. Recent studies could not confirm the efficacy of theoretically promising drugs such as rituximab [1,2] and bortezomib [3]. Preliminary reports on new compounds like complement inhibitors [4,5] or IL-6 receptor antibodies [6] are partially

doi:10.1111/tri.13525

encouraging. However, their efficacy needs to be confirmed by randomized controlled trials. To date, a generally accepted, clearly defined treatment algorithm based on high-quality evidence does not exist [7]. In a recent review, Böhmig and colleagues summarized the underlying pathogenesis, the clinical impact as well as currently available options and future concepts for the treatment of late ABMR [8]. Apart from that, it is becoming more and more clear that graft survival following diagnosis of ABMR may not only be dependent on the applied treatment protocol, but also on the underlying functional and morphological parameters at diagnosis [9]. Consequently, before subjecting an individual to an intensified immunosuppressive anti-ABMR treatment protocol, a risk-benefit analysis based on independent predictors of graft survival should be performed, in order to avoid high-dose immunosuppressive treatment, when the anticipated graft survival benefit is questionable. Accordingly, relevant predictors of graft survival should be incorporated in future ABMR trials at early stage already. In our present study, we aimed to identify specific clinical, pathological, and immunological parameters including treatment that may independently predict graft survival when ABMR is evident in a cohort of 54 well-characterized patients.

Patients and methods

Patient characteristics

Between January 2005 and November 2015, we treated 54 consecutive renal allograft recipients with clinically relevant, biopsy-proven ABMR. After informed consent, all patients were treated according to our current standard of care in accordance with the ethical standards of the declarations of Helsinki 2000 and Istanbul 2008. Following the treatment of groups of 10–12 patients, we critically analyzed clinical outcome of the respective group and modified our protocol for the upcoming patients. Meanwhile, five different treatment groups resulted (Figure 1). Group 1 (RLP, $n = 10$) was treated with a fixed dose of rituximab (500 mg i.v.), a fixed low dose of 30 g intravenous immunoglobulins (IVIG) and six sessions of plasmapheresis (PPH). Group 2 (BLP, $n = 11$) received one cycle of bortezomib (4× 1.3 mg/m^2 i.v.) together with low-dose IVIG (30 g) and PPH (6×). Patients of group 3 (BHP, $n = 11$) received high-dose IVIG treatment (1.5 g/kg) together with bortezomib (4× 1.3 mg/m^2 i.v.) and PPH (6×). Group 4 patients (RBHP, $n = 10$) were treated with a combination of rituximab (500 mg i.v.) and bortezomib (4× 1.3 mg/m^2 i.v.) together with high-dose IVIG (1.5 g/kg) and PPH (6×). In group 5 (CHP, $n = 12$), patients were treated with six i.v. cyclophosphamide pulses (15 mg/kg adapted to age and renal function) at 3-week intervals, PPH (6×), and high-dose IVIG (1.5 g/kg). All patients additionally received three i.v. pulses of 500 mg methylprednisolone. We excluded three patients of the original groups: In group 1 (RLP), two patients with non-HLA antibodies (HLAab) were excluded; in group 5 (CHP), one patient, who refused completion of cyclophosphamide treatment for personal reasons after two cyclophosphamide pulses, was excluded. Patients received triple maintenance immunosuppression including low-dose steroids, tacrolimus, and mycophenolic acid. Because not all patients (45/54, 83.3%) received tacrolimus maintenance treatment, tacrolimus was included as independent variable in the analyses. Clinical outcome of the individual groups including side effect profiles has already been described [10–13]. Here, we assessed all 54 patients together by using univariate and multivariate analyses, in order to investigate, which parameters predict graft survival following diagnosis. All patients achieved a minimum follow-up of 24 months. We included (i) relevant patient characteristics, (ii) renal function, (iii) HLAab status at diagnosis, (iv) baseline renal biopsy results, and (v) the applied treatment regimen in our model. The study was approved by the institutional Ethics Committee of the Charité hospital (EA1/048/14).

Matched-pair analysis

In order to generate two matched control groups, one consisting of patients with no rejection and one consisting of patients with T cell-mediated rejection (TCMR), a cohort of potential patients was identified in our web-based electronic patient record system "TBase" [14] in analogy to the ABMR treatment cohort: renal transplantation between January 01, 1990, and December 31, 2015, reproducible graft function and either biopsy-proven TCMR between December 31, 2004, and December 31, 2017, or no rejection episode. Patients of this cohort were then matched with patients of the ABMR treatment cohort with regard to gender, age at transplantation (±5 years), mode of transplantation (living donor or deceased donor), time after transplantation (±5 years), and time of rejection (±5 years) in case of TCMR.

