Transpl Int (2000) 13 [Suppl 1]: S112–S116

Luigi Boschiero
Roberto Foroni
Michele Tonini
Giusto Ancona

An objective method for detecting time-dependent effects in graft survival

L. Boschiero (✉) · R. Foroni · M. Tonini · G. Ancona
Centro Trapianti Renali,
Ospedale Civile Maggiore,
Piazzale Stefani 1, 37126,
Verona, Italy
e-mail: bos@iol.it
Fax: + 39-45-8072006

Abstract The proportional hazards model has become increasingly important in the analysis of censored survival data after transplantation. Neverthless, in clinical transplantation it is still undefined how the influence of covariates changes over time. The additive regression model is an alternative (or extension) to the Cox model. It results in plots (Aalen plots) that may give information on the effect of covariates over time by way of the cumulative regression function plots. A total of 386 primary cadaveric kidney transplants performed between 1984 and 1996 were included in our analysis. The follow-up period ranged from 24 to 156 months. According to Aalen, an additive regression model was used and plots for the detection of time-dependent effects of covariates were determined. Patients dying with functioning grafts were classed as graft failures. Factors potentially affecting graft outcome, such as sex, donor and recipient's age, HLA A-B match, cold ischaemia time (CIT), delayed graft function (DGF), serum creatinine at 1 month (Cr1), rejection episodes within 3 months (R3), and type of brain death (BD), were considered. The slopes of the plots by donor age, DGF, HLA A-B match, R3, Cr1 and BD appear to have a significant influence throughout the observation period, with different time-dependent effects on graft survival. Slopes for DGF, Cr1, and age of donor are positive (increased hazard), while slopes for HLA match and BD are negative (decreased hazard). Estimated regression functions for DGF, donor age and Cr1 show a prompt slope (within 3 months); the covariate R3 has a clear influence for about 5 years, and then seems to disappear; and BD appears to have a consistent effect over the entire period. The additive regression model with Aalen plots represents a useful technique in the analysis of survival data after kidney transplantation. Some covariates, such as R3, may often lose their effects on graft survival, with a relevant clinical impact. Others have a clear and additive influence over the entire period (BD), while the effects of donor age, DGF and CR1 each appear to have a prompt effect in the outcome.

Key words Kidney transplantation · Survival analysis · Additive regression model · Aalem plots

Introduction

Many factors undoubtedly affect the kidney graft survival rate. Some of them are well documented, such as donor age, HLA matching, ischaemia/reperfusion injury, and acute vascular rejection [3, 9, 13, 15]; some are suspected but difficult to measure, such as the "centre effect", especially for heterogeneous populations. Many studies on the effects on transplant survival have been published, and the proportional hazard model is the most commonly used regression model for survival data analysis after transplantation. In this model, the ef-

Table 1 Demographic characteristics of donors and transplant recipients (*BD* brain death, *CIT* cold ischaemia time, *CVA* cardiovascular accident, *DGF* delayed graft function)

	Mean	SD	No.	%
Donor age	35	15		
Recipient age	42	10		
Donor sex (M/F)			249/137	65/35
Recipient sex (M/F)			256/130	66/34
BD				
Trauma			206	53.4
CVA			158	40.9
Others			22	5.6
% PRA				
< 50%			368	95
> 50%			18	5
No. with HLA A-B match				
0			5	1.3
1			91	23.7
2			177	45.8
3			83	21.6
4			29	7.6
DGF				
Yes			145	32
No			262	68
CIT (hours)	12	5		
s-Creatinine (Crl)	372	310		
Acute rejection				
Yes			169	44
No			217	56

Table 2 Causes of graft loss in study population

	No. of patients	%
Acute rejection	48	43
Death with functioning graft	36	32
Chronic rejection	22	20
Recurrent disease	3	2.3
Primary nonfunction	2	1.8
Noncompliance	1	0.9

fect of covariates on survival time can be assessed using Cox analysis with a constant ratio of graft failure risk in particular prognostic groups [4].

Nevertheless, the way the influence of covariates changes over the time in renal transplantation is still undefined. The additive regression model [1] is an alternative (or an extension) to the Cox model. The resulting plots (Aalen plots) may give information on the effect of covariates over time by way of the cumulative regression function plots. Plots are obtained by estimating the instantaneous contributions of covariates to the hazard at each distinct failure time and summing up the resulting estimates. The slope of such plots indicates whether a specific covariate has a constant or a time-dependent effect. Slopes are positive when covariate increases correspond to hazard increases, and negative when covariate increases correspond to hazard decreases, while a cumulative-sums slope approaches zero when a covariate has no effect on the hazard.

