Transpl Int (2004) 17: 78–88
DOI 10.1007/s00147-003-0664-5

ORIGINAL ARTICLE

Katarzyna Lietz
Zbigniew Lewandowski
Mieczyslaw Lao
Leszek Paczek
Zbigniew Gaciong

Pretransplant and early posttransplant predictors of chronic allograft nephropathy in cadaveric kidney allograft—a single-center analysis of 1112 cases

Received: 12 November 2002
Revised: 18 July 2003
Accepted: 4 August 2003
Published online: 6 November 2003

M. Lao · L. Paczek
Transplantation Institute, The Medical University of Warsaw, Warsaw, Poland

Z. Lewandowski ·
Department of Epidemiology, The Medical University of Warsaw, Warsaw, Poland

K. Lietz · Z. Gaciong (✉)
Department of Internal Medicine and Hypertension, The Medical University of Warsaw, 1A Banacha Street, 02-097, Warsaw, Poland
E-mail: zgaciong@amwaw.edu.pl
Tel.: +48-22-6597506
Fax: +48-22-6593373

Abstract This retrospective series reviews risk factors for chronic allograft nephropathy (CAN) based on the 10-year experience of a single institution. One thousand one hundred and twelve primary cadaveric renal transplant recipients whose graft survived for more than 6 months were followed for a mean of 4.6 years. The data were analyzed using the multivariate Cox proportional hazards model. CAN was defined as an irreversible rise of serum creatinine (SCr) by 30% in the absence of other causes and occurred in 42% of the patients. The risk of CAN was significantly increased in patients who experienced late rejections. Recipients of organs from donors that were older than 50 years and from such who died secondary to cerebrovascular accident were at increased risk of incurring CAN. Early markers of progression to CAN found at 6 months after transplantation included SCr levels of greater than 1.8 mg/ml, proteinuria, hypoalbuminemia, and hypertension. In conclusion, immune and non-immune factors affect progression to CAN in renal allograft recipients.

Keywords Chronic allograft nephropathy · Transplantation

Introduction

The development of new treatment modalities and better patient selection have remarkably improved early results in kidney transplantation, but showed little impact on late graft loss, which has remained nearly the same for the last 20 years [1]. Chronic allograft nephropathy (CAN) has become the most common cause of late graft dysfunction (LGD) and, eventually, renal allograft loss beyond the first year after transplantation. Despite significant improvements in patient care and the introduction of new, more potent immunosuppressive drugs, the incidence of CAN has remained unchanged.

CAN results from a combination of chronic immunological process induced by histoincompatibility and superimposed non-antigenic mechanisms, such as drug toxicity, donation-induced damage, metabolic disturbances, and hemodynamic injury [2, 3]. The insult to the transplanted organ facilitates the action of cytokines, growth substances, and enzymes, which promote proliferative and remodeling processes leading to scarring of the kidney allograft and, ultimately, to end-stage allograft failure [4, 5]. A typical histopathological manifestation of CAN, such as tubular atrophy, interstitial fibrosis, and arterial intimal thickening, may develop in various states of disease and probably represents a common tissue response to injury caused by many factors. Hence, the term chronic allograft nephropathy (CAN) was introduced to replace the previously used term chronic rejection [3].

The issue of CAN has many clinical implications, and multiple clinical studies have sought potential risk factors for CAN [1, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. However, in most of them immunological and

non-immunological factors, which are mutually related and dependent, were analyzed separately. Our retrospective study includes a multivariate analysis of time to onset of first symptoms of CAN, comparing the relative importance of immunological and non-immunological factors in a group of recipients of primary cadaveric renal transplants over 10 years in a single center.

Patients and methods

Patient population

This retrospective analysis included 1112 of 1508 consecutive adult recipients of primary cadaveric renal allografts between January 1987 and January 1997 at the Warsaw Transplant Center whose allograft survived for at least 6 months. Patients who died ($n=29$) or returned to hemodialysis ($n=172$) before the sixth month after transplantation were excluded from analysis. Patients that received combined pancreatic-renal transplants who were followed after surgery by other centers as well as those with incomplete records for more than 6 months following surgery or such who were lost to follow-up were excluded from the study ($n=195$). All patients were followed until death, return to dialysis, or July 1, 1998.

Patient characteristics

All 1112 primary kidney allograft recipients were Caucasian, predominantly male (59%), and were a mean 37.9 ± 10.6 years of age. The cause of end-stage renal disease included chronic glomerulonephritis (69%), chronic pyelonephritis (8%), polycystic kidney disease (6%), diabetes (5%), and reflux nephropathy (1.5%). The mean time on dialysis prior to transplantation was 2.04 ± 1.7 years (range: 0–15.1 years). The kidneys were obtained from cadaveric donors, 72% of which were male, with a mean age of 35.9 ± 12.6 years and with traumatic brain death (60.1%) prevailing over cerebrovascular deaths (31.9%) and other causes (7.85%). The mean total ischemia time was 26.0 ± 9 h. The peak value for panel-reactive antibodies (PRA) was greater than 20% for 294 patients (26.7%); however, immediately before transplantation only 7% of the recipients showed a PRA value of greater than 20%. The mean degree of HLA mismatches was 1.26 ± 0.6 for the HLA-A locus, 1.20 ± 0.6 for the HLA-B locus, and 1.31 ± 0.6 for the HLA-DR locus. Pretransplant chronic anemia was treated with recombinant human erythropoietin (rHu-EPO) in 24% of the patients, 66% were continued on blood transfusion therapy until transplantation, and 10% did not require therapy. Delayed graft function was observed in 36.2% of the recipients.

Immunosuppressive regimens

A total of 771 patients transplanted after 1992 received a triple immunosuppressive regimen comprising cyclosporine (CyA), azathioprine (Aza), and prednisone (Pred), with the exception of 174 patients with pretransplant leukopenia and/or increased aminotransferase activity who were given the combination of Pred + CyA only. One hundred sixty-seven patients transplanted prior to 1992, when CyA was not widely available in our country, received the combination of Pred + Aza. CyA was given at an initial dose of 8 mg/kg per day, which was adjusted to maintain a whole blood trough level between 150 and 250 ng/ml (Imx, Abbott). Pred was given in tapering doses of 1–2 mg/kg per day immediately after the operation to 0.5 mg/kg per day at 3 months. Aza was given at an initial dose of 3 mg/kg per day and maintained at a dose of 1.0–1.5 mg/kg per day, adjusted to white blood cell and platelet counts. In addition, 70 high-risk recipients (i.e., diabetic nephropathy as primary cause of end-stage renal disease, sensitized with PRA > 50%, recipient age over 55 years) received i.v. OKT3 or antithymocyte globulin (ATG) for 7–14 days after transplantation. Treatment of acute rejection consisted of high-dose methylprednisolone (1000 mg daily bolus i.v. for 3–4 days). The non-responders were given Minnesota antilymphocyte globulin (ALG) or ATG (Upjohn) (10–15 mg/kg per day i.v. for 10–14 days) or OKT3 (5–10 mg/day i.v. for 10 days).