HLA diagnostics

Renal transplantation was performed at the Charité hospital based on a negative complement-dependent

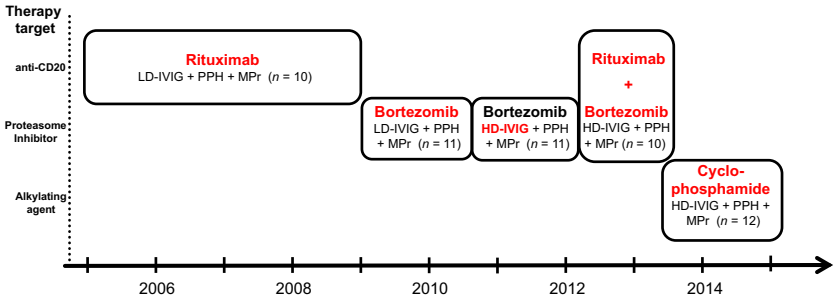

Figure 1 Timeline of the applied ABMR treatment regimen. Changes compared with the previous regimen are depicted in red, unchanged parts of the regimen are depicted in black. Abbreviations: HD-IVIG, high-dose IVIG; LD-IVIG, low-dose IVIG; MPr, methylprednisolone; and PPH, plasmapheresis.

cytotoxicity crossmatch (CDC-XM) with and without dithiothreitol using T- and B-lymphocytes with current and historical serum. In addition, graft allocation was based on a negative virtual crossmatch by considering current and historical unacceptable antigens as defined by Luminex®-based single antigen bead assays (>1000 mean fluorescence intensity (MFI) units). In case of retransplants, all repeat mismatches regardless of detectable antibodies were defined as unacceptable. Consequently, only patients with true *de novo* donor-specific HLA antibodies (DSA) were included.

After transplantation, serum samples were screened for HLAab by the Luminex® bead-based assay LABScreen® Mixed (One Lambda, Canoga Park, CA, USA). In addition, HLAab specificities were determined by LABScreen® single antigen beads assay (One Lambda). As an indicator for the antibody level, the normalized MFI was used. HLAab were considered positive when exceeding an MFI value of 500. The DSA showing the highest MFI at the time of ABMR diagnosis (DSAmax) and the MFI sum of all DSA (DSAsum) were recorded.

Renal biopsy

Renal biopsies were taken on indication only. All patients presented with clinically relevant allograft dysfunction post-transplant manifesting as an otherwise unexplained increase of serum creatinine (≥0.3 mg/dl), proteinuria (≥1 g/day), or primary nonfunction in the early phase after transplantation. Renal allograft pathology was performed by two experienced nephropathologists (K.W., B.R.). The diagnosis of ABMR was based on the presence of circulating DSA and significant allograft pathology according to the definitions of the Banff 2013 classification [15]. Biopsies taken before publication of the Banff 2013 classification were retrospectively scored.

Follow-up

Patients were regularly followed up in our outpatient clinic. Serum creatinine was measured monthly during the first 6 months after diagnosis, and then quarterly until 24 months after diagnosis. If no creatinine was measured at a particular time point, no data were imputed. The estimated glomerular filtration rate (eGFR) was calculated according to the chronic kidney disease epidemiology collaboration (CKD-EPI) formula [16]. If the patient was on dialysis treatment, an eGFR of 0 ml/min/1.73 m^2 was imputed for the respective time point. Laboratory values were extracted from our web-based electronic patient record system "TBase" [14].

Statistical methods

The data set includes only few missing values in the three parameters donor age ($n = 2$), DSA class I ($n = 1$) and vascular fibrous intimal thickening ($n = 4$), otherwise it is complete. Renal allograft survival was defined as the interval between diagnosis of ABMR and return to maintenance dialysis treatment. For comparison of clinical characteristics at diagnosis between treatment groups the Fisher's exact test for categorical variables and a median regression model for continuously distributed variables were used. The approximate linear association between the outcome variables and independent variables was visually tested by generalized additive models. The risk of graft loss at 24 months after diagnosis was univariately and multivariably investigated by a Cox proportional hazard model. The proportional hazard assumption was tested by using the Schoenfeld residuals after fitting a Cox proportional hazard model. Model calibration was assessed by the goodness of fit test as suggested by Gronnesby and Borgan. The Harrel's C statistics (ranging from 0.5 to 1, 0.5 = prediction by chance, 1 = perfect prediction) was calculated to evaluate the predictive power of each parameter with respect to graft loss in the multivariable model. A Cox proportional hazard model was performed to investigate differences in graft survival between patients with a diagnosis of ABMR and patients with a diagnosis of TCMR or no rejection. The change (slope) in eGFR

