Multivariable analysis of risk factors in renal transplantation

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CHAPTER 1

Introduction and Aim of this thesis

Introduction

The first human to human kidney transplant was performed in 1933 in Kiev by Voronoy (1). The graft failed after 4 days probably because of blood group ABO incompatibility. After this first attempt, many efforts were made in different centres worldwide but none proved to be successful. In France, in 1952 a mother donated a kidney to her son, the first living related transplantation (2). This graft functioned for 21 days but was then rejected. The first real success was in 1954 between 23-year-old monozygotic twins (3). From then on successes alternated with failures and success rates could be directly derived. Transplant immunology and knowledge about immuno suppression was developing rapidly in the following years and results improved further. Many factors are supposed to be involved in the success rate of renal transplantation. These can be subdivided into non-immunological and immunological factors. One example of the former is recipient age. Patient survival is frequently found to be worse in the elderly. However, most, but not all studies agree that graft survival of the elderly is equal to or better than that of the young (4-19). An explanation of the improved results with age could be that the elderly reject their graft less aggressively than young recipients. The results from kidney transplantation from elderly donors are found to be inferior to those from young donors, probably as a result of the decrease in the absolute number of functioning glomeruli with age (20-23). For both variables it holds that in the studies on this subject age groups are arbitrarily and not uniformly chosen and that the end point of observation is not always the same in different studies. There are many other examples of non-immunological factors supposed to influence survival after renal transplantation. The influence of recipient race on the risks of graft and patient failure is controversial (24-33). Recipients from African descent are more often found to suffer from kidney rejection and graft loss compared to recipients from Caucasian or Asian descent (24,26,29,31,33). In univariable analyses, time spent on renal replacement therapy and also the type of therapy is found to influence graft survival: the longer the therapy, the less favourable the outcome (34,35). First transplants do better than subsequent transplants (36).

Living donor transplantation, either from related or from unrelated donors (spouses) has a better prognosis concerning graft function and survival than postmortal donor transplantation (37-45). Postmortal or cadaveric donors can be subdivided into heart-beating and non-heart-beating donors. Although the latter are associated with poor initial graft function, the long-term allograft survival has been reported not to be very different from the results of heart beating donor organ transplantation (36,46-47). The cause of donor death may also be influential. Grafts from a donor that suffered from an intracerebral haemorrhage seem to have a worse prognosis concerning the incidence of delayed graft function compared to other (traumatic) causes of donor death (24,48,49). In these studies the composition of the preservation fluids used are described to cause a difference in the occurrence of delayed graft function and graft survival (48-51). Increased cold ischemia times are found to influence graft survival, but there is no agreement on the importance of this risk in the whole constellation of other risk factors (37,38,52-60). In univariable analyses, it has been shown that donor creatinine clearance is associated with recipient graft function in the high-risk donor population (61-63). It is probable that other donor related factors preceding and during nephrectomy, e.g. presence of donor proteinuria, brain death, use of vasopressants, cardiovascular instability and differences in anaesthesics during kidney procurement may influence the eventual results of individual transplantations but the extent is not yet known (64-69)

The results of studies on the influence of immunological factors on the graft failure risk are variable. At the beginning blood group ABO incompatibility used to be looked upon as a major obstacle for successful transplantation (1,70,71). However, recently ABO incompatible kidney transplantation has been shown to have acceptable results, although these are inferior to the results of ABO compatible transplantation (72,73). Besides, on the argument that blood-group A2 donor kidneys are less immunogenic, it has been shown that these kidneys, when donated to blood group B or O recipients are less vigorously rejected and acceptable, though inferior results can be obtained (74-76). The influence of the number of HLA-mismatches (human leukocyte antigen) between donor and recipient has been a matter of debate for a long time (77-84). Both univariable (39,77,79,80) and multivariable analyses (36,38,40,78,81,82,83) have been carried out, and in some studies economic arguments were taken into account (77,78). The benefit of laborious and time-consuming matching is appreciated by some (78,81,80,82,84) and called into question by others (38,77,79,83). Both peak and current PRA have been associated with an increased risk of graft failure in some

studies (24,40,85-88), while not confirmed in others (89,90).
The problem with trying to match for HLA is the extremely polymorphic nature of the HLA system. One way of increasing the number of patients who can receive a well matched kidney is to match for shared or public antigens (CREGS= cross reactive groups). In retrospective analyses a positive effect of CREG matching is found on graft survival. In these studies matching for MHC (major histocompatibility complex) class I CREGs resulted in reduced graft rejection and superior graft outcome with better access for more patients (including minorities) to well-matched transplants (91-93). However, especially in the European population this effect may be a reflection of adequate matching for HLA (92).
In the early seventies an association was found between the administration of pretransplant blood transfusions and improved postmortal kidney graft survival (94,95). In the ensuing years there were many reports on a beneficial effect of blood transfusions and the favourable effect of HLA matched transfusions (24,96-98). A possible explanation for this favourable effect could be selection or a reduction of immunologic responsiveness or induction of allograft tolerance (99,100). Recently, probably as a result of improved patient care and immunosuppression, the clinical importance of the transfusion effect has declined possibly because of improved graft survival in patients who did not receive transfusions (79,101).
Once transplantation is performed, a new variety of secondary variables can be defined concerning their influence on long-term graft failure. Examples are: delayed graft function (DGF), acute rejection, protocol biopsies, recipient hyperlipidemia, serum creatinine, blood pressure and antihypertensive therapy and last but not least patient compliance. There is a strong correlation between post-transplant proteinuria and/or an elevated serum creatinine and a bad prognosis regarding long-term transplant function and survival (102-105). Posttransplant hyperlipidaemia occurs in 60-80% and cardiovascular death in 40-60% of the patients (106). In patients with a renal graft myocardial infarctions occur 25 times more often compared to the normal population (107,108). In spite of this, there is still discussion on whether renal transplant patients with high serum cholesterol levels should be treated, as there is no conclusive evidence of a direct relationship between serum cholesterol level and cardiovascular death in this multi-risk patient population (109-111). Another important variable influencing graft survival is recipient blood pressure after renal transplantation. An elevated recipient blood pressure is supposed to have a negative influence on survival (112, 113). The incidence of delayed graft function has been studied thoroughly and is found to

only influence graft function, and not survival by some authors (114). Other studies suggest that DGF influences graft survival (115,116), especially in the presence of acute rejection (117,118). Most investigators agree that delayed graft function is supposed to occur more often with prolonged cold preservation times and probably is a multi-factorial clinical circumstance (114,116-119). Not only the mere incidence but also the severity and the number of acute rejection episodes influence long-term graft survival (45,114,118,120-122). The status of graft function at the time of discharge from the hospital after renal transplantation was shown to be a strong predictor of 1-year graft survival (24). Another very important variable that influences graft survival is patient compliance. Although it is hard to check, patient compliance was shown to be positively associated with allograft survival when compared to non-compliance (123-125)

Apart from all these variables, the results of renal transplantation are improving all the time, probably as a result of improved diagnostics, immunosuppressants, treatment for concomitant disease and experience (126,127).

Because of the diversity of all these variables, their influence on different parts of the transplantation process and the improvement of results in time, analysis is complicated, especially since selection and interaction between variables interfere with the results.

Aim of this thesis:

The aim of this thesis is to make a distinction between potentially important variables in their influence on the risk of failure after renal transplantation. Which of the known variables really influence our results? Which variables can be neglected? Most studies on this subject are univariable analysis. In these studies selection and inter-dependency of the influence of variables cannot be taken into account. Besides, the importance of the influence of different variables is studied in different analyses and cannot be compared. Multivariable analysis with the Cox proportional hazards analysis offers best prospects to compare the influences of different variables on the failure risk. In the next chapter we describe how the Cox deals with our question and how risk factors are defined.

Our principal motivation was to come to an understanding of the reasons for failure after renal transplantation. With this knowledge we could try to decrease the prevalence of high risk or combination of high risk factors in recipients.

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CHAPTER 2

The Cox (Proportional Hazards Analysis) in words, Examples in the renal transplantation field.

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Submitted

Introduction

Many factors are involved in the continuously improving results of renal transplantation. However, because of the diversity of these variables it is difficult to ascertain their individual contribution to the improved renal transplant survival rate.

Cox Proportional hazards versus Kaplan Meier analysis.

Do we need the Cox?

In survival analysis primary outcome measure is the time it takes for an event to occur. For example, we are interested in the time between renal transplantation and graft loss or death. Observation time is the time between transplantation and the event or the (arbitrary) end of the study. In the latter observation time is "censored"; i.e. observation stops without the occurrence of an event. Survival analysis is a statistical technique that takes these censored observation times into account by considering that the patient has been at risk all the time he was under observation. Special statistical methods that can be used for analysis of time-to-event data are the Kaplan Meier analysis and the Cox Regression model (1,2). The occurrence of an event can also be studied within a fixed time frame without considering time as a variable; logistic regression analysis is then the appropriate tool.

Up to a few years ago, the Kaplan Meier analysis was most often used. This is an univariable analysis that has two important drawbacks (3,4). Firstly, only the influence of categorical variables can be estimated because the Kaplan Meier analysis can not deal with continuous variables. When a variable is continuous (e.g. age) it can be subdivided into a manageable number of categories. One disadvantage of this procedure is that information gets lost as grouping is arbitrary and the number of subjects per category decreases when the number of categories increases. Secondly, the importance of a specific variable cannot be tested if adjustment for the whole set of other relevant variables is required. Although it is possible to perform a stratified Kaplan Meier analysis, data must be subdivided into distinct covariate patterns with enough observations per pattern. This is often impossible. E.g. when organs from *elderly donors* are found to negatively influence survival after transplantation, selection could have influenced the results. In cases when older donor organs are relatively more often *postmortal* organs that are preferentially transplanted to *older recipients,* a large part of the poor outcome can be explained by the latter two variables but the individual influence of each variable cannot be unravelled.

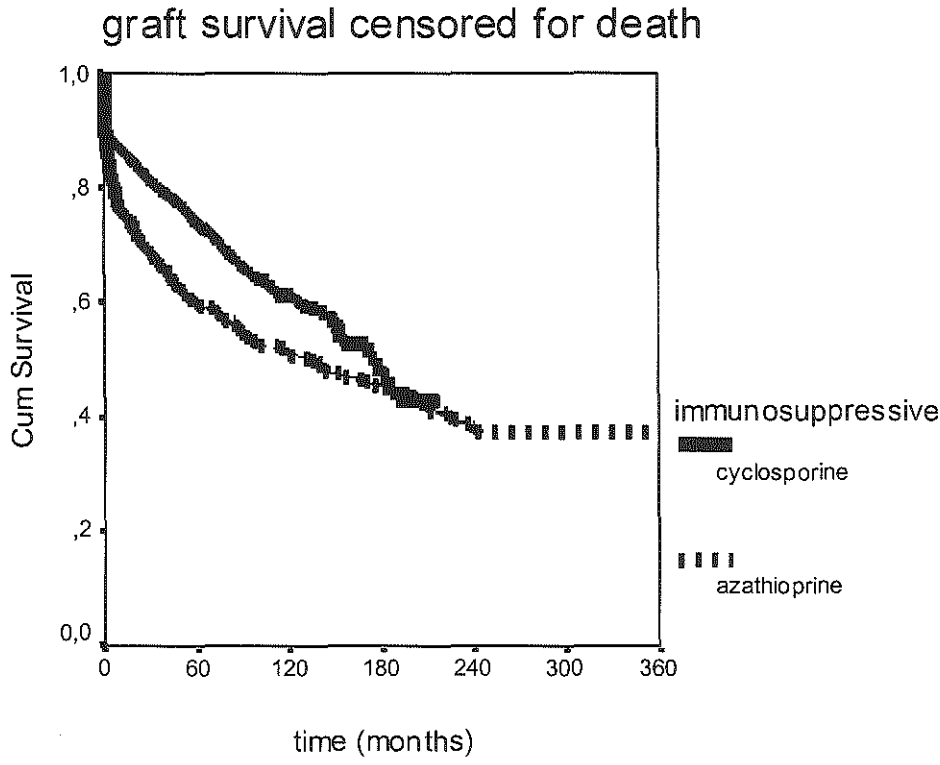

***Figure 1**: Kaplan Meier curves for graft survival censored for death from the population transplanted with azathioprine versus cyclosporine as primary immunosuppression.*

Figure 1 shows the Kaplan Meier curves for primary immunosuppression after renal transplantation (azathioprine or cyclosporine). At the start the results of cyclosporine are much better but in time the curves cross. Does the nephrotoxicity of cyclosporine play a role here? The azathioprine treated population had been transplanted long before the cyclosporine population. Was the first population favourably selected compared to the latter? Is cyclosporine really better or are we actually looking at an ameliorated survival due to improvements in techniques and

in many other aspects of transplantation and medicine in general? It is evident that the Kaplan Meier analysis can not answer these questions.
Moreover, the Kaplan Meier is a non-parametric analysis that can only test global differences in survival curves between patient groups. It is not possible to estimate the magnitude of the difference.
In the Cox proportional hazards (PH) analysis the effect of a number of independent variables (both continuous and categorical) on survival can be studied simultaneously. The Cox PH analysis is semi-parametric; it estimates the magnitude of the effect of the individual variables. However, it is not possible to directly estimate the level of the hazard itself in a parameterised way. This means that only relative risks or rate ratios (RR) can be calculated, not absolute risks.
The Cox PH model uses the hazard function to estimate the RR of failure. The hazard is a function of time. It is not a probability but an event rate (e.g. death) per unit of time. The hazard at time t, *h(t),* only approximates the probability of a patient dying at time t during a small one unit time interval, given that he has survived up to time t. The cumulative hazard function H(t) (the sum of the hazards over time until time t) can be derived from the survival function S(t) and vice versa because the relation can be expressed as H(t) = -log(S(t)). Both are essentially identical ways of describing survival in a population. The hazard rate h(t) is the proportional rate of decline of the survival function per unit of time.
As any statistical model the Cox has a number of assumptions. For example, the name "Proportional Hazards" implies the assumption that for two different patients in the population at risk, the ratio of their hazards (RR) will be constant throughout time. Example: the RR for graft failure of a patient with a female donor is 1.3 compared to that of a patient with a male donor (table 1). This means that at any time during observation the graft failure risk of the first patient is assumed to be 1.3 times that of the second patient.

***Table 1**: Multivariable Cox Proportional Hazards Analysis*

	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death			
Recipient age (per year)	0.9849	0.9769 - 0.9929	0.0002
Donor gender	1.3258	1.1004 - 1.5974	0.0030
Type of immunosuppressive	0.7807	0.5277 - 1.1549	0.0031
Type of immunosuppressive*time	2.1314	1.3679 - 3.3210	

How does the Cox work?

Three characteristics: For the Cox PH analysis we need three characteristics for all patients in the study: the time on study, occurrence (or absence) of the event and variables (risk factors). The *time on study* is calculated from transplantation until the event sought for (time-to-event or uncensored times) or until the moment of analysis (censored times). For most standard statistical analyses survival times are not amenable. Firstly because the survival time may actually be longer than the observation time e.g. because patients were lost to follow up or were transplanted only shortly before the observation time was stopped. The second feature is that survival time often follows a distribution that is skewed (to the right), implying that long survival times may occur.

The *events* studied must be clearly defined and a date should be present. The events in the renal transplantation population are graft failure and death. If a patient is lost to follow-up the date he was last seen holds for the date of censoring. When the risk of graft failure censored for death is studied, then the patients who died are censored at the date of death. This is not regarded as an event. Conversely, this holds true for patient failure (censored for graft failure). The reason for censoring is that the event "graft failure" is very different from the event "death" and so are the variables that influence them. Addition of all events in "over-all graft failure" will give some general idea about over-all survival of the kidney transplant patient but interpretation of the influences of the variables may be hard as some variables will have opposing influences on the two events. This has been shown for recipient age; increasing recipient age is associated with an increasing risk of death but with a decreasing risk of graft failure censored for death.

