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Change in Estimated GFR and Risk of Allograft Failure in Patients Diagnosed With Late Active Antibody-mediated Rejection Following Kidney Transplantation

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Background. There are challenges in designing adequate, well-controlled studies of patients with active antibody-mediated rejection (AMR) after kidney transplantation (KTx). **Methods.** We assessed the functional relationship between change in estimated glomerular filtration rate (eGFR) following the diagnosis of AMR and the risk of subsequent death-censored graft failure using the joint modeling framework. We included recipients of solitary KTx between 1995 and 2013 at 4 transplant centers diagnosed with biopsy-proven active AMR at least 1 year post-KTx, who had a minimum of 3-year follow-up. **Results.** A total of 91 patients across participating centers were included in the analysis. Of the 91 patients, n = 54 patients (59%) met the death-censored graft failure endpoint and n = 62 patients (68%) met the all-cause graft failure composite endpoint. Kaplan-Meier death-censored graft survival rates at 12, 36, and 60 months postdiagnosis of AMR pooled across centers were 88.9%, 58.9%, and 36.4%, respectively. Spaghetti plots indicated a linear trend in the change in eGFR, especially in the first 12 months postdiagnosis of active AMR. A significant change in eGFR was observed within the first 12 months postdiagnosis of active AMR, getting worse by a factor of –0.757 mL/min/1.73 m^2 per month during the 12-month analysis period (a delta of –9.084 mL/min/1.73 m^2 at 1 y). Notably, an extrapolated 30% improvement in the slope of eGFR in the first 12 months was associated with a 10% improvement in death-censored graft failure at 5 years. **Conclusions.** If prospectively validated, this study may inform the design of pivotal clinical trials for therapies for late AMR.

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INTRODUCTION

Kidney transplantation (KTx) is the preferred therapeutic option for patients with end-stage renal disease (ESRD). Despite improvements in short-term outcomes posttransplant, more significant improvements in long-term graft outcomes are required.[1] After an intermediate duration of good allograft function, recipients, and their allograft continue to suffer from a multitude of problems, including immunologic complications.[1] Several studies have shown antibody-mediated rejection (AMR) to be an important cause of graft dysfunction and late graft loss.[2,3]

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AMR can occur at any time after KTx and may be classified into 1 of 2 types, depending on the histopathologic findings: active or chronic active AMR.[4] Despite this classification, active and chronic active AMR are part of a continuum of injury which, left untreated, results in the loss of the transplanted allograft.[4,5] The continuum of injury produced by active AMR is triggered by inflammation following interaction between recipient donor-specific antibodies (DSAs) and donor HLA or non-HLA peptides expressed on the surface of the transplant vascular endothelium.[4-6] Regardless of how the process is initiated, this ongoing injury eventually manifests as transplant glomerulopathy and loss of functional renal mass that leads to a decline in renal function.[4-6]

The purpose of this study was to evaluate the relationship between change in renal function, as measured by estimated glomerular filtration rate (eGFR) and risk of allograft failure in patients diagnosed with active or chronic active AMR following KTx. Results can be used to quantify a clinically meaningful change in eGFR, as it relates to a clinically meaningful change in allograft survival, which can be used to inform the design of a phase 3 clinical trial.

MATERIALS AND METHODS

Study Design and Objective

This is a historical cohort study. The objective was to evaluate the relationship between change in eGFR following the diagnosis of active AMR and subsequent risk of allograft failure using data pooled across different data sources from the University of Wisconsin Madison;[7] the University of Manitoba, Winnipeg, Manitoba;[8] and the University Hospital Vall d'Hebron and the Hospital del Mar, Barcelona.[9]

Study Population

Recipients of living or deceased donor, solitary renal transplant (index) between January 1995 and August 31, 2013, who were diagnosed with biopsy-proven (initial) active AMR at least 1 year posttransplant and had a minimum of 3-year follow-up were eligible (unless patient lost allograft or died). Patients had to be aged between 15 and 75 years, inclusive, at the time of diagnosis of active AMR with eGFR ≥25 mL/min/1.73 m^2 and positive anti-HLA DSA (class I or class II positive). The eGFR threshold of 25 mL/min/1.73 m^2 was selected in consideration of clinical trials for the treatment of AMR, which would likely exclude cases with low kidney function. In addition, patients had to have at least 1 serum creatinine at or just before the diagnosis of active AMR and at least 2 measures of serum creatinine postdiagnosis of active AMR. Recipients of multiple organs and ABO-incompatible organs, as well as those patients lost to follow-up within the first 3 years postdiagnosis of AMR and whose allograft status was unknown, were excluded. The initial diagnosis of active AMR was confirmed by biopsy-proven criteria according to the Banff 2015 criteria.[10] The initial screening for study entry was based on surveillance (Manitoba) or indication (proteinuria, rise in Scr, de novo DSA) biopsies (Wisconsin, Hospital Vall d'Hebron, Hospital del Mar). All biopsies were reviewed locally, according to the recent Banff criteria. Antibody-detection methods were also determined locally, using single-antigen bead assays at each center (Manitoba, WI) or a central lab (Hospital Vall d'Hebron, Hospital del Mar). The study was approved by the ethics boards of all 4 institutions.

Study End Points

The primary outcome variable was time to death-censored graft failure, defined as graft loss (ie, the need for permanent dialysis, allograft nephrectomy, or retransplantation) or eGFR <15 mL/min/1.73 m^2. The eGFR component of the primary outcome variable consisted of the first occurrence of 2 follow-up eGFR values of <15 mL/min/1.73 m^2 occurring a minimum of 3 days (72 h) apart. Time to death-censored graft failure was right-censored if the patient was alive with a functioning graft at the date of the last known follow-up or end of the study period (August 31, 2017) or if the patient died with a functioning graft, and the eGFR component of the primary outcome variable definition was not met (ie, confirmed eGFR <15 mL/min/1.73 m^2). The secondary outcome variable was time to all-cause graft failure, defined as graft loss (ie, the need for permanent dialysis, allograft nephrectomy, retransplantation) or eGFR <15 mL/min/1.73 m^2 or patient death occurring at any time over the course of patient follow-up postdiagnosis of active AMR. The eGFR component of the secondary outcome variable consisted of the first occurrence of 2 follow-up eGFR values of <15 mL/min/1.73 m^2 occurring a minimum of 3 days (72 h) apart. Time to all-cause graft failure was right-censored if the patient was alive with a functioning graft at the date of last known follow-up or end of the study period (August 31, 2017), and the eGFR component of the secondary outcome variable definition (ie, eGFR <15 mL/min/1.73 m^2) was not met.