Materials and methods

A consecutive series of 386 primary cadaveric kidney transplants performed at our centre from 1984 to 1996 were included in the analysis. Technical failures (7 cases) were excluded. The follow-up period was 24–156 months. Patients who died with functioning grafts were classed as graft failures. Factors potentially affecting graft outcome, such as sex (1 = male, 2 = female), donor and recipient age, number of HLA A-B matches (HLA-DR matching, not available for all patients, was not considered), cold ischaemia time (CIT, h), delayed graft function (DGF, need for postoperative dialysis), serum creatinine at 1 month (Crl), acute rejection episodes within 3 months (R3, 0 = no, 1 = yes,) and type of brain death (BD, 1 = head injury, 2 = CVA, 3 = other causes) were considered. Current panel-reactive antibody (% PRA) was not included in the analysis, because only a few patients were hyperimmunized (% PRA > 50%). The baseline immunosuppressive regimen included double therapy with cyclosporin A microemulsion, (initial dose 10–14 mg/kg per day, then adjusted to maintain trough levels of 300–400 ng/ml for 2 months, and 200–300 ng/ml thereafter); and methylprednisolone (0.5 mg/kg per day for the 1st month, then 0.25 and 0.12 mg/kg per day after months 2 and 3, respectively). Azathioprine was added later, and only if clinically indicated. Rejections were diagnosed by clinical symptoms, increased blood urea and creatinine, echo Doppler evaluation and histological evidence of rejection on biopsy. All patients with graft dysfunction underwent renal graft biopsy where not clinically contraindicated. Unresponsive (vascular) rejections were treated with OKT3, acute interstitial rejections with pulse corticosteroid therapy (10 mg/kg for 3 days). All study patients were seen in our transplant centre for follow-up evaluations. Only patients with a minimum follow-up of 2 years were analysed. Graft failure was defined as irreversible acute or chronic rejection, patient death with functioning graft, primary nonfunction, and irreversible recurrent disease with graft loss. A normalized statistical test (TST test) for the detection of the overall effect of a covariate was used to test whether regression function slopes were significantly different from zero. Values were compared with a standard normal distribution (*P*-value). An additive regression model (Aalen model) was used, and time-dependent effects of covariates were plotted [2].

Results

Study population characteristics are shown in Table 1. Between 1984 and 1996, 112 events (graft failures) in 386 consecutive transplant recipients (29%) were seen. Acute and chronic rejections and deaths with functioning graft accounted for 63% and 32% of all grafts lost during this study period, respectively (Table 2).

Estimated cumulative regression functions (CRF) by additive regression model are show in Figs. 1 and 2.

Slopes by donor age, DGF, HLA A-B match, R3, Crl and BD showed significant effects throughout the obser-