The number of *variables* that can be included in the study is limited but does not depend on the number of patients but on the number of events occurring in these patients. As a rule of thumb the number of variables that can be included in a study equals 10% or the square root of the number of events (that which is lower). Variables are categorical when they are subdivided in a small number of categories. In (nominal) categorical variables there is no ordinal relationship between the categories e.g.: blood group, gender, race, living related or post-mortal transplantation. In continuous variables there is a relationship between the different values a variable can take on. Examples are donor and recipient age, serum creatinine, cholesterol and quantity of proteinuria. The number of previous transplantations can be introduced as a continuous variable because 3 previous transplants are more than two. However, we can not be sure that the difference

between the third and second transplantation is as much as that between the second and first. In that case the number of previous transplants can be introduced as a categorical variable. In the Cox model, as in any model, the independent or predictor variable is often called covariate or explanatory variable.

Testing the proportional hazards (PH) assumption: As discussed above, one of the assumptions of the Cox model is that for any two patients, the ratio of their hazards across time is a constant. This "PH" assumption must be tested for each variable. Whether this assumption is true for a given categorical variable can be seen in a so-called "log-minus-log survival plot" or, to a lesser extent, in the Kaplan Meier curve (4). For example: figure 1 shows the Kaplan Meier curves of type of primary immunosuppression. The lines cross suggesting that survival is better with cyclosporine in the first period after transplantation, but after a few years graft survival of the azathioprine treated population is better. The hazards assumption does not hold and in the Cox regression analysis stratification for type of immunosuppression is an optimal way of adjusting for it as a confounder.

Estimation of the influences of the variables: The first step is the generation of an entire survival curve for each variable studied. The "fitting" of a Cox Model to the data and the subsequent estimation of the relative contribution of all variables is complex. For all variables in the model a so-called B coefficient is estimated so that the fit of the model to the data is optimal. This regression coefficient B tells us how much a given variable contributes to the risk of the event investigated (5,6,7). It is through this coefficient B that the variable increases the hazard above the baseline hazard. For the estimation of the B coefficients in the Cox model algorithms are available in many statistical software packages (4). The exponent of the B-coefficient (Exp(B)) is the factor by which the hazard rate has to be multiplied for a unit increase in the independent variable. For categorical variables the RR is equal to the Exp(B). The RR denotes the risk to have an event in the presence of a variable compared to the risk in the absence of that variable. For example, the RR of a female donor kidney is 1.32 compared to that of a male donor (table 1). For continuous variables Exp(B) represents a large number of risks. For example, the influence of recipient age on the graft failure rate is studied (table 1). Exp(B) is found to be 0.9849, p=0.0002. As Exp(B) is smaller than 1 this means that the graft failure risk decreases with increasing recipient age. The hazard rate decreases by 1 - 0.9849=0.0151 or 1.51% when a person is 1 year older. This appears to be a very slight decrease but it holds for a difference of just one-year. The RR of an arbitrary age range can be calculated relatively to a chosen reference age of e.g.

20 years, by the formula: RR= Exp(B*(age-20)). The regression line from this equation is shown in figure 2.

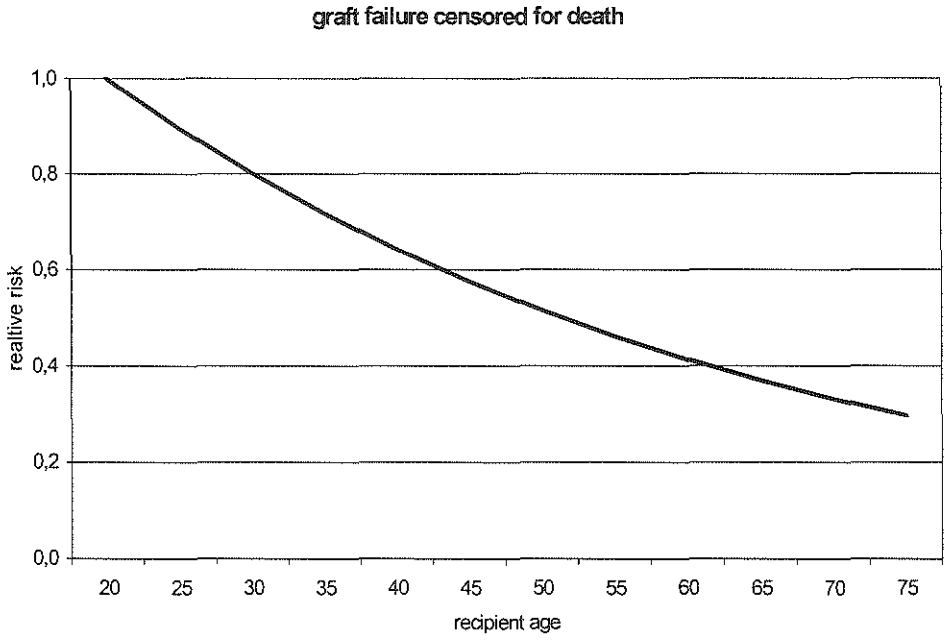

***Figure 2**: The influence of recipient age on the RR for graft failure censored for death. Reference age is 20 years.*

Now we see that the graft failure risk of a 60-year-old is indeed much lower (only 40%) compared to that of a 20-year-old recipient. In order to estimate the risk of a constellation of risk factors the RR belonging to all individual risk factors are multiplied. E.g. the above mentioned 60-year-old receives a kidney from a female donor. The RR for graft failure censored for death from a female donor is 1.3 (table 1). The risk of graft failure is now: 0.4 • 1.3 =0.52 compared to the reference (1 in a 20-year-old recipient with a male donor).

Methods of variable selection: In the Cox's PH model, as in any model, there are several methods for variable selection. In backward selection, all variables are entered into the model in a single step. Then the variables are examined for removal on the basis of their p-value. Variables are removed one by one, step by

step. The variable to be removed at a step is the one with the highest p-value in the model fitted at that step. The algorithm stops when no more variables meet entry or removal criteria.

P-values are obtained by fitting two models, a complete model and a restricted model without the variable whose contribution is going to be tested. The fit of both models is compared through the so-called likelihood ratio test. E.g. the model is analysed first with recipient age, secondly recipient age is deleted from the model and the likelihood ratio of the first to the second model is calculated. The higher this ratio, the higher the probability that recipient age is an important predictor of the event sought for (graft failure or death).

Independent influence: This is not a statistical term but it means absence of confounding and interaction. Confounding is the phenomenon that the effect of an independent variable on the outcome may be hidden or exaggerated due to its correlation with another independent variable that is not included in the model. For example: When in the US the risk of graft failure is studied in a model including race, the results of renal transplantation in African Americans are found to be worse compared to that of Caucasians. However, as in the US African Americans often have a lower socio-economic status, their access to health care and medication is impaired compared to whites. Part of the renal failures will be related to the latter and not to their race.

Interaction or effect modification: This is a higher order phenomenon. It means that the effect of a certain variable is not constant but is modified by another variable. The mathematical product of both variables can be included and its coefficient tested in the model. If this product variable has a significant p value the influence of one variable is dependent on the value of the other. Now the regression equation gets more complicated: two variables and their interaction term. For example: If we find proteinuria after renal transplantation to interact with native kidney disease this means that the influence of proteinuria on the risk of graft failure is different for the categories of native diseases. The graft failure risk of proteinuria in a patient with SLE can be much larger compared to that of a patient with congenital hereditary disease as his native disease.

Time-dependency: Sometimes, the effect of covariates appears to be time-dependent which means that their effect changes during the study period implying that the PH assumption does not hold. E.g. we can test whether the difference in graft survival between patients on cyclosporine (CsA) or on azathioprine (AZA) is constant in time or whether the risk of CsA is lower at the start (risk ratio<1) but increases thereafter (ratio increasing). Time-dependency can be tested

categorically (comparing periods) or continuously (looking for a time-trend). When comparing the first 4 years after transplantation with the period thereafter we see that type of immunosuppression and its time interaction variable influence the graft failure risk (p=0.0031, table 1).
With azathioprine as the reference category (RR=1) the RR for failure in patients on cyclosporine is 0.78 (78% of the risk of patients on azathioprine) in the first period. Thereafter this risk ratio increases to 0.78·2.13=1.66 (166% of the risk of the patients on azathioprine. Table 1). If we look carefully at figure 1 we could have foreseen this by comparing the slopes of both curves. An explanation could be the known nephrotoxicity of cyclosporine that takes some time to develop.

How do we read the output of the Cox?
The p-value tells us about the statistical importance of the variable in explaining the risk of failure. E.g. when recipient age has a p-value of <0.0001 and HLA identity has a p-value of 0.0251, this means that the influence of recipient age is much less likely to be caused by chance than the effect of HLA. It does *not* tell us *anything* about the magnitude (strength) of the influence from a clinical point of view. The RR is indicative for the magnitude (strength) of the influence.
A theoretical example: suppose kidney anatomy is found to be a very important variable influencing the graft failure risk with a p-value of 0.0001. The RR of a kidney with an abnormal anatomy could be found as 1.0005 (=0.05%) compared to that of a normal anatomy with a RR of 1. Although the difference in risk is very significant, the clinical relevance of this finding is very low, as the RR itself is negligible.

The Kaplan Meier provided us with unequivocal graphs, but also with the Cox regression model an entire survival curve can be generated for each combination of independent variables. However, it should be borne in mind that for the construction of the survival curve from a subgroup all other variables are set to their over-all mean.
In summary, the advantages of the Cox PH analysis compared to the Kaplan Meier analysis are described. The most important advantage is its potential to evaluate the effect of a certain risk factor after having incorporated the (confounding) effect of other relevant risk factors on survival. In addition, the magnitude of this effect on survival can be estimated and tested and confidence intervals can be calculated. For clinical purposes the Cox model is a very powerful statistical tool for non-randomised studies, where the comparisons we are interested in can be adjusted

for other important variables that might influence survival. In randomised studies, due to chance, (slight) imbalances between allocated treatment groups are almost invariably present. A Cox model can be a priori specific to adjust for confounding due to these imbalances. In a complex, observational study concerning a multi-factor influenced population as the renal transplant population, the use of the Cox model is mandatory to unravel the influences of the different variables on the failure risk.

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CHAPTER 3

Renal graft survival in native and not native European recipients.

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Abstract

Most studies on the influence of recipient race on kidney transplant-survival have been performed in the U.S.A. Generally they show a lower survival in African-Americans compared to Caucasians. As Rotterdam has gradually become a multi-ethnic society we were able to study the effect of origin on kidney-survival. From all 805 transplantations that were carried out in our centre between July 1983 and July 1997, the study population was restricted to recipients of a primary postmortal kidney graft with cyclosporine as primary immunosuppressive. Patients were divided into two main groups according to origin: European (N=399) and non-European (N=110). No statistical differences were found for mean donor age, sex-distribution and the total number of HLA-A and DR mismatches. Non-Europeans had significantly more mismatches on their HLA-B locus (p=0,01) and recipient age was lower (p=0,003). They appeared to have more often hypertension and less often hereditary diseases as their original disease. The causes of death and the causes of transplant failure were not different. In the multivariable Cox proportional hazards analysis European or non-European origin did not turn out to be an independent predictor of graft survival (2 categories, p=0,25).

We conclude that in our centre the prognosis after kidney transplantation is comparable for Europeans and non-Europeans.

Introduction

The impact of ethnical origin on graft and patient survival after kidney-transplantation is still controversial. Most studies were done in the United States, analysing the results in black Americans versus whites (2,5,12,13,17,18,20,27,29,31,32,33). Only a few studies addressed the effect in other races and no studies were done to evaluate the results in non-Europeans living in Europe (3,6,9,21,24,33). Contrary to the American situation, our medical health system assures equal care for all inhabitants, largely excluding economic circumstances as an important factor influencing graft survival.

A part of our not native population has an impaired (or absent) mastery of the Dutch language, causing misunderstandings e.g. regarding medication. This could possibly have a negative influence on graft survival. We studied the differences in patient characteristics and graft survival between European (Eur) and non European (non Eur) renal transplant recipients.

Materials and Methods

Patients:

At the University Hospital Rotterdam-Dijkzigt 805 kidney-transplantations were carried out between July 1983 and July 1997. At the moment of analysis all had at least half a year of follow-up. Of these transplantations 509 were primary transplants with a postmortal donor. These were divided into 2 categories according to origin of the recipient: Eur (n=399) and non-Eur (n=110). The non-Eur population included patients from African descent (Surinam-Creoles (n=15), Antillians (n=9), and Cape Verdians (n=13)); Asian descent (Surinam-Hindustani (n=29), Surinam-Chinese (n=2), Indonesian (n=11) and Chinese (n=2)): Arabian descent (Moroccans (n=11) and Middle-Eastern (n=2)) and from Turkey (n=16).

Donor origin is not reported in Europe but estimations are that almost all are Caucasian because of various (including religious) objections to postmortal organ donation in many of the non-European communities. In the Netherlands the National Health Service assures that health care is equally accessible for the entire population.

Immunosuppression:

Steroids were given from the first day of transplantation and were slowly tapered to 10 mg of prednisone daily. Cyclosporine (CsA) was used as primary immunosuppression in all patients. CsA was started at a dose of 2 mg/kg i.v., later 4 mg/kg orally, while the same corticosteroid scheme was given. No patient received triple therapy on a routine basis.

Statistical analysis:

Kidney transplant survival was defined as alive with a functioning graft. There were no exclusions for technical or nonimmunologic failures. Potential associations with graft survival were analysed by means of the Cox proportional hazards regression analysis. Recipient and donor age, gender and blood group, HLA mismatches on A, B and DR, recipient origin, original disease and transplantation year were analysed as independent variables. Patient characteristics were compared with an unpaired t-test when they were continuous. Discrete variables were tested with a Chi-square test. A P-value < 0.05 was considered significant. All data are presented as mean $\pm$ SD.

Table 1: *Characteristics of the patient populations*

		Europeans	non-Europeans	p
N=		399	110	
Age recipient (years)		49 ± 12	45 ± 13	0.003
Age donor (years)		38 ± 16	39 ± 17	ns
Gender recipient (m/f)		224/175	67/43	ns
Gender donor (m/f)		247/147	63/42	ns
Recipient blood group (%)				
	A	46%	34%	0.023
	B	10%	20%	0.003
	AB	5%	8%	ns
	O	39%	38%	ns
Donor blood group (%)				
	A	44%	33%	0.030
	B	8%	15%	0.024
	AB	2%	4%	ns
	O	46%	48%	ns
Observation time (months)		54 ± 45	44 ± 39	0.0433

Un-paired t-test and Chi-square test. Mean ±sd.
M=male, f=female, ns=not significant

Results

The characteristics of the two main groups are presented in table 1.

Donor age and donor and recipient gender were not different between the groups. Recipient age was significantly lower in the non-Eur population. Blood group A was significantly less and blood group B was more prevalent in the non-Eur compared to the Eur recipients. The same holds true for the donor blood groups. Table 2 shows that the number of HLA-mismatches on the A and DR locus was not different between the groups, but non Europeans had significantly more mismatches on HLA B.

***Table 2**: Comparison of HLA matching in the European and the non-European population (Unpaired t-test. Mean±sd).*

		Europeans	non-Europeans	p
Mean HLA mismatch				
	A	0,71 ± 0,65	0,84 ± 0,70	ns
	B	0,79 ± 0,61	0,95 ± 0,64	0.015
	DR	0,48 ± 0,56	0,46 ± 0,54	ns

In non Eur hypertension was more frequently observed (table 3). This was caused by a higher incidence, though not significant, of hypertension in the African and Arabian population. Congenital and hereditary diseases were less frequently seen in the non Eur compared to the Eur. This was reflected in all non Eur subgroups. The causes of graft failure and the causes of death were not different between the groups (Tables 4 and 5).