Table 1. Patient characteristics at diagnosis

	Group 1 (RLP) $n = 10$	Group 2 (BLP) $n = 11$	Group 3 (BHP) $n = 11$	Group 4 (RBHP) $n = 10$	Group 5 (CHP) $n = 12$	P value	Total $n = 54$
Demographic factors							
Donor age (years), median (interquartile range)	50.5	46.5	54.0	51.0	37.5	0.797	49.5 (IQR 35.3–58.8)
Recipient age (years), median (interquartile range)	47.0	26.0	45.0	32.0	46.0	0.050	40.0 (IQR 27.5–49.5)
Number of transplant, *n*							
1st	8	7	11	8	12	0.106	46
2nd	2	3	0	2	0		7
3rd	0	1	0	0	0		1
Living donation, *n*	5	5	7	3	6	0.694	26
Interval between transplantation and diagnosis (months), median (interquartile range)	23.0	57.0	20.0	41.0	39.5	0.334	34.0 (IQR 6.8–84.3)
Tacrolimus maintenance therapy, *n*	10	6	10	8	11	0.058	45
Renal function at diagnosis							
eGFR (CKD-EPI; mL/min/1.73 m^2), median (interquartile range)	5.8	22.7	24.9	28.6	23.2	0.057	22.5 (IQR 10.5–31.9)
Proteinuria (mg/day), median (interquartile range)	1066.0	713.0	1270.0	847.0	721.0	0.922	900 (IQR 447–2655)
HLA antibodies at diagnosis							
DSAmax (MFI), median (interquartile range)	7924.5	7872.0	8365.0	4155.5	4504.0	0.476	5192 (IQR 2173–10 872)
DSAsum (MFI), median (interquartile range)	9055.0	8316.0	8365.0	5035.0	7283.5	0.859	8035 (IQR 2755–14 087)
Patients with DSA class I, *n*	4	7	5	4	3	0.485	23
Patients with DSA class II, *n*	8	9	9	7	10	0.961	43
Number of DSA, *n*	1.5	2.0	1.0	1.5	1.5	0.095	2.0 (IQR 1.0–2.0)
Banff parameters at baseline biopsy, median (interquartile range)							
Inflammation (i)	1.0	1.0	2.0	1.0	2.0	0.071	2.0 (IQR 1.0–2.0)
Tubulitis (t)	1.0	1.0	1.0	0.5	2.0	0.104	1.0 (IQR 0.0–2.0)
Intimal arteritis (v)	0.0	0.0	0.0	0.0	0.0	0.443	0.0 (IQR 0.0–1.0)
Glomerulitis (g)	0.5	1.0	2.0	2.0	2.0	0.786	2.0 (IQR 0.0–2.3)
Peritubular capillaritis (ptc)	1.0	0.0	2.0	2.0	2.0	0.060	2.0 (IQR 0.0–2.0)
C4d	1.0	1.0	0.0	0.0	0.0	0.520	1.0 (IQR 0.0–1.0)
Chronic glomerulopathy (cg)	1.5	3.0	0.0	1.5	0.0	0.172	1.0 (IQR 0.0–3.0)
Mesangial matrix increase (mm)	0.5	1.0	0.0	1.0	0.0	0.220	0.5 (IQR 0.0–2.0)
Arteriolar hyalinosis (ah)	0.5	2.0	2.0	2.0	1.5	0.230	2.0 (IQR 0.0–3.0)

Table 1. Continued.

	Group 1 (RLP) $n = 10$	Group 2 (BLP) $n = 11$	Group 3 (BHP) $n = 11$	Group 4 (RBHP) $n = 10$	Group 5 (CHP) $n = 12$	*P* value	Total $n = 54$
Vascular fibrous intimal thickening (cv)	1.0	2.0	2.0	2.0	1.0	0.024	2.0 (IQR 1.0–2.0)
Interstitial fibrosis (ci)	0.0	0.0	0.0	1.0	0.0	0.451	0.0 (IQR 0.0–1.0)
Tubular atrophy (ct)	0.0	0.0	0.0	1.0	0.0	0.451	0.0 (IQR 0.0–1.0)

BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); CKD-EPI, chronic kidney disease epidemiology collaboration[16]; DSA, donor-specific HLA antibodies; DSAmax, DSA showing the highest MFI; DSAsum, the MFI sum of all DSA; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MFI, mean fluorescence intensity; RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

from diagnosis to 24-month follow-up was investigated by generalized linear mixed models with time (included as random effect) and the interaction of time with treatment group as independent variables. Potential predictor variables were entered into the generalized linear mixed model to test their effect on the change in eGFR over the 24 months observation interval. Standardized regression coefficients (ranging from −1 to 1) were reported to compare the strength of association between the variables in the multivariable analysis. Variable selection in the multivariable analysis of 24 months graft survival and change in eGFR were based on a stepwise backward selection and bootstrapping. Fifty bootstrap samples were drawn, and in each sample, the backward variable selection was performed. Variables selected in at least 43 (>85%) samples were included in the final model. This approach was chosen to get a stable model in the presence of a small sample size and the limitations of the backward variable selection in the source sample. The incidence of adverse events was reported by the number of patients with at least one adverse event and the number of adverse events per patient-years. Poisson regression models were used to compare the incidence and incidence rates of adverse events between treatment groups. Differences in hemoglobin reduction, leukocyte counts, and platelets counts were tested by linear regression. The result of the global test was reported, pairwaise group comparisons were only performed if the global test reached the level of significance. All statistical analyses were conducted with STATA 12.1 (StataCorp LLC, College Station, Texas, USA).