Follow-up

Patients were followed from the date of diagnosis of active AMR until the earliest of the following: graft failure (permanent dialysis, retransplantation, or nephrectomy); death; lost to follow-up; or end of the study period (August 31, 2017).

Estimated Glomerular Filtration Rate

Although several estimated measures of renal function have been developed, the modification of diet for renal disease 4-variable equation has been shown to better predict renal function, especially at low levels of creatinine clearance.[11] For pediatric patients (15 ≤ age < 18 y), the Schwartz equation[12] was used.

Prognostic Factors

Prognostic factors evaluated for potential inclusion in risk-adjusted multivariable regression models were based on factors known to be associated with a change in renal function and graft failure composite end points. These included:

- Factors measured at the time of KTx: type of donor (deceased versus living donor), donor age, recipient gender, race/ethnicity, delayed graft function, primary cause of ESRD, anti-HLA DSA positive, and type of antibody induction immunosuppression (thymoglobulin, alemtuzumab, basiliximab, daclizumab, antilymphocyte globulin, or other or none)

- Factors measured at the time of diagnosis of active AMR: age of the recipient (in y); length of time from KTx to the diagnosis of active AMR (in mo); C4d positive stain (yes versus no); anti-HLA DSA positive (class I only, class II only, or both class I and class II positive); baseline eGFR (at time of diagnosis of active AMR); medication nonadherence, as noted by a physician; recurrent disease posttransplant before the diagnosis of active AMR; and pathological features of acute inflammation (ptc>0 and g>0) and chronic injury (cg>0); type of treatment for active AMR (plasmapheresis, intravenous immunoglobulins, rituximab, bortezomib, other, or none); and type of maintenance immunosuppression used at time of diagnosis of active AMR (tacrolimus, cyclosporine, mycophenolate, azathioprine, sirolimus, everolimus, belatacept, and corticosteroids)

Data Sources

Medical records and databases at participating centers were reviewed by their research staff to identify eligible patients and obtain data required for the analyses. Deidentified data were incorporated into a standardized database, which was provided to a third party for data review, query generation, and analysis. All information recorded in the database was to be consistent with the Investigator's source documentation for the study patients. There was no direct access to source data or documents. A standardized data collection format was instituted to minimize potential bias from the imbalance of potential confounding variables, incomplete data, inconsistent definition of active AMR, and variable indications for biopsy.

Statistical Methods

Continuous variables were summarized overall (ie, pooled) and by center by presenting the number of nonmissing observations, mean, SD, median, 25th and 75th percentiles, and minimum and maximum. Categorical variables were summarized overall and by center by presenting the number of patients and percentage for each category. Median follow-up was estimated using the reverse Kaplan-Meier method. Missing demographic and baseline data were treated as missing; no method for imputation was utilized.

Because follow-up testing did not necessarily occur at the same time for all patients, times were defined using assessment windows (Appendix II, SDC, http://links.lww.com/TP/B930). These study visits were defined to reflect routine practices at participating centers for assessing renal function following the diagnosis of active AMR.

Observed eGFR values were graphically displayed over time using locally weighted scatterplot smoothing (LOESS) curves with 95% confidence limits. The LOESS is a nonparametric regression method that uses local weighted regression to fit a smooth curve through points in a scatterplot.[13] The main advantage of this method is that it makes very little assumptions about the form of the relationship between the biomarker and time. Plots were used as a general guideline to assess the functional relationship of eGFR over time for modeling purposes.

The relationship between change in eGFR and risk of graft failure was assessed using the joint modeling framework.[14,15] Details of the joint model are provided in Appendix I, SDC, http://links.lww.com/TP/B930. Briefly, the joint model allows the simultaneous modeling of a longitudinal (repeatedly measured over time) outcome such as eGFR (referred to as the longitudinal submodel) and a time-to-event outcome such as time to death-censored graft failure (referred to as the failure time submodel). The longitudinal submodel can be used to estimate an individual's eGFR profile as well as predict their slope (ie, how their eGFR changes over time). This dynamic process is then linked to the failure time submodel to ascertain whether an individual's "true" eGFR trajectory is associated with risk of death-censored graft failure. The main advantage of the joint model over traditional approaches (eg, separate analyses of each outcome or time-varying Cox's hazard model) is the correct treatment of time-varying variables that are subject to measurement error, such as eGFR. Modeling the longitudinal measures accounts for this error, which enables unbiased estimation of the relationship between the biomarker and time to event outcome. Residual diagnostics were used to assess model assumptions such as normality and misspecification of the mean response structure (linear versus nonlinear).

Joint models were used to provide conditional survival beyond 1 year following diagnosis of active AMR for a set of hypothetical eGFR dynamic characteristics. Predictions were used to determine the change in eGFR during the 1-year time horizon associated with a clinically meaningful difference in graft survival beyond 1 year. The 1-year time frame was chosen for the eGFR profile, because this is the length of follow-up that is typically used for pivotal trials. The set of hypothetical individual scenarios was based on the distribution of the individual slopes estimated from the longitudinal eGFR model. For each individual profile, the conditional survival beyond 1 year was estimated. Survival rates for individuals with a stable eGFR profile were provided as the basis for quantifying a clinically meaningful difference in death-censored and overall graft survival because of the progressive decrease in eGFR from baseline at 1 year.

Analysis Sets

The relationship between change in eGFR and risks of death-censored graft failure and all-cause graft failure was evaluated using 2 a priori defined analysis sets: (1) Primary analysis set: it is defined as the set of all eGFR values observed across the entire course of a patient's follow-up, and (2) supplementary analysis set: it is defined as the set of eGFR values restricted to the first 12 months postdiagnosis of active AMR. The joint model, therefore, ignores eGFR assessments beyond 1 year postdiagnosis of active AMR when evaluating the effect of change in eGFR and risks of death-censored and all-cause graft failure.

Analyses were performed using SAS for Windows statistical software (SAS, Cary, NC; version 9.4), except where other software was deemed more appropriate (eg, R). Joint modeling was performed in R using the JM package (for the joint modeling of longitudinal and time-to-event data). $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics

A total of n = 183 patients were identified across 4 participating centers for potential inclusion in the analysis: 14 (8%) from Barcelona (Universitat Autonoma de Barcelona, n = 8 and Hospital del Mar, Barcelona, n = 6), 47 (26%) from Manitoba, and 122 (67%) from Wisconsin. Of these,

TABLE 1.