Clinical data

The following recipient pretransplant variables were analyzed: age, gender, major cause of end-stage renal disease, bilateral nephrectomy, duration of hemodialysis prior to transplantation, number of blood transfusions and administration of rHu-EPO, and PRA (peak reactivity and at the time of transplantation). Donor factors included: age, gender, cause of death, degree of HLA-A, -B, and -DR mismatches, and duration of ischemia time. The following variables were monitored at 3 and 6 months after transplantation: blood pressure, serum creatinine (SCr) and albumin concentration, and 24-h urine protein excretion. Delayed graft function (DGF) was defined as the need for hemodialysis within the first week following transplantation. The diagnosis of acute rejection was based on clinical symptoms and/or verified by biopsy according to the standard histological criteria [20].

Late graft dysfunction (LGD)

Late graft dysfunction was defined as a gradual and irreversible rise in SCr concentration. SCr measurements were obtained within the first week after transplantation, at 3 and 6 months, and then annually at years 1–9. The year at which the SCr level irreversibly increased by 30% from the base level found at 3 months after transplantation was used as end-point for survival analysis. Irreversibility was determined if the rise in SCr was maintained for at least 1 year. If the rise in SCr to above 30% of the baseline was noted in the last year of the follow-up and it was not certain whether the increase was temporary, then the record was censored at the previous observation year for statistical purposes.

Chronic allograft nephropathy (CAN)

A subgroup of patients with LGD related to CAN was identified. Renal graft biopsies were performed in 52% ($n=555$) of the patients with worsening renal function. In these patients the presence of CAN was defined histologically based on standard criteria [5], and they were assigned to either the CAN group or censored from the study if other causes of LGD were present (i.e., recurrence of disease, late acute rejection, etc.). The remaining patients with LGD who declined or who were not offered biopsy or for whom a representative specimen could not be obtained were evaluated clinically using laboratory findings and radiological/ultrasound assessment. Patients with evidence of other causes of LGD (i.e., renal artery stenosis, etc.) were censored from the study.

Since CyA nephrotoxicity is difficult to distinguish from CAN clinically and histologically, patients with suspected irreversible CyA nephrotoxicity were included in the CAN group. Patients with suspected temporary CyA nephrotoxicity, defined by a temporary increase in SCr without histological evidence of rejection and/or return of SCr to previous levels upon CyA dose reduction, were censored from the study.

Statistical analysis

Differences between groups were examined using Student's t-test, χ^2-test, and the log-rank method for comparing Kaplan-Meier actuarial survival plots [20]. The risk factors for development of CAN were examined by means of univariate and multivariate Cox' proportional hazards model [21]. The covariates, which correlated with end-points on univariate analysis ($P < 0.15$), were entered into the multivariate Cox' analysis model. Results were considered significant for P values below 0.05. Values are reported as mean ± SD. The clinical information was analyzed using SAS statistical software, version 7.0 (SAS Institute).

Results

Patient and graft survival

The overall 1-, 5-, and 10-year graft survival at our center was 86.1%, 63.7%, and 50% ($n = 1508$). Among the selected group of 1112 patients whose graft survived for 6 months, the respective graft survival rates were 97.3%, 72.4%, and 55%, with a mean follow-up of 4.56 ± 2.5 years (range: 0.5–10 years). Beyond 6 months after transplantation, 291 (26%) of these patients returned to dialysis and 80 (7%) died with a functioning graft.

The incidence of CAN and its influence on patient survival

AN was diagnosed clinically in 467 patients (42%), of whom half ($n = 233$) had a biopsy performed that revealed histological evidence of CAN. The median time to the first clinical symptoms of CAN was 2.67 years. As shown in Fig. 1, the development of CAN correlated with decreased recipient survival after transplantation. Among patients whose graft survived for 6 months, the 5-year recipient survival was 89% in the CAN group and 94% for those who did not develop CAN (log-rank test, $P = 0.005$).

Acute rejection increases the risk of CAN

Episodes of acute rejection constitute the strongest independent risk factor for accelerated progression to CAN (Table 1). Sixty-two percent of the patients (686/

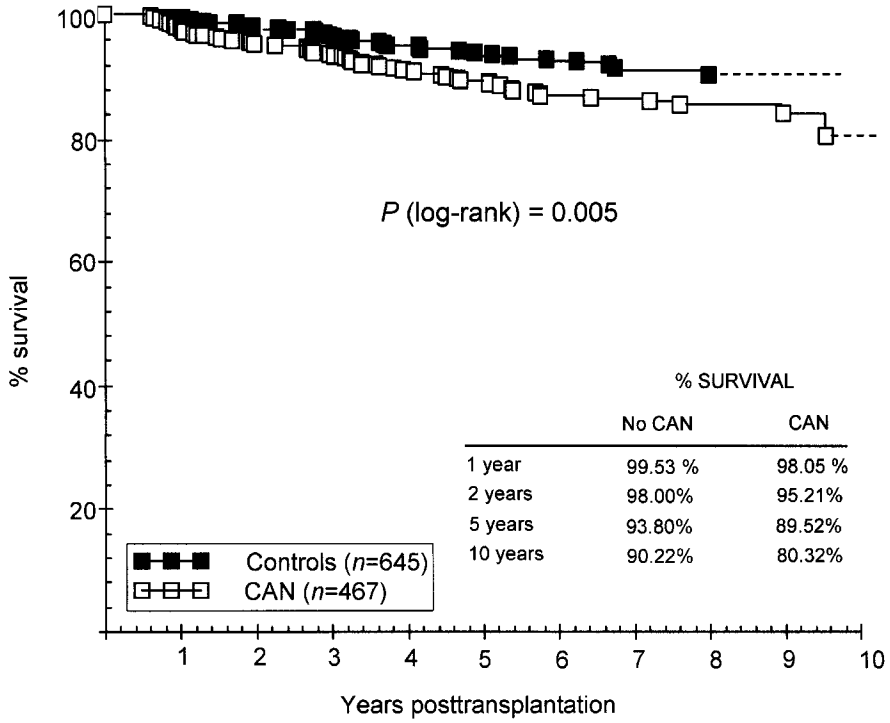

Fig. 1 Progression to chronic allograft nephropathy (*CAN*) correlates with decreased recipient survival after kidney transplantation (log-rank test, $P = 0.005$)