Results

Patient characteristics

We analyzed a well-characterized population of 54 consecutive renal allograft recipients with a diagnosis of biopsy-proven ABMR established between 2005 and 2015 in order to identify predictors of graft survival at the time of diagnosis. All patients had *de novo* DSA and none of the patients had undergone desensitization pretransplant. In all patients, the indication for renal biopsy was clinically relevant allograft dysfunction, in other words, there was no case of "subclinical rejection". Follow-up time was at least 24 months in all patients. Stepwise modifications of the treatment protocol following groups of 10–12 patients resulted in five different treatment groups (Figure 1). Triple maintenance immunosuppression remained unchanged during

Table 2. Important adverse events during the first year after diagnosis

	Group 1 (RLP) $n = 10$	Group 2 (BLP) $n = 11$	Group 3 (BHP) $n = 11$	Group 4 (RBHP) $n = 10$	Group 5 (CHP) $n = 12$	*P* value
Hemoglobin reduction > 1 g/dL (baseline - nadir; patients)	8 (80%)	9 (81.8%)	10 (90.9%)	9 (90%)	8 (66.7%)	0.576
Hemoglobin reduction (baseline - nadir; g/dL)	1.7 (IQR 1.0–2.4)	2.1 (IQR 1.6–3.9)	3.1 (IQR 1.6–3.8)	3.3 (IQR 2.1–4.3)	2.4 (IQR 0.5–6.0)	0.785
Leukopenia (patients)	7 (70.0%)	6 (54.6%)	5 (45.5%)	6 (60.0%)	7 (58.3%)	0.654
Leukocytes at nadir (cells/nL)	3.0 (IQR 1.1–4.6)	3.5 (IQR 2.5–5.2)	4.1 (IQR 2.7–6.8)	3.5 (IQR 2.6–4.4)	3.3 (IQR 2.7–5.3)	0.538
Thrombocytopenia (patients)	7 (70.0%)	10 (90.9%)	7 (63.6%)	10 (100.0%)	6 (50.0%)	0.326
Platelets at nadir (cells/nl)	107 (IQR 101–222)	106 (IQR 96–137)	107 (IQR 82–156)	85 (IQR 70–118)	143.5 (IQR 109–188)	0.081
Infection (patients)	5 (50.0%)	7 (63.6%)	2 (18.2%)	8 (80.0%)	7 (58.3%)	0.350
Infection episodes (events/events per 100 patient-years*)	10/151.4	12/163.2	9/85.6	12/120.0	9/75.6	0.332
Nausea (patients)	0 (0.0%)	0 (0.0%)	2 (18.2%)	2 (20.0%)	3 (25.0%)	0.998
Vomiting (patients)	0 (0.0%)	0 (0.0%)	2 (18.2%)	1 (10.0%)	0 (0.0%)	0.991
Diarrhea (patients)	1 (10.0%)	2 (18.2%)	7 (63.6%)	5 (50.0%)	4 (33.3%)	0.531
Polyneuropathy (patients)	0 (0.0%)	0 (0.0%)	2 (18.2%)	3 (30.0%)	0 (0.0%)	0.993
Allergic reaction (patients)	1 (10.0%)	1 (9.1%)	1 (9.1%)	2 (20.0%)	5 (41.7%)	0.557
Hospitalization (patients)	6 (60.0%)	7 (63.6%)	5 (45.5%)	8 (80.0%)	7 (58.3%)	0.719
Hospitalization (events/events per 100 patient-years*)	9/122.4	10/95.1	19/190.0	8/67.2	7/58.3	0.075

*Patient-years: Group 1 (RLP): 6.6; Group 2 (BLP): 7.4; Group 3 (BHP): 10.5; Group 4 (RBHP): 10.0; and Group 5 (CHP): 11.9. BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), highdose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

Table 3. Predictors of graft loss at 24 months after diagnosis—univariate analysis