Summary of patient disposition and availability of serum creatinine measurements

Variable	Barcelona cohort N	Manitoba cohort N	Wisconsin cohort N	Pooled N
No. of patients provided for potential inclusion in the primary analysis	14	47	122	183
No. of patients included in the primary analysis	9	27	55	91
No. of patients excluded from the primary analysis	5	20	67	92
Reason for exclusion (any, multiple): age <15 y at diagnosis of active AMR				
Diagnosis of active AMR <1 y after transplant	0	4	0	4
<3 y of follow-up postdiagnosis of active AMR, unless patient lost graft or died	2	2	7	11
Fewer than required number of SCr measurements	0	2	12	14
<3 visit windows with SCr measurements,	4	5	9	18
1 at diagnosis and 2 postdiagnosis of active AMR	4	7	23	34
eGFR <25 mL/min/1.73 m^2 at time of diagnosis of active AMR	0	11	48	59
No. of serial measures for SCr per subject:				
Mean (SD)	9.6 (4.4)	19.2 (10.7)	12.2 (5.8)	14.0 (8.2)
Median (25th–75th percentiles)	8 (6–11)	18 (11–26)	11 (7–16)	12 (8–18)
Min, max	5, 18	4, 46	3, 28	3, 46

AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate; SCr, serum creatinine.

a total of 91 patients were included in the primary analysis: 9 (10%) from Barcelona, 27 (30%) from Manitoba, and 55 (60%) from Wisconsin. Reasons for excluding patients from the primary analysis are provided in Table 1. The most common reason for exclusion was having an eGFR <25 mL/min/1.73 m^2 at the time of diagnosis of active AMR. The median number of serial serum creatinine measurements per patient up to end of follow-up across centers was 12, ranging from 8 in Barcelona to 18 in Manitoba. During each specified time window through 1 year following the diagnosis of AMR, at least 61 (67%) subjects had serum creatinine measurements. The median number of serial serum creatinine measurements per patient during the first-year post diagnosis of active AMR across centers was 6. At 1 year following AMR diagnosis, 75 (82%) subjects had serum creatinine measurements for analysis.

Prognostic Risk Factors Measured at Time of Transplantation

Summary of risk factors measured at time of index KTx is provided in Table 2. The distribution of risk factors was similar across the centers. Overall, the mean age of the recipient at the time of transplantation (SD) was 39.4 (15.1) years, 27.5% were female, and 86.8% were white. The most common primary cause of ESRD was "glomerulopathy" (37.4%). Over half of the patients received a kidney allograft from a deceased donor (54.9%). Induction therapy included alemtuzumab (29.7%), basiliximab (27.5%), or thymoglobulin (20.9%) alone or in combination. No induction therapy was used in 24.2% of patients. Only 11.0% of patients experienced delayed graft function. Anti-HLA DSA class I or class status was unknown, because it was untested in most of the patients with the exception of Manitoba; all patients (n = 27 or 100%) were negative.

Prognostic Risk Factors Measured at Time of Diagnosis of AMR

Summary of risk factors measured at the time of diagnosis of AMR is provided in Table 3. The mean (SD) age of the patients at the time of diagnosis of AMR was 45.6 (15.4) years. Distribution of risk factors was similar across the centers except for the timing of the diagnosis of AMR posttransplant and the observed eGFR at baseline (d 0). Median time from transplant to the initial diagnosis of AMR was higher in the Barcelona cohort (89 mo) compared to the Manitoba and Wisconsin cohorts (61 and 68 mo, respectively), whereas the mean eGFR at the time of diagnosis was higher in the Manitoba cohort (56.8 mL/min/1.73 m^2) compared to the Barcelona and Wisconsin cohorts (44.7 and 39.93 mL/min/1.73 m^2, respectively). Overall, DSA status was class I only, class II only, or both class I and class II in 22.0%, 54.9%, and 23.1% of patients, respectively. None of the patients had evidence of recurrent disease.

Histological scores at the time of AMR diagnosis are summarized in Table 4 and are generally similar across all cohorts. Most of the patients (77%) had evidence of chronic tissue injury, that is, cg scores > 0. However, whereas all patients in the Wisconsin and Barcelona cohorts had the evidence of chronic tissue injury, only 22% of patients in the Manitoba cohort had chronic tissue injury. Overall, evidence of acute inflammation (ptc > 0 and/or g > 0) was found in 90.1% of the patients. The most commonly used agents for maintenance immunosuppression were corticosteroids (95.6%), mycophenolate (93.4%), and tacrolimus (60.4%). The most commonly used treatments for AMR were intravenous immunoglobulins (60.4%), methylprednisolone (Solumedrol, 51.6%), and rituximab (25.3%). Plasmapheresis was used sparingly (6.6%). Over one-third of the patients (37.4%) received no treatment of AMR.

Survival Outcomes

The unadjusted Kaplan-Meier death-censored and overall graft survival curves are displayed in Figure 1. Median (25th–75th) follow-up was 61.7 (47.7–91.8) months. Of the 91 patients, n = 54 patients (59%) met the death-censored graft failure composite endpoint. Two-thirds of these patients experienced a decline in eGFR to <15 mL/min/1.73 m^2, whereas the remaining subjects met the criteria for graft loss (need for permanent dialysis, allograft nephrectomy, or retransplantation). The median time to reach the composite endpoint of death-censored graft failure pooled across centers was 46.2 months. Of the 91 patients, n = 62 patients (68%) met the all-cause graft failure composite

TABLE 2.

Summary of prognostic risk factors measured at the time of index kidney transplantation