Table 1 Relationship between early and late recurrent episodes of acute rejection and progression to chronic allograft nephropathy (*RR* risk ratio, *CI* confidence interval)

Acute rejection episodes	RR	CI	P-value
No rejection	1	–	–
Before 3 months after transplantation only	1.14	0.865–1.510	0.35
After 3 months after transplantation only	3.10	2.368–4.072	< 0.001
Before and after 3 months after transplantation	3.60	2.814–4.607	< 0.001

1112) experienced at least one episode of acute rejection. Over half of the rejectors (53%) eventually developed CAN, in contrast to only 25% of those who did not reject ($P < 0.001$). Late rejections indicate a more than three times higher risk of CAN than rejections occurring within the first 3 months after transplantation, as illustrated in Fig. 2. Recurrent episodes in both the early and late postoperative period correlate with CAN; however, repetitive rejections beyond the third month after transplantation markedly increased the risk of CAN by the factor of 2.6, 3.3, and 5.5, respectively, for one, two, and more than two rejections (Table 2).

The influence of immunosuppressive regimen on CAN

In the studied population, initial immunosuppression consisted of a triple-drug (Pred + CyA + Aza, $n = 771$, 69%) or double-drug regimen (Pred + Aza, $n = 167$, 15%; and Pred + CyA, $n = 174$, 16%). Within 6 months after transplantation, 18% (212/1112) of the recipients required modification of the initial therapy. Ultimately, at 6 months after transplantation, 778 (70%) recipients were placed on triple-drug maintenance therapy, 93 (8%) were treated with Pred + Aza, and 241 (22%) received Pred + CyA only.

According to the results of multivariate analysis, neither the initial immunosuppressive protocol nor the three basic regimens maintained continuously for 3 and 6 months after transplantation were shown to be independent risk factors for CAN. In univariate analysis only, CyA-based therapy (Pred + Aza + CyA and Pred + CyA) maintained for at least 3 months after transplantation delayed the onset of CAN and was superior to Aza-based protocols (RR = 1.35, $P = 0.04$), as shown in Fig. 3. Furthermore, in patients who received a CyA-based regimen for the first 3 months after transplantation, the cumulative rejection frequency within that period declined to 0.63, in comparison to 0.94 in the Aza group ($P < 0.001$).

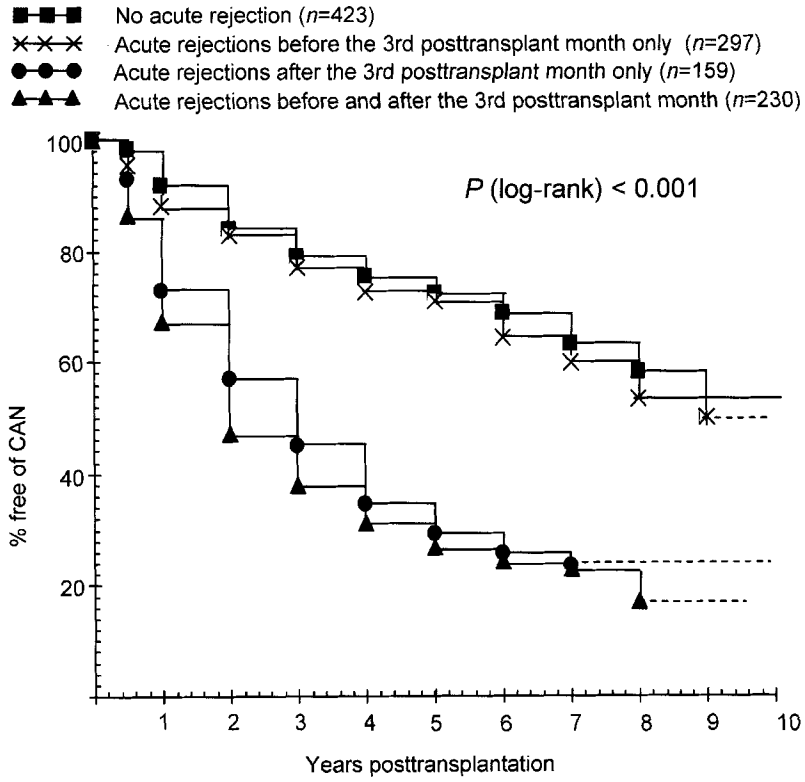

Fig. 2 Late episodes of acute rejection increase the risk of progression to chronic allograft nephropathy (*CAN*) in contrast to early episodes of acute rejection

Table 2 Relationship between number of acute rejection episodes and progression to chronic allograft nephropathy (*RR* risk ratio, *CI* confidence interval)

No. of acute rejection episodes	Before 3 months after transplantation, RR (CI)	*P*-value	After 3 months after transplantation, RR (CI)	*P*-value
0	1	–	1	–
1	1.18 (0.96–1.45)	0.12	2.56 (2.04–3.23)	< 0.001
2	1.68 (1.28–2.19)	< 0.001	3.29 (2.56–4.28)	< 0.001
> 2	1.75 (1.16–2.65)	0.008	5.50 (4.13–7.33)	< 0.001