	HR	95% CI	*P* value
Demographic factors			
Donor age (per year)	1.00	0.98; 1.03	0.810
Recipient age (per year)	1.00	0.97; 1.02	0.743
Number of transplant (per transplant)	1.39	0.57; 3.37	0.473
Living donor (yes versus no)	0.39	0.15; 1.02	0.054
Interval between transplantation and diagnosis (per year)	1.05	0.97; 1.14	0.214
Tacrolimus maintenance therapy (yes versus no)	2.11	0.48; 9.10	0.317
Renal function at diagnosis			
eGFR (CKD-EPI; per ml/min/1.73 m^2)	0.97	0.94; 1.00	0.058
Proteinuria (per 500 mg/day)	1.12	0.99; 1.27	0.072
HLA antibodies at diagnosis			
DSAmax (per 3000 MFI)	1.01	0.84; 1.23	0.905
DSAsum (per 3000 MFI)	0.96	0.85; 1.09	0.573
DSAmax (>10 000 MFI)	0.93	0.37; 2.55	0.880
DSAsum (>10 000 MFI)	0.87	0.35; 2.19	0.769
DSA Class I (yes versus no)	1.30	0.53; 3.19	0.572
DSA Class II (yes versus no)	1.39	0.41; 4.74	0.601
Number of different DSA (per DSA)	0.99	0.63; 1.55	0.967
Banff parameters at baseline biopsy (per grade, 0–3)			
Inflammation (i)	0.81	0.51; 1.27	0.356
Tubulitis (t)	0.76	0.49; 1.18	0.221
Intimal arteritis (v)	1.00	0.59; 1.70	0.994
Glomerulitis (g)	1.04	0.72; 1.49	0.834
Peritubular capillaritis (ptc)	0.80	0.54; 1.20	0.284
C4d	1.03	0.43; 2.47	0.951
Chronic glomerulopathy (cg)	1.30	0.94; 1.82	0.116
Mesangial matrix increase (mm)	1.05	0.68; 1.63	0.810
Arteriolar hyalinosis (ah)	1.75	1.12; 2.74	0.015
Vascular fibrous intimal thickening (cv)	0.82	0.51; 1.32	0.413
Interstitial fibrosis (ci)	1.45	0.91; 2.31	0.122
Tubular atrophy (ct)	1.45	0.91; 2.31	0.122

CI, confidence interval; CKD-EPI, chronic kidney disease epidemiology collaboration[16]; DSA, donor-specific HLA antibodies; DSAmax, DSA showing the highest MFI; DSAsum, the MFI sum of all DSA; eGFR, estimated glomerular filtration rate; HR, hazard ratio; and MFI, mean fluorescence intensity.

the study period. The fact that not all patients received tacrolimus maintenance immunosuppression (45/54, 83.3%) was considered by inclusion of tacrolimus as independent variable in the analyses. Tacrolimus trough levels (7.0 ng/ml (IQR 6.2–9.2), $P = 0.095$) and daily mycophenolic acid doses (mycophenolate mofetil equivalent: 1498 mg (IQR 1196–1906), $P = 0.129$) were not different between groups. Relevant patient characteristics, renal function, and HLAab status at diagnosis as well as the scoring of the baseline renal biopsy are shown in Table 1. We found no significant differences between treatment groups except for the "cv score" (vascular fibrous intimal thickening) ($P = 0.024$), which was significantly higher in group 3 as compared to group 1 ($P = 0.002$) and group 5 ($P = 0.014$). During the 24-month follow-up period none of the patients died, none of the patients developed cancer except for two patients with nonmelanoma skin cancer in group 3 (BHP), and 20/54 patients (37%) returned to maintenance dialysis treatment. No significant differences between treatment groups were found concerning important adverse events such as blood count disorders, infection episodes, gastrointestinal and neurological side effects, as well as allergic reactions and hospitalizations (Table 2).

Predictors of graft loss

In the univariate analysis, only the "ah score" (arteriolohyalinosis) showed a significant influence on the occurrence of graft loss at 24 months after diagnosis (HR 1.75; 95% CI 1.12; 2.74; $P = 0.015$) (Table 3). The risk

Table 4. Influence of group affiliation on graft loss at 24 months after diagnosis—univariate analysis

	HR	95% CI	*P* value
Group 1 (RLP)		(reference)	
Group 2 (BLP)	0.56	0.18; 1.76	0.317
Group 3 (BHP)	0.15	0.03; 0.73	0.019
Group 4 (RBHP)	0.34	0.10; 1.19	0.091
Group 5 (CHP)	0.14	0.03; 0.66	0.013

BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); CI, confidence interval; HR, hazard ratio; RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); and RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