Variable	Barcelona cohort (N = 9)	Manitoba cohort (N = 27)	Wisconsin cohort (N = 55)	Pooled (N = 91)
Recipient age, y				
Mean (SD)	35.4 (17.4)	34.9 (17.9)	42.2 (12.6)	39.4 (15.1)
Median (25th–75th percentiles)	38 (22, 46)	37 (16, 48)	40 (31, 54)	40 (27, 51)
Recipient gender, n (%)				
Female	5 (55.6%)	5 (18.5%)	15 (27.3%)	25 (27.5%)
Male	4 (44.4%)	22 (81.5%)	40 (72.7%)	66 (72.5%)
Recipient race/ethnicity, n (%)				
White	9 (100.0%)	21 (77.8%)	49 (89.1%)	79 (86.8%)
Black	0 (0.0%)	1 (3.7%)	4 (7.3%)	5 (5.5%)
Asian	0 (0.0%)	0 (0.0%)	2 (3.6%)	2 (2.2%)
Other	0 (0.0%)	5 (18.5%)	0 (0.0%)	5 (5.5%)
Type of donor, n (%)				
Deceased	9 (100.0%)	12 (44.4%)	29 (52.7%)	50 (54.9%)
Living	0 (0.0%)	15 (55.6%)	26 (47.3%)	41 (45.1%)
Donor age, y				
Mean (SD)	26.7 (15.1)	32.9 (13.0)	37.6 (13.8)	35.1 (14.0)
Median (25th–75th percentiles)	26 (17, 28)	30 (27, 42)	41 (30, 48)	36 (27, 46)
DGF, n (%)				
No	6 (66.7%)	27 (100.0%)	48 (87.3%)	81 (89.0%)
Yes	3 (33.3%)	0 (0.0%)	7 (12.7%)	10 (11.0%)
Primary cause of ESRD, n (%)				
Glomerulopathy	2 (22.2%)	15 (55.6%)	17 (30.9%)	34 (37.4%)
Vascular nephropathy	2 (22.2%)	3 (11.1%)	0 (0.0%)	5 (5.5%)
Diabetes	0 (0.0%)	2 (7.4%)	8 (14.5%)	10 (11.0%)
Polycystic kidney disease	0 (0.0%)	2 (7.4%)	6 (10.9%)	8 (8.8%)
Hypertension	1 (11.1%)	2 (7.4%)	3 (5.5%)	6 (6.6%)
Other	4 (44.4%)	3 (11.1%)	21 (38.2%)	28 (30.8%)
Anti-HLA DSA (class I or class II), n (%)				
Negative	0 (0.0%)	27 (100.0%)	0 (0.0%)	27 (29.7%)
Positive	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Unknown	9 (100.0%)	0 (0.0%)	55 (100.0%)	64 (70.3%)

DGF, delayed graft function; DSA, donor-specific antibody; ESRD, end-stage renal disease.

TABLE 3.

Summary of prognostic risk factors measured at time of diagnosis of active AMR

Variable	Barcelona cohort (N = 9)	Manitoba cohort (N = 27)	Wisconsin cohort (N = 55)	Pooled (N = 91)
Age, y				
Mean (SD)	41.8 (17.2)	40.4 (19.7)	48.7 (11.9)	45.6 (15.4)
Median (25th–75th percentiles)	46 (27, 50)	39 (21, 60)	48 (40, 57)	47 (34, 58)
Timing of initial diagnosis of active AMR posttransplantation, mo				
Mean (SD)	76.3 (34.8)	66.6 (41.0)	77.6 (46.5)	74.2 (43.8)
Median (25th–75th)	89 (45, 107)	61 (34, 91)	68 (41, 105)	63 (37, 104)
DSA class category, n (%)				
Class I only	1 (11.1%)	6 (22.2%)	13 (23.6%)	20 (22.0%)
Class II only	7 (77.8%)	16 (59.3%)	27 (49.1%)	50 (54.9%)
Class I and class II	1 (11.1%)	5 (18.5%)	15 (27.3%)	21 (23.1%)
C4d positive stain, n (%)				
No	6 (66.7%)	10 (37.0%)	1 (1.8%)	17 (18.7%)
Yes	3 (33.3%)	14 (51.9%)	54 (98.2%)	71 (78.0%)
Unknown	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Technique used to determine C4d positive stain, n (%)				
Light microscopic	9 (100.0%)	0 (0.00%)	55 (100.0%)	64 (70.3%)
Immunofluorescence	0 (0.00%)	27 (100.0%)	0 (0.00%)	27 (29.7%)
eGFR (observed) at AMR diagnosis, mL/min/1.73 m^2				
Mean (SD)	44.7 (15.4)	56.8 (18.2)	39.9 (13.6)	45.4 (16.9)
Median (25th–75th)	39.1 (37.2–53.1)	54.0 (42.9–64.6)	36.8 (29.2–46.4)	41.8 (31.8–53.0)

AMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate.

TABLE 4.

Histological scores at time of diagnosis of initial active AMR

Variable	Barcelona cohort (N = 9) n (%)	Manitoba cohort (N = 27) n (%)	Wisconsin cohort (N = 55) n (%)	Pooled (N = 91) n (%)
Glomerulitis, g score				
0	1 (11.1%)	11 (40.7%)	17 (30.9%)	29 (31.0%)
1	2 (22.2%)	9 (33.3%)	21 (38.2%)	32 (35.2%)
2	3 (33.3%)	4 (14.8%)	13 (23.6%)	20 (22.0%)
3	3 (33.3%)	0 (0.00%)	4 (7.3%)	7 (7.7%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Peritubular capillaritis, ptc score				
0	2 (22.2%)	3 (11.1%)	13 (23.6%)	18 (19.8%)
1	3 (33.3%)	0 (0.00%)	24 (43.6%)	27 (29.7%)
2	3 (33.3%)	17 (63.0%)	17 (30.9%)	37 (40.7%)
3	1 (11.1%)	4 (14.8%)	1 (1.8%)	6 (6.6%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Tubulitis, t score				
0	9 (100.0%)	9 (33.3%)	45 (81.8%)	63 (69.2%)
1	0 (0.00%)	13 (48.1%)	9 (16.4%)	22 (24.2%)
2	0 (0.00%)	0 (0.00%)	1 (1.8%)	1 (1.1%)
3	0 (0.00%)	2 (7.4%)	0 (0.00%)	2 (2.2%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Intimal or transmural arteritis, v score				
0	7 (77.8%)	23 (85.2%)	49 (89.1%)	79 (86.8%)
1	0 (0.00%)	0 (0.00%)	5 (9.1%)	5 (5.5%)
2	0 (0.00%)	0 (0.00%)	1 (1.8%)	1 (1.1%)
3	0 (0.00%)	1 (3.7%)	0 (0.00%)	1 (1.1%)
Missing	2 (22.2%)	3 (11.1%)	0 (0.00%)	5 (5.5%)
Inflammation, i score				
0	6 (66.7%)	5 (18.5%)	37 (67.3%)	48 (52.7%)
1	3 (33.3%)	10 (37.0%)	13 (23.6%)	26 (28.6%)
2	0 (0.00%)	5 (18.5%)	3 (5.5%)	8 (8.8%)
3	0 (0.00%)	4 (14.8%)	2 (3.6%)	6 (6.6%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Double contour, cg score				
0	0 (0.0%)	18 (66.7%)	0 (0.00%)	18 (19.8%)
1	2 (22.2%)	5 (18.5%)	14 (25.5%)	21 (23.1%)
2	3 (33.3%)	0 (0.00%)	13 (23.6%)	16 (17.6%)
3	4 (44.4%)	1 (3.7%)	28 (50.9%)	33 (36.3%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Interstitial fibrosis, ci score				
0	1 (11.1%)	5 (18.5%)	1 (1.8%)	7 (7.7%)
1	4 (44.4%)	10 (37.0%)	34 (61.8%)	48 (52.7%)
2	3 (33.3%)	7 (25.9%)	16 (29.1%)	26 (28.6%)
3	1 (11.1%)	2 (7.4%)	4 (7.3%)	7 (7.7%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Tubular atrophy, ct score				
0	1 (11.1%)	1 (3.7%)	4 (7.3%)	6 (6.6%)
1	6 (66.7%)	12 (44.4%)	33 (60.0%)	51 (56.0%)
2	2 (22.2%)	8 (29.6%)	14 (25.5%)	24 (26.4%)
3	0 (0.00%)	3 (11.1%)	4 (7.3%)	7 (7.7%)
Missing	0 (0.00%)	3 (11.1%)	0 (0.00%)	3 (3.3%)
Fibrous intimal thickening, cv score				
0	4 (44.4%)	10 (37.0%)	10 (18.2%)	24 (26.4%)
1	1 (11.1%)	11 (40.7%)	31 (56.4%)	43 (47.3%)
2	2 (22.2%)	3 (11.1%)	7 (12.7%)	12 (13.2%)
3	0 (0.00%)	0 (0.00%)	6 (10.9%)	6 (6.6%)
Missing	2 (22.2%)	3 (11.1%)	1 (1.8%)	6 (6.6%)