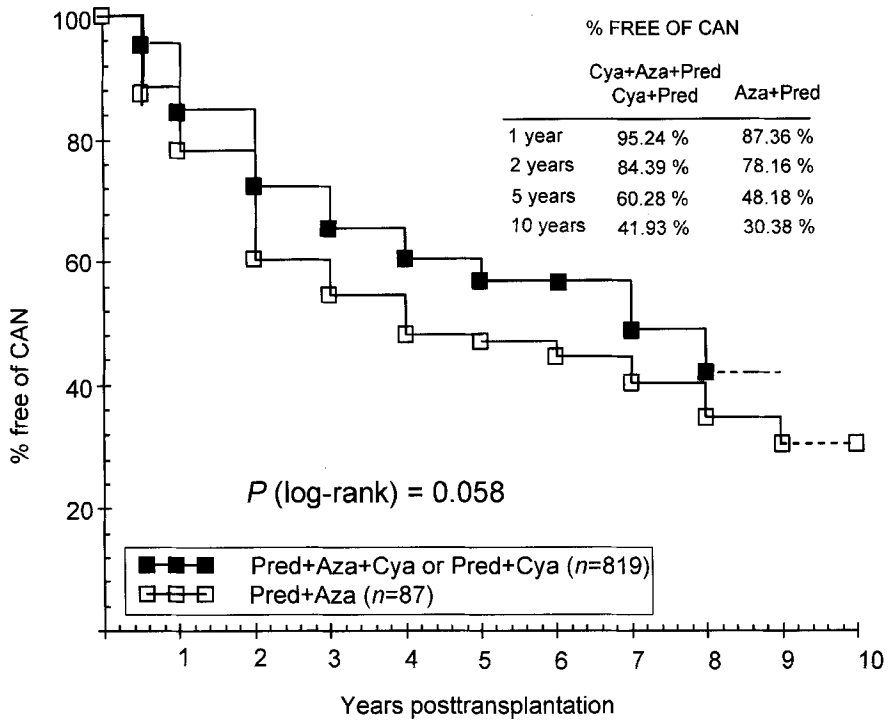

Fig. 3 Cyclosporine-based immunosuppressive regimens (prednisone [*Pred*] + azathioprine [*Aza*] + cyclosporine [*CyA*] and prednisone + cyclosporine) maintained for at least 3 months after transplantation are superior to azathioprine-based protocols in preventing the development of chronic allograft nephropathy (*CAN*) (log-rank test, $P = 0.058$)

Table 3 Comparison of dosage of immunosuppressive drugs in the population of 684 patients who received prednisone + cyclosporine + azathioprine maintenance therapy for at least 6 months after transplantation (*CAN* chronic allograft nephropathy)

Time after transplantation	Prednisone (mg/day, mean + SD)			Azathioprine (mg/day, mean + SD)			Cyclosporine (mg/day, mean + SD)		
	No CAN ($n = 418$)	CAN ($n = 266$)	*P*	No CAN ($n = 418$)	CAN ($n = 266$)	*P*	No CAN ($n = 418$)	CAN ($n = 266$)	*P*
0	31.5 ± 6.6	31.5 ± 7.2	–	133 ± 64	141 ± 47	–	372 ± 109	355 ± 94	*
3 months	16.4 ± 5.5	17.3 ± 5.7	*	95 ± 34	99 ± 45	–	245 ± 74	233 ± 72	*
6 months	13.1 ± 4.6	13.9 ± 4.8	*	97 ± 35	100 ± 40	–	224 ± 64	215 ± 61	*
12 months	11.6 ± 5.2	13.0 ± 5.2	**	92 ± 38	95 ± 42	–	206 ± 65	191 ± 65	**

*$P < 0.05$, **$P < 0.001$

Table 4 Relationship between median doses of cyclosporine (*CyA*) and frequency of acute rejection episodes in the population of 684 patients who received prednisone + cyclosporine + azathioprine maintenance therapy for at least 6 months after transplantation

Months after transplantation	Median CyA dose (mg/day)	Frequency of rejections	*P*-value
0–3	< 250	0.67	–
	> 250	0.57	0.05
3–6	< 200	0.13	–
	> 200	0.09	0.06
6–12	< 200	0.22	–
	> 200	0.15	0.02

In order to further investigate the potential effects of CyA-based therapy, a group of 684 patients was identified that had been consistently treated with the triple-drug protocol for at least 6 months after transplantation. The mean dosage of Pred, Aza, and CyA was defined at 0, 3, 6, and 12 months after transplantation, and the mean values were compared between the groups that developed CAN. The results shown in Table 3 indicate that recipients who were at risk of CAN received lower doses of CyA ($P < 0.001$) and higher doses of Pred ($P = 0.04$) throughout the first year after transplantation. Lower median doses of CyA at 3, 6, and 12 months after transplantation also correlated with higher frequency of acute rejection episodes experienced in the respective time periods (Table 4).

Pretransplant risk factors for CAN

The following pretransplant risk factors were found to be significant by multivariate analysis using the Cox proportional hazards model for time to CAN: recipient age greater than 50.1 years (RR = 0.598, $P = 0.009$), treatment of chronic anemia with rHu-EPO (RR = 0.57, $P = 0.001$), donor age greater than 50.1 years (RR = 1.69, $P < 0.001$), and donor death due to cerebrovascular accident (RR = 1.37, $P = 0.05$) (Table 5). Pretransplant factors that were not found to be significant included etiology of end-stage renal disease (glomerulonephritis, diabetes mellitus, polycystic kidney disease), bilateral nephrectomy, years of hemodialysis prior to transplantation, number of blood transfusions, DGF, and total ischemia time greater than 24 h. Among the immunological risk factors, neither a peak PRA value of more than 20% nor a complete mismatch at the HLA-A, -B, and -DR loci were independent risk factors for CAN. In univariate analysis only higher degrees of mismatch in the HLA-A, -B, and -DR loci were associated with an increased risk of CAN (three mismatches: RR = 1.4, $P = 0.11$; four mismatches: RR = 1.64, $P = 0.01$; five mismatches: RR = 1.66, $P = 0.02$; and complete mismatch: RR = 1.93, $P = 0.02$).

Ischemia time

As mentioned previously, total ischemia time, which mainly comprises cold ischemia time, did not influence the risk of CAN in the studied population. In order to investigate whether warm ischemia time had any impact on the development of CAN, an analysis of actuarial survival was performed in the group of 745 patients with available data. As shown in Fig. 4, the harvesting of kidneys from heart-beating donors protects against CAN (log-rank test, $P = 0.001$). No correlation was found between CAN and cold ischemia time (CIT) of greater than 24 h or warm anastomosis time (WIT2) of greater than 30 min.