***Table 3**: Causes of renal failure in the European and the non-European population (chi-square test).*

	Europeans	non-Europeans	p
glomerulonephritis	27%	24%	ns
interstitial disease	20%	13%	ns
congenital or hereditary	17%	7%	0.009
hypertension, renovasc.	11%	22%	0.003
diabetes mellitus	6%	9%	ns
systemic disease	4%	7%	ns
unknown	15%	18%	ns

***Table 4**: Numbers and causes of transplant failure in the European and the non-European population (chi-square test).*

		Europeans	non-Europeans	p
N=		399	110	
Number of failures		114	29	ns
Failure cause (%)				
	never functioning graft	9%	7%	ns
	surgical complications	25%	10%	ns
	acute rejection	30%	38%	ns
	recurr. original disease	7%	0%	ns
	chronic rejection	13%	24%	ns
	other	12%	14%	ns
	infection	4%	7%	ns

***Table 5**: Numbers and causes of death in the European and the non-European population (chi-square test).*

		Europeans	non-Europeans	p
N=		399	110	
Number of deaths		124	24	ns
Cause of death				
	unknown	18%	25%	ns
	cardiac	30%	25%	ns
	vascular	14%	9%	ns
	infectious	17%	25%	ns
	gastro-intestinal	4%	4%	ns
	treatment stopped	2%	0%	ns
	accidental	5%	4%	ns
	other causes	10%	8%	ns

In the multivariable Cox analysis recipient origin was not a significant predictor of graft survival (p=0.25). Figure 1 shows the survival curves of Eur and non-Eur recipients. The other variables that were independent predictors of graft survival were recipient and donor age, original disease, recipient gender and year of transplantation.

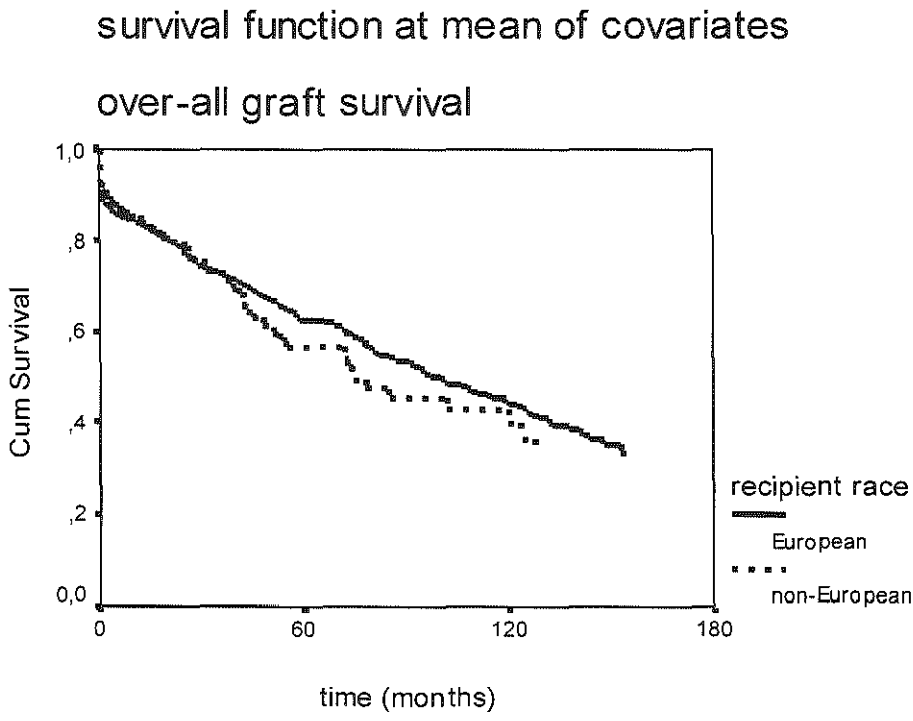

Figure 1: *Survival curves (Cox) of Europeans (n=399) and non-Europeans (n=110). Difference not significant (p=0,25).*

Discussion

Despite the early report of Opelz in 1977 (23), showing an apparent negative impact of black race on kidney transplant outcome in the U.S.A., the debate on the influence of race on kidney graft survival has not been settled. Several studies demonstrated the existence of a racial disparity in transplant outcome especially in long-term graft

survival (4,27). Most of these studies have focussed on black race since they were performed in the United States. Some indicated reduced transplant survival in blacks compared to whites (2,9,12,13,18,20,21,24,27,29,34), whereas others did not report any difference (3,4,5,7,17,18,31,32,33). The results of kidney transplant survival in other non-black races compared to whites have appeared only sporadically in the literature (3,6,9,21,24,33). The issue of race effect is attracting increasing attention because it has been shown that it might be influencing long term rather than short term graft survival (4). Several factors have been suggested to explain the results of kidney transplantation in the black population; such as a higher prevalence of hypertension (4), poor compliance (2,15,21), stronger immune-responsiveness in blacks (14), less often living related kidney donation (22) and socio-economic variables (2,8,10,16). A higher degree of HLA-polymorphism has also been demonstrated in blacks (19,35). This might cause a higher error rate in serologic HLA-DR determinations (30). Moreover the most frequently occurring HLA-haplotypes in blacks are different from those occurring in the Caucasians (1,11,30). This might explain why blacks have a smaller chance to get equally HLA-matched kidneys from a predominantly Caucasian donor supply (19). In many of the studies HLA-matching is relatively poor compared to European standards and blacks often have worse matching compared to Caucasians (4,9,21,25,26). Butkus showed that although overall kidney graft survival in blacks was worse than in whites there was no difference in graft survival between blacks and whites at any level of HLA-mismatching (2).

In our patient population graft survival was equal for European and non-European recipients. Except for a difference in recipient age and number of HLA mismatches on B, the risk variables for graft loss were comparable in both groups. As could be expected blood group B was more prevalent in the non-Eur recipients. This was reflected in the donor population which indicates that non-Eur with blood group B did not receive blood group O kidneys more often than Eur. Hypertension and renovascular disease were more common causes of renal failure in non-Eur. This could be expected because hypertension is more prevalent among originally African people. In addition, poor treatment of hypertension in the countries of origin leads to a higher incidence of terminal renal failure. However, the original disease hypertension, by itself does not have an independent influence on renal graft survival (28). In contrast congenital and hereditary diseases were less frequently observed in the non-Eur, possibly because they lead to death at childhood in less developed countries. Number and causes of graft failure and number and causes of deaths were not different. The difference between the European and the non-European identity did not have an

independent influence on graft survival (figure 1). This means that Eur and non-Eur patients with a first postmortal kidney transplant have the same prospects. The other variables that showed an independent influence on graft survival are discussed elsewhere (28).

In conclusion, our single centre data do not support the concept that kidney graft survival is worse in non-European compared to European recipients.

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CHAPTER 4

The vanishing importance of age in renal transplantation.

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Abstract

Background: The growing number of patients awaiting a kidney transplant raises questions about allocation of kidneys to the elderly and about the use of elderly donors. In all reported studies analysing the influence of age on the outcome after renal transplantation, age is investigated as a categorical variable.

Methods: We studied age both as a categorical (Kaplan-Meier) and as a continuous (Cox) variable in all 509 CsA treated recipients of a primary postmortal kidney graft transplanted between July 1983 and July 1997. For the Kaplan-Meier analysis the population was divided into three comparably sized age groups: 17-43 years (n=171), 44-55 years (n=169) and 56-75 years (n=169).

Results: In the Kaplan Meier analysis patient survival was better and graft survival censored for death was worse in the younger patients. Over-all graft survival (endpoint is death or graft failure) was not significantly influenced by age. In the Cox proportional hazards analysis, transplantation year turned out to be an important, independent variable influencing all end points. As the influence was not linear, three periods were defined in which the relative risk remained stable: 1983-1990, 1991-1993 and 1994-1997. In the second period the relative risk of transplant failure or death was 49% of that in the first period. In the third period the relative risk had decreased to 22% of that in the first period. Recipient age and donor age were significant predictors of over-all transplant failure and of graft failure censored for death. There was no interaction between these variables and transplantation year. Within each transplantation period, an increased recipient age increased the relative risk of over-all graft failure, whereas the risk of graft failure censored for death decreased. The influence of donor age on both end-points followed a J-shaped curve with a minimum at 30 years. Recipient age was a significant predictor of patient failure. The influence of increasing either recipient or donor age was counteracted by the improving results in time.

Conclusion: Considering the improving results in time there are, at this moment no arguments for an age restriction for kidney transplant recipients or donors.

Introduction

The impact of age on graft and patient survival after kidney transplantation has been the subject of investigation for many years. In general the dialysis population is steadily getting older. As the supply of donor kidneys remains insufficient the life expectancy of elderly patients raises the question whether allocating kidneys to them is justified. In many studies patients were included regardless of their primary immunosuppression (4-10). Other studies were done in patient groups treated with

either AZA (1-3) or CsA (11-16) only. In all studies age was investigated as a categorical variable. Our aim is to study the long-term effect of age, as a continuous variable, on kidney transplant-survival in a homogeneous population using a computerised database and well controlled management protocols. These results, obtained by the Cox proportional hazards analysis, are compared with those obtained by the Kaplan-Meier analysis.

Materials and Methods

Patients:

At the University Hospital Rotterdam-Dijkzigt 805 kidney-transplantations were carried out between July 1983 (introduction of CsA) and July 1997. The analysis was done in January 1998, all patients having at least six months of follow-up. Of these transplantations 509 were primary transplants with a postmortal donor and cyclosporine (CsA) as primary immunosuppression. For the Kaplan-Meier analysis the population was divided into three comparably sized age groups: youngest: 17-43 years (n=171, median=35, mean=33.8 years), middle age: 44-55 years (n=169, median=50, mean=49.8 years) and eldest: 56-75 years (n=169, median=61, mean=61.7 years).

Immunosuppression:

CsA was used as primary immunosuppressive and was given on the basis of 12-hours trough levels (target level 100-150 ng/ml). Steroids were given from the first day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily half a year after transplantation. No patient received triple drug therapy on a routine basis. During the whole period studied patients were included in immunosuppressive treatment protocols. Although, in some a reduction in the number of acute rejection episodes occurred, none of them resulted in an increase in graft survival so none of them was included in our basical immunosuppressive protocol.

Statistical analysis:

"Over-all graft survival " was defined as alive with a functioning graft (endpoint being either death or graft failure). For "graft survival censored for death" death was censored and for "patient survival" graft failure was censored. For the analysis of patient survival, death occurring within three months after graft failure was included in the analysis. There were no exclusions for technical or nonimmunologic failures. For comparison two analyses were done: the Kaplan-Meier analysis and the Cox proportional hazards regression analysis. Survival curves were constructed according to the method of Kaplan and Meier. Potential associations

with survival were analysed by means of the Cox proportional hazards regression model. Recipient and donor age, gender and blood group, HLA-mismatches on A, B and DR locus, difference in age between donor and recipient, matching for gender, recipient race, original disease and transplantation period were analysed as independent variables. Variables were selected by backward elimination using likelihood ratio tests. For categorical variables indicator coding was used with the first category as reference. The reference categories were: 1983-1990 as transplantation period, male gender (both recipient and donor), blood group O (both recipient and donor), zero HLA mismatches on A, B and DR locus and Caucasian race. Glomerulonephritis was used as the reference category for original disease in the analysis for patient survival, congenital and hereditary diseases in the analysis for over-all graft survival. For categorical variables the relative risk (RR) was equal to e^B, where B is the regression coefficient for that variable. Both donor and recipient age were analysed as continuous variables increasing per year. As a reference value, the relative risk of a 20 year old (both recipient and donor) was settled to 1. The combination of regression coefficient B and age in the regression equation ($RR=e^{B*(age-20)}$), resulted in the regression line showing the influence of age on the relative risk. When the effect followed a second-degree course the regression equation was $RR=e^{B1*(age-20)+B2*(age*age-20*20),}$ where B1 is the coefficient for age as a linear variable and B2 as a second-degree variable. Patient characteristics were compared with an unpaired t-test (continuous variables) or with a Chi-square test (discrete variables). A p-value <0.05 was considered significant. The analyses were performed with SPSS for Windows version 7.52.

Results

Mean recipient age and SD of the 509 kidney transplantations under investigation was 48.4 ± 12.5 years, 58% was male. Mean donor age and SD was 38.5 ± 16.7 years, 62% was male. Over 99% of HLA matches were available. The percentage of non-Europeans in the recipient population was 22%.

Kaplan-Meier analysis: There was no significant difference between the three age groups for recipient gender, donor age, blood group and gender and the number of mismatches on the HLA-A, B and DR locus. Table 1 shows the original diseases.

Table 1: *Original disease (%)*

	age (years)			
	<44	44-55	>56	p
Glomerulonephritis	35	24	20	0.007
congenital or hereditary	9	21	16	0.008
diabetes mellitus	6	5	10	0.22
hypertension, renovasc	10	12	18	0.10
interstitial disease	18	21	17	0.50
systemic disease	6	4	3	0.24
Infectious	0	1	0	0.36
Unknown	16	12	16	0.19

Chi-square test. p is overall value comparing all three groups.

Glomerulonephritis was a more frequent cause of renal failure in the youngest compared to the middle and eldest age groups (p=0.028 and p=0.003 respectively). Congenital and hereditary diseases were less often the cause of renal failure in the youngest compared to the middle and elderly age groups (p=0.0019 and p=0.043 respectively).

As shown in figure 1 patient survival was significantly worse in the elderly ($p<0.0001$) whereas graft survival censored for death was significantly better with increasing age as is shown in figure 2 ($p=0.0285$).