AMR, antibody-mediated rejection.

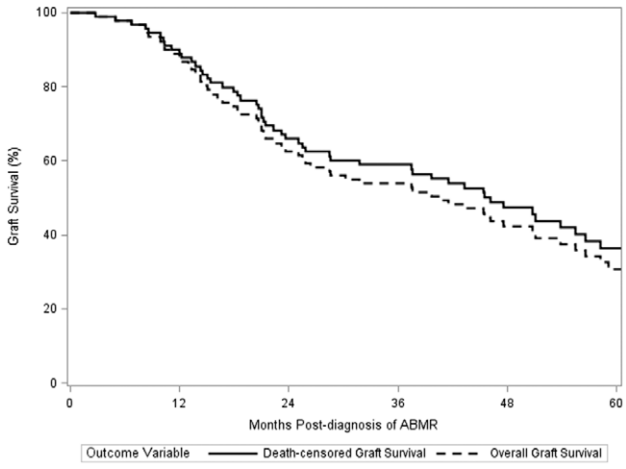

FIGURE 1. Kaplan-Meier (unadjusted) death-censored and overall graft survival. This is a plot of the unadjusted Kaplan-Meier death-censored (solid line) and overall graft survival (dashed line) mo postdiagnosis of AMR. AMR, antibody-mediated rejection.

endpoint. Death-censored graft survival and overall graft survival at 12, 36, and 60 months postdiagnosis of AMR pooled across centers were 88.9%, 58.9%, and 36.4%, and 87.9%, 53.8%, and 30.7%, respectively (Figure 1).

Association Between eGFR Slope and Graft Failure

To determine whether time-dependent changes in eGFR could predict outcomes, we first analyzed the scatterplots of pooled longitudinal eGFR data with LOESS smooth curves for death-censored graft failure endpoint (Figure 2A) and all-cause graft failure composite endpoint (Figure 2B). Close inspection of the plots suggested a potential linear trend in the change in eGFR over time, especially in patients whose graft failed. This linear relationship appeared to be more pronounced in the first 12 months postdiagnosis of active AMR.

Joint Modeling of the Primary Analysis Set

We next used joint modeling studies to better define the relationship between change in eGFR and risks of death-censored graft failure and all-cause graft failure, using 2 a priori defined analysis sets: (1) Primary analysis set is defined as the set of all eGFR values observed across the entire course of a patient's follow-up, and (2) Supplementary analysis set is defined as the set of eGFR values restricted to the first 12 months postdiagnosis of active AMR. Joint modeling of the primary analysis set yielded interesting findings. Residual plots (not displayed) suggested a systematic piece-wise linear trend in the change in eGFR postdiagnosis of active AMR. A scatterplot of the individual predicted slopes based on the longitudinal submodel by follow-up, according to whether the patient met the death-censored graft failure endpoint, is provided in Figure 3. Slopes with the largest negative values (steeper decline in eGFR) are notable in the first 12 months postdiagnosis of active AMR. Similar results were obtained with the joint model fit of longitudinal eGFR and time to all-cause graft failure. For this reason, results described below and survival prediction are based on the joint modeling of the supplementary analysis set (ie, the set of eGFR values observed within the first 12 mo postdiagnosis of active AMR, ignoring eGFR values after 12 mo).

Joint Modeling of the Supplementary Analysis Set

Results of the fit of the joint longitudinal submodel for predicting the risk of death-censored graft failure composite endpoint using the supplementary analysis set are provided in Table 5. Results of the fit of the joint longitudinal submodel for predicting the risk of all-cause graft failure composite endpoint are provided in supplemental Table S1A, SDC, http://links.lww.com/TP/B930. Three of the evaluated factors were significantly associated with baseline eGFR (d 0). Patients in the Manitoba cohort had a higher baseline eGFR compared to patients in the Wisconsin cohort, whereas no difference in baseline eGFR was observed in patients in the Barcelona cohort. Patients with class II, only anti-HLA DSA at the time of diagnosis of active AMR, had higher baseline eGFR versus patients with both class I and class II anti-HLA DSA at the time of diagnosis of active AMR. Increasing donor age was associated with lower baseline eGFR (ie, decrease in eGFR at baseline of 0.317 mL/min/1.73 m^2 for every y increase in donor age). No other factors were found to be significantly associated with baseline eGFR.

These studies determined a significant change in eGFR (by a factor of −0.757 mL/min/1.73 m^2 per mo) during the 12-month assessment period following AMR, which translates to a delta of −9.084 mL/min/1.73 m^2 at 1 year postdiagnosis of active AMR. None of the risk factors evaluated in the linear mixed effects submodel significantly altered the negative slope change (ie, 2-way interaction terms with time were not statistically significant).