Table 5 Multivariate analysis of pretransplant risk factors for progression to chronic allograft nephropathy (*RR* risk ratio, *CI* confidence interval, *rHu-EPO* recombinant human erythropoietin)

	RR	CI	P
Recipient age > 50.1 years	0.598	0.406–0.883	0.009
Pretransplant administration of rHu-EPO	0.575	0.410–0.806	0.001
Donor age > 50.1 years	1.695	1.279–2.247	< 0.001
Donor death: cerebrovascular accident vs trauma	1.376	0.995–1.903	0.053

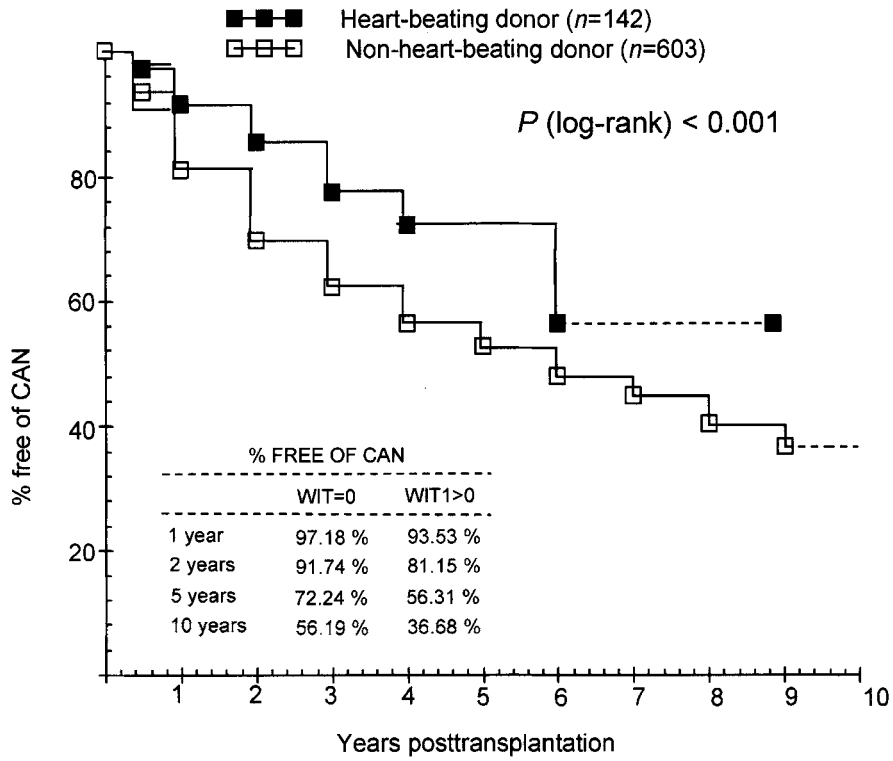

Fig. 4 Harvesting of kidneys from heart-beating donors (warm ischemia time [*WIT*] = 0) protects against chronic allograft nephropathy (*CAN*) (log-rank test, $P = 0.001$)

Table 6 Univariate analysis of early posttransplant predictors of chronic allograft nephropathy ($n = 1112$) (*RR* risk ratio, *CI* confidence interval)

Posttransplant marker	(%)	3 months after transplantation, RR (CI)	*P*-value	(%)	6 months after transplantation, RR (CI)	*P*-value
Serum creatinine (mg/dl)						
< 1.2	29	1	–	28	1	–
1.2–1.8	49	1.45 (1.15–1.82)	0.002	46	1.45 (1.13–1.87)	0.004
> 1.8	22	2.23 (1.72–2.90)	< 0.001	26	3.36 (2.60–4.35)	< 0.001
Proteinuria	34	1.03 (0.77–1.37)	0.84	29	1.54 (1.15–2.08)	0.004
Hypoalbuminemia	13	1.30 (0.94–1.81)	0.11	9	1.43 (0.96–2.14)	0.08
Hypertension	59	0.98 (0.82–1.19)	0.89	39	1.18 (0.977–1.43)	0.08

Early posttransplant markers of CAN

We next sought to investigate the prognostic value of early postoperative markers of CAN, such as SCr level, proteinuria (urinary protein > 0.5 g/day), hypoalbuminemia (serum albumin concentration < 3.5 g/dl), and hypertension requiring therapy with at least two antihypertensive agents. Univariate analysis of these parameters at 3 and 6 months after transplantation was performed (Table 6). Three months after transplantation, only the SCr concentration was predictive of CAN, with levels of 1.2–1.8 mg/dl indicating a 1.45-fold higher risk of CAN and levels exceeding 1.8 mg/dl indicating a 2.2-fold higher risk ($P = 0.009$ and $P < 0.001$, respectively). At 6 months after transplantation, all of the above factors were predictive of CAN: SCr levels of 1.2–1.8 mg/dl (RR = 1.45, $P = 0.004$) and over 1.8 mg/dl (RR = 3.36, $P < 0.001$), proteinuria (RR = 1.54, $P = 0.004$), hypoalbuminemia (RR = 1.43, $P = 0.08$), and hypertension (RR = 1.18, $P = 0.08$). Hypoalbuminemia was present in 13% of the renal transplant recipients at 6 months after transplantation, and serum albumin levels inversely correlated with the amount of urinary protein excretion (correlation coefficient = –0.18, $P < 0.001$). Fifty percent of the patients with hypoalbuminemia had proteinuria, as opposed to 26% of the patients with normal albumin levels ($P = 0.03$).

Discussion

Of 1112 renal allograft recipients studied in the present series whose graft survived for at least 6 months after transplantation, 42% developed clinical symptoms of CAN within a median time of 2.7 years after surgery. The study used standard criteria for the clinical diagnosis of late graft dysfunction, as assessed by an irreversible increase in SCr concentration in relation to the SCr level at 3 months after transplantation [1]. The definitive diagnosis of CAN was based on typical histopathological findings, including tubular atrophy, interstitial fibrosis, and fibrous intimal thickening, and graded according to the Banff classification [5]. Histological evaluation was available for 52% of the studied population. In the remaining patients, clinical symptoms, laboratory findings, and radiological/ultrasound assessment were used to identify other causes of late graft dysfunction.

The process of CAN inevitably leads to graft loss [6], but the rate of progression to complete graft insufficiency varies with the individual. In order to assess the dynamics of progression to CAN, the primary end-point in our statistical analysis was the time to onset of CAN symptoms.

The most consistently cited immunological predictor of CAN and late renal allograft loss is the history of acute rejection episodes. A single episode of acute rejection occurring early after transplantation either does not affect progression to CAN [11] or increases the risk only slightly [12]; however, repeated, late, or vascular rejection episodes signify the greatest risk of adverse outcomes [7, 8, 9, 10, 13]. This was confirmed in our series, where a single episode of early acute rejection did not affect progression to CAN, in contrast to a three times higher risk for even one rejection episode occurring beyond the third month after transplantation (Fig. 1). Similarly, recurrent episodes in the early postoperative period contributed only modestly to CAN (RR = 1.7), while recurrent rejections beyond the third month after transplantation indicated a significant risk of CAN, namely 2.6, 3.3, and 5.5 for one, two, and more than two rejections, respectively (Table 2).