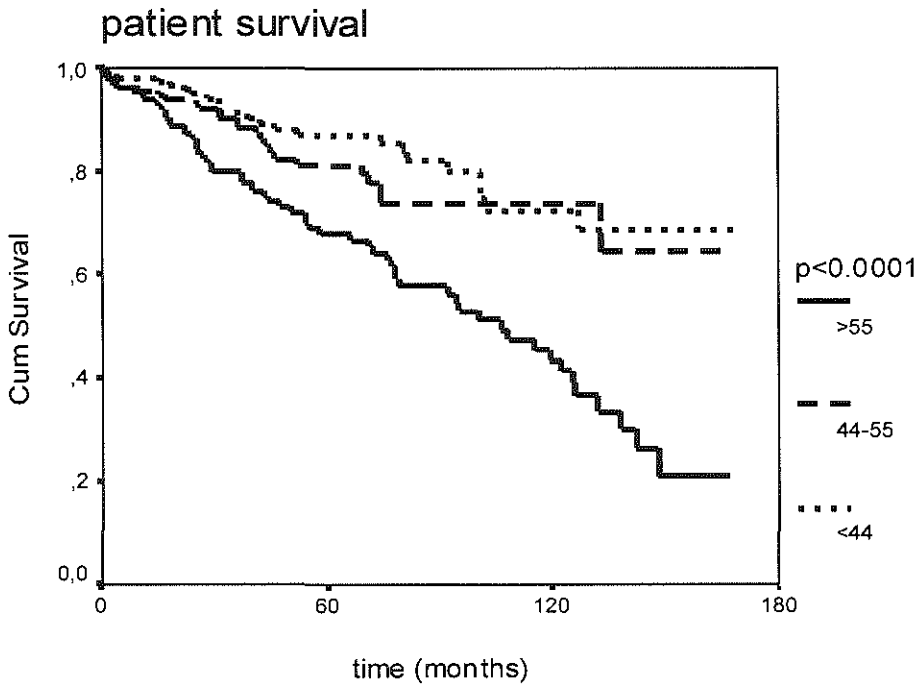

Figure 1: *Patient survival in the three recipient age groups.*

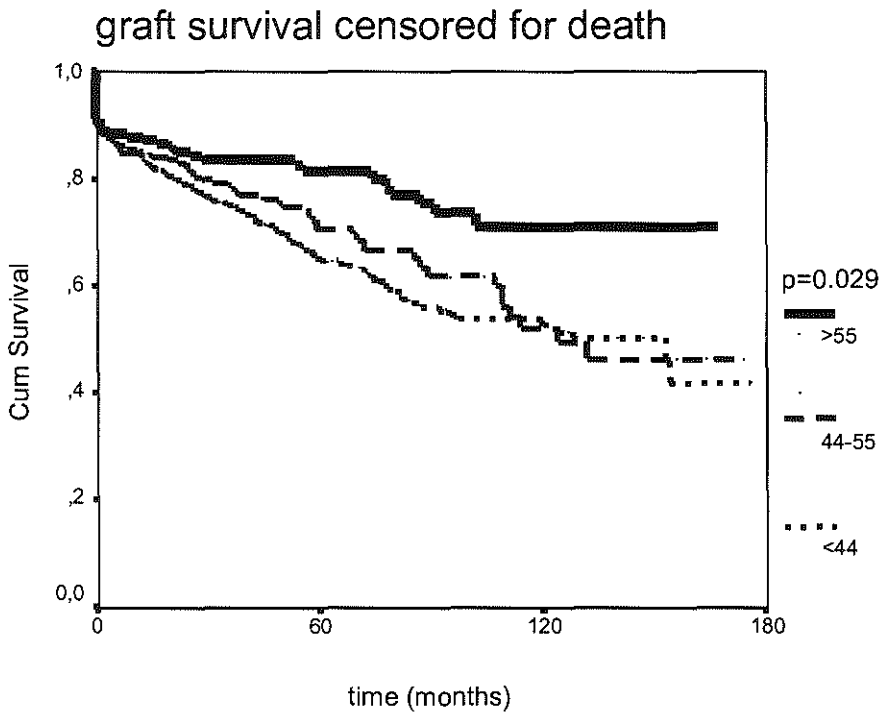

Figure 2: *Graft survival censored for death in the three recipient age groups. See figure 1.*

Due to these opposing effects, over-all graft survival of the three age groups was not significantly different (figure 3; p=0.38). Numbers and causes of graft failure are shown in table 2.

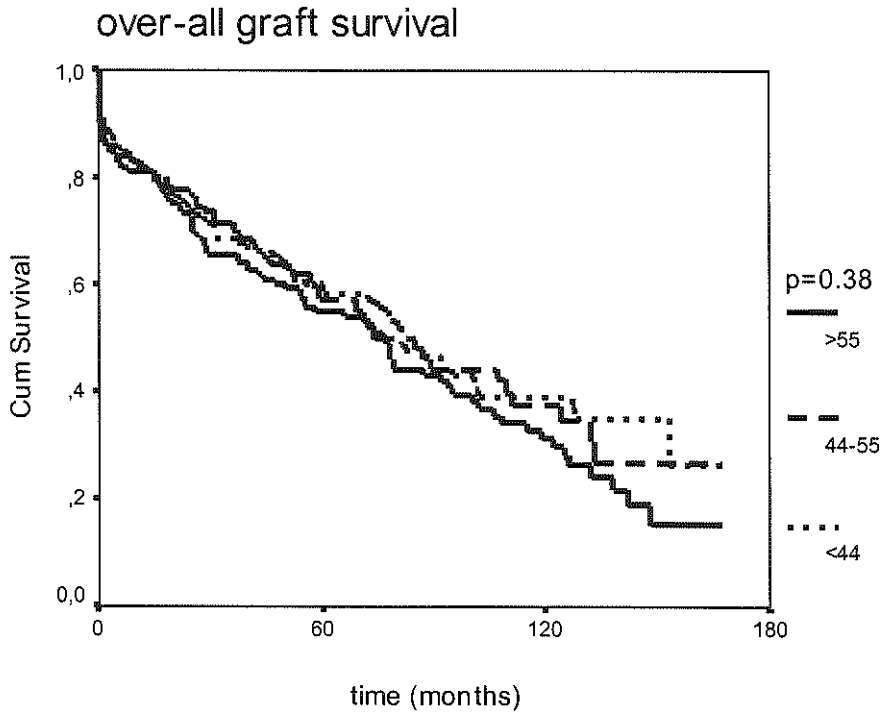

Figure 3: *Over-all graft survival in the three recipient age groups. See figure 1.*

Table 2: *Numbers and causes of graft failure*

	age (years)			
	<44	44-55	>56	p
Numbers of failures	62	47	34	0.004
Failure causes (%)				
never functioning graft	7	11	9	0.73
surgical complications	16	28	24	0.34
acute rejection	42	25	21	0.06
recurr. original disease	8	4	3	0.51
chronic rejection	14	19	12	0.64
infection	5	0	12	0.05
other	8	13	19	0.21

see table 1

There is a significant difference between the three age groups concerning the number of failures (p=0.004). When comparing the number of graft failures in the youngest age group (n=62) to those in the eldest (n=34) the difference was even more striking (p=0.001). Although there is a trend towards a lower occurrence of acute rejection as the cause of graft failure with increasing age the difference was not significant. The occurrence of infection as the cause of graft failure was significantly different between the three groups (p=0.05) and this was caused by a significant difference between the middle compared to the oldest age groups (p=0.016). All other causes of failure were not significantly different between the groups. Table 3 shows the numbers and causes of death.

Table 3: *Numbers and causes of death*

	age (years)			
	<44	44-55	>56	p
Numbers of death	32	44	72	<0.0001
Cause of death (%)				
unknown	22	21	17	0.78
cardiac	19	34	31	0.32
vascular	19	11	12	0.61
infectious	25	9	21	0.15
gastro-intestinal	3	9	1	0.12
treatment stopped	3	0	3	0.52
accidental	6	5	4	0.90
other causes	3	11	11	0.38

see table 1

As could be expected the occurrence of death was significantly different between the three groups ($p<0.0001$). This was most noticeable between the oldest and the middle age group ($p=0.0013$) and the oldest and the youngest age groups ($p<0.0001$). The causes of death were not significantly different between the groups.

Cox Proportional Hazards Analysis: Transplantation year turned out to be an independent covariate influencing patient survival, graft survival censored for death and over-all graft survival (table 4).

***Table 4**: Results of the multivariable Cox proportional hazards analysis.*

	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death			
Recipient age (per year)	0.9779	0.9623-0.9937	0.0063
Donor age (per year)	0.9224	0.8805-0.9664	0.0002
Square of donor age (per year)	1.0013	1.0006-1.0019	
Transplantation year			0.0013
1983-1990	1.000		
1991-1993	0.4205	0.2238-0.7900	0.0071
1994-1997	0.2323	0.0828-0.6516	0.0055
Patient failure			
Recipient age (per year)	1.0537	1.0348-1.0729	<0,0001
Donor age (per year)			ns
Transplantation year			0.0087
1983-1990	1.000		
1991-1993	0.5707	0.3308-0.9845	0.0438
1994-1997	0.2903	0.1139-0.7403	0.0096
Over-all graft failure			
Recipient age (per year)	1.0144	1.0022-1.0267	0.0204
Donor age (per year)	0.9656	0.9296-1.0030	0.0177
Square of donor age (per year)	1.0006	1.0001-1.0011	
Transplantation year			<0.0001
1983-1990	1.000		
1991-1993	0.4871	0.3201-0.7411	0.0008
1994-1997	0.2219	0.0926-0.4400	0.0001

The relative risk of over all graft failure is one when it concerns a 20 year old male recipient with glomerulonephritis as original disease, who received a kidney from a 20 year old donor in the transplantation period 1983-1990.

As the influence of transplantation year was not linear three periods were defined in which the relative risk was stable: 1983-1990, 1991-1993 and 1994-1997. The first period was used as the reference category with a relative risk of 1. Table 4 shows the dramatic influence of time on the relative risk of losing the graft and/or death in the second and third period compared to the first. E.g. in the second period the relative risk of over-all graft failure decreased to 49% of that in the first period and in the third period even further to 22% of that in the first period.
Recipient age was an independent variable influencing patient survival, graft survival censored for death and over-all graft survival in a multivariable Cox proportional hazards analysis (table 4). An exp(B) of 1.0144 for over-all transplant failure meant that any increase in recipient age by one year increased the relative risk by 1.44%. When assuming that the relative risk of a 20-year-old was 1 in the period studied, and the relative risks of all other variables remained the same, the relative risk increased to 1.33 in a 40-year-old and to 2.04 in a 70-year-old recipient ($RR=e^{B*(age-20)}$). With this formula the solid line in figure 4 was produced. There was no interaction between recipient age and transplantation period. When combining the influences of transplantation period and recipient age on the relative risk of over-all graft failure both dashed lines in figure 4 were obtained (RR recipient age ·RR transplantation period).

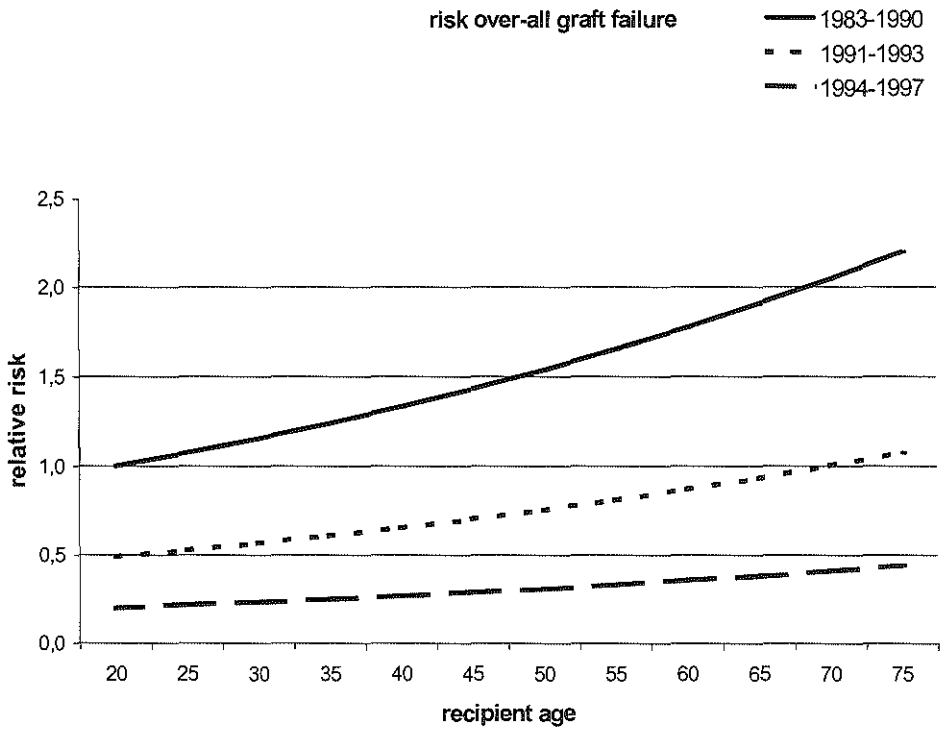

Figure 4: *Combined influence of recipient age and transplantation period on the relative risk of over all graft failure.*

The solid line is the baseline showing the influence of age when the RR of all other variables is 1. As the RR of transplantation period 1983-1990 is 1, the solid line represents the RR of age in the first transplantation period. When the RR of the transplantation period is not equal to 1, as in periods 1991-1993 and 1994-1997 (table 4), the RR of the age baseline is multiplied by the matching RR's (0.49 respectively 0.22) so that both dashed lines are obtained.

The influence of recipient age on the RR for graft failure censored for death decreased with increasing age (table 4: p=0,0063, figure 5).

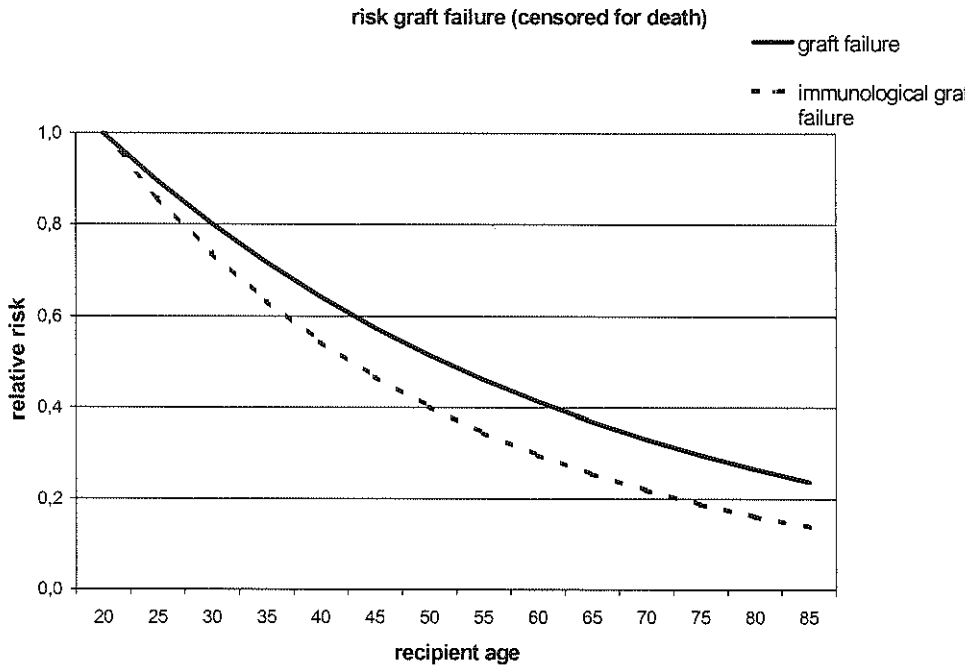

Figure 5: *The influence of recipient age on the RR for graft failure censored for death and on the RR for immunologic graft failure censored for death.*

When restricting to immunologic failure (acute and chronic rejection) the decrease of the RR with age was even steeper. The influence of recipient age on patient failure was, as could be expected, increasing with age ($p<0,0001$, graph not shown).

Donor age also was an independent variable influencing the risks of over-all graft failure and graft failure censored for death (table 4). The effect followed a second-degree course resulting in a J-shaped regression line. Figure 6 shows that the best over-all graft survival results were obtained by a 15-40 year-old donor kidney.

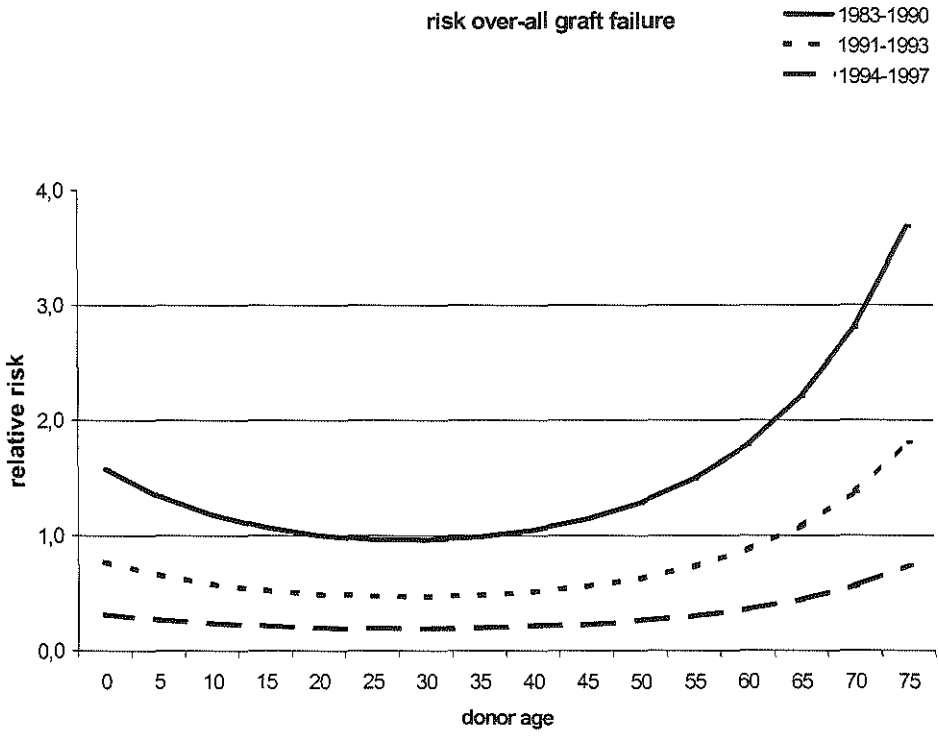

Figure 6: *Combined influence of donor age and transplantation period on the relative risk of over all graft failure.*
Legend: See figure 4.