Relationship Between Predicted and Observed eGFR

The longitudinal submodel for eGFR was used to estimate individual baseline eGFR as well as predict individual slope. Figure 4 shows the relationship between model-predicted ("true") and observed eGFR values at the time of AMR diagnosis (d 0). Plot suggest good agreement between the predicted (model-based adjusted) (mean = 45.6) and the observed baseline eGFR (mean = 45.4). The prognostic significance of baseline predicted eGFR and individual slopes with the risk of death-censored graft failure composite endpoint were evaluated in the joint submodel of the event process. Change in eGFR (individual slope parameterization) was included as a time-dependent covariate. Results are presented in Table 5. Baseline eGFR and slope (change in eGFR during the first-year postdiagnosis of active AMR) were significantly associated with the risk of death-censored graft failure (hazard ratio = 0.880 per unit increase in baseline eGFR [$P < 0.0001$] and hazard ratio = 0.107 per unit increase in slope [$P < 0.0001$]). Results for all-cause graft failure are provided in supplemental Table S1B, SDC, http://links.lww.com/TP/B930. Hazard ratios <1 indicate that the risk of death-censored graft failure decreases as baseline eGFR increases or as the rate of change in eGFR during the 12-month observation period becomes more stable. Therefore, reducing the rate of change in eGFR in the first 12 months postdiagnosis of active AMR should improve death-censored graft survival.

Predicted Outcomes Based on eGFR Slope

We next conducted analyses to predict graft survival based on the joint modeling studies. Table 6 provides

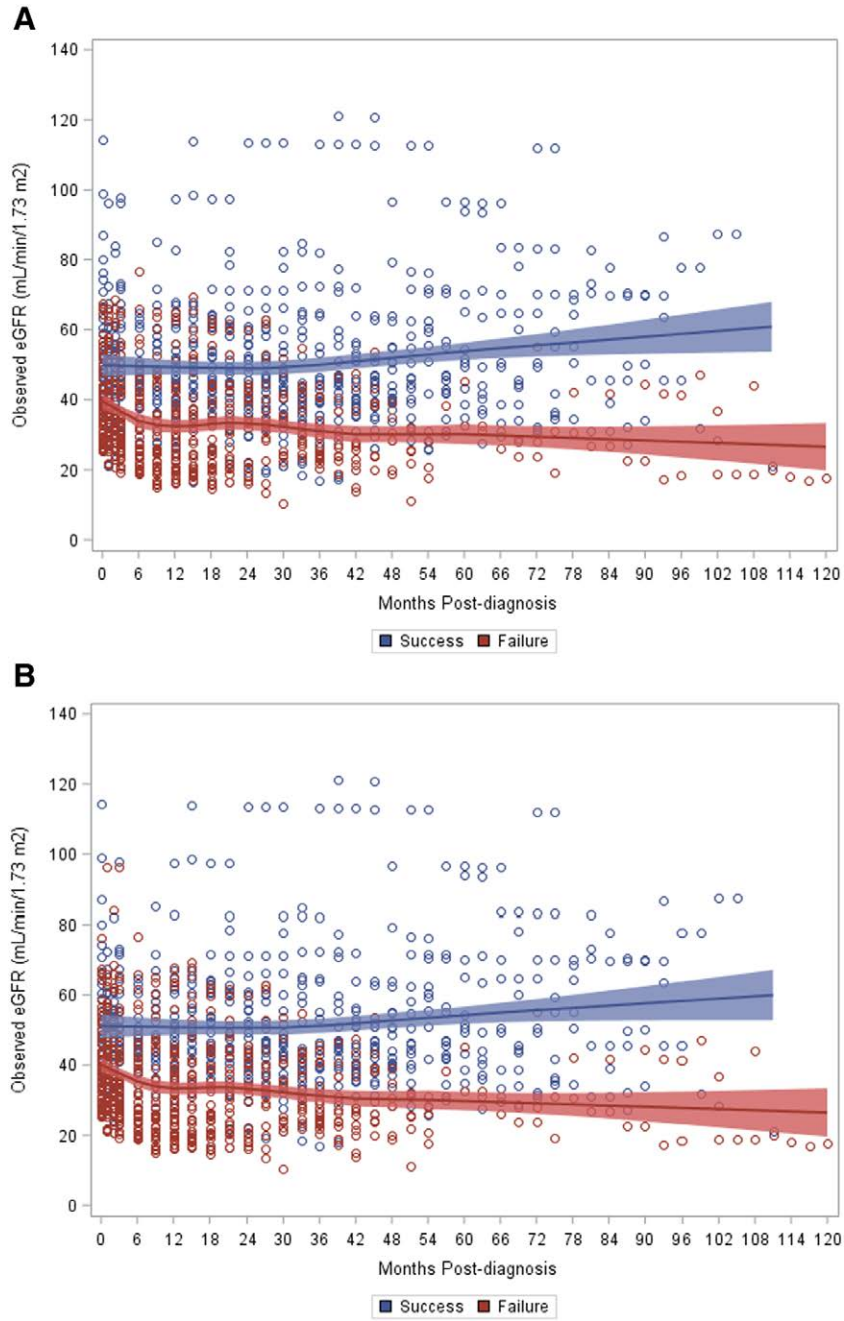

FIGURE 2. Scatterplots of observed longitudinal measures of eGFR over time by kidney allograft status. This is a plot of observed estimated GFRs over time postdiagnosis of active antibody-mediated rejection. (A) is a scatterplot of eGFRs stratified on whether the individual's graft met the criteria for death-censored graft failed (ie, graft loss or eGFR <15 mL/min/1.73 m^2; blue-colored lines) or not (red colored lines). LOESS plots are superimposed. Shaded area represents the 95% confidence limits for the predicted LOESS mean values. (B) is a scatter plot of observed estimated GFRs stratified on whether the individual's graft met the criteria for all-cause graft failure (ie, graft loss, eGFR <15 mL/min/1.73 m^2 or patient death; blue colored lines) or not (red-colored lines). LOESS plots are superimposed. Shaded area represents the 95% confidence limits for the predicted LOESS mean values. eGFR, estimated GFR; GFR, glomerular filtration rate; LOESS, locally weighted scatterplot smoothing.

a summary of the joint model predicted conditional event-free survival (ie, freedom from graft loss or eGFR <15 mL/min/1.73 m^2) using the supplemental analysis set. The mean eGFR profile (second row) reflected a 19.9% decline from baseline (AMR diagnosis) to month 12 post-AMR diagnosis and was associated with predicted event-free survival rates of 86.2%, 67.4%, 53.0%, and 27.0% at 2, 3, 4, and 5 years post-AMR diagnosis, respectively.