of graft loss was significantly lower in group 3 (BHP) (HR 0.15; 95% CI 0.03; 0.73; $P = 0.019$) and group 5 (CHP) (HR 0.14; 95% CI 0.03; 0.66; $P = 0.013$) as compared to group 1 (RLP) (Table 4). For subsequent multivariate Cox regression analysis, we excluded the variables "living donor" and "arteriolohyalinosis", because these variables showed a high correlation with "eGFR at diagnosis" ($r = 0.71$) and the "cg score" (chronic glomerulopathy) ($r = 0.81$), respectively, indicating intercollinearity, a phenomenon, which is known to interfere with multivariate analyses. No evidence of intercollinearity ($r < 0.5$) was found between other variables. In addition, we combined the "g score" (glomerulitis) and the "ptc score" (peritubular capillaritis) to a common "microvascular inflammation (mvi) score". We found that an increase of eGFR at diagnosis reduced the risk of graft loss (HR 0.94; 95% CI 0.89; 0.98; $P = 0.018$) (Table 5). In other words, with every mL of eGFR increase at diagnosis, the risk of graft loss at 24 months after diagnosis decreased by 6%. On the other hand, microvascular inflammation (HR 1.37; 95% CI 1.01; 1.88; $P = 0.048$) and chronic glomerulopathy (HR 1.57; 95% CI 1.01; 2.58; $P = 0.045$) both increased the risk of graft loss by 37% and 57%, respectively, with every grade of Banff score increase (Table 5). The interval between transplantation and diagnosis had no significant influence on graft loss (HR 1.11; 95% CI 0.99; 1.24; $P = 0.065$). The multivariable model resulted in a predictive power of 0.78 (c-statistic) and was well calibrated ($P = 0.895$). The proportional hazard assumption was not violated ($P = 0.428$). Further analysis showed that "eGFR at diagnosis" had the highest predictive power of these three parameters (c-statistics) followed by the "cg score" and the "mvi score" (Table 5). Concerning group affiliation, we found that the risk of graft loss was significantly lower in group 5 (CHP) as compared to group 1 (RLP) (HR 0.10; 95% CI 0.02; 0.54; $P = 0.008$) and group 2 (BLP) (HR 0.16; 95% CI 0.02; 0.99; $P = 0.049$) and in group 4 (RBHP) as compared to group 1 (RLP) (HR 0.21; 95% CI 0.05; 0.94; $P = 0.041$) (Table 6). Notably, graft survival improved stepwise along with the stepwise modifications of our treatment protocol (HR = 0.62, 95% CI 0.44; 0.87; $P = 0.006$). A significant (>10%) decrease of DSAmax (HR 0.62; 95% CI 0.25; 1.52; $P = 0.294$) or DSAsum (HR 0.49; 95% CI 0.20; 1.21; $P = 0.121$) following treatment was not associated with an improved graft survival at 24 months after diagnosis.

In order to underline the clinical impact of ABMR on graft survival, we performed a matched-pair analysis in which we compared graft survival including death with functioning graft of patients with a diagnosis of ABMR with patients with a diagnosis of TCMR and with patients with no rejection (Figure 2). Both control groups were matched with the ABMR group with regard to age, sex, time after transplantation, and mode of transplantation. Statistical analysis showed that graft survival was significantly better in patients without

Table 5. Predictors of graft loss at 24 months after diagnosis—multivariate analysis

	HR	95% CI	*P* value	c-statistics
eGFR at diagnosis (CKD-EPI; per ml/min/1.73 m^2)	0.94	0.89; 0.98	0.018	0.65
Chronic glomerulopathy (cg) (per grade, 0–3)	1.57	1.01; 2.58	0.045	0.61
Microvascular inflammation (mvi) (g+ptc, per grade, 0–6)	1.37	1.01; 1.88	0.048	0.53

CI, confidence interval; CKD-EPI, chronic kidney disease epidemiology collaboration[16]; eGFR, estimated glomerular filtration rate; g, glomerulitis; HR, hazard ratio; ptc, peritubular capillaritis. c-statistics = Harrel's C (range 0.5–1), measure for the predictive power of a risk factor for graft loss at 24 months.

Table 6. Influence of group affiliation on graft loss at 24 months after diagnosis—multivariate analysis

	Group 1 (RLP)			Group 2 (BLP)			Group 3 (BHP)			Group 4 (RBHP)		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Group 1 (RLP)		-										
Group 2 (BLP)	0.60	0.17; 2.11	0.425		-							
Group 3 (BHP)	0.26	0.04; 1.53	0.136	0.43	0.07; 2.75	0.390		-				
Group 4 (RBHP)	0.21	0.05; 0.94	0.041	0.36	0.07; 1.74	0.100	0.82	0.13; 5.09	0.832		-	
Group 5 (CHP)	0.10	0.02; 0.54	0.008	0.16	0.02; 0.99	0.049	0.38	0.04; 2.92	0.352	0.46	0.08; 2.71	0.392

BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); CI, confidence interval; HR, hazard ratio; RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); and RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

rejection ($P < 0.001$) and in patients with TCMR ($P = 0.010$) as compared to patients with ABMR.

Predictors of eGFR

When we used the "eGFR slope during 24 months after diagnosis" instead of "graft loss after 24 months" as dependent variable in the multivariate analysis, we observed similar results. As before, an increase of eGFR at diagnosis (beta 0.46; 95% CI 0.22; 0.69; $P < 0.001$) exerted a positive effect, while an increase of the "cg score" (beta −5.47; 95% CI −8.44; −2.51; $P < 0.001$) and the interval between transplantation and diagnosis (beta −1.79; 95% CI −2.73; −0.85; $P < 0.001$) exerted a negative effect on the eGFR slope. Concerning group affiliation the eGFR slope was significantly better in group 5 (CHP) as compared to group 1 (RLP) (beta 10.70; 95% CI 1.95; 19.45; $P = 0.017$) and group 2 (BLP) (beta 11.21; 95% CI 2.73; 19.69; $P = 0.010$) (Table 7). Again, we observed a stepwise improvement of the eGFR slope along with the stepwise modifications of our treatment protocol (beta = 4.64, 95% CI 3.68; 5.62; $P = 0.001$) (Figure 3).