Although acute rejection constitutes a predictor of CAN, the attempt to reduce recipient alloreactivity by means of efficient immunosuppression, better donor/recipient HLA matching [13, 14], and lower sensitization with anti-HLA antibodies prior to transplantation [12] has not been found to be an important determinant of CAN. In our study, the degree of HLA-A, -B, and -DR mismatch and a pretransplant PRA value of greater than 20% did not contribute to accelerated CAN. It is possible that the lack of association between pretransplant sensitization with anti-HLA antibodies and progression to CAN may result from an immunomodulatory effect of mono- or polyclonal antilymphocyte serum induction administered in some of the high-risk patients (PRA > 50%).

Minimizing the risk of acute rejection remains an important approach to prevent CAN. However, the triple immunosupressive regimen containing CyA that

was shown to effectively reduce acute rejection particularly within the first months after surgery was paradoxically not reported to affect the progression of CAN [12, 15]. Analogously, our study revealed that the initial protocols with Pred + Aza increased the risk of CAN only modestly (1.3-fold), as compared to CyA-containing regimens (Pred + Aza + CyA and Pred + CyA), and the results were confined to univariate analysis only.

Nevertheless, the importance of achieving and maintaining sufficient and consistent levels of immunosuppression cannot be discounted in preventing CAN. Previous investigations have shown that higher CyA or tacrolimus levels may protect against immune injury and, thereby, CAN [16, 17, 18]. In our study of a selected group of 684 patients treated consistently with CyA for at least 6 months after surgery, the recipients of higher doses of CyA experienced a lower frequency of acute rejection both in the early (< 3 months) and late (3–12 months) period after transplantation. Moreover, treatment with higher doses of CyA in the early posttransplant period was characteristic of patients who did not develop clinical symptoms of CAN. Conversely, patients treated with initially lower doses of CyA were at increased risk of acute rejection and CAN. Also, the latter group was noted to have received higher average doses of steroids, likely reflecting the adjustment of steroid therapy required to treat more frequent rejections in this group. It is possible that higher doses of steroids might later contribute to metabolic complications in these patients and independently affect the progression of CAN.

Although higher doses of CyA seem beneficial, several studies on administration versus withdrawal of calcineurin inhibitors point to a tenuous balance between the adequate and toxic effects of these drugs [19, 22]. CyA and tacrolimus toxicity, particularly in the early posttransplant period, was reported to increase the risk of CAN [17, 18]. In addition, poor patient compliance with the immunosuppressive medication may play an important role in the development of CAN; however, our data were insufficient to allow meaningful conclusions.

The lack of association with immune factors traditionally known to predispose to acute rejection and the minimal role of CyA-based immunosuppressive regimens indicate that other nonimmunological processes may be involved in the progression to CAN. There are two major categories of donor antigen-independent factors: pretransplant factors that affect initial renal function (e.g., donor/recipient age, pretransplant hypertension or vascular disease, brain death, ischemia and reperfusion, donor/recipient size mismatch) and such occurring after transplantation (e.g., hypertension, lipid disorders, drug toxicity, and recurrent disease).

The negative influence of nonimmune parameters on graft function concentrates on either reduction of number of nephrons or direct injury to the tissue [23]. Certain donor variables may all influence the initial number of nephrons in a donor kidney and participate in the development of CAN, such as older donor age [24, 25], hypertension [26, 27], and disproportion between metabolic demands of the recipient and physiological capabilities of the donor kidney [26, 27]. In our study, organs obtained from donors that were older than 50 years were at a 1.7-fold higher risk of incurring CAN.

In cadaveric organ recipients, injuries contracted during the transplantation process seem to be the major factor affecting transplant outcome adversely. This is evidenced by inferior survival rates of cadaveric kidneys as compared to organs from living donors [28]. The biological consequences of brain death and their impact on the transplant organ are profound and not well understood. During brain death a variety of inflammatory mediators and acute phase proteins [29] are released, which may affect the transplanted organ. Increased sympathetic system activity and production of catecholamines may even contribute to local hypoperfusion of the organ in an otherwise normotensive environment [30, 31, 32]. The cause of death appears relevant in that organs from donors who died of cerebrovascular accident [33] develop CAN sooner than such from donors who died of trauma. In our series, donor death due to cerebrovascular accident increased the risk of CAN 1.4-fold.

Ischemia/reperfusion injury during the procurement of cadaveric kidneys is a serious factor implicated not only to increase the incidence of DGF or primary nonfunction [34, 35], but also to affect long-term outcome of the transplant [36]. Ischemia was demonstrated to stimulate rapid infiltration of the host leukocyte population with up-regulation of inflammatory mediators [37] and MHC class II expression resulting in increased immunogenicity of the organ [38, 39]. In rat models, brain death and reperfusion injury was associated with more rapid rejections and accelerated CAN [39, 40, 41]. In the current series, prolonged cold ischemia time did not correlate with CAN; however, harvesting kidneys from non-heart-beating donors substantially accelerated progression to CAN (Fig. 4).

Among the nonimmune pretransplant recipient factors, such as recipient demographics, etiology of end-stage renal disease, years of hemodialysis prior to transplantation, and pretransplant blood transfusions, only recipient age of more than 50 years and administration of rHu-EPO were found to correlate with CAN. Reports on the association between older recipient age and CAN are inconsistent [42, 43]. In our study older recipient age appeared to be protective against CAN. This may be explained by the naturally occurring senescence of T-cell function with secondarily reduced alloreactivity, as reported in heart and renal transplant recipients [43, 44], but is more likely the effect of

administration of monoclonal anti-T-cell antibodies to all recipients older than 50 years, who constituted a high-risk group of patients. Pretransplant hypertension, a commonly cited recipient pretransplant risk factor for progression to CAN, was not found to be a significant predictor of CAN.