Relative to the mean eGFR decline, a 30% improvement in monthly eGFR decline was associated with higher predicted event-free survival rates of 89.4%, 74.2%, 61.9%, and 37.2% at 2, 3, 4, and 5 years post-AMR diagnosis (Figure 5). As shown in Figure 5, further improvements in monthly eGFR decline were associated with higher predicted event-free survival rates, especially at later time points post-AMR diagnosis. A stable eGFR was associated with predicted event-free survival rates of 94.4%, 85.7%,

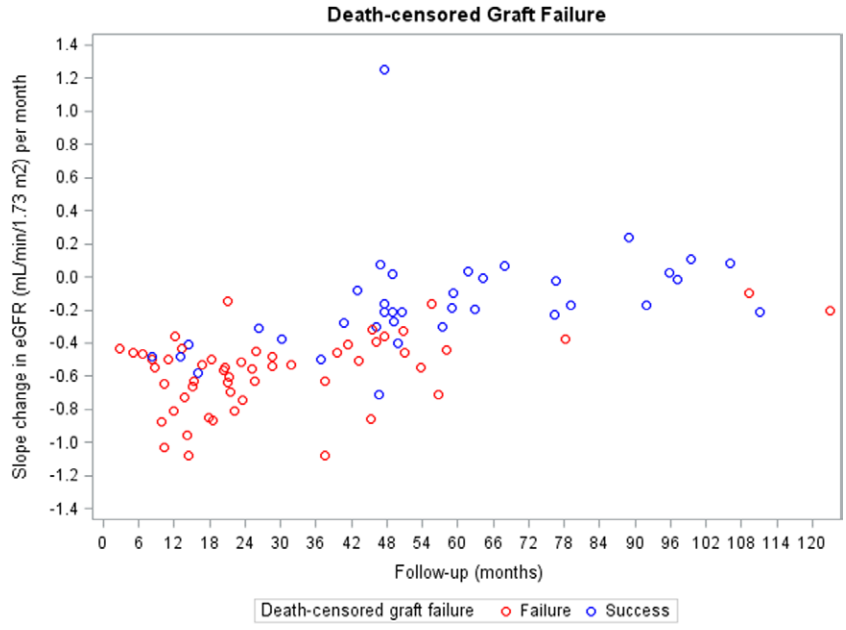

FIGURE 3. Scatterplot of individual slope change in eGFR. based on linear mixed effects model by individuals follow-up time. This is a scatterplot of individual slopes (rate of change in eGFR per mo), derived from the linear mixed effects longitudinal submodel, by the length of individual follow-up time (in mo). Blue-colored circles denote individuals whose grafts failed based on the criteria for death-censored graft failure (graft loss or eGFR <15 mL/min/1.73 m^2). Red-colored circles denote individuals whose grafts did not fail. eGFR, estimated glomerular filtration rate.

TABLE 5.

Joint modeling results of the estimated GFR longitudinal process and risk of death-censored graft failure composite endpoint (supplementary analysis set). A, linear mixed effects submodel for the longitudinal eGFR process. B, Cox's hazards submodel for time to death-censored graft failure composite endpoint

Linear mixed effects model for longitudinal eGFR process	Comparison	Estimate	SE	*P*	Event process	Comparison	Hazard ratio	95% Confidence interval	*P*
Variable					Variable				
Intercept		50.884	7.869	<0.0001	Estimated GFR (predicted value) at time of diagnosis	Per unit increase	0.880	0.830, 0.933	<0.0001
Time	Per mo	−0.757	0.104	<0.0001	Slope	Per mo increase in eGFR	0.107	0.034, 0.334	<0.0001
Prognostic factors									
Site	Barcelona vs Wisconsin cohort	−0.458	5.881	0.938					
	Manitoba vs Wisconsin cohort	11.499	3.848	0.003					
Donor type	Deceased vs living	−0.865	3.060	0.777					
Donor age	Per y increase	−0.317	0.108	0.003					
Length of time from kidney transplantation to AMR diagnosis	Per mo increase	−0.028	0.033	0.401					
C4d positive stain at time of AMR diagnosis	Yes vs no/unknown	−0.350	4.394	0.937					
Anti-HLA DSA class at time of AMR diagnosis	Class I only vs class I and class II	5.753	4.258	0.177					
	Class II only vs class I and class II	7.240	3.566	0.042					

The longitudinal submodel only included eGFR values observed during the first 12 mo postdiagnosis of active AMR.
AMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated GFR; GFR, glomerular filtration rate; SE, standard error.

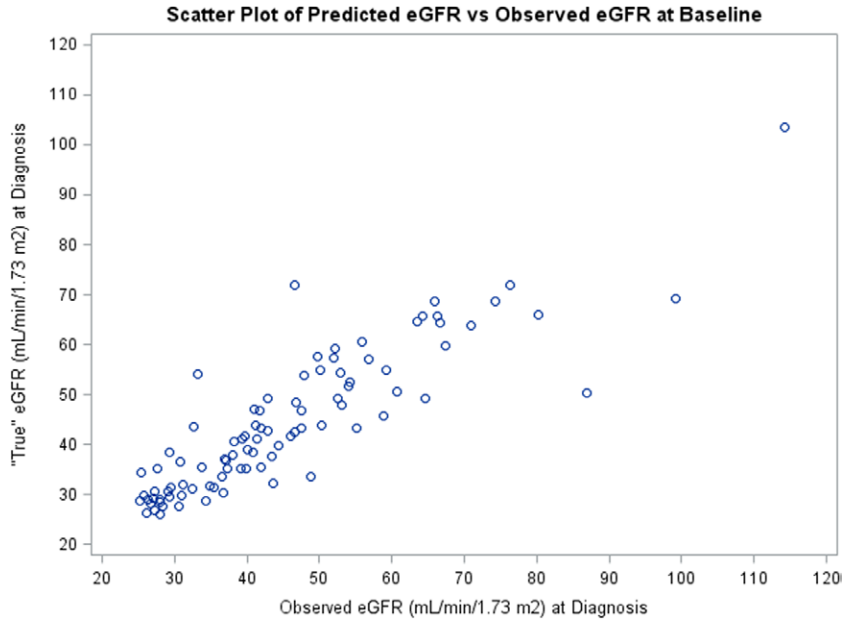

FIGURE 4. Model-predicted vs observed eGFR at diagnosis of active AMR (baseline eGFR at d 0). This is a scatterplot of the model-based prediction of eGFR for each individual at time of diagnosis of active AMR and their observed value at time of diagnosis of active AMR. Model-predicted eGFRs are based on the fit of the linear mixed effects longitudinal submodel. AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate.

TABLE 6.

