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# Intercept and Slope Analysis of Risk Factors in Chronic Renal Allograft Nephropathy

*Yvo W. J. Sijpkens, Aeilko H. Zwinderman, Marko J. K. Mallat, Henk Boom, Johan W. de Fijter, and Leendert C. Paul*

In the Leiden cohort of cadaveric renal transplants, the authors compared the risk factors of a low intercept, defined as a creatinine clearance lower than 50 ml/min at 6 months, and of a negative slope of the reciprocal creatinine concentrations after 6 months. Two hundred of 654 grafts (31%) failed to reach optimal function because of old donor age, female gender of the donor, histoincompatibility, delayed graft function, or acute rejection episodes in the first 6 months. Forty-four percent of all grafts displayed progressive deterioration of function over time. The association with younger recipient age, sensitization, class I histoincompatibility, baseline immunosuppression, and late acute rejection episodes suggests an underlying immunological process, very likely activated through the indirect antigen-presenting route. The negative impact of proteinuria and diastolic hypertension at 6 months on the slope is compatible with their role as progression factors. Although associations are not necessarily causally related and interventions do not necessarily result in an improvement in outcome, it is conceivable that better matching, optimal immunosuppression, and a more aggressive anti-hypertensive and antiproteinuric treatment result in improvement of long-term graft survival.

## Introduction

Chronic rejection is the most prominent cause of chronic allograft nephropathy and graft loss beyond the initial 6 posttransplant months. Clinically, it results in chronic graft dysfunction, which is characterized by a decline in glomerular filtration rate over months or years, often in combination with proteinuria and hypertension.<sup>1</sup> The histopathology of chronic rejection shows arterial intimal thickening, transplant glomerulopathy, glomerulosclerosis, interstitial fibrosis, and tubular atrophy.<sup>2</sup> Several risk factors have been identified, but it is not clear how they operate in the process of graft attrition. In the present study, we defined transplants with a low intercept or a negative slope and compared their risk profiles.

## Materials and Methods

We analyzed the course of the renal function in 654 patients transplanted between 1983 and 1996 who survived at least 6 months.<sup>3</sup> The endogenous creatinine clearance at 6 months was used to categorize the intercept of the graph that describes graft function over time as lower or higher than 50 ml/min. Chronic decline in function was modeled by 1 or 2 least-squares-fitted regression lines,<sup>4</sup> which determines the slope of the graft function over time. A negative slope significantly different from zero categorized transplants as having a decline in function beyond 6 months. Using logistic regression techniques, we assessed the risk profiles of a low intercept and a negative slope, respectively.

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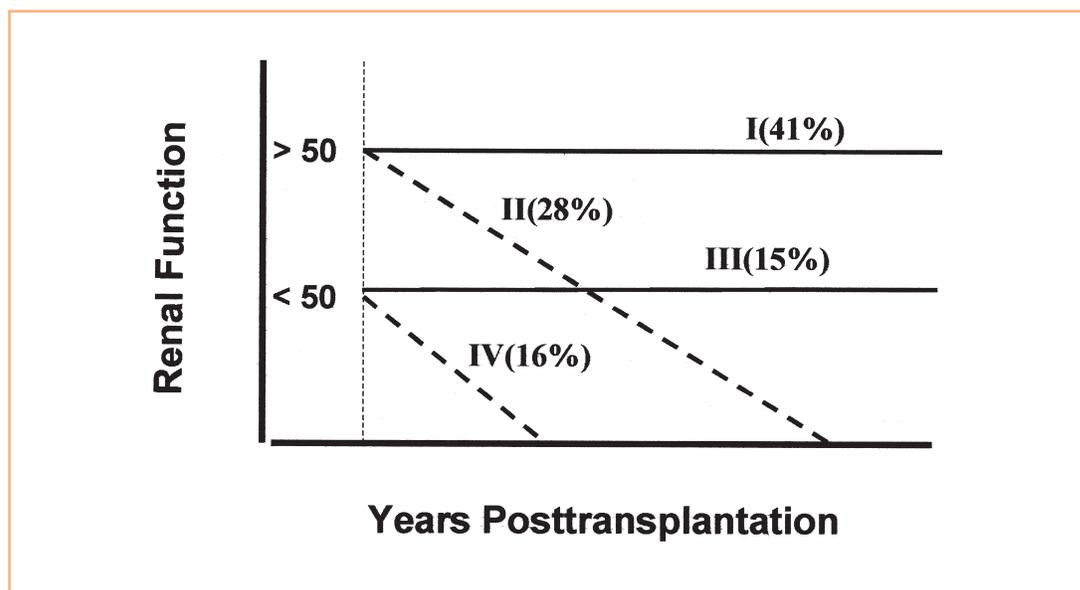


Figure 1. 604 transplants categorized according to intercept (endogenous creatinine clearance at 6 months) and slope of reciprocal creatinine concentrations after 6 months posttransplant: I > 50 ml/min, stable function (n = 246); II > 50 ml/min, decline in function (n = 171); III < 50 ml/min, stable function (n = 92); IV < 50 ml/min, decline in function (n = 95).

## Results

Four patterns of “evolution of graft function over time” were created (Fig. 1). Forty-one percent of grafts resumed and maintained optimal function postimplantation (creatinine clearance > 50 ml/min), whereas another 28% achieved a similar function but experienced functional deterioration afterward. At 6 months, the remaining 31% had a creatinine clearance < 50 ml/min; about half of these maintained this function, whereas the remaining grafts displayed progressive loss of function. We found a significant correlation between a low intercept and a negative slope in a chi-square test ( $P = 0.025$ ). Furthermore, the slope, expressed as a decline of the reciprocal creatinine concentration per month, was significantly steeper in the group with a low intercept ( $-1.09$ ) compared with the group with a high intercept ( $-0.44$ ). Figure 2 shows the graft survival censored for patient death in the groups with a negative slope stratified by the intercept. Univariate analysis (Table 1) showed that old donor age, female gender of the donor, histoincompatibility of major histocompatibility complex (MHC) class I antigens, delayed graft function, or acute rejection episodes were associated with a low

intercept. Donor age, odds ratio (OR) 1.45 (1.27-1.65) per 10 years increase; CREG matching, OR 0.77 (0.66-0.91) per shared CREG; and acute rejection, OR 3.09 (2.02) for episodes within the first 2 months, and OR 7.73 (4.26-14.04) for episodes between months 2 and 6 posttransplantation, were independent factors in multivariate analysis. Younger recipient age, sensitization, year of transplantation, repeat transplantation, histoincompatibility, baseline immunosuppression, late acute rejection episodes, diastolic blood pressure, and proteinuria at 6 months were associated with progressive loss of renal function. In multivariate analysis, transplantation in the 1980s, OR 2.77 (1.95-3.93); human leukocyte antigen (HLA) matching, OR 0.81 (0.68-0.96) per shared antigen; and acute rejection episodes between months 2 and 6 posttransplantation, OR 1.91 (1.09-3.33), were independently predictive of a negative slope.

## Discussion

Although the zenith of renal function posttransplantation may be after the 1st year,<sup>5</sup> this intercept and slope analysis allows an interesting insight into the evolution of graft function over time. In the

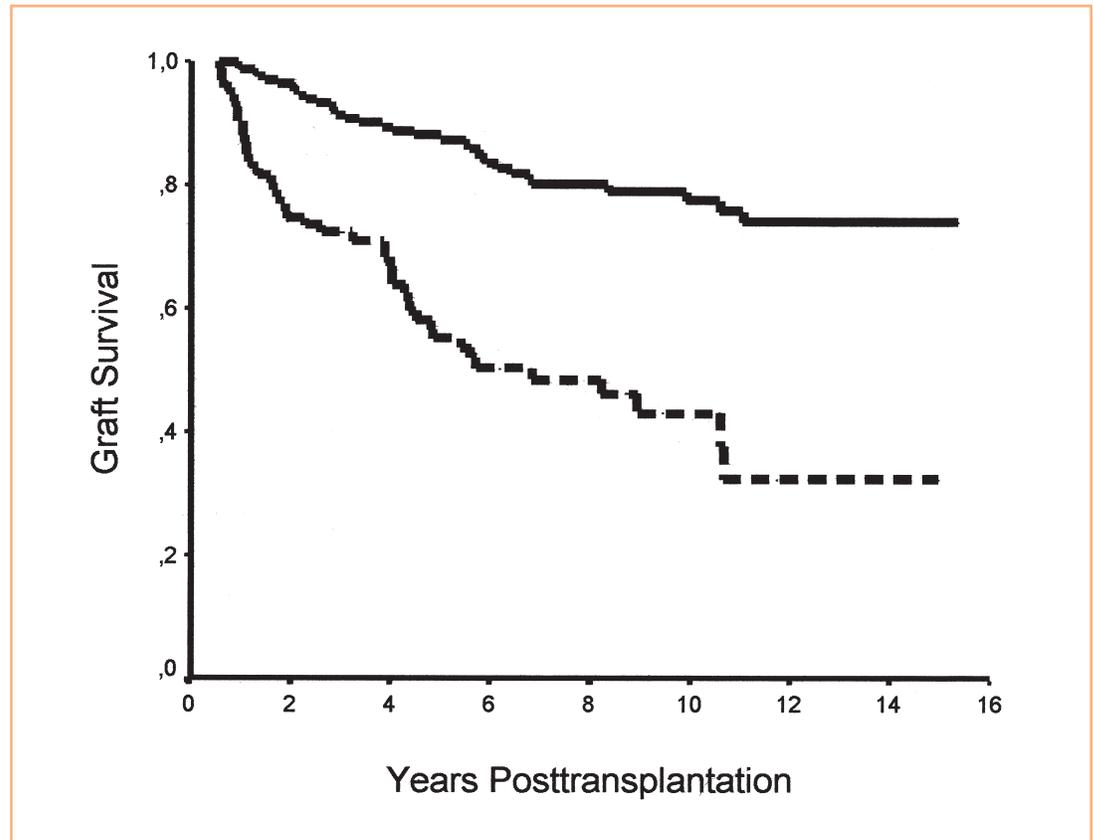


Figure 2. Graft survival censored for patient death of transplants with a creatinine clearance at 6 months > 50 ml/min and a decline in function after 6 months (solid line) and transplants with a creatinine clearance at 6 months < 50 ml/min and a decline in function after 6 months (dashed line). Log rank test:  $P < 0.001$ .

present study, old donor age, female gender of the donor, and delayed graft function were the discriminating factors correlating with a low intercept, which was defined as a creatinine clearance of less than 50 ml/min at 6 months posttransplantation. Kidneys from old or female donors may have a lower renal mass. Moreover, old donor kidneys are more likely to develop delayed graft function and to undergo acute rejection episodes.<sup>6,7</sup> We have previously shown that delayed graft function is associated with an impaired long-term outcome, dependent on the level of renal function,<sup>8</sup> and is therefore an intercept problem. Grafts with delayed function also have a higher likelihood of acute rejection episodes.<sup>8</sup> Acute rejection episodes had a strong impact on the intercept. Interstitial and vascular rejection had a similar effect. Previous studies have

shown that acute vascular rejections are of prognostic significance, but graft loss in this group occurs early after transplantation.<sup>9</sup> Other investigators have also noted the lack of correlation of acute rejection pathology and survival beyond 6 months posttransplantation.<sup>10</sup> Multivariate analysis showed that acute rejection episodes occurring later in the initial 6 months had a stronger impact on the intercept than the histopathological type of acute rejection. Moreover, in contrast to early acute rejection episodes, late acute rejection activity appeared to be the link between a low intercept and a negative slope. We recently confirmed previous data that patients undergoing acute rejection episodes > 60 days have an increased incidence of chronic rejection.<sup>3,11</sup> In our well-matched cohort of renal transplants sharing cross-reactive groups of MHC

Table 1 | UNIVARIATE ANALYSIS OF RISK FACTORS FOR A LOW INTERCEPT AND FOR A NEGATIVE SLOPE

	LOW INTERCEPT	NEGATIVE SLOPE
Recipient age (per 10 years increase)	1.10 (0.96-1.26)	0.84 (0.74-0.96)*
Panel reactive antibodies (per 10% increase)	1.04 (0.96-1.12)	1.09 (1.01-1.18)*
Donor age (per 10 years increase)	1.49 (1.32-1.68)*	1.03 (0.92-1.15)
Donor gender (female)	1.88 (1.34-2.64)*	0.78 (0.56-1.08)
Year of transplantation (83-89 vs. 90-97)	0.95 (0.68-1.33)	2.69 (1.29-3.76)*
Repeat transplant	1.40 (0.91-2.16)	1.70 (1.10-2.61)*
HLA-A,B,DR mismatches (per antigen)	1.05 (0.91-1.22)	0.99 (0.86-1.15)
HLA-A,B,DR shares (per antigen)	0.90 (0.76-1.06)	0.82 (0.70-0.96)*
CREG mismatches (per group)	1.27 (1.09-1.49)*	1.02 (0.88-1.19)
CREG shares (per group)	0.80 (0.69-0.92)*	0.84 (0.73-0.96)*
Azathioprine versus cyclosporine	0.95 (0.52-1.75)	2.25 (1.26-4.02)*
Delayed graft function	1.90 (1.30-2.78)*	0.81 (0.56-1.19)
Acute interstitial rejection episodes < 6 months	3.67 (2.45-5.51)*	0.90 (0.62-1.30)
Acute vascular rejection episodes < 6 months	4.20 (2.54-6.96)*	1.31 (0.81-2.12)
Acute rejection episodes 0-2 months	2.68 (1.81-3.95)*	0.93 (0.66-1.31)
Acute rejection episodes 3-6 months	8.04 (4.62-14.0)*	1.74 (1.03-2.95)*
Systolic RR 6 months (per 10 mmHg increase)		1.06 (0.98-1.15)
Diastolic RR 6 months (per 10 mmHg incr.)		1.26 (1.07-1.51)*
Dipstick proteinuria > 1+		2.72 (1.33-5.55)*
Creatinine 6 months (per 10 mmol/l increase)		1.01 (0.99-1.04)

## LOW INTERCEPT:

Creatinine clearance at 6 months lower than 50 ml/min.

class improved long-term graft survival<sup>3</sup> and influenced both the intercept and the slope. This observation is consistent with the hypothesis that an immunological mechanism is involved in chronic rejection, as is the association with late acute rejection episodes. Although freedom from chronic rejection is associated with lack of donor-specific immune reactivity, patients with chronic rejection often have circulating T lymphocytes that recognize incompatible donor MHC peptides presented on recipient antigen-presenting cells<sup>12</sup> or anti-HLA antibodies or anti-kidney antibodies.<sup>13,14</sup> We hypothesized that sharing HLA antigens down-regulates the conventional MHC-directed response, which is also attenuated by immunosuppressive treatment.<sup>3</sup> However, the correlation between the degree of MHC matching and graft loss from chronic rejection is variable,<sup>15-17</sup> suggesting that the specificity of the immune response might also be directed to non-MHC determinants. Therefore, it is also conceivable that chronic rejection results from an immune reaction against damaged tissues and that such responses result in perpetuation of chronic inflammation and impairment of the tissue repair process, in turn resulting in excessive fibrosis and tissue remodeling.<sup>18</sup>

The discriminating impact of recipient age, sensitization, and repeat transplant on the slope confirms the importance of an antigen-driven process in the attrition of graft function. Young age is associated with noncompliance<sup>19</sup> but also with a state of heightened immune response to alloantigens.<sup>20</sup> Losing a kidney increases the risk of broad sensitization, with a subsequent risk of acute and chronic rejection.<sup>21</sup> Patients using baseline immunosuppression with azathioprine and prednisone were transplanted in the early 1980s and therefore had a longer follow-up to develop a negative slope, whereas patients randomized to conversion from cyclosporine to azathioprine at 6 months did not have an inferior graft survival.<sup>22</sup>

The level of renal function, proteinuria, and hypertension are nonimmunological risk factors that have also been identified as risk factors in the progression of native kidney diseases. In experimental animals, reduction in renal mass results in an increase in the glomerular capillary hydrostatic pressure and glomerular filtration rate in the remaining nephrons—a result of afferent arteriolar dilatation.<sup>23</sup> It has been proposed that increased hydrostatic pressure results in glomerulosclerosis and progressive renal damage. Using a rat model, we

demonstrated that following reduction in renal mass or transplantation, the glomerular hydrostatic pressure increases in some rat strains but not others.<sup>24</sup> We have previously shown that the rate of human long-term graft loss depends on the level of function as assessed by the creatinine clearance.<sup>8</sup> The present analysis confirms that if functional deterioration occurs, the rate of decline is different for grafts with a creatinine clearance of < 50 ml/min, compared with grafts with a clearance of > 50 ml/min at 6 months posttransplantation.

Hypertension is an established progression factor in renal transplantation, as it is in native kidney diseases.<sup>25</sup> The association between the diastolic blood pressure and the slope of reciprocal creatinines has been reported previously.<sup>26</sup> Experimental data in a rat renal transplant model of chronic kidney graft rejection are consistent with the hypothesis that this effect is mediated by glomerular hypertension.<sup>24,27</sup> Lowering of the blood pressure has a beneficial effect on the rate of progression of chronic rejection in animals,<sup>28</sup> but there are very few clinical data available.

The amount of proteinuria has traditionally been considered a marker of the severity of a renal disease. Recent studies indicate, however, that proteins filtered through the glomerular capillaries may have intrinsic renal toxicity, which plays a role in the progression of renal damage,<sup>29</sup> and that the amount of urinary protein excretion correlates with the tendency of a given disease to progress.<sup>30</sup> Dietary protein restriction, ACE inhibitors, and other antihypertensive drugs are capable of limiting the progressive decline in glomerular filtration rate to the extent that they effectively lower the urinary protein excretion rate in native kidney disease<sup>31</sup> and in chronic rejection.<sup>32</sup>

The combination of a decline in renal function, hypertension, and proteinuria characterizes the syndrome of chronic transplant dysfunction, which might begin weeks to months after transplantation. Chronic rejection is the most important cause of chronic transplant dysfunction, which is supported by the impact of histoincompatibility of class I antigens and late acute rejection episodes on both the intercept and the slope in this study. Graft survival is further modified by pure intercept factors such as donor age, female gender of the donor and

delayed graft function, and the presence of hypertension and proteinuria as progression factors. Better matching, optimal immunosuppression, and a more aggressive antihypertensive and antiproteinuric treatment should improve long-term graft survival.

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## NEGATIVE SLOPE:

The slope of the reciprocal of the serum creatinine over time after 6 months shows a significant decline and is different from zero.

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