Discussion

In our present study, we aimed to identify predictors of graft survival following diagnosis of ABMR. Therefore, we investigated a group of 54 consecutive, well-characterized patients with clinically relevant, biopsy-proven ABMR at our center. In agreement with Viglietti *et al.* [9], our results indicate that both eGFR at diagnosis and chronic glomerulopathy are significant predictors of renal allograft loss. In addition, microvascular inflammation and the applied treatment regimen also seem to influence graft loss. Because microvascular inflammation corresponds with later transition into chronic glomerulopathy [17,18], the observed effect of microvascular inflammation on graft loss seems to be plausible. Extending the results of Viglietti *et al.*, our results indicate that the predictive value of eGFR, chronic glomerulopathy, and microvascular inflammation is independent of the applied treatment regimen, and that treatment with cyclophosphamide plus high-dose IVIG may be superior compared to treatment with single-dose rituximab or one cycle of bortezomib plus low-dose IVIG regarding graft loss and eGFR slope. The question, whether higher doses of rituximab, bortezomib, or IVIG would have resulted in improved outcome cannot be answered based on our results, but should be addressed in future. Nevertheless, these results

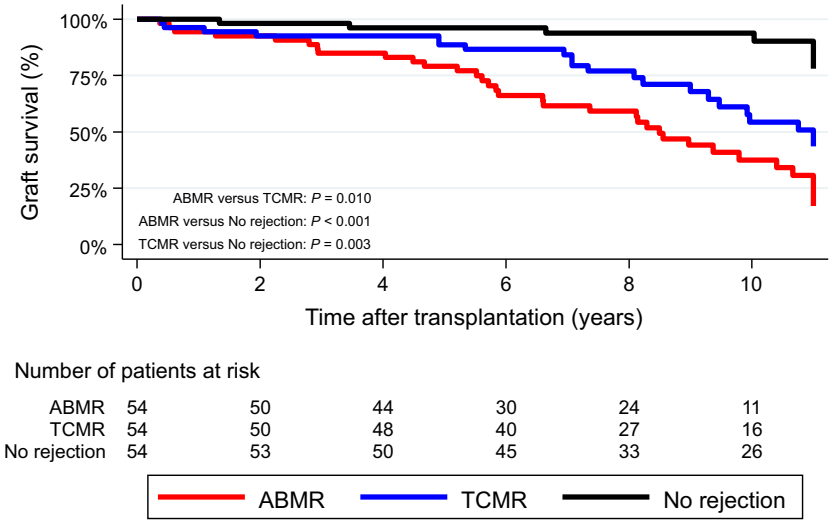

Figure 2 Graft survival starting at the time of transplantation. Matched-pair analysis comparing patients with antibody-mediated rejection (ABMR), T cell-mediated rejection (TCMR), and no rejection episode. Note, death with functioning graft was not censored. Kaplan Meier plot with log-rank test.

confirm that i.v. cyclophosphamide may be a valuable and cost-effective treatment option [13]. The fact that graft survival and eGFR slope improved stepwise along with the modifications of our treatment regimen is important as it retrospectively supports and justifies our therapeutic approach. The effect of the time interval between transplantation and diagnosis was just above the significance threshold in the analysis of graft loss, but clearly below the significance threshold in the analysis of the eGFR slope. Therefore, the time interval between transplantation and diagnosis may play a role as pointed out by Walsh *et al.* [19], although Viglietti *et al.* [9] did not observe such a correlation. We could not detect a significant effect of chronic vascular or interstitial changes on graft loss or eGFR slope. Although we are not able to prove this hypothesis, it is tempting to speculate that the prognostic relevance of chronic glomerular changes may be functionally more relevant and may thereby overrule the prognostic relevance of chronic vascular or interstitial changes in the setting of ABMR. Of note, all of our patients developed *de novo* DSA after transplantation whereas the cohort of Viglietti *et al.* [9] included more than one-third of patients with preformed DSA.

Against our anticipation, the level and dynamics of HLAab at the time of diagnosis and while treatment, respectively, did not predict allograft outcome, whereas renal function, morphological changes, and the applied treatment regimen seem to be more robust predictors. The detection of *de novo* DSA indicates emerging alloimmunization against the graft but the level of DSA in the periphery may not necessarily correlate with the amount of antibodies damaging the graft. In addition, the level of HLAab in the periphery may lag desensitization measures. We can only speculate about the potential significance of the complement-fixing ability of DSA over the MFI level alone in predicting outcome because we do not have the data available. However, based on current literature, the MFI level is intimately linked to and predicts the complement-fixing ability of DSA [20]. Thus, we would not assume that testing for C1q- or C3d-fixing ability would have contributed significantly to improve our prediction model.