The results deteriorated with increasing age and deteriorated slightly when younger donor kidneys were used. There was no interaction between transplantation period and donor age. Figure 6 also shows the combined influence of transplantation period and donor age. As shown in table 4 and in figure 7, the influence of donor age on the graft failure risk censored for death was not linear either and also followed a second degree function with the lowest RR at a donor age of 30 years (solid line).

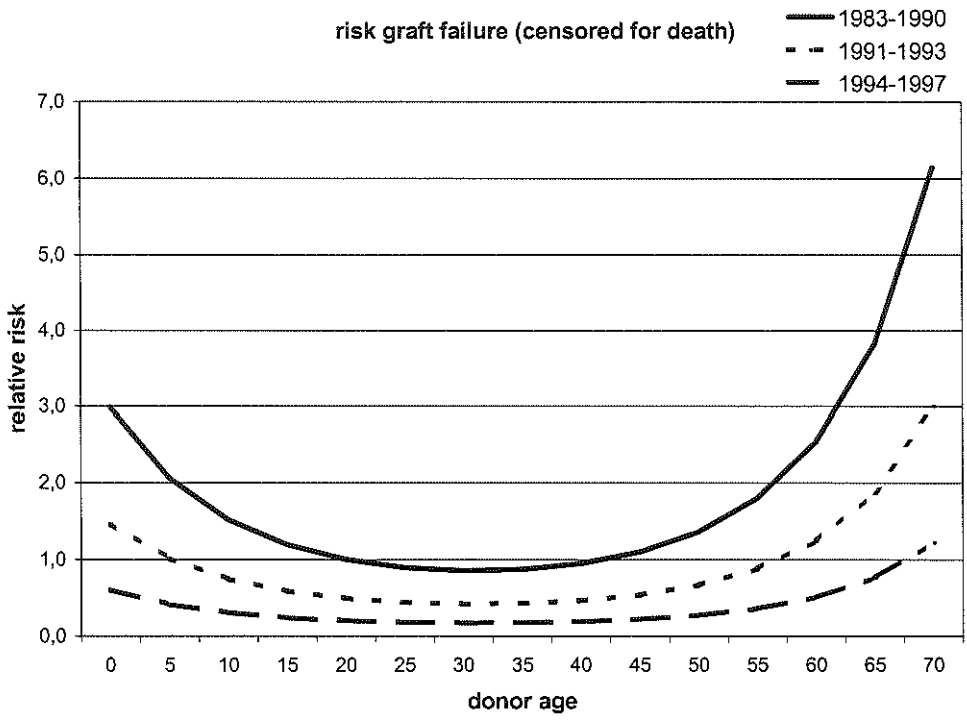

Figure 7: *Influence of donor age on the RR for graft failure censored for death. Legend: see figure 4.*

When combining the influences of donor age and transplantation period on the risk of graft failure censored for death, both dashed lines in figure 7 are obtained.
Recipient gender had a significant influence on over-all graft survival (RR=0.7366, p= 0.0453) and on patient survival (RR=0.6325, p=0.0254), women having the lowest risks. Original disease had a significant influence on over-all graft survival (p=0.0256). Congenital and hereditary diseases turned out to have the lowest risk of over-all graft failure and was chosen as the reference category. Significantly higher relative risks were found for diabetes mellitus (RR=2.3223; p=0.0070), hypertension (RR=1.8616; p=0.0284) and systemic diseases (RR=3.2874; p=0.0008). Original disease also had a significant influence on patient survival

(p=0.0413). Glomerulonephritis had the lowest risk of death and was used as the reference category for original disease in the analysis regarding patient survival. Significantly higher risks were found for diabetes mellitus (RR=1.8122; p=0.0346) and systemic diseases (RR=2.5653; p=0.0040). All other variables tested did not have an independent influence on survival in the multivariable analysis.

Discussion

In this retrospective study concerning 509 consecutive kidney transplants the Kaplan-Meier analysis shows that in this CsA treated population patient survival declines with increasing age. However, graft survival censored for death is significantly better with increasing age. As over-all graft survival is a composition of both patient and graft survival censored for death, this is similar for all age groups. The Cox regression analysis also shows that an increase in recipient age is associated with a decrease in patient survival and an increase in graft survival censored for death. However, in contrast to the Kaplan-Meier analysis increasing age is associated with a decrease in over-all graft survival. The influence of the improving kidney transplantation results over the years exceeds by far the influence of age. For example: the relative risk of a 20 year old patient transplanted between 1983 and 1990 is equal to that of a 70 year old transplanted between 1991 and 1993 (RR=1). Therefore, the raw comparison of age groups by means of the Kaplan-Meier analysis thus is confounded if the distribution of recipient age depends on year of transplantation. When elderly patients, who were transplanted recently (with the benefits of improving results) were compared with a younger population that had been transplanted a longer time ago, the Kaplan-Meier curves would fail to show the actual difference in over-all graft survival between age groups that would have been found after proper adjustment for transplantation year. So, apart from the age of dichotomy "young versus old", the distribution of the moment of transplantation determines the outcome of the Kaplan-Meier analysis. Both the Kaplan-Meier and the Cox proportional hazards analysis show an increased graft survival censored for death with increasing recipient age. This is in agreement with the trend towards a lower occurrence of graft loss due to acute rejection in the elderly. If we define "immunological graft failure" as caused by acute or chronic rejection and censor for both patient death and non-immunological failure we find a decrease in exp(B) to 0.9696 whereas it was 0.9779 in graft survival censored for death. This means that the relative risk of immunological graft failure decreases even more steeply with age than that of graft failure censored for death (figure 5). An explanation might be that the elderly show a broad immune

incompetence reflected in a decreased incidence of acute rejection, as has also been shown by Tesi (13) and others (17-21). The Cox proportional hazards analysis demonstrates that this effect does not start at arbitrarily chosen "elderly" age but is a constant factor increasing from the youngest age groups onwards.
The influence of donor age, on the relative risk of both graft failure censored for death and over-all graft failure, was not linear but followed a J-shaped course with a minimum at a donor age of 30 years. Thereafter there is an increase in relative risk of over-all transplant failure with age but this is surpassed by the improving results in time. The influence of donor age on over-all graft failure is a reflection of that on graft failure censored for death as the influence on patient death is not significant. Considering the improving results in time and their dramatic influence on over-all graft survival, outweighing the influence of increasing recipient and donor age, we can state that if ever there was a reason for age restrictions it was in the past. As there is no interaction between donor and recipient age, this analysis does answer the question whether we should give elderly kidneys to young or to elderly recipients: There is no advantage or disadvantage for either the young or the old. From the view of donor shortage it is customary to give them to the elderly because they have lower metabolic demands so function and demands are in equilibrium. Moreover, elderly reject less often and less aggressively so damage to the elderly kidney is limited in the elderly recipient (figure 5). However, the RR of over-all graft failure in a combination of an elderly donor and recipient is very high. This over-all RR is composed of two parts; the risks of graft failure censored for death and of patient failure. As the RR of graft failure censored for death decreases with increasing recipient age but increases with increasing donor age these risks may balance each other out in an old-old combination. However, the death risk of the elderly recipient is unopposed. This should be borne in mind when deciding to give an elderly kidney to an elderly individual who happens to suffer from complicating ailments and diseases. Especially when it concerns a donor with known cardiovascular disease, a moderate renal function or other variables with an expected negative influence on future renal function.
As could be expected females have a lower relative risk of death than men. Their decreased relative risk of over-all graft failure is a reflection of this phenomenon. It is remarkable that systemic diseases have such a large impact on both patient and over-all graft survival. Diabetes mellitus is well known to result in a lower life expectancy, and thus in a worse over-all graft survival.

We conclude that, although recipient and donor age influence the outcome of kidney transplantation, the age effects have become an issue of minor importance in view of the dramatically improved results over time, and in comparison with other independent predictors of over-all graft survival.

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CHAPTER 5

Cholesterol as an independent predictor of outcome after renal transplantation.

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Abstract

Background: The debate on the role of high serum cholesterol levels in kidney transplanted patients on cardio-vascular disease or chronic vascular rejection has not yet been settled.

Methods: We studied the influence of serum cholesterol at one year after transplantation on the failure risk in all 676 recipients of a kidney graft who survived the first year with a functioning graft. Other variables included in this analysis were: donor/recipient age and gender, original disease, race, number of HLA-A and -B mismatches, previous transplants, postmortal or living related transplantation and transplantation year. At one year after transplantation we included serum cholesterol, serum creatinine, proteinuria and hypertension.

Results: In the Cox proportional hazards analysis serum cholesterol at one year after transplantation turned out to be an important, independent variable influencing all end points (adjusted for all other variables in the model). The influence on **graft failure censored for death** was log-linear and there was interaction with serum creatinine at one year. The adverse effect of elevated serum cholesterol levels on the graft failure rate decreased with increasing serum creatinine levels. The influence of serum cholesterol on the rate ratio (RR) for **patient failure** was linear too and here there was interaction with recipient age. The negative influence of serum cholesterol on the RR for patient failure decreased with increasing recipient age. Increasing serum cholesterol levels influenced the risk of **over-all graft failure** and there was interaction with recipient age. As recipient age had interaction with donor age and serum creatinine the influence of all four variables together on the RR was estimated. It is shown that the RR for over-all graft failure in young recipients of a renal transplant significantly increased with higher cholesterol levels, whereas there was hardly any influence on the RR for elderly recipients. The risk increased proportionally with increasing serum creatinine levels.

Conclusion: Serum cholesterol levels have an independent influence on graft, patient and over-all graft failure.

Introduction

In the seventies the Framingham study demonstrated a logistic relationship between serum cholesterol levels and the incidence of coronary-vascular diseases (1). In primary intervention studies treatment induced a relative decrease in cardiovascular death but the absolute numbers of death were small (6). Secondary prevention studies showed a significant decrease in cardiovascular death in the population treated with cholesterol lowering medication (2,3,4,5). In patients with a renal graft hyperlipidaemia occurs in 60-80% and cardiovascular death in 40-60% of the patients (8,9). In patients with a renal graft myocardial infarctions occur 25 times more often compared to the normal population (10,11). In spite of this, there is still discussion whether renal transplant patients with high serum cholesterol levels should be treated, as there is no conclusive evidence of a direct relationship between serum cholesterol level and cardiovascular death in this multi-risk patient population (12,13,14). Our aim is to study the effect of serum cholesterol, as a continuous variable, on long-term graft, patient and over-all graft survival.

Materials and Methods

Patients:

At the University Hospital Rotterdam-Dijkzigt 883 kidney-transplantations were performed from the start in 1971 until January 1994. The analysis was done in January 1999, all patients having at least 5 years of follow-up. To evaluate the long-term risk factors we studied those patients that were alive with a functioning graft, one year after transplantation (n=676). Serum cholesterol, creatinine and data regarding the presence of proteinuria and hypertension between one and two years after transplantation were gathered. Hypertension was defined as a diastolic blood pressure above 95 mm Hg and/or a systolic blood pressure above 200 mm Hg at two or more visits, or the use of antihypertensive medication. Proteinuria was defined as urinary protein excretion above 0,15 g/l at more than 2 visits. Patients were not routinely treated with cholesterol lowering medication.

Immunosuppression:

Initially Azathioprine (AZA) was given as primary immunosuppressive therapy in a dose of 2-3 mg/kg body weight. From July 1983 onwards Cyclosporine (CsA) was used as primary immunosuppressant and was given on the basis of 12-hours trough levels. Steroids were started the day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily 6 months after

transplantation. No patient received triple drug therapy on a routine basis. Throughout the study period patients were included in several immunosuppressive treatment protocols. Although some of these treatments resulted in a significant reduction in the number of acute rejection episodes, none of them resulted in an increase in one-year graft survival.

Statistical analysis:

"Over-all graft survival " was defined as alive with a functioning graft (endpoint being either death or graft failure). For "graft survival" death was censored and for "patient survival" graft failure was censored. There were no exclusions for technical or non-immunologic failures. Potential associations with survival were analysed by means of the Cox proportional hazards regression model. The variables included in the study were cholesterol, creatinine, proteinuria and hypertension at one year after transplantation. We also included recipient and donor age and gender, HLA-mismatches on A and B locus, recipient race, original disease and transplantation period. HLA-DR mismatches were excluded from this analysis because they were not available in transplants before 1983. The gain in graft survival by matching for HLA-DR appears to be due to its effect in the first 5 months following transplantation. After this period this influence is negligible (15). As all our patients survived the first year we felt that omission of DR matching was acceptable. We also introduced participation to an immunosuppressive trial directly after transplantation as a variable. Categories were patients in the same trial. All patients not participating and the controls were the reference category. Interactions between variables were tested using likelihood ratio tests: for hierarchical reasons all lower order terms and main effects remained in the model if the interaction was significant. For categorical variables indicator coding was used with the first category as reference. The reference categories were: 1983-1990 as transplantation period, male gender (both recipient and donor), zero HLA mismatches on the A and B locus and Caucasian race. Glomerulonephritis was used as the reference category for original disease. For categorical variables the rate ratio (RR) of a category versus the reference was equal to e^B, where B is the regression coefficient for that category. Both donor and recipient age were analysed as continuous variables measured in years. As a reference value, the rate ratio (RR) of a 20 year old (both recipient and donor) was settled to 1. Concerning the one-year-variables: cholesterol was analysed as a continuous variable, the RR of a cholesterol of 6 mmol/l was set to 1. Creatinine was also analysed as a continuous variable, the RR of 75 μmol/l being 1. The RR of continuous variables is per unit of the variable considered. Proteinuria and hypertension were analysed as categorical variables, presence compared to

absence, the latter being the reference category. Because the influence of AZA and CsA on survival appeared not to satisfy the proportional hazards assumption, we chose to stratify for immunosuppressive medication. In order to get an impression about the precision of the estimated relationships the confidence intervals were also calculated. The analyses were performed with SPSS for Windows version 7.52. P-values ≤ 0.05 were considered significant.

Results

In the observed population of 676 patients, 220 received AZA and 456 CsA as primary immunosuppressant. One or more variables were missing in 18 patients (2,7%). Mean recipient age was 41.8 ± 13.1 years. In only 4 patients the recipient age was over 70 years. Mean donor age 33.5 ± 15.7 years. At one year 95% of the patients had a serum creatinine below 300 μmol/l (median150 μmol/l, range 53-1240 μmol/l). A serum cholesterol lower than 11 mmol/l was found in 96% of the patients. Mean serum cholesterol was not significantly different between the primarily AZA and the CsA treated population (respectively 7,0 ± 2,0 and 7,2 ± 1,8 mmol/l). Proteinuria was found in 33,8% of the patients. Hypertension was present in 67,7% of the patients.

Cox Proportional Hazards Analysis: Adjusted for all other variables in the model, serum cholesterol was an independent covariate influencing patient survival, graft survival censored for death and over-all graft survival (table 1).