Predicted conditional event-free survival (event-free survival = freedom from graft loss or eGFR <15 mL/min/1.73 m^2) using 12 mo of eGFR data (supplementary analysis set)

	Anticipated eGFR (mL/min/1.73 m^2)				Predicted survival[a]			
Scenario	Baseline	12 mo	% Change[b]	Per mo change	2 y	3 y	4 y	5 y
Mean eGFR	45.576	36.492	–19.932%	–0.757	0.862	0.674	0.530	0.270
30% improvement in slope[c]	45.576	39.217	–13.952%	–0.530	0.894	0.742	0.619	0.372
50% improvement in slope[c]	45.576	41.034	–9.966%	–0.379	0.912	0.781	0.672	0.441
75% improvement in slope[c]	45.576	43.305	–4.983%	–0.189	0.930	0.823	0.731	0.524
Stable eGFR	45.576	45.576	0.000%	0.000	0.944	0.857	0.781	0.600

[a]Predicted survival is conditional on being event-free at 12 mo following active AMR diagnosis.
[b]% change in eGFR from baseline at 12 mo following active AMR diagnosis.
[c]Relative to the mean eGFR profile (with an average slope change of –0.757/mo during the first 12 mo postdiagnosis of AMR).
AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate.

78.1%, and 60.0% at 2, 3, 4, and 5 years post-AMR diagnosis, respectively.

DISCUSSION

The results of our study confirm the significant impact of AMR on graft dysfunction and graft loss. The mean decrease in eGFR during the first year following a diagnosis of AMR in our cohort was nearly 20%. A diagnosis of AMR also was associated with a high rate of graft failure, with approximately one-third of patients experiencing death-censored graft failure after 3 years. We further found a strong relationship between the eGFR trajectory during the first year following AMR diagnosis and the risk of subsequent graft failure. This association was independent of patient characteristics assessed at the time of transplantation and at the time of AMR diagnosis. In fact, the only other factor associated with the risk of graft failure, in addition to the slope of eGFR, was the baseline eGFR at the time of AMR diagnosis. Even relatively small improvements in the slope of eGFR were associated with significantly better cumulative event-free survival. A 30% improvement in slope, which corresponds in our cohort to an average decrease from 20% to 14% during the first year, was associated with a predicted 3.2% absolute decrease in graft failure events during the next year and 10% fewer events 4 years later. A larger decrease in the 1-year slope of 50% correlates with a predicted 5% absolute decrease in graft failures 1 year later and 17% four years later.

Current challenges in clinical trial design in long-term studies after KTx include the use of clinical end points, surrogate end points, and biomarkers.[16-21] A clinical endpoint is an outcome or variable representing a measure of how a patient feels, functions, or survives.[22] The current gold standard clinical end points in renal transplantation are patient and graft survival measured at appropriate time points.[22] A biomarker is an objectively measured characteristic that is evaluated as an indicator of normal biological or pathogenic processes or pharmacologic responses to an intervention. It may allow for faster, more efficient clinical trials but greatly depends on the quality of data supporting its use and the setting in which applied. And a surrogate

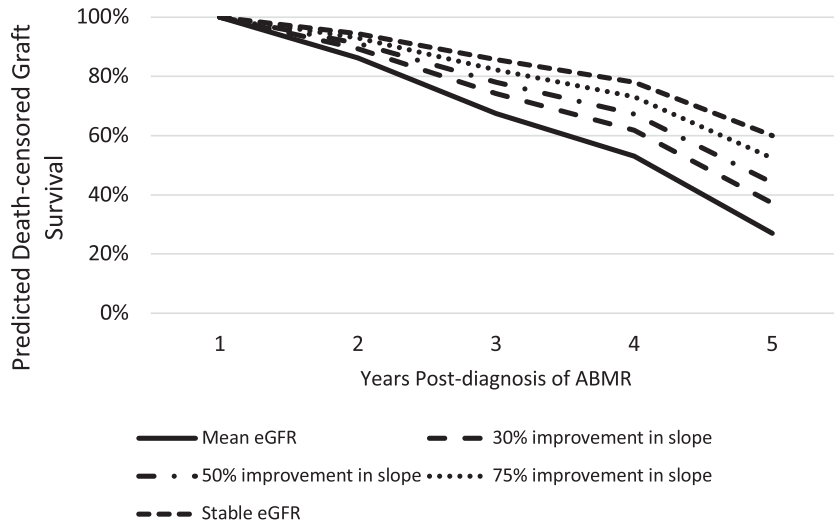

FIGURE 5. Predicted conditional event-free survival rates using 12 mo of eGFR data. This is a plot of the joint model predicted conditional event-free survival (ie, freedom from graft loss or eGFR <15 mL/min/1.73 m^2) rates using different eGFR trajectories at 12 mo postdiagnosis of AMR. The 30%, 50%, and 75% improvement in slope curves are relative to the observed slope of –0.757, which corresponds to the mean eGFR profile observed in the data. The conditional event-free survival projections for stable eGFR are based on a scenario wherein the eGFR remains constant (no change) at 12 mo postdiagnosis of AMR. AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate.

endpoint is a biomarker intended to substitute for a clinical endpoint and expected to predict clinical benefit.[22] The challenges in clinical trial design in renal transplantation, including studies addressing AMR, reside in our inability to predict individual patient outcome in a safe, efficient, and accurate manner. The results of our investigation have important implications for clinical practice in patients diagnosed with AMR. The significant decrease in graft function following AMR that we observed implies that close monitoring of patients is required to identify patients at the highest risk. And the strong association between short-term eGFR slope decline and subsequent risk of graft failure demonstrates that such monitoring can significantly improve prognostication. Unfortunately, few treatment options currently exist to modify the eGFR trajectory following AMR diagnosis.

Our study has shortcomings including relatively small sample size, lack of validation of this model in an external dataset, our inability to include all pathological and clinical data including proteinuria that could impact outcomes, and limited information on the generalizability of the findings to T cell–mediated rejection or recurrent disease. However, these limitations may be mitigated by the multicenter approach to an important problem, using granular data, and the overall goal to assess change in eGFR and its effect on graft failure irrespective of treatment. Beyond the ongoing debate regarding the optimal approach for determining GFR and the need for accurate serial measurements, declining GFR over time may be more predictive of late allograft failure and, therefore, a better surrogate endpoint.[18] The observed relationships between the decrease in eGFR during the first year and the subsequent risk of graft failure also have far-reaching implications for clinical research. The transplant community may be able to use these results to define groups of patients who have a better prognosis, and ultimately determine why they have a better prognosis. In addition, our study can be used to estimate the degree of eGFR change, which corresponds to a clinically meaningful improvement in the hard endpoint of graft failure. The raw data and this modeling exercise were utilized for the design of the Interleukin 6 Blockade Modifying Antibody-Mediated Graft Injury and Estimated Glomerular Filtration Rate (eGFR) Decline Trial (NCT03744910). In summary, this work provides data that, if prospectively validated, may inform the design of pivotal clinical trials that aim to test therapies for late AMR.

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