Limitations: We are aware of the fact that this is a retrospective study on patients with a diagnosis of ABMR, who underwent different treatment protocols. However, the number of patients investigated ranges among the upper 20% of similar studies [7], the population is well-characterized, the data set is nearly complete, maintenance immunosuppression remained unchanged throughout the study period, and multivariate analyses provided significant results. We included patients with early and late ABMR as well as patients with acute active and chronic active ABMR. This heterogeneity may be regarded as a disadvantage. However, as it is not unequivocally clear at present, which parameters predict graft survival following diagnosis of ABMR, the heterogeneity of our cohort bears the opportunity to reveal potential predictors of graft survival that might have remained undetected otherwise. Finally, we cannot exclude some kind of unconscious era effect during a 10-year observation period, although the involved team of pathologists, immunologists, and nephrologists as well as standard procedures including the indication for renal biopsy and outpatient follow-up remained unchanged.

In summary, our finding that "eGFR at diagnosis" and the "cg score" proved to be significant predictors of

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Table 7. Influence of group affiliation on eGFR slope between months 0 and 24 after diagnosis—multivariate analysis

	Group 1			Group 2			Group 3			Group 4		
	Beta	95% CI	*P* value	Beta	95% CI	*P* value	Beta	95% CI	*P* value	Beta	95% CI	*P* value
Group 1 (RLP)		-			-			-			-	
Group 2 (BLP)	−0.51	−9.38; 8.36	0.911									
Group 3 (BHP)	5.81	−3.80; 15.42	0.236	6.32	−3.45; 16.08	0.205						
Group 4 (RBHP)	6.13	−3.28; 15.54	0.202	6.64	−2.66; 15.94	0.162	0.32	−8.62; 9.25	0.944			
Group 5 (CHP)	10.70	1.95; 19.45	0.017	11.21	2.73; 19.69	0.010	4.89	−3.69; 13.47	0.264	4.57	−4.15; 13.29	0.304

BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); CI, confidence interval; RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); and RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

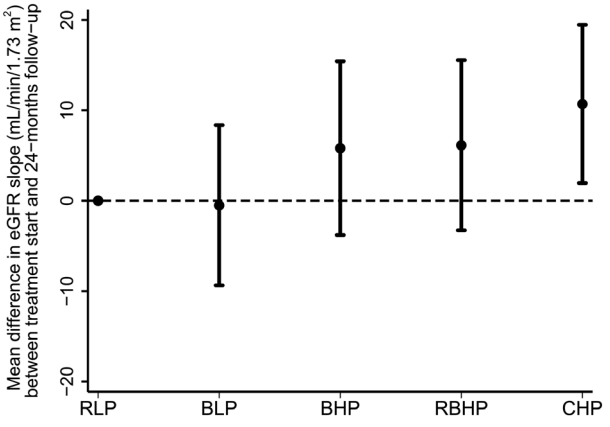

Figure 3 Change in eGFR [16] between treatment start and 24-months follow-up (mean and 95% CI). Abbreviations: BHP, bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); BLP, bortezomib (4× 1.3 mg/m^2 i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×); CHP, cyclophosphamide (6× 15 mg/kg i.v. adapted to age and renal function), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); RBHP, rituximab (500 mg i.v.), bortezomib (4× 1.3 mg/m^2 i.v.), high-dose IVIG (1.5 g/kg i.v.), and plasmapheresis (6×); and RLP, rituximab (500 mg i.v.), low-dose IVIG (30 g i.v.), and plasmapheresis (6×).

both, graft loss and eGFR slope, confirms and extends the existing evidence inasmuch as both predictors seem to be independent of the applied treatment regimen. Therefore, our results may help to further delineate predictors of graft survival and graft function at the time of diagnosis of ABMR, that is, when the decision for treatment is made. Our results also indicate that our approach to stepwise adapt and modify the treatment protocol including the administration of cyclophosphamide was beneficial. In future, randomized controlled trials taking into account important predictors of graft survival are needed, in order to define and improve common therapeutic standards of care.

Authorship

JW and MD: involved in conception, design, analysis and interpretation of the data, drafting and revising of the article, final approval of the version to be published. JK: involved in the analysis and interpretation of the data, drafting and revising of the article, final approval of the version to be published. NL, KW, BR, FH, LL and FB: involved in the analysis and interpretation of the data, revising of the article, final approval of the version to be published. KB: involved in conception, analysis and interpretation of the data, revising of the article and final approval of the version to be published.

Funding

The authors received no funding for this work.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

[Correction added on 3 November 2020, after first online publication: Projekt Deal funding statement has been added.]

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