Table 1: *Results of the multivariable Cox proportional hazards analysis. The relative risk of over-all graft failure is one when it concerns a 20 year old recipient with a congenital or hereditary disease as original disease, who received a kidney from a 20 year old donor in the transplantation period 1971-1982. Serum creatinine is 75 μmol/l and serum cholesterol is 6 mmol/l, while there is no proteinuria.*

	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death			
Serum cholesterol (mmol/l)	1.3349	1.1505-1.5488	0.0001
Serum creatinin (umol/l)	1.0174	1.0111-1.0238	<0.0001
cholesterol*creatinin	0.999	0.9982-0.9999	0.0218
Donor age (per year)	0.9865	0.9765-0.9966	0.009
Donor gender	1.3688	1.0162-1.8436	0.0388
Number of transplantations	1.3670	1.0749-1.7386	0.0108
Proteinuria	2.4630	1.8203-3.3324	<0.0001
Original disease			0.0003
Diabetes Mellitus	1.000		
Systemic diseases	6.0482	1.9058-19.1942	0.0023
Patient failure			
Serum cholesterol (mmol/l)	1.5290	1.1024-2.1205	0.0109
Recipient age (per year)	1.1262	1.0696-1.1858	<0.0001
cholesterol*recipient age	0.9933	0.9867-1.000	0.0498
Recipient gender	0.6852	0.4879-0.9624	0.0292
Proteinuria	2.2741	1.6460-3.1418	<0.0001
Original disease			0.0290
Glomerulonephritis	1.000		
Diabetes Mellitus	3.3662	1.8418-6.1524	0.0001
Over-all graft failure			
Serum cholesterol (mmol/l)	1.2231	0.7273-2.0571	0.4477
Serum creatinin (umol/l)	1.0385	1.0205-1.0568	<0.0001
Recipient age (per year)	0.9582	0.7536-1.2183	0.7274
Recipient age squared (per year)	1.0017	0.9989-1.0044	0.2429
Donor age (per year)	0.8895	0.8301-0.9531	0.0009
recipient age*cholesterol recipient age squared*cholesterol			0.0127
recipient age*creatinin recipient age squared*creatinin			0.0001
recipient age*donor age recipient age squared*donor age			0.0067
Proteinuria	2.2189	1.7673-2.7860	<0.0001
Original disease			0.0279
Congenital, hereditary	1.000		
Systemic diseases	1.9670	1.0449-3.7025	0.0361

The influence of serum cholesterol on either of the end-points did not turn out to be time-dependent.

Both cholesterol and creatinine were independent variables influencing **graft failure censored for death**, adjusted for all other variables in the model. There was a negative interaction between them. Because of this the effect of both variables on the hazard rate has to be considered simultaneously. This simultaneous risk is defined here as a continuous function of serum cholesterol for four levels of creatinine (75, 150, 250 and 300 μmol/l). It is expressed relatively to a cholesterol level of 6 mmol/l and a creatinine level of 75 μmol/l. Hence for these levels the rate ratio is one (unity). Figure 1 shows the regression lines of the influence of the interaction of cholesterol and creatinine.

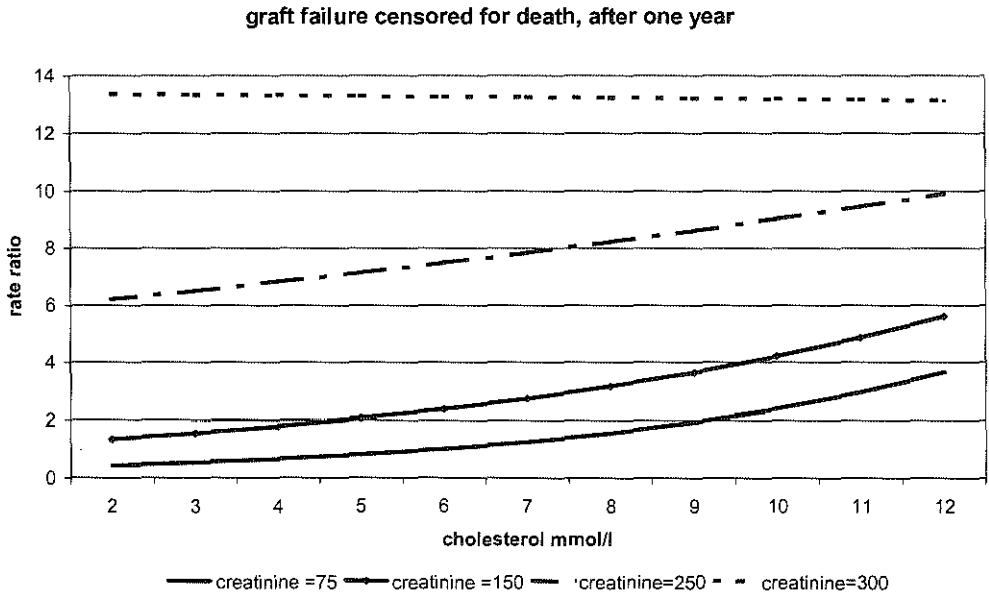

Figure 1: *Simultaneous influence of serum cholesterol and serum creatinine on the relative risk of graft failure censored for death.*
The solid line is the regression line obtained with the regression equation when the RR for creatinine is 1. The influence of other values of serum creatinine on the influence of cholesterol on the RR is plotted according to the regression equation.

The proportional increase in the RR between a serum cholesterol of 6 and 12 is largest in patients with the best renal function (at a creatinine of 75 μmol/l the risk increases from 1 to 3.8; a proportional increase of 3.8). In patients with a bad renal function (creatinine 300 μmol/l) the line is horizontal; increasing serum cholesterol levels do not influence the RR. It is clear that in patients with a good renal function the negative influence of high serum cholesterol is larger than in patients with a bad kidney function.

Both serum cholesterol and recipient age were independent variables influencing the RR for **patient death,** adjusted for all other variables in the model. There was interaction between these variables. Therefore the effect of both variables on the hazard rate was considered. The simultaneous risk is defined as a continuous function of serum cholesterol for four ages (20, 40, 50 and 60 years). It is expressed relatively to a cholesterol level of 6 mmol/l and a 20-year-old patient. Figure 2 shows the influence of the interaction of cholesterol and recipient age as RR relatively to the rate of a 20-year-old with cholesterol of 6 mmol/l.

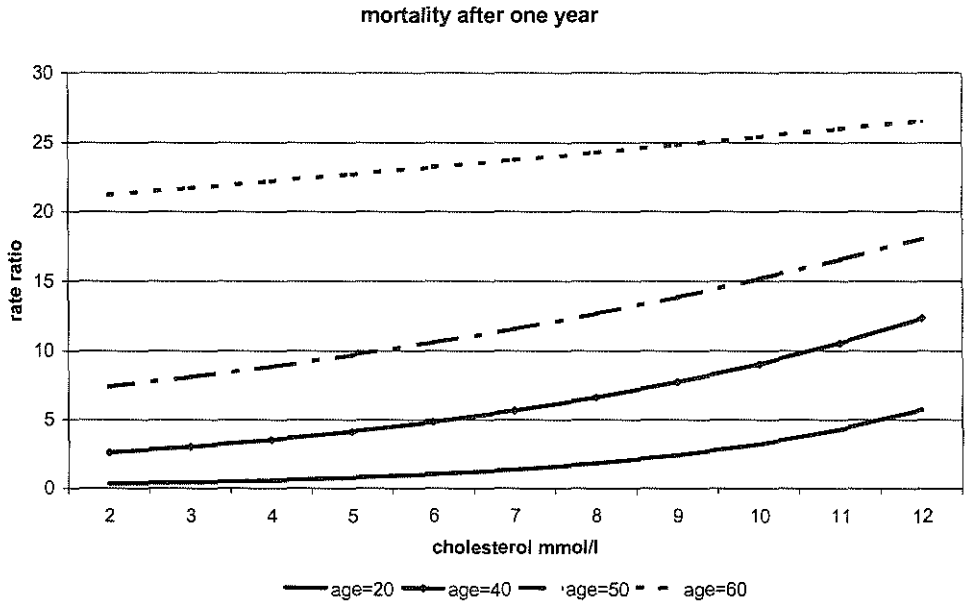

Figure 2: *Simultaneous influence of serum cholesterol and recipient age on the relative risk of death.*
The solid line is the regression line obtained with the regression equation when the RR for recipient age is 1. The influence of other recipient ages on the influence of cholesterol on the RR is plotted according to the regression equation.

The negative influence of high serum cholesterol is largest in the youngest patients. In the elderly the rate increase caused by cholesterol is outweighed by the larger rate caused by other factors associated with higher recipient age.
Cholesterol, creatinine and donor age, recipient age and the square of recipient age amongst others, predicted **over-all graft failure**. Chi square likelihood ratio tests showed a significant influence by the interaction terms: cholesterol*recipient age together with cholesterol*recipient age squared, by creatinine*recipient age together with creatinine*recipient age squared and by donor age*recipient age together with donor age*recipient age squared. Because of these interactions the simultaneous effect of all these variables together was considered. Again the risk is considered as a continuous function of serum cholesterol but now for four donor and recipient age combinations (20 and 60 years), all four for two serum creatinine values (75 and 250 μmol/l). The RR is expressed relatively to a cholesterol level of 6 mmol/l in a 20 year old recipient and donor combination with a serum creatinine of 75 μmol/l. Figure 3 shows the regression lines of the influence of cholesterol and its interactions with recipient age, recipient age squared and donor age at a serum creatinine of 75 μmol/l (at one year after transplantation).
The over-all graft failure rate is largest in young recipients of a young donor kidney; it is lower for young recipients of an old donor kidney. The influence of increasing serum cholesterol levels is significant and proportionally almost equal for both groups. The over-all graft failure rate is low for elderly recipients and is hardly dependent upon the age of the donor. There is no influence of cholesterol on the failure rate of old recipients of either a young or old donor kidney.

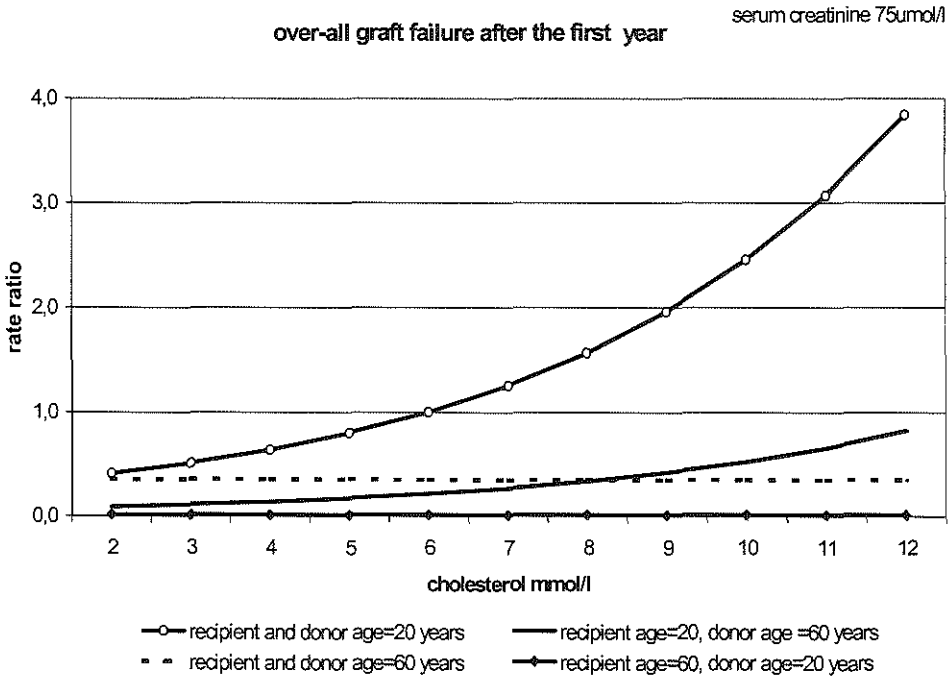

Figure 3: *Simultaneous influence of serum cholesterol, recipient age, recipient age squared and donor age on the relative risk of over all graft failure at a fair renal function with a serum creatinine of 75 μmol/l.*

The solid line with the open circles is the baseline showing the influence of cholesterol when the RR of all other variables is 1. When recipient and/or donor age is not 20, the influence is plotted according to the regression equation for the different ages.

Figure 4 shows the regression lines of the influence of cholesterol and its interactions with recipient age, recipient age squared and donor age at a serum creatinine of 250 μmol/l. The risks for young recipients have increased even further whereas those of elderly recipients have hardly changed compared to those in figure 3.

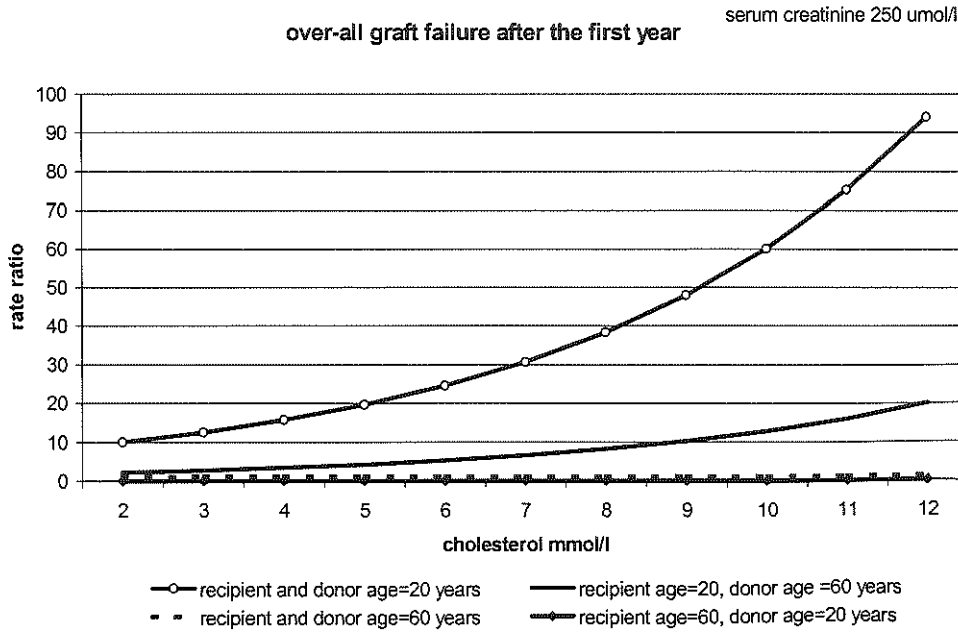

Figure 4: *Simultaneous influence of serum cholesterol, recipient age, recipient age squared and donor age on the relative risk of over all graft failure at a bad renal function with a serum creatinine of 250 μmol/l.*
The solid line with the open circles is the baseline showing the influence of cholesterol when the RR of all other variables, except that for serum creatinine, is 1. When recipient and/or donor age is not 20, the influence is plotted according to the regression equation for the different ages.

As could be expected, the confidence intervals of the relationships found to be significant, appeared to be very wide (not shown); especially for extreme combinations of values for the explanatory variables.
Other factors having an independent influence on transplant failure were donor gender and number of previous transplants (table 1). Recipient gender had an independent influence on patient failure (table 1). Both, proteinuria and original disease had a significant influence on all three end points (table 1). All original diseases not mentioned in the table did not differ significantly from the reference category in their influence on the failure rate. Participation to an immunosuppressive trial in the non-control group did not significantly influence the failure rate.
The presence of hypertension did not have an independent influence on any of the end-points studied. All other variables tested did not have an independent influence on failure in the Cox proportional hazards analysis.