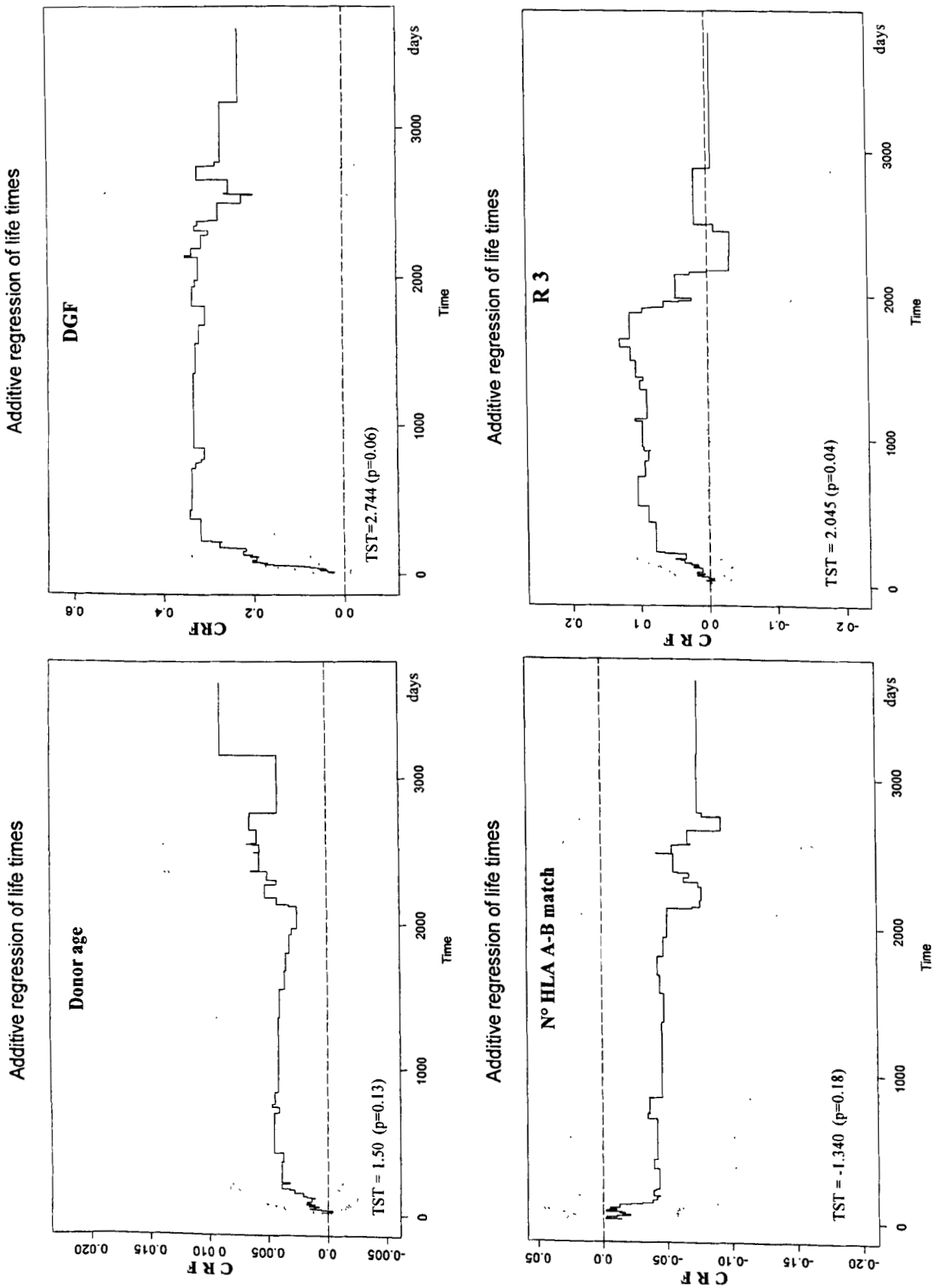

Fig. 1 Cumulative regression function for donor age, DGF, HLA A-B match and R3

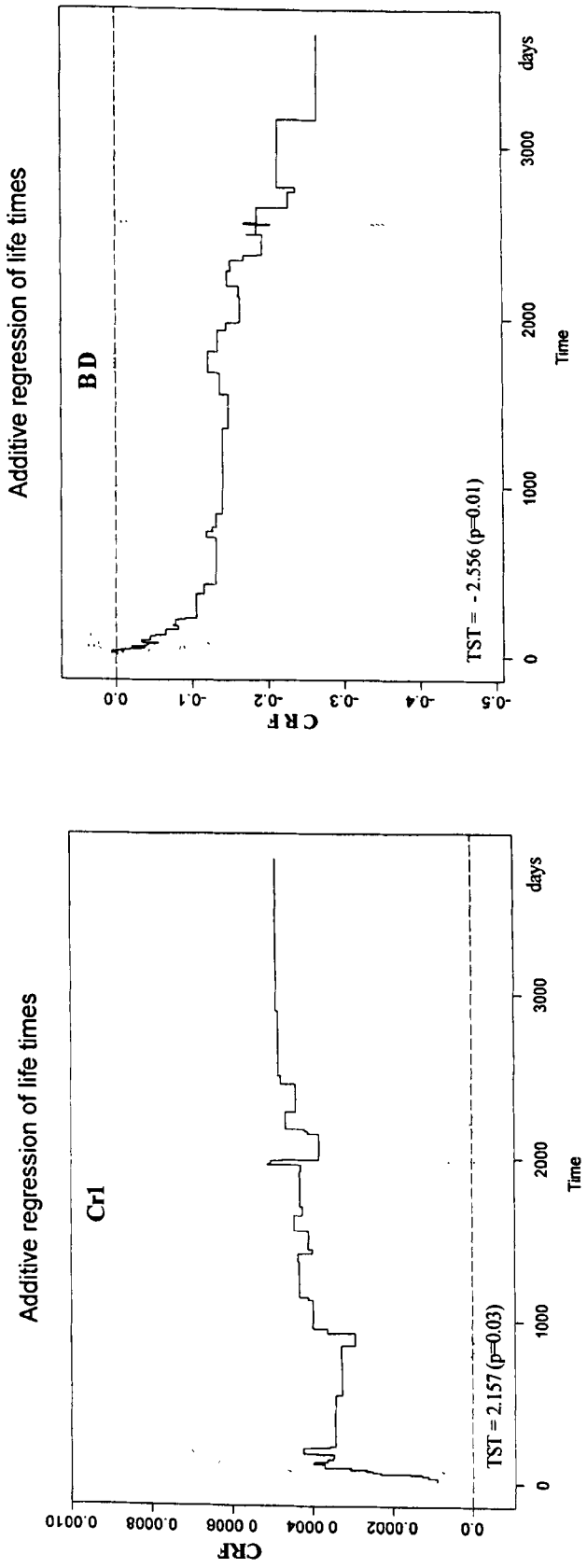

Fig. 2 Cumulative regression function for Cr1 and BD

Table 3 Linear model TST test

	TST test	*P*
BD	-2.556	0.01
R3	2.045	0.04
DGF	2.744	0.006
Crl	2.157	0.03
CIT	-1.30	0.19
Donor age	1.50	0.13
Recipient age	0.868	0.38
Donor sex	0.615	0.53
Recipient sex	-0.213	0.83
No. of HLA A-B matches	-1.340	0.18

vation period, with different time-dependent effects on graft survival. Slopes were positive for DGF, Crl, and age of donor (increased hazard), and negative for HLA match and BD (decreased hazard). Estimated regression functions for DGF, donor age and Crl showed a steep slope (within 3 months), while the covariate R3 showed a clear effect for about 5 years, disappearing afterwards. BD showed a consistent effect throughout the entire study period. Table 3 shows the linear model statistical test (TST) and corresponding *P*-values for all covariates. Four covariates only (BD, R3, DGF, and CR1) strongly affected graft survival. The cumulative regression function for BD covariate showed a steady decrease, indicating a lower risk for graft survival in recipients of kidney grafts from donors who had died of CVA. Acute rejection episodes within 3 months, delayed graft function and elevated creatinine values at 1 month significantly affected graft outcomes. High HLA A-B matches had positive effects on graft survival in the observation period, although the difference did not reach significance ($P = 0.180$); the effect of more advanced donor and recipient age was negative, though not significantly so ($P = 0.132$ and 0.385, respectively).

Discussion

This study examined the impact of a new method for the detection of time-dependent effects of various covariates in survival data after renal transplantation. The additive regression model revealed a strong impact of four covariates on graft outcome: type of brain death, delayed graft function, early acute rejection, and kidney graft function at 1 month.

Clinical evidence shows that ischaemia/reperfusion injuries affect long-term graft survival [15]. In addition, the more satisfactory short- and long-term performance of kidney grafts taken from living related and unrelated donors than of cadaver kidneys [12] led to the assumption that cadaveric donor-specific events, particularly brain death, may also influence later graft outcome [10, 11]. Our results suggest that catastrophic central injury

affects graft survival with a consistent effect over the whole period, and that the risk is lower when donors have died of CVA.

Acute rejection episodes are thought to be particularly important for the development of chronic rejection and graft failure [8]. However, questions such as the importance of rejection time and whether all acute episodes are associated with similar risk are still controversial [5, 7]. In our study, rejection episodes in the first 3 months affected kidney graft outcome for a prolonged period (5 years), after which they seemed to disappear, with allogeneic-independent factors becoming increasingly important [14].

The impact of DGF on graft outcome in kidney transplant recipients is currently under debate [6, 16]. At least two reasons could explain the shorter graft survival than of early functioning grafts: (1) a higher incidence of early acute rejection, and (2) a significant loss of functioning nephrons caused by hyperfiltration damage after long-lasting DGF. These findings suggest that the long-term prognosis of these grafts may be poor. Our results show that DGF had a prompt and negative effect on graft survival, with no further changes after the first 6 months.

Prompt graft function associated with lower serum creatinine levels in the early period is a useful marker of good renal function at 3 years [3]. In our analysis, creatinine levels at 1 month were significantly associated with long-term graft survival, with increased risk of graft failure for higher early values.

To define how the influence of covariates changes over the time, we used the additive regression model with Aalen plots [1, 2], which represents a useful technique for the analysis of censored survival data and a viable alternative to more established approaches, such as the Cox model and others. From a practical standpoint, the graphic representation of the cumulative regression functions is attractive, because it provides a direct perception of data and a picture of how effects and the model fit in with change over time. Clearly some caution is required when estimate plots are interpreted, especially in later periods, when few patients remain in the risk set. In our analysis, some covariates, such as R3, often lose their effects on graft survival, followed by a corresponding clinical impact. Others have a clear and additive influence throughout the entire period (BD), while the effect of donor age, DGF and CR1 on the outcome is prompt. In conclusion, graft survival failure is a dynamic process, affected by several factors, some of which are known and some still unknown. Clinicians may benefit from the use of statistical mathematical methods, which help in predicting the effect of one or more variables on graft survival and in verifying their influence on graft or recipient survival. Further and more extensive analyses with this statistical method in renal transplants are desirable.

References

1. Aalen OO (1989) A linear regression model for the analysis of life times. Stat Med 8: 907–925
2. Aalen OO (1993) Further results on the non-parametric linear regression model in survival analysis. Stat Med 12: 1569–1588
3. Cecka JM, Cho YW, Terasaki PI (1992) Analyses of the UNOS Scientific Renal Transplant Registry at three years – early events affecting transplant success. Transplantation 53: 59–63
4. Cox DR (1972) Regression model and life table (with discussion). J R Stat Soc 34: 197–227
5. Hariharan S, Alexander JW, Schroeder TJ, First MR (1996) Impact of first acute rejection episodes and severity of rejection on cadaveris renal allograft survival. Clin Transplant 10: 538–541
6. Koning OH, Rutger Ploeg J, Hajo van Bockel J, Groenewegwn M, Fokko Van Der Woude J, Guido Persijin G, Hermans J for the European Multicenter Study Group (1997) Risk factors for delayed graft function in cadaveric kidney transplantation. Transplantation 63: 1620–1628
7. Leggat JE, Akinlolu OO, Leichtman AB, Port FK, Wolfe RA, Turenne MN, Held PJ (1997) Long-term renal allograft survival: prognostic implication of the timing of acute rejection episodes. Transplantation 63: 1268–1272
8. Matas AJ, Gillingham KJ, Payne WD, Najarian JS (1994) The impact of an acute rejection episode on long-term renal allograft survival ($t_{1/2}$). Transplantation 57: 857–861
9. Pirsch JD, Ploeg RJ, Gange S, D'Alessandro AM, Knechtle SJ, Sollinger HW, Kalayoglu M Belzer FO (1996) Determinants of graft survival after renal transplantation. Transplantation 61: 1581–1586
10. Pratschke J, Wilhelm MJ, Kusuka M, Basker M, Cooper DKC, Hancock WW, Tilney NL (1999) Brain death and its influence on donor organ quality and outcome after transplantation. Transplantation 67: 343–348
11. Takada M, Kari Nadeau C, Hancock WW, Mackenzie HS, Shaw GD, Waaga AM, Chandraker A, Sayegh Mh, Tilney NL (1998) Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Transplantation 65: 1533–1542
12. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S (1995) High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med 333: 333 –338
13. Terasaki PI, Gjertson DW, Cecka JM, Takemoto S, Cho YW (1997) Significance of the donor age effect on kidney transplants. Clin Transpl 11: 366–372
14. Terasaki PI, Koyama H, Cecka JM, Gjertson DW (1994) the hyperfiltration hypothesis in human renal transplantation 57: 1450–1454
15. Tilney NL, Guttmann RD (1997) Effects of initial ischemia/reperfusion injury on the transplant kidney. Transplantation 64: 945–947
16. Troppmann C, Killingham Kj, Benedetti E (1995) Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. Transplantation 59: 962–966