In our study, pretransplant treatment of chronic anemia with rHu-EPO significantly detained the progression of CAN. This is consistent with the previous study by Vella et al. that investigated the long-term effects of pretransplant rHu-EPO, in which the projected half-life of renal allografts that survived for 1 year increased from 14.8 years in controls to 19.8 years in rHu-EPO-treated patients [45]. There are several reports that de novo therapy with rHu-EPO or conversion from blood transfusion to rHu-EPO therapy decreases the risk of allostimulation with repeated transfusions and reduces the degree of allosensitization in patients undergoing transplantation [45, 46, 47]. Several other immunomodulatory properties have been postulated to reduce host alloreactivity in rHu-EPO-treated patients, such as direct suppression of T- and B-cell clonal proliferation [47, 48, 49, 50, 51, 52] and induction of anti-idiotypic anti-HLA antibodies [53]. However, it has not been determined whether these properties of rHu-EPO can affect the propensity to develop CAN. In our study, due to the shortage in rHu-EPO availability in the 1980 s, only 24% of the patients requiring treatment of chronic anemia were switched from blood transfusion therapy to rHu-EPO. Although the selection of patients who received rHu-EPO was conducted randomly, it may have been biased and, as in all retrospective analyses, these results must be interpreted with caution.

Among early posttransplant antigen-independent factors, delayed allograft function immediately after transplantation was demonstrated to be associated with poorer graft survival [54]. In our series delayed graft function, formerly defined as the need for dialysis after transplantation, did not correlate with progression to CAN. However, the level of SCr as early as 3 and 6 months after transplantation, at which time graft function is thought to be stable, was a sensitive marker of long-term deterioration of renal allograft function. The cut-off value for SCr concentration of greater than 1.2 mg/dl was found to correlate with progression to CAN.

Hypertension, proteinuria, hypoalbuminemia, and hyperlipidemia are the most often cited posttransplant metabolic factors associated with poor graft survival [55]. In our study hypertension, proteinuria, and hypoalbuminemia as early as 6 months after transplantation increased the relative risk of CAN (Fig. 4). The metabolic risk factors may reflect both the cause and the consequences of progressive CAN. For instance, risk factors of CAN such as proteinuria and hypoalbuminemia most likely reflect nephrosis in patients with CAN. Unwanted side effects of immunosuppression may also cause metabolic complications, accelerate the progression of CAN, and increase patient mortality from cardiovascular disease [56, 57]. In our study, patients with CAN exhibited inferior long-term survival, indicating that the metabolic risk factors associated with CAN may contribute to an increased risk of cardiovascular complications in these patients. This study contains limitations associated with a retrospectively performed study and should therefore be interpreted with caution.

In conclusion, (i) progression to CAN depends on the combination of host alloreactivity and donor antigen-independent factors, of which many depend on donor/recipient selection, (ii) the new immunosuppressive agents such as CyA fail to alter progression to CAN despite reducing the rate of acute rejection, and (iii) certain metabolic parameters estimated as early as 3 and 6 months after transplantation accelerate the development of CAN and may affect patient survival.

References

1. Cecka M. Clinical outcome of renal transplantation. Factors influencing patient and graft survival. Surg Clin North Am 1998; 78:133.
2. Paul LC. Chronic allograft nephropathy: an update. Kidney Int 1999; 56:783.
3. Fellström BC, Larsson E. Pathogenesis and treatment perspectives of chronic graft rejection (CGR). Immunol Rev 1993; 134:83.
4. Häyry P. Chronic rejection: an update on the mechanism. Transplant Proc 1998; 30:3993.
5. Solez K, Axelsen RA, Benediktsson H, et al. International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. Kidney Int 1993; 44:411.
6. Modena FM, Hostetter TH, Salahudeen AK, Najarian JS, Matas A, Rosenberg ME. Progression of kidney disease in chronic renal transplant rejection. Transplantation 1991; 52:239.
7. Koyama H, Cecka JM. Rejection episodes. Clin Transpl 1992: 391.
8. Matas AM, Gillingham KJ, Payne WD, Najarian JS. The impact of an acute rejection episode on long-term renal allograft survival ($t_{1/2}$). Transplantation 1994; 57:857.
9. Basadonna GP, Matas AJ, Gillingham KJ, et al. Early versus late acute renal allograft rejection: impact on chronic rejection. Transplantation 1993; 55:993.
10. Tejani A, Cortes L, Stablein D. Clinical correlates of chronic rejection in pediatric renal transplantation. A report of the North American Pediatric Renal Transplant Cooperative Study. Transplantation 1996; 61:1054.

11. Isoniemi H, Kyllönen L, Eklund B, et al. Acute rejection under triple immunosuppressive therapy does not increase the risk of late first cadaveric renal allograft loss. Transplant Proc 1995; 27:875.
12. Massy ZA, Guijarro C, Wiederkehr MR, Ma JZ, Bertram LK. Chronic renal allograft rejection: immunologic and nonimmunologic risk factors. Kindey Int 1996; 49: 518.
13. Almond PS, Matas A, Gillingham K, et al. Risk factors for chronic rejection in renal allograft recipients. Transplantation 1993; 55: 752.
14. Held PJ, Kahan BD, Hunsicker LG, et al. The impact of HLA mismatches on the survival of first dcadaveric kidney transplants. N Engl J Med 1994; 331: 765.
15. Kahan BD. Cyclosporine: a base for therapy present and future. Transplant Proc 1993; 25: 508.
16. Salomon DR. An alternative view minimizing the significance of cyclosporine nephrotoxicity and in favor of enhanced immunosuppression for long-term kidney transplant recipients. Transplant Proc 1991; 23: 2115.
17. Nickerson P, Jeffery J, Gough J, et al. Identification of clinical and histopathologic risk factors for diminished renal function 2 years posttransplant. J Am Soc Nephrol 1998; 9:482.
18. Isoniemi HM, Ahonen J, Tikkanen MJ, et al. Long-term consequences of different immunosuppressive regimens for renal allografts. Transplantation 1993; 55:494.
19. Thiel G, Bock A, Spondlin M, et al. Long-term benefits and risks of cyclosporin A (Sandimmun): an analysis at 10 years. Transplant Proc 1994; 26:2493.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53:457.
21. Cox DR. Regression models and life tables (with discussion) J R Stat Soc 1972; B34:187.
22. Beckingham IJ, O'Rourke JS, Stubington SR, Hinwood M, Bishop MC, Rigg KM. Impact of cyclosporin on the incidence and prevalence of chronic rejection in renal transplants. Ann R Coll Surg Engl 1997; 79:138.
23. Brenner BM, Cohen RA, Milford EL. In renal transplantation, one size may not fit all. J Am Soc Nephrol 1992; 3: 162.
24. Kim HC, Suk J, Joo I, Park SB, Cho WH, Park CH. Risk factors for chronic rejection in renal allograft recipients. Transplant Proc 1999; 28:1456.
25. Längle F, Sautner T, Grünberger T, et al. Impact of donor age on graft function in living-related kidney transplantation. Transplant Proc 1992; 24: 2725.
26. Hostetter TH. Chronic transplant rejection. Kidney Int 1994; 46: 266.
27. Lee LS, Auersvald LA, Claus EB, et al. Body size mismatch between donor and recipient and the development of chronic rejection in renal transplantation. Transplant Proc 1997; 29:111.
28. Cecka JM. The UNOS Scientific Renal Transplant Registry. Clin Transpl 1996: 1.
29. Takada M, Nadeau KC, Hancock WW, et al. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Transplantation 1998; 65:1533.
30. Power BM, van Heerden PV. The physiological changes associated with brain death: current concepts and implications for the treatment of the brain-dead donor. Anaesth Intensive Care 1995; 23:26.
31. Herijgers P, Leunens V, Tjandra-Maga TB, Mubagwa K, Flameng W. Changes in organ perfusion after brain death in the rat and its relation to circulating catecholamines. Transplantation 1996; 62:330.
32. Mertes PM. Physiology of brain death. In: Tilney NL, Strom TB, Paul LC, eds. Transplantation biology: cellular and molecular aspects. Philadelphia, Lippincott, 1996: 275.
33. Gjertson DW. A multi-factor analysis of kidney graft outcomes at one and five years posttransplantation: 1996 UNOS update. Clin Transpl 1996: 343.
34. Lagiewska B, Pacholczyk M, Szostek M, Walaszewski J, Rowinski W. Hemodynamic and metabolic disturbances observed in brain-dead organ donors. Transplant Proc 1996; 28:165.
35. Tilney NL, Guttman RD. Effects of initial ischemia/reperfusion injury on the transplanted kidney. Transplantation 1997; 64:945.
36. Koo DD, Welsh KI, McLaren AJ, Roake JA, Morris PJ, Fuggle SV. Cadaver versus living donor kidneys: impact of donor factors on antigen induction before transplantation. Kidney Int 1999; 56:1551.
37. Takada M, Nadeau KC, Shaw GD, Marquette KA, Tilney NL. The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. J Clin Invest 1997; 99:2682.
38. Kusaka M, Pratschke J, Wilhelm MJ, et al. Activation of proinflammatory mediators in rat renal isografts by donor brain death. Transplantation 2000; 69:405.
39. Pratschke J, Wilhelm MJ, Kusaka M, et al. Accelerated rejection of rat renal allografts from brain-dead donors. Ann Surg 2000; 232:263.
40. Wilhelm MJ, Pratschke J, Beato F, et al. Activation of the heart by donor brain death accelerates acute rejection after transplantation. Circulation 2000; 102:2426.
41. Pratschke J, Markus JW, Laskowski I, et al. Influence of donor brain death on chronic rejection of renal transplants in rats. J Am Soc Nephrol 2001; 12:2474.
42. Meier-Kriesche HU, Ojo AO, Cibrik DM, et al. Relationship of recipient age and development of chronic allograft failure. Transplantation 2000; 70:306.
43. Cameron JS. Renal transplantation in the elderly. Int Urol Nephrol 2000; 32:193.
44. John R, Lietz K, Schuster M, et al. Older recipient age is associated with reduced alloreactivity and graft rejection after cardiac transplantation. J Heart Lung Transplant 2001; 20:212.
45. Vella JP, O'Neill D, Atkins N, Donohoe JF, Walshe JJ. Sensitization to human leukocyte antigen before and after the introduction of erythropoietin. Nephrol Dial Transplant 1998; 13:2027.
46. Friedlaender MM, Azem O, Rubinger D, Brautbar C. Effect of human recombinant erythropoeitin therapy on panel-reactive antibodies in chronic dialysis patients. Isr J Med Sci 1996; 32: 730.
47. Pfaffl W, Gross JH, Neumeier D, Nattermann U, Samtleben W, Gurland HJ. Lymphocyte subsets and delayed cutaneous hypersensitivity in hemodialysis patients receiving recombinant human erythropoetin. Contrib Nephrol 1988; 66:195.
48. Imiela J, Korczak-Kowalska G, Malecki R, Nowaczyk M, Stepien-Sopniewska B, Gorski A. Immunomodulatory action of human recombinant erythropoietin in man. Immunol Lett 1993; 35:271.
49. Kimball PM, Kerman RH. Erythropoietin: a potential immunomodulator? Transplant Proc 1991; 23: 336.
50. Kimata H, Yoshida A, Ishioka C, Masuda S, Sasaki R, Mikawa H. Human recombinant erythropoietin directly stimulates B cell immunoglobulin production and proliferation in serum free medium. Clin Exp Immunol 1991; 85: 151.
51. Steffensen G, Aunsholt NA, Povlsen JV. Evidence that treatment of ESRD patients with recombinant human erythropoietin induces immunosuppression without affecting the distribution of peripheral blood mononuclear cell subpopulations. Clin Nephrol 1996; 45: 98.

52. Grimm PC, Sinai-Trieman L, Sekiya NM, et al. Effects of recombinant human erythropoietin on HLA sensitization and cell-mediated immunity. Kidney Int 1990; 38: 12.
53. Grimm PC, Sekiya NM, Robertson LS, Robinson BJ, Ettenger RB. Recombinant human erythropoietin decreases anti-HLA sensitivity and may improve renal allograft outcome: involvement of anti-idiotypic antibody. Transplant Proc 1991; 23:407.
54. Lechevallier E, Dussol B, Luccioni A, et al. Posttransplantation acute tubular necrosis: risk factors and implications for graft survival. Am J Kidney Dis 1998; 32:984.
55. Matas AJ, Gillingham KJ, Humar A, Dunn DL, Sutherland DER, Najarian JS. Immunologic and nonimmunologic risk factors: different risk factors for cadaver and living donor transplantation. Transplantation 2000; 69:54.
56. Massy ZA, Kasiske BL. Post-transplant hyperlipidemia: mechanisms and management. J Am Soc Nephrol 1996; 7:971.
57. Guijarro C, Massy ZA, Kasiske BL. Clinical correlation between renal allograft failure and hyperlipidemia. Kidney Int 1995; 48: S56.