Discussion

Charlesworth did the first investigations on the influence of serum cholesterol in patients with a renal transplant in 1974 (16). He found that a pre-transplant hypercholesterolaemia was associated with a significantly worse renal function after one year. Most recent studies use post transplant serum cholesterol levels for the evaluation of the effect of cholesterol on graft survival. Some of them use graft survival as an end point, finding a significant effect of serum cholesterol (17,18), while others do not (19). Others use (histologically confirmed) chronic vascular rejection as read out point and do (20) or do not find a significant effect (21,22).
Divakar found that a longer survival after transplantation was associated with a lower LDL and a higher HDL cholesterol (23). In a prospective, randomised, controlled study, Katznelson treated 24 patients, directly after renal transplantation, with pravastatin and compared them to 24 untreated patients (24). He concluded that acute rejection therapy was significantly less often necessary and that the histological findings were less severe in the treated population. After 3 months there was no difference in renal function. Two prospective studies in heart transplantation patients showed that the HMG-CoA reductase inhibitor treated population suffered from less severe rejection episodes, had a better one-year graft survival and less often chronic rejection (25,26).
In the present retrospective study concerning 658 kidney transplants the Cox regression analysis shows that serum cholesterol is a serious risk factor for patient,

graft and over-all graft failure. Figure 1 shows the combined influence of increasing cholesterol levels and kidney function on **graft failure**. As the proportional increase in RR between cholesterol values of 6 and 12 mmol/l is largest at the best renal function (creatinine 75 μmol/l), compared to the worst renal function, the disadvantageous effect of elevated serum cholesterol levels is largest in recipients with a fair renal function. As the RR of a bad kidney function by itself is much larger than that of high serum cholesterol, the latter is outweighed in this situation. However, in the interpretation of the results in patients with a bad renal function it should be borne in mind that in our population 95% of the patients had a serum creatinine below 300 μmol/l. Extrapolation of the results to higher creatinine values is hardly reliable. Hence, as far as graft failure is concerned, treatment of elevated levels of serum cholesterol is worth being considered in patients with a fair renal function.

Figure 2 shows the combined influence of cholesterol and recipient age on the **RR for death**. The largest proportional increase in RR is found in the youngest age groups, Thus the influence of high serum cholesterol on the risk of death is largest for the young recipients. The RR of high recipient age is much larger than that of high cholesterol levels, so that serum cholesterol levels do not influence the RR of elderly persons. Treatment of elevated cholesterol levels in order to prevent patient death is indicated in the young and middle aged.

The influence of cholesterol and its interaction with recipient age and recipient age^2 (=age squared), combined with the influences of donor age and serum creatinine, on **over-all graft failure** is shown in figures 3 and 4. As the proportional increase in RR with increasing serum cholesterol levels is largest for young recipients, it is clear that cholesterol is much more harmful to the young, compared to the elderly recipient. This holds true for both a good and a bad renal function.

Young recipients appear to do worse than elderly. A bad renal function one-year after transplantation is less unfavourable if the recipient is elderly. Although there is a higher risk of failure after transplantation with elderly donor kidneys, this analysis shows that when an elderly donor kidney managed to function at least until one year after transplantation the risk of over-all graft failure has decreased substantially below that of young donor kidneys (8). This analysis also shows that the worst possible combination is a young recipient and a young donor. And that the risk of elderly recipients is low and hardly influenced by donor age, cholesterol or renal function after one year.

Comparison of figures 3 and 4 shows that a high serum creatinine causes a larger increase in risk of over-all graft failure in the young compared to the elderly. This is

probably caused by the higher metabolic demands of the young, resulting in hyperfiltration and graft loss.
The confidence interval of an interaction term is influenced by the confidence intervals of all variables in the term; this explains why they are wide. Especially for extreme combinations of values for the various explanatory variables (including their interactions) the uncertainty in the predictive power of the model should be considered.
Hypertension did not turn out to be an important variable influencing the failure rate. A reason for the lack of significance could be that our criteria for hypertension are too strict, resulting in this diagnosis in two third of our population. Probably a well-controlled hypertension should not be estimated as hypertensive.
From our investigation it is clear that there is no "safe" cholesterol value as the influence is log-linear and the risk increases with increasing cholesterol values. Unfortunately, there is no proof as to whether treatment of elevated serum cholesterol levels reduces the RR for any of the endpoints in the long term. The prevalence of atherosclerotic disease is high in the renal transplant population. Hyperlipidaemia is one component of the risk pattern for coronary heart disease that is shown to be important in the general population. Many studies showed that cholesterol-lowering therapy reduces morbidity and mortality from coronary heart disease. There is no obvious reason for not extrapolating this evidence to renal transplant recipients. A fascinating subject for the future will be the long-term influence of cholesterol lowering therapy on both patient and graft survival.
In conclusion: Serum cholesterol is an independent risk factor for patient, graft and over-all graft failure after renal transplantation. The effect is log-linear so that there is no optimal value; the lower, the better. Because of interaction with recipient age, the risks for patient and over-all graft failure in patients with high serum cholesterol are largest in the youngest population, decreasing with age. The influence of cholesterol on the chance of graft failure censored for death is influenced by the serum creatinine levels; the largest influence is shown in patients with the best renal function.
Treatment of patients with a renal graft and an elevated serum cholesterol level seems to be indicated with the aim of conserving renal function and preventing death.

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CHAPTER 5

The influence of cholesterol on mortality after transplantation is age dependent.

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Abstract

Background: There is still no consensus on the treatment of elevated serum cholesterol in patients with a renal transplant. In the general population treatment is age dependent.

Methods: We studied the influence of serum cholesterol one year after transplantation in all 676 recipients of a kidney graft, transplanted in Rotterdam, that survived and functioned for at least one year. The other variables included in this analysis are: donor and recipient age and gender, original disease, race, number of HLA-A and B mismatches, number of previous transplantations, postmortal or living related transplantation and transplantation year. At one year after transplantation the following variables were included: serum cholesterol, serum creatinine, proteinuria and hypertension.

Results: In the Cox proportional hazards analysis serum cholesterol at one year after transplantation turned out to be an important, independent variable influencing patient failure. The influence was linear but there was interaction with recipient age. The negative influence of serum cholesterol on the RR for patient failure decreased with increasing recipient age. E.g. the proportional increase of the RR of a 20-year-old with serum cholesterol of 12 mmol/l compared to that of cholesterol of 6 mmol/l was 6. In a 60-year-old with cholesterol of 12 mmol/l the proportional increase of the RR was only 1,2 compared to a contemporary with cholesterol of 6 mmol/l.

Conclusion: Serum cholesterol levels have an independent influence on patient failure. The RR is influenced by recipient age, so that the negative effect of increasing cholesterol levels in the elderly is overruled by the RR of age, and disappears.

Introduction

A relationship between serum cholesterol and cardiovascular mortality has been proven for the general population (3,4,5,9,10,11). Apart from serum cholesterol, elderly people have a larger risk of cardiovascular mortality as their over-all death risk is raised. It is customary to combine the influences of age, serum cholesterol and other variables, on the death risk in deciding whether to treat patients with elevated cholesterol or not (1,6). We wondered whether age should be included, because then the population treated is a selection of patients with a largely age-related high risk.

The population that receives a renal transplant is known to have an increased risk of cardiovascular mortality (2,7,8,12). We studied the additional risk of an elevated

serum cholesterol level on the death risk of different age groups in our renal transplant population.

Materials and Methods

Patients:

At the University Hospital Rotterdam-Dijkzigt 883 kidney-transplantations were carried out from the start in 1971 until January 1994. The analysis was done in January 1999, all patients having at least 5 years of follow-up. One year after transplantation 676 patients were still alive with a functioning graft. They were included in this analysis. From all these patients serum cholesterol, creatinine and information regarding the presence of proteinuria and hypertension between one and two years after transplantation was gathered. Patients were not routinely treated with cholesterol lowering medication.

Immunosuppression:

Initially Azathioprine (AZA) was given as primary immunosuppressive in a dose of 2-3 mg/kg body weight. From July 1983 onwards Cyclosporine (CsA) was used as primary immunosuppressant and was given on the basis of 12-hours trough levels. Steroids were given on the day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily 6 months after transplantation. No patient received triple drug therapy on a routine basis.

Statistical analysis:

"Patient survival" was censored for graft failure. There were no exclusions for technical or non-immunologic failures. Potential associations with survival were analysed by means of the Cox proportional hazards regression model. The variables included in the study were cholesterol, creatinine, proteinuria and hypertension at one year after transplantation, as well as recipient and donor age and gender, HLA-mismatches on A and B locus, recipient race, original disease and transplantation period. HLA-DR mismatches were excluded from this analysis because they were not available in transplants before 1983. Variables were selected by backward elimination using likelihood ratio tests. For categorical variables indicator coding was used with the first category as reference. For categorical variables the rate ratio (RR) of a category versus the reference was equal to e^B, where B is the regression coefficient for that category. Both donor and recipient age were analysed as continuous variables measured in years. As a reference value, the rate ratio (RR) of a 20 year old (both recipient and donor) was settled to 1. Concerning the one-year-variables: cholesterol was analysed as a continuous variable, the RR of cholesterol of 6 mmol/l was set to 1. Creatinine was also

analysed as a continuous variable, the RR of 75 μmol/l being 1. The RR of continuous variables is per unit of the variable considered. Because the influence of AZA and CsA on survival appeared not to satisfy the proportional hazards assumption we chose to stratify for immunosuppressive medication. The analyses were performed with SPSS for Windows version 7.52.

Results

In the observed population of 676 patients, 220 were transplanted with AZA and 456 with CsA as primary immunosuppressive. From 18 patients one or more variables were missing (2,7%).

Cox Proportional Hazards Analysis: Serum cholesterol turned out to be an independent covariate influencing the RR for **patient death**. Because there was interaction with recipient age, the effect of both variables on the hazard rate was considered. The simultaneous risk is defined as a continuous function of serum cholesterol for three ages (20, 40 and 60 years). It is expressed relatively to a cholesterol level of 6 mmol/l in a contemporary with a renal transplant. Figure 1 shows the influence of the interaction of cholesterol and recipient age as a RR relatively to the rate of a contemporary with cholesterol of 6 mmol/l.

As the proportional increase in the RR is highest in the youngest recipients, the negative influence of high serum cholesterol is largest in the youngest patients.

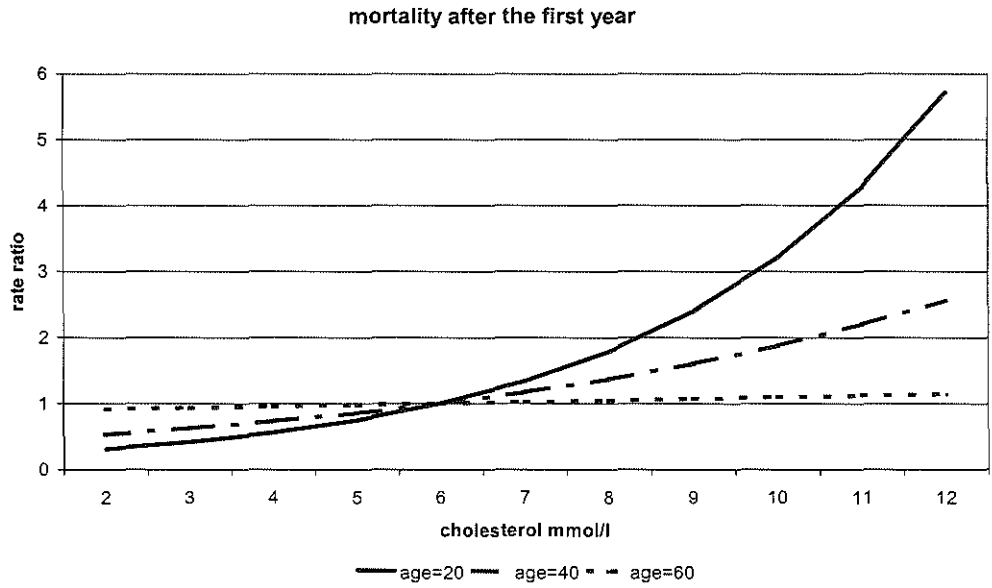

Figure 1: *Simultaneous influence of serum cholesterol and recipient age on the relative risk of death.*
The solid line is the baseline showing the influence of cholesterol when the RR of all other variables is 1. The RR of serum cholesterol of 6 mmol/l is settled to 1, whatever the recipient age. The influence of cholesterol on the RR at different ages is calculated.

Discussion

Results from studies in the general population regarding the influence of serum cholesterol on the chances of death resulted in a generally accepted consensus concerning treatment of persons with elevated serum cholesterol levels (1,6). In the Netherlands it is customary to treat uncomplicated patients with serum cholesterol levels above 8 mmol/l with cholesterol lowering medication. Below this value treatment is dependent upon six variables: cholesterol/HDL ratio, age, gender, diabetes, smoking habits and hypertension. It is remarkable that in this scoring system the indication for treatment increases with age. This is because the risk is calculated by means of the equation coefficients of all 6 variables mentioned. The calculated risk is the 10 years chance on a cardiovascular event. However, this risk increases with age, independent of any other risk factor. Moreover, age cannot be treated. So, it is questionable whether this variable should be weighed so heavily. Whether the risks of the other variables add to that of increasing age is more interesting. Apart from the age related risk, does an equally elevated serum cholesterol result in the same risk at different ages? Is smoking for an elderly person as dangerous as for a young person? The population we studied is not a general population, but as cardiovascular disease is such a prominent cause of death it is an interesting population to study these influences (2,7,8,12).

In this retrospective study concerning 658 kidney transplants the Cox regression analysis shows that serum cholesterol is a serious risk factor for patient death, but the effect is different at different ages. The largest proportional increase in RR is found in the youngest age groups, so the influence of high serum cholesterol on the risk of death is largest for the young recipients (figure 1). Serum cholesterol levels do not influence the RR of elderly persons, so although their death risk is larger than that of young patients the serum cholesterol level does not add to this effect. Our conclusion for this patient population is that treatment of elevated cholesterol levels in the hope to prevent patient death seems to be indicated especially in the young and middle aged.

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CHAPTER 6

Proteinuria after renal transplantation affects not only graft survival but also patient survival.

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Abstract

Background: It has been shown that proteinuria is associated with an increased risk of renal failure. Moreover, proteinuria is associated with an increased death risk in patients with diabetes mellitus or hypertension and even in the general population.

Methods: We studied the influence of the presence of proteinuria at one year after renal transplantation on the risk of either graft failure or death in all 722 recipients of a kidney graft in our centre, surviving at least one year with a functioning graft. Proteinuria was analysed both as a categorical (presence versus absence) and as a continuous variable (quantification of 24 hour urine). Other variables included in this analysis were: donor/recipient age and gender, original disease, race, number of HLA-A and -B mismatches, previous transplants, postmortal or living related donor transplantation and transplantation year. At one year after transplantation we included: proteinuria, serum cholesterol, serum creatinine, blood pressure and the use of antihypertensive medication.

Results: In the Cox proportional hazards analysis proteinuria at one year after transplantation (both as a categorical and continuous variable) turned out to be an important, independent variable influencing all end points. The influence of proteinuria as a **categorical** variable on **graft failure censored for death** showed no interaction with any of the other variables. There was an adverse effect of the presence of proteinuria on the graft failure rate (RR=2.03). The influence of proteinuria as a **continuous** variable showed interaction with original disease. The presence of glomerulonephritis, hypertension and systemic diseases as original disease significantly increased the risk of graft failure with an increasing amount of proteinuria at one year. The influence of proteinuria as a **categorical** variable on the rate ratio for **patient failure** was significant and there was no interaction with any of the other significant variables (RR=1.98). The death risk was almost twice as high for patients with proteinuria at one year compared to those without. The influence of proteinuria as a **continuous** variable was significant too and there was no interaction either. The death risk increased with an increasing amount of proteinuria at one year. Both the risks for cardiovascular and for non-cardiovascular death were increased.

Conclusion: Proteinuria after renal transplantation not only increases the risk of graft failure but also the risk of death.

Introduction

In renal transplant patients, proteinuria is known to decrease the prognosis regarding graft survival (1,2,3). The use of ACE inhibitors is shown to decrease proteinuria but its influence on graft survival is not known (4,5,6). Guijarro showed that hypoalbuminaemia is common after renal transplantation and that low serum albumin is a strong, independent risk factor for all-cause mortality (7). Recently, an increasing number of publications showed a positive association between proteinuria and cardiovascular or all-cause mortality in non-transplanted diabetics, patients with essential hypertension and in the general population (8-22).
In a previous study we found a significant influence of proteinuria as a categorical variable on the failure risk after transplantation (23). We wondered whether this influence was dependent on the amount of proteinuria or on its mere presence.
We studied the influence of proteinuria and other variables on the graft failure and death risks after renal transplantation.

Materials and Methods

Patients:

At the University Hospital Rotterdam "Dijkzigt" 1007 kidney-transplantations were performed from the start in 1971 until January 1995. The analysis was carried out in January 2000, all patients having at least 5 years of follow-up. To evaluate the long-term risk factors we studied those patients that were alive with a functioning graft, one year after transplantation (n=722). Serum cholesterol, creatinine and data regarding the presence of proteinuria and hypertension between one and two years after transplantation were gathered. The influences of the systolic and diastolic blood pressure and the use of antihypertensive medication were analysed. Patients were not routinely treated with cholesterol lowering medication.
Proteinuria was analysed in two ways: as a binary categorical variable (present / absent), presence defined as urinary protein excretion above 0,20 g/l at more than 2 successive visits. Secondly, proteinuria was analysed as a continuous variable (g/24 hours, detection limit 0,20 g/l). Methods: From 1971 onwards the biuret procedure was used, which was replaced by the completely automated method with benzethonium chloride reagent at the end of the eighties. Micro-albuminuria was not routinely determined in this retrospective analysis.

Immunosuppression:

Initially Azathioprine (AZA) was given as primary immunosuppressive therapy in a dose of 2-3 mg/kg body weight. From July 1983 onwards Cyclosporine (CsA) was used as primary immunosuppressant and was dosed on the basis of 12-hours

trough levels. Steroids were started on the day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily 6 months after transplantation. No patient received triple drug therapy on a routine basis. Throughout the study period patients were included in several immunosuppressive treatment protocols. Although some of these treatments resulted in a significant reduction in the number of acute rejection episodes, none of them resulted in a significant increase in one-year graft or patient survival.

Statistical analysis:

"Graft survival" was censored for death. "Patient survival" was censored for graft failure; observation was until three months after graft failure. There were no exclusions for technical or nonimmunologic failures. Potential associations with survival were analysed by means of the Cox proportional hazards regression model. The variables included in the study were cholesterol, creatinine, proteinuria and blood pressure at one year after transplantation. We also included recipient and donor age and gender, HLA-mismatches on A and B locus, recipient race, original disease and transplantation period. HLA-DR mismatches were excluded from this analysis because they were not available in transplants performed before 1983. Variables were selected by backward elimination using likelihood ratio tests. For categorical variables, indicator coding was used with the first category as reference. The reference categories were: 1971-1979 as transplantation period, male gender (both recipient and donor), zero HLA mismatches on the A and B locus and Caucasian race. Congenital/hereditary disease was used as the reference category for original disease. For categorical variables the rate ratio (RR) of a category versus the reference was equal to e^B, where B is the regression coefficient for that category. Both donor and recipient age were analysed as continuous variables measured in years. As a reference value, the rate ratio (RR) of a 20 year old (both recipient and donor) was settled to 1. Concerning the one-year-variables: cholesterol was analysed as a continuous variable, the RR of a cholesterol of 6 mmol/l was set to 1. Creatinine was also analysed as a continuous variable, the RR of 75 µmol/l being 1. The RR of continuous variables is per unit of the variable considered. Proteinuria was both analysed as a continuous and as a categorical variable (0 g/24 hours resp. absence were the reference categories). Systolic and diastolic blood pressure were both analysed as continuous variables (reference categories 140 resp. 90 mm Hg). Use of antihypertensive medication was introduced as a categorical variable, absence being the reference category. Because the influence of AZA and CsA on survival appeared not to satisfy the proportional hazards assumption, we chose to stratify for immunosuppressive medication.

The analyses were performed with SPSS for Windows version 8.0. P-values ≤ 0.05 were considered significant.

Table 1: *Patient characteristics. Categorical variables.*

proteinuria	31% positive
donor origin	12% living (un) related
use of antihypertensives	60%
number of transplants	82,7% first, 13.2% second, 3.9% third
race	83.9% caucasian, 4.8% african, 5.7% asian, 3.9% turkish, 1.7% arabian
gender	60% males
donor gender	64.7% males
transplantation period	19.4% before 1979, 47.5% between 1980-1989, 33.1% after 1989
original disease	12.3% congenital, 39% glomerulonephritis, 4.6% diabetes; 8.2% hypertension, 22.1% TIN/obstruction; 2.8% systemic diseases, 11.1% unknown.

Results:

One year after transplantation proteinuria was present in almost one third of the patients (table 1).

Patients with congenital or hereditary diseases as their original disease had significantly less often proteinuria at one year after transplantation (p=0.001). There was an over-representation of glomerulonephritis in the proteinuric population compared to the other original diseases (p=0.039). The increased number of failures in the proteinuric population was represented by an increase in acute and chronic rejection and in recurrence of original disease (all p<0.0001). However, within the failed population chronic rejection was the main cause of failure in both the proteinuric and the non-proteinuric population (54 resp. 43.1% difference not significant). In the failed proteinuric population acute rejection occurred more often but not significantly. Recurrence of original disease, as the cause of failure appeared significantly more often in the proteinuric compared to the non-proteinuric population (13.8 resp. 5.3% p=0.042). There were very weak correlations between proteinuria and systolic blood pressure (R=0.128), between

systolic blood pressure and serum creatinine (R=0.138) and between proteinuria and creatinine (R=0.101). Patient characteristics from both the categorical and the continuous variables are shown in tables 1 and 2 respectively.

Table 2: *Patient characteristics. Continuous variables.*

	mean	standard dev	median	minimum	maximum	5th percentile	95th percentile
recipient age	42	13.2	42	16	73	21	64
donor age	34	15.8	33	1	73	10	59
creatinine	163	112	139	53	1270	80	306
cholesterol	7.2	2.8	6.9	3	19	4.5	10.5
systolic pressure	146	20.4	140	90	240	115	180
diastolic pressure	90	11.6	90	40	130	70	110
proteinuria g/day	0.7	1.8	0	0	17	0	4.25
HLA-A mismatches	0.73	0.58	1	0	2	0	2
HLA-B mismatches	0.62	0.62	1	0	2	0	2

Ages in years, creatinine in μmol/L, cholesterol in mmol/L, blood pressure in mm Hg

In the Cox proportional hazards analysis, proteinuria turned out to be an independent variable influencing all end points. As a *categorical variable*, proteinuria had an independent influence on the **graft failure rate censored for death**. Presence was associated with a RR of 2.03 (table 3).

***Table 3**: Results of the Cox proportional hazards analysis.*

	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death			
Proteinuria categorical	2.0326	1.4973-2.7594	<0.0001
Proteinuria g/day			
Original disease			<0.0001
proteinuria*original disease			
Proteinuria (g/day) in			
hypertension	1.4076	1.1467-1.7279	0.0011
systemic diseases	1.5601	1.2259-1.9853	0.0003
glomerulonephritis	1.1705	1.0827-1.2655	0.0001
congenital/hereditary disease			ns
diabetes mellitus			ns
TIN/obstruction			ns
Donor age (per year)	0.9423	0.9147-0.9707	
Recipient age	0.9400	0.9126-0.9683	<0.0001
donor age*recipient age	1.0011	1.0004-1.0018	
Systolic blood pressure	1.0176	1.0075-1.0277	0.0006
Patient failure			
Proteinuria categorical	1.9779	1.4375-2.7214	<0.0001
Proteinuria g/day	1.1647	1.0767-1.2600	0.0001
Recipient age (per year)	1.0681	1.0534-1.0829	<0.0001
Original disease			0.0023
Hereditary diseases	1.000		
Diabetes Mellitus	3.1676	1.7310-5.7963	0.0002
Hypertension	2.1194	1.2277-3.6589	0.0070
Recipient race			0.0077
Turkish	3.8530	1.7267-8.5973	0.0010

TIN=tubulo-interstitial nephritis

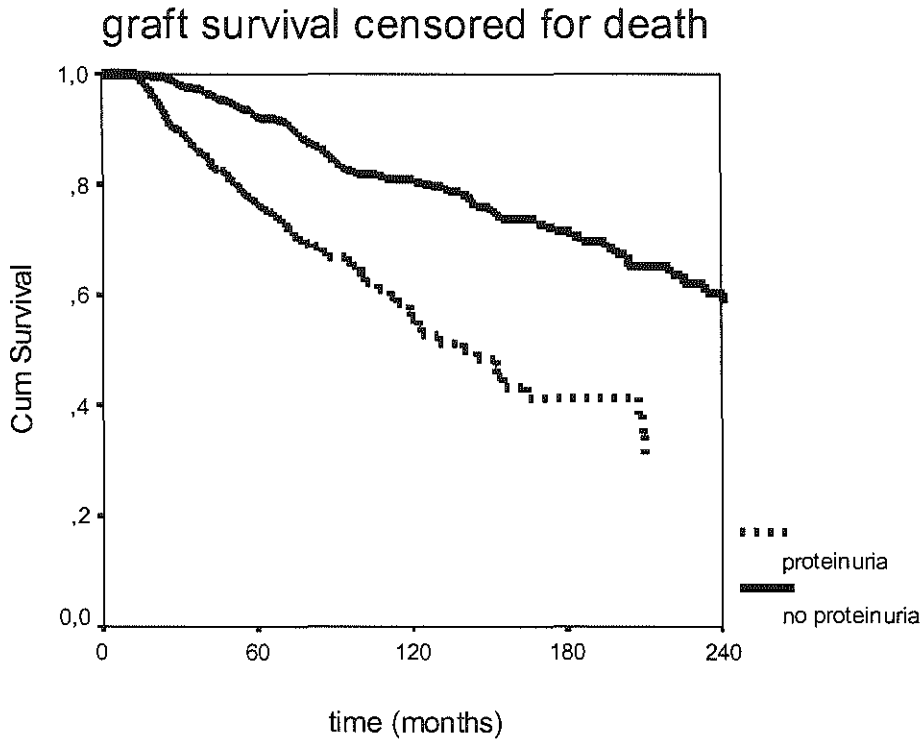

Figure 1: *Graft survival curve obtained by the Cox analysis.*

The different graft survival lines for proteinuria are shown in figure 1. There was no interaction with any of the other variables. Original disease also had a significant influence independent of all other variables. This influence primarily concerned systemic diseases. The influence of proteinuria as a *continuous variable* on the graft failure rate censored for death showed interaction with original disease as shown in table 3. The influence was significant for hypertension, glomerulonephritis and for systemic diseases (table 3, figure 2).

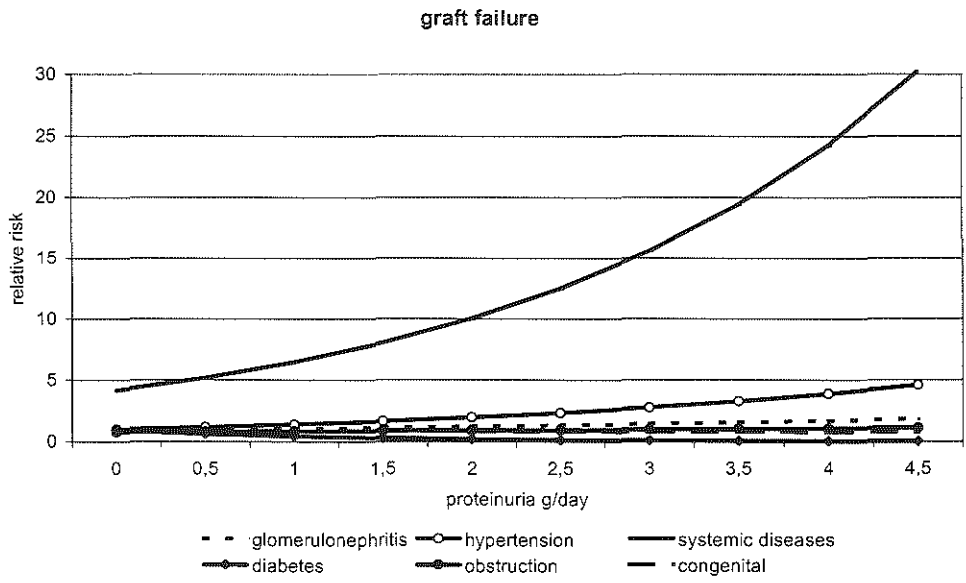

Figure 2: *Calculated relative risk of graft failure with increasing amounts of proteinuria for the different original diseases. The reference combination chosen for the combination of original disease and proteinuria is congenital/hereditary diseases and 0 g/day.*

As the results of both analyses (proteinuria as a continuous and as a categorical variable) largely overlapped concerning significance, regression coefficients and interactions, the results of the second analysis are not shown. Donor and recipient age showed a significant influence on the graft failure rate and there was interaction between them. The risk decreased with increasing donor and/or recipient age (table 3, figure not shown).

Systolic blood pressure was a significant predictor of the graft failure risk (table 3). The regression lines are shown in figure 3. It is clear that the risk of graft failure increases with increasing systolic blood pressure. The influence of diastolic blood pressure was not significant in either of the analyses.

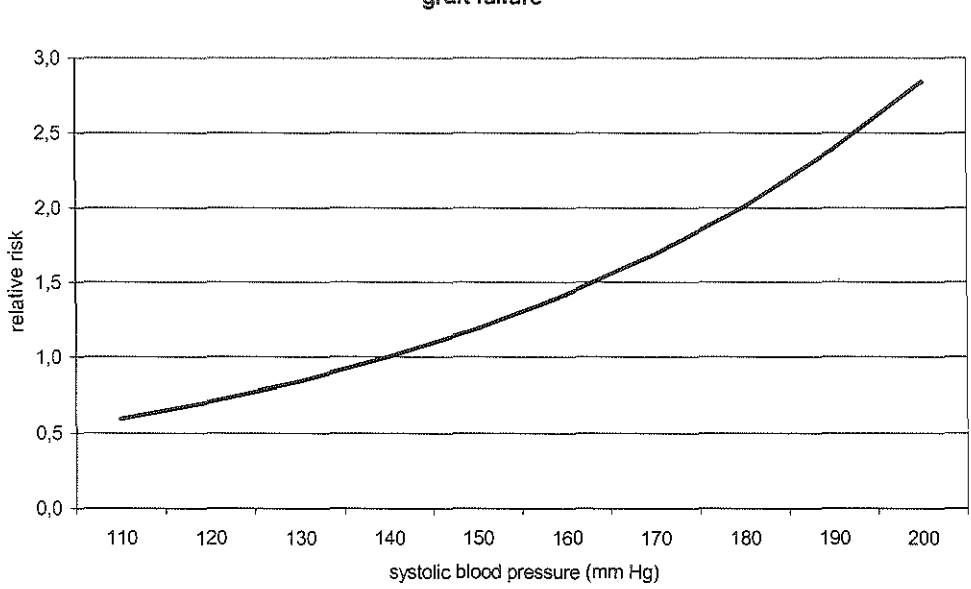

Figure 3: *Calculated relative risk of graft failure with increasing systolic blood pressure (mm Hg). The reference value is 140 mm Hg.*

As shown before, serum creatinine, cholesterol and donor gender were all significant factors influencing the graft failure rate and there was interaction between them (data not shown, 23). Their mutual influence resulted in an increasing risk with increasing creatinine and/or cