High tacrolimus clearance is a risk factor for acute rejection in the early phase after renal transplantation

Erlend J. Egeland, MSc,[1] Ida Robertsen, PhD,[1] Monica Hermann, PhD,[1,2]

Karsten Midtvedt, MD, PhD,[3] Elisabet Størset, MSc,[3,4] Marte T. Gustavsen, MSc,[3]

Anna V. Reisæter, MD, PhD,[3] Rolf Klaasen, MSc,[5] Stein Bergan, PhD,[1,5]

Hallvard Holdaas, MD, PhD,[3] Anders Hartmann, MD, PhD,[3] Anders Åsberg, PhD,[1,3]

[1]School of Pharmacy, University of Oslo, Oslo, Norway

[2]Stord/Haugesund University College, Norway

[3]Department of Transplant Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway

[4]Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[5]Department of Pharmacology, Oslo University Hospital, Oslo, Norway

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Correspondence: Erlend J. Egeland, MSc. Pharm, School of Pharmacy, University of Oslo, Postbox 1068 Blindern, 0316 Oslo, Norway. (e.j.egeland@farmasi.uio.no)

ABBREVIATIONS:

ATG, Anti-thymocyte globulin

AUC, Area under the curve

BPAR, Biopsy-proven acute rejection

CYP, Cytochrome P450

DGF, Delayed graft function

dnDSA, *De novo* donor-specific antibodies

DSA, Donor specific antibodies

HLA, Human leukocyte antigen

IPV, Intra-patient variability

IvIg, Intravenous immunoglobulin

LC-MS/MS, Liquid chromatography-tandem mass spectrometry

NPV, Negative predictive value

PPV, Positive predictive value

PRA, Panel reactive antibodies

ROC, Receiver operator characteristic

TDM, Therapeutic drug monitoring

ABSTRACT

Background

Patients with high tacrolimus clearance eliminate more drug within a dose interval compared to those with low clearance. Delays in dosing time will result in transient periods of lower concentrations in high- versus low clearance patients. Transient subtherapeutic tacrolimus concentrations may induce acute rejection episodes.

Methods

A retrospective study in all renal transplant patients treated with tacrolimus at our centre from 2009 to 2013 was conducted. The association between individually estimated tacrolimus clearance (daily tacrolimus dose [mg] / trough concentration [μg/L]) and biopsy-proven acute rejection (BPAR) the first 90 days post-transplantation was investigated.

Results

In total, 638 patients treated with oral tacrolimus were included in the analysis. Eighty-five (13.3%) patients experienced BPAR. Patients were stratified into four groups according to their estimated clearance. The patients in the high clearance group had significantly higher incidence of BPAR (20.6%) with a HR of 2.39 (95% CI; 1.30-4.40) compared to the low clearance group. Clearance estimate (as a continuous parameter) showed a hazard ratio of 2.25 (95% CI; 1.70-2.99) after adjusting for other risk factors. There were no significant differences in neither trough concentrations the first week after transplantation nor time to target trough concentration between patients later experiencing BPAR or not.

Conclusion

High estimated clearance is significantly associated with increased risk of BPAR the first 90 days post-transplantation, and may predict an increased risk of rejection in the early phase following renal transplantation.

INTRODUCTION

Even with the current immunosuppressive regimens 10 to 20% of renal transplant recipients develop early acute rejections.[1,2] Tacrolimus is the cornerstone in the immunosuppressive regimen after renal transplantation. Underdosing of tacrolimus increases the risk of acute rejection and development of *de novo* donor specific antibodies (dnDSA).[3] Overdosing on the other hand is associated with side effects such as renal toxicity and post-transplantation diabetes mellitus.[4,5] Hence, both over- and under dosing are potentially detrimental for long-term graft survival. To assure individually optimal doses of tacrolimus, therapeutic drug monitoring (TDM) utilizing tacrolimus trough concentration measurements is routinely applied at most transplant centres.[6]

Tacrolimus is a critical dose drug with a high intra- and interindividual pharmacokinetic variability.[7,8] High intrapatient variability (IPV) of tacrolimus has in several studies been related to impaired long-term outcome.[9-13] The high IPV may be due to many factors, such as co-medication, non-adherence and food.[14] Non-adherence in renal transplant recipients is estimated to be approximately 20 to 25%, and is associated with impaired long-term outcomes.[15-18] Almost every transplant patient will in daily life take their immunosuppressive drugs at irregular time intervals or even miss a dose at some occasions. Saint-Marcoux *et al.* investigated the pharmacokinetic consequences of a missed or delayed dose of tacrolimus using population pharmacokinetic modelling.[19] They showed that missing one dose would lead to a potentially clinically relevant temporary lower tacrolimus exposure (area under the curve (AUC)) in patients with high tacrolimus clearance as compared to those with low clearance. Such temporary subtherapeutic immunosuppressive

episodes may trigger the immune system and potentially lead to an acute rejection episode or promote development of dnDSA.

The aim of the present analysis was to investigate the association between individual tacrolimus clearance, controlled for actual trough concentrations obtained, and incidence of biopsy-proven acute rejection (BPAR) the first 90 days following renal transplantation.

MATERIALS AND METHODS

A single centre, retrospective study was conducted. Data from all adult tacrolimus treated renal recipients followed for a minimum of 8 weeks post-transplantation at Oslo University Hospital Rikshospitalet, Norway, transplanted between January 2009 and December 2013 were included.

Oral tacrolimus was initiated on the day of transplantation, starting with 0.04 mg/kg twice daily in standard-risk patients and 0.05 mg/kg twice daily in high-risk patients. TDM was applied and dose adjustments aimed to reach target whole blood trough concentrations of 3 to 7 µg/L in standard risk patients,[20] and 8 to 12 µg/L (days 0-30) and 6 to 10 µg/L (after day 30) in high-risk patients. High immunological risk was defined as presence of donor-specific antibodies, panel reactive antibodies (PRA) >20% at transplantation or ABO incompatibility between donor and recipient. Before 2012 allocation to either tacrolimus or ciclosporin was based on an algorithm (patients with diabetes and those older than 55 years at the time of transplantation received ciclosporin) resulting in a roughly 50:50 distribution. From January 2012 all patients received tacrolimus except recipients already treated with ciclosporin before transplantation.

Induction treatment consisted of 20 mg intravenous basiliximab on day 0 and 4 after transplantation and 250 mg (standard risk) or 500 mg (high risk) intravenous methylprednisolone on day 0. A single dose of 375 mg/m^2 rituximab was given to DSA-positive and ABO-incompatible patients four weeks prior to transplantation (living donor) or at transplantation (deceased donor). From day 0 to 4, 400 mg/kg intravenous human globulins (IvIg) were given to DSA-positive patients, while ABO-incompatible patients were given a single dose of 500 mg/kg IvIg at transplantation. Patients with PRA >20% which were DSA-negative were given anti-thymocyte globulin induction (ATG, Genzyme) at time of transplantation.

As maintenance therapy in addition to tacrolimus, all patients received 750 mg oral mycophenolate mofetil twice daily and prednisolone once daily, initiated at 20 mg (80 mg in high-risk patients), and tapered to 10 mg at 4 weeks in standard risk recipients and 8 weeks in immunologic high-risk recipients.

The clinical follow up after transplantation was performed at the transplant centre for the first 8 to 10 weeks, after which the patients were transferred to their local nephrology unit. If there was suspicion of rejection patients were referred back to the transplant centre for an ultrasound guided graft biopsy. Tacrolimus trough concentrations were measured 3-4 times per week early after transplantation and were gradually reduced to once a week after about eight weeks. All patients were routinely scheduled for a comprehensive investigation at 8 weeks post-transplantation. During this investigation both measured glomerular filtration rate (iohexol plasma clearance) and an oral glucose tolerance test were performed. The study was approved by the Regional Committee for Medical Research Ethics and was performed in accordance with the declaration of Helsinki. The clinical and research activities being reported are consistent with the Principles of the Declaration

of Istanbul as outlined in the “Declaration of Istanbul on Organ Trafficking and Transplant Tourism”.

Analysis of tacrolimus trough concentrations

All blood samples for tacrolimus whole-blood concentration measurement were drawn immediately before the morning dose. Concentrations were determined using the chemiluminescent microparticle immunoassay (CMIA, analysed on the Architect Instrument; Abbott Laboratories, Abbot Park, IL).[21] The assay was consistently applied throughout the study period. The lower limit of quantification was 1.0 µg/L. The coefficients of variation of the between series imprecision were 6% at 2 µg/L and 3.5% at 7.2 µg/L, respectively.

Acute rejection

In this study, only BPARs are reported. Suspicion of acute rejection was based on an unexplained increase in serum creatinine of >20 %. If there was suspicion a renal core biopsy was obtained and classified according to the Banff criteria.[22] Between 6 and 8 weeks posttransplant, a protocol biopsy was scheduled and subclinical acute rejections diagnosed in these biopsies were also included in the final analyses. Rejections were treated with intravenous methylprednisolone given in daily pulses introduced at 500 mg, and followed by doses of 250 mg/day for up to three days. Oral prednisolone doses were also increased to 30 mg/day and were tapered by 5 mg every other week. Steroid-resistant rejections or vascular rejections were treated with CD3 monitored intravenous ATG (Genzyme).[23]

Estimation of tacrolimus clearance

The ability of each patient to eliminate tacrolimus was estimated by dividing the total daily tacrolimus dose with the morning trough concentrations (daily dose [mg] / trough concentration [µg/L]). The mean of all whole-blood concentrations obtained from 7 days prior to 2 days after the in-depth investigation day 8 weeks after transplantation were used. For simplicity, this variable will be referred to as "clearance", and reported without units (mg μg^{-1} L^{-1}), throughout the manuscript. Rejection treatment with intravenous methylprednisolone may induce cytochrome P450 (CYP)-enzymes, which in turn may lead to an overestimation of tacrolimus clearance.[24] Clearance for BPAR-patients was therefore also estimated from three tacrolimus doses and trough concentrations prior to the day of initiation of acute rejection treatment.

Statistical analysis

The outcome in the present analysis was the association between tacrolimus clearance and BPAR the first 90 days after transplantation. Patients were stratified into four groups (low, below average, above average and high) according to the quartiles of estimated tacrolimus clearance. A Kaplan-Meier analysis with log-rank test and a Cox regression analysis comparing hazard ratio of the below average, above average and high clearance groups individually versus the low clearance group were performed.

To further assess the independent effect of potential risk factors on acute rejection, a multivariate Cox-regression was performed using time to first BPAR as a main outcome. Estimated clearance (on a continuous scale) and other clinically important risk factors such as delayed graft function (DGF; dialysis treatment first 7 days after

transplantation),[1,2] HLA-DR mismatch (1 or 2 mismatches),[2] immunologic high-risk[25] and gender were included in the model.

Receiver operator characteristic (ROC) curve analyses were performed to identify clearance cut-offs for patients at increased risk of BPAR the first 90 days after transplantation. One cut-off was made by finding the clearance value with the highest sum of specificity and sensitivity, and another was made by its possibility to detect 20% of the patients experiencing BPAR. The prevalence of BPAR was used to calculate the PPV and negative predictive value (NPV).

Differences between continuous variables were analysed with the student's t-test, and differences between categorical variables with the Chi-squared test. *P*-values less than 0.05 were considered to be statistically significant. All analyses were done in R version 3.2.3.[26]

RESULTS

Patients

A total of 1198 adult patients were transplanted from January 2009 to December 2013, and 717 of these received oral tacrolimus. A total of 465 patients received ciclosporin, 4 received an investigational study drug and 3 received everolimus instead of tacrolimus. Eight patients had missing data and 1 patient had a non-functional transplant. Seventy-nine of the 717 tacrolimus treated patients (10.8%) were early transferred to their local hospital and did not attend the 8-week comprehensive investigation at our transplant centre. Distributions of selected demographical data of the remaining 638 patients included in the analysis are shown in Table 1. During the first 90 days post transplantation a total of 85 (13.3%) patients experienced BPAR, after a median (interquartile range) time of 8 days (5-31). Sixty-five of the 537 (12.1%) standard-, and 20 of 101 (19.8%) high immunological risk patients experienced BPAR, respectively.

BPAR risk factors

The recipients were categorised into low, below average, above average and high tacrolimus clearance groups with mean estimated clearance of 0.38 ± 0.10, 0.62 ± 0.06, 0.85 ± 0.08 and 1.48 ± 0.54 units, respectively. The Cox regression with the categorical clearance groups showed that the below average group had a HR of 0.87 (95% CI, 0.41-1.83; P=0.72), above average HR of 1.65 (95% CI, 0.86-3.14; P=0.13 and the high clearance group had a HR of 2.39 (95% CI, 1.30-4.40; P<0.006) for BPAR the first 90 days post-transplant. All groups were compared to the low clearance group. This is depicted visually in a Kaplan-Meier plot in Figure 1.

Table 2 shows the results from the multivariate Cox-analysis. Tacrolimus clearance, DGF, immunological high risk and male gender were all shown to be independent risk factors for BPAR in the multivariable model.. The mean hazard ratio (HR) for having a BPAR was 2.46 (95% CI, 1.84-3.29; *P*<0.001) for a 1 unit increase in estimated clearance.

There were no differences in mean tacrolimus trough concentrations at week 1 or 8 after transplantation between patients with or without BPAR in neither standard- nor high immunological risk recipients (Table 3). The mean trough concentration before BPAR-diagnosis was within the therapeutic range for the standard risk patients. In the immunologic high-risk group the mean trough concentration before BPAR was within target range, and not significantly different from values at week 1 (*P*=0.33) nor week 8 (*P*=0.63) after transplantation.

Time to reach target tacrolimus concentrations (i.e >3.0 µg/L for standard immunological risk- and >8.0 µg/L for high immunological risk patients) for the low, below average, above average and high clearance groups were 1.4 ± 0.79, 2.06 ± 2.48, 2.44 ± 3.10 and 3.06 ± 4.14 days, respectively. There was no difference in days to reach target for patients having and not having BPAR (2.22 ± 2.29 vs 2.24 ± 3.04 *P*=0.95).

ROC-analysis showed a clearance cut-off value of 0.71 units to maximise the sum of sensitivity and specificity. The sensitivity was 68% and specificity 48%. With a prevalence of BPAR of 13.3% the PPV and NPV were 17.9% and 91.4%, respectively, and 323 of the 638 patients had a clearance above 0.71 units. The BPAR-free survival curve of this risk stratification is shown in Figure 2. An optimised cut-off for detecting 20% of patients having BPAR was 1.45 units. The PPV was

29.5% and the corresponding NPV was 88.4%. Applying this cut-off identified 17 of the 85 (20%) patients with BPAR. The BPAR-free survival curve is shown in Figure 3.

DISCUSSION

The major finding in this study was that renal transplant recipients with high tacrolimus clearance had a higher risk of having an acute rejection episode in the early phase after renal transplantation. A plausible explanation could have been that patients with high tacrolimus clearance had lower trough concentrations, but no such difference was identified between recipients experiencing BPAR or no-BPAR in neither standard- nor in high immunological risk patients. Similar to what have been shown in previous studies, we found that DGF, male gender and immunological high risk were independent risk factors for acute rejection in the early phase following transplantation.[1]

The multivariate analysis showed that a 1 unit higher estimated clearance resulted in an independent doubling of the risk of BPAR the first 90 days post-transplantation. E.g. a patient receiving a daily tacrolimus dose of 7.5 mg to reach a trough concentration of 5.0 µg/L will have more than twice the risk of BPAR compared to a patient receiving a dose of 2.5 mg to achieve the same trough concentration.

The times to reach the target trough concentrations were different between the clearance groups. Patients in the low clearance group reached the target trough concentrations faster than the patients in the high clearance groups. Theoretically this could have been an explanation for the difference in risk of BPAR seen between the groups. But there were no significant difference in time to target between patients experiencing, and not experiencing BPAR. The induction therapy including basiliximab may be sufficient to overshadow this initial small difference in target achievement. To our knowledge this is the first study investigating the association between patients' individual tacrolimus clearance and clinical outcomes after renal

transplantation. Based on the St-Marcoux *et al.* publication[19] it is likely that patients with high tacrolimus clearance compared to patients with low clearance have a higher risk of triggering the immune system with a delayed or skipped dose of tacrolimus. Unfortunately we do not have adherence data from our patients, but the median time from transplantation to BPAR in the present study was only 8 days (Figure 1). In this period all patients were hospitalised in the surgical ward with an anticipated adherence close to 100%. Non-adherence is therefore not likely to be the main reason for the findings in the present study.

A high estimated clearance may also be due to a low oral bioavailability which generates a higher dosage-need to achieve target trough concentrations. An alternative hypothesis to our findings is that there is a correlation between tacrolimus oral bioavailability and intralymphocyte concentrations (site of immunosuppressive action). Many of the same mechanisms that lead to a low bioavailability (e.g. P-glycoprotein and CYP-enzymes) are also present in lymphocytes and might lead to a low intralymphocyte tacrolimus concentration.[27]

Disregarding the mechanism behind the present findings, individual clearance estimates would be a fast and easily obtainable clinical risk factor to identify recipients that need special attention with regards to tacrolimus therapy. Setting a cut-off at about 1.5 units has the potential to identify a significant proportion of the patients with increased risk of acute rejection.

$$\text{High risk of BPAR} = \frac{\text{Daily tacrolimus dose (mg)}}{\text{Trough concentration } (\mu\text{g L}^{-1})} > 1.5 \text{ units}$$

A potential for optimising therapy in high risk patients identified by high clearance estimates may be intensified information and education on the importance of strict

adherence, or even switching these patients to an extended release tacrolimus formulation to minimise episodes of under immunosuppression.[28] The 1.5 unit cut-off has a PPV of 29.5%, which is not very high. But our suggested interventions for an individual patient, in case of a clearance estimate above the cut-off, are non-invasive. In the case of a false positive test, the patient gets a more thorough follow-up, which is not associated with any increased risk for the individual patient.

CYP3A5 genotype is associated with tacrolimus clearance and patients expressing the functional protein need about twice as high dose to obtain the same trough concentrations as patients not expressing functional CYP3A5.[29] A previous publication from our centre[30] found that 15% of our patient population express functional CYP3A5, which is in concordance with other estimates from Caucasian populations.[31] Unfortunately we do not have *CYP3A5* genotype data on the patients included in the present analysis, which is an obvious limitation to our study. However, continuous clearance estimates from all patients were used in the multivariate analysis which means that a relative increase in clearance gives an increased risk in every patient. Therefore will the vast majority of patients that do not express functional CYP3A5 still have the same increased of risk of BPAR associated with high clearance as those expressing functional CYP3A5. Approximately half of the patients had an estimated clearance above 0.71 units and had a higher risk of BPAR compared to patients with clearance below this threshold as shown in Figure 2. Thus it is unlikely that the effect shown in the present study could be explained solely by *CYP3A5*-genotype.

Concomitant drugs influencing CYP3A will affected the clearance estimates. We do not have data on co-medications given to the patients in the study. But influence of CYP3A inducers or inhibitors is a minor problem because it is the phenotype that

matters. In addition, a previous study from our centre found that of 102 subsequent transplantations from January to June 2014, only 1 patient started to use a CYP3A-inducing drug after transplantation.[32]

Glucocorticoid doses given for acute rejection treatment may have biased the results in the present analysis due to the effect on tacrolimus clearance via induction of CYP-enzymes.[24] The results remained however similar, irrespective of whether clearance was estimated from the 8-week investigational day or the day before initiation of acute rejection treatment.

The strengths of this study include a large number of patients transplanted at a single centre that underwent uniform clinical treatment and –investigations.

The study has several limitations, some of which has been addressed in previous sections. The majority of patients are followed at our transplant centre for 8 to 10 weeks post-transplant, but 79 patients were early transferred to their local hospital. We cannot rule out that this has introduced a small bias in the data sample, but the decision to transfer patients is not based on any known factors that directly influence either tacrolimus clearance or risk of acute rejection.

Ethnicity may play a role in interindividual variability of drug metabolism and response.[33] We do not register ethnicity of patients transplanted at our centre. The study population is however very homogenous, and there is a considerable over-weight of Caucasian patients.

All tacrolimus concentrations used in this study were measured with a CMIA immunoassay. This is not the current gold standard, but it correlates well (R^2=0.97

with liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) [34] The immunoassay did however measure an average of 18% higher tacrolimus concentrations, probably due to cross reactivity with metabolites. This small overestimation of tacrolimus concentrations does not affect the main results in this study since the immunoassay was consistently applied through the entire study period, but somewhat different cut-off values will be expected if using alternative analytical methods.

In order to increase the generalisability of our results we included all standard- and high-risk patients transplanted in the period between January 2009 and December 2013 treated with tacrolimus. Since the standard- and high-risk patients have different target trough concentrations, the Cox-regression analysis did not include trough concentration as a variable. In a subanalysis of the 537 standard risk patients we performed a similar multivariate Cox-regression as seen in Table 2, also including mean trough concentrations day 1-7 posttransplant for all patients (Table S1, SDC). This model overall showed the same results as seen in the whole population and lower tacrolimus trough concentrations were not associated with increased risk of BPAR.

CONCLUSION

Estimated tacrolimus clearance is significantly and independently associated with an increased risk of BPAR the first 90 days following renal transplantation in a real-life setting with both standard- and high immunological risk patients. A cut-off of 1.5 units can identify patients with increased risk of acute rejection in the early phase following transplantation. The causality of this association is not known, *CYP3A5*-genotype

may potentially explain part of the association, though expression of CYP3A5 may not fully substitute high estimated clearance as a risk factor.

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Table 1: Demographic data at transplantation.

	BPAR	No BPAR	*P*
Number of patients	85	553	
Age (years)	50.0 ± 14.0	50.3 ± 14.6	0.86[b]
Gender (male)	66 (78%)	361 (65%)	0.024[a]
Preemptive tx	20 (24%)	164 (30%)	0.25[a]
Dialysis (months)[c]	23 ± 22	20 ± 18	0.25[b]
DGF	20 (24%)	31 (6%)	<0.001[a]
Donor age (years)	49.6 ± 15.4	50.8 ± 15.3	0.51[b]
Cold ischemia (hours)	10.8 ± 6.5	9.9 ± 6.3	0.22[b]
HLA-DR mismatches (1 or 2):	64 (75%)	360 (65%)	0.064[a]
Living donor	25 (29%)	194 (35%)	0.31[a]
Immunologic high risk[d]	20 (24%)	81 (15%)	0.037[a]

Continuous data are presented as mean±SD, and categorical data as n (% of group total).
[a]Chi-squared test.
[b]Student's *t*-test
[c]Excluding patients with preemptive transplantation
[d]Defined as presence of donor specific antibodies and/or panel reactive antibodies >20% and/or ABO-incompability at transplantation
BPAR; Biopsy-proven acute rejection, DGF; Delayed graft function, HLA; Human leukocyte antigen, Tx; Transplantation

Table 2: Multivariate Cox-regression model of risk factors associated with biopsy-proven acute rejection the first 90 days post transplantation

	B) Multivariate analysis		
	Hazard ratio	95% CI	*P*
Clearance[a,b]	2.25	1.70-2.99	<0.001
DGF	3.25	1.85-5.53	<0.001
HLA-DR mismatch (1 or 2)	1.65	0.99-2.72	0.051
Immunologic high-risk[c]	1.88	1.12-3.15	0.018
Male gender	1.92	1.14-3.21	0.014
Cold ischemia (hours)	1.01	0.98-1.05	0.50

[a]Estimated by (daily tacrolimus dose[mg] / trough concentration[µg/L]) at 8 weeks or before BPAR-treatment.
[b]Continuous values from all patients
[c]Defined as presence of donor specific antibodies and/or PRA >20% and/or ABO-incompability at transplantation
DGF: Delayed graft function, HLA: Human Leukocyte Antigen, Tx: Transplantation

Table 3: Mean tacrolimus concentrations in standard- and high immunological risk patients

	Standard risk			High risk		
	BPAR	No BPAR	*P*	BPAR	No BPAR	*P*
	n=68	n=486		n=20	n=81	
Tac week 1[a]	6.4 ± 2.1	6.3 ± 2.5	0.71	7.3 ± 2.4	7.3 ± 2.3	0.98
Tac week 8[b]	7.2 ± 2.0	6.8 ± 1.8	0.07	8.4 ± 1.8	8.8 ± 1.7	0.39
Tac before BPAR[c]	5.9 ± 1.8	NA		8.0 ± 2.6	NA	

Data presented as mean ± SD, and comparisons are done with Students *t*-test
[a]Mean of all measured concentrations
[b]Mean of all concentrations measured between 7 days before to 2 days after the 8-week investigational day
[c]Mean of the 3 concentrations measured before steroid pulse treatment
All concentrations are in µg/L
BPAR; Biopsy proven acute rejection, Tac; Tacrolimus

Figure 1 Legend

Figure 1. Kaplan-Meier freedom from first biopsy-proven acute rejection the first 90 days post-transplant by low, below average, above average and high tacrolimus clearance

Figure 2 Legend

Figure 2. Kaplan-Meier freedom from first biopsy-proven acute rejection during the first 90 days post-transplant above and below a 0.71 unit cut-off

Figure 3 Legend

Figure 2. Kaplan-Meier freedom from first biopsy-proven acute rejection during the first 90 days post-transplant above and below a 1.5 unit cut-off

Figure 1

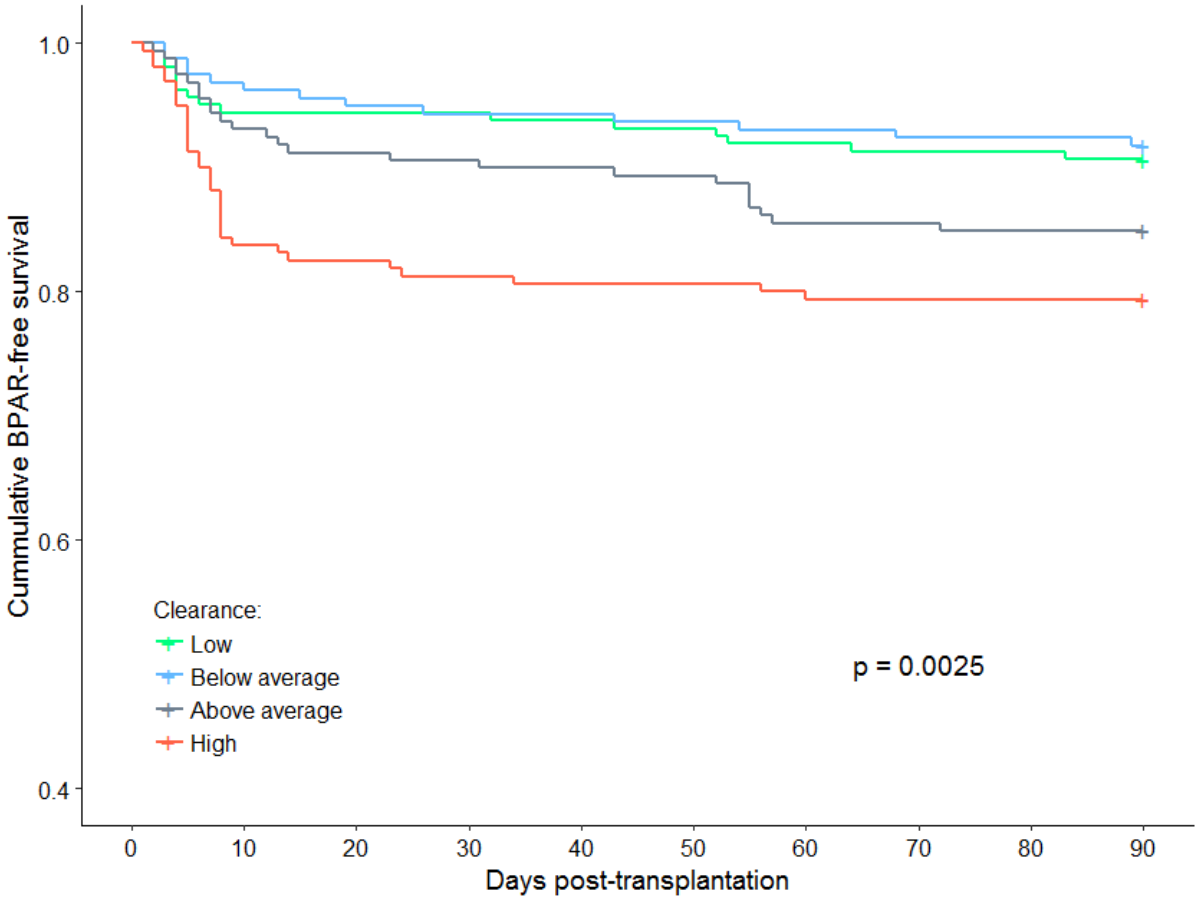

1.0
0.8
0.6
0.4
Cummulative BPAR-free survival
Clearance:
Low
Below average
Above average
High
p = 0.0025
0
10
20
30
40
50
60
70
80
90
Days post-transplantation

Figure 2

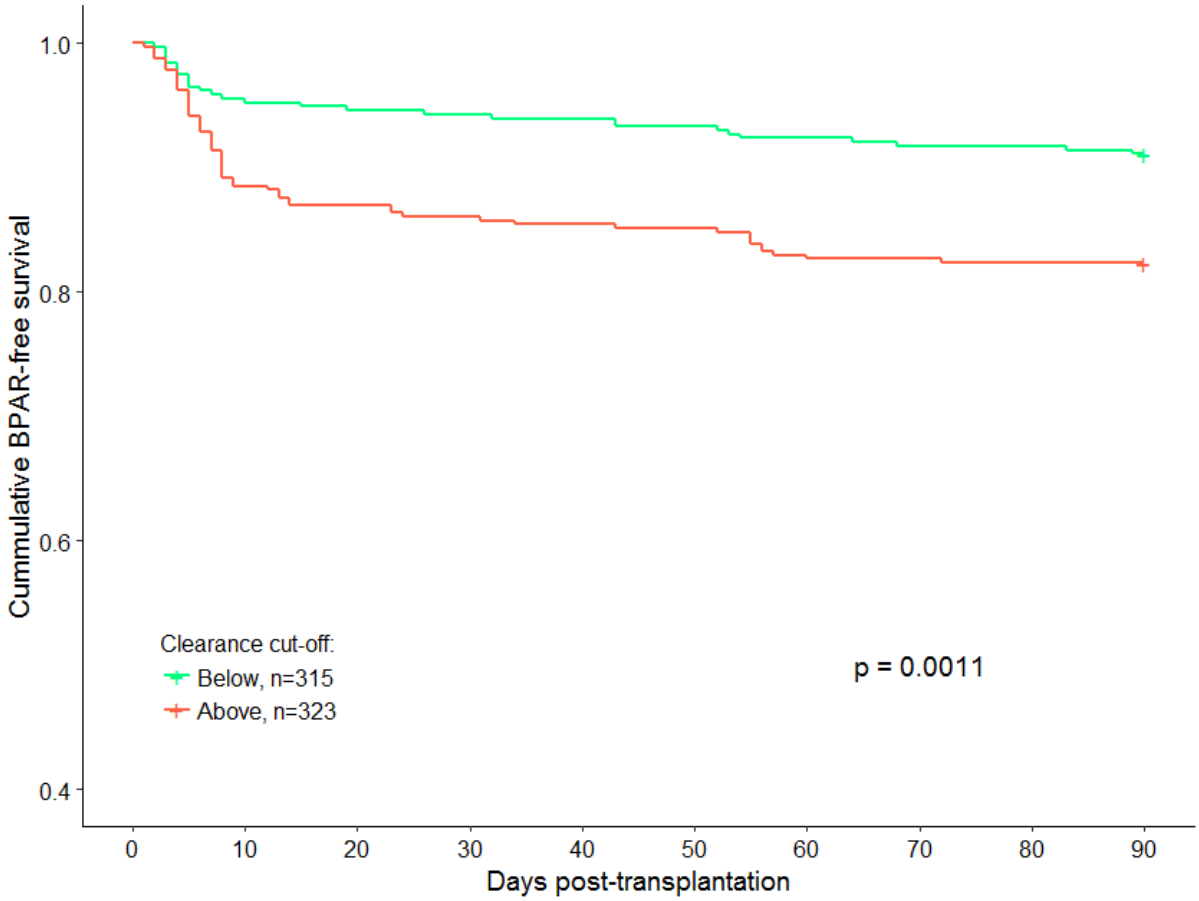
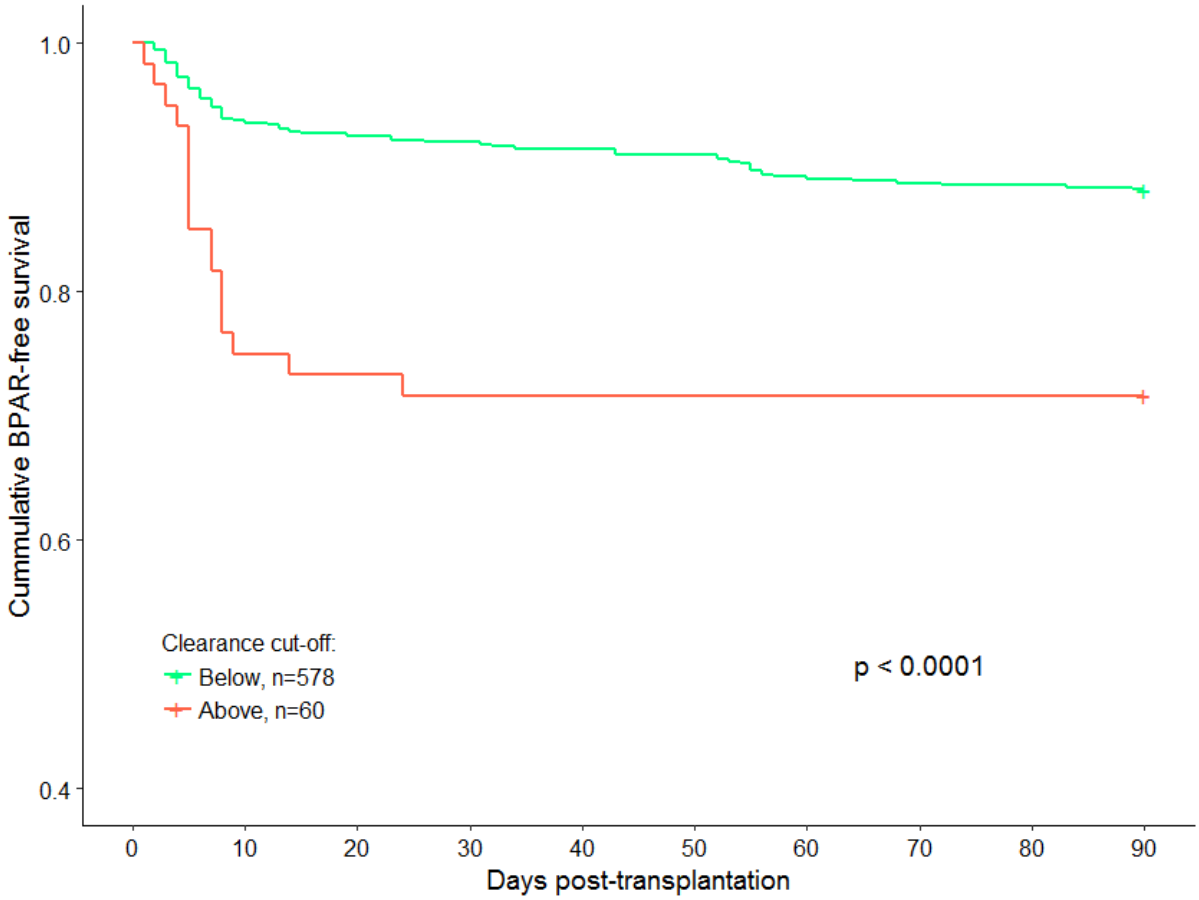

1.0
0.8
0.6
0.4
Cummulative BPAR-free survival
Clearance cut-off:
Below, n=315
Above, n=323
p = 0.0011
0
10
20
30
40
50
60
70
80
90
Days post-transplantation

Figure 3


1.0
0.8
0.6
0.4
Cummulative BPAR-free survival
0
10
20
30
40
50
60
70
80
90
Days post-transplantation
Clearance cut-off:
Below, n=578
Above, n=60
p < 0.0001

SUPPLEMENTARY DIGITAL CONTENT

Table S1, SDC: Multivariate cox-regression model of risk factors associated with biopsy-proven acute rejection the first 90 days post transplantation in standard immunological risk patients

	B) Multivariate analysis		
	Hazard ratio	95% CI	*P*
Clearance[a,b]	2.98	2.06-4.31	<0.001
DGF	1.91	0.90-4.03	0.092
HLA-DR mismatch (1 or 2)	1.63	0.90-4.03	0.11
Male gender	2.71	1.33-5.52	0.006
Tacrolimus trough concentration week 1[c]	1.13	1.02-1.24	0.019
Cold ischemia (hours)	0.99	0.95-1.03	0.64

[a]Estimated by (daily tacrolimus dose[mg]/ trough concentration[µg/L]) at 8 weeks or before BPAR-treatment.
[b]Continuous values from all patients
[c]Mean of all measured concentrations, in µg/L
DGF: Delayed graft function, HLA: Human Leukocyte Antigen, Tx: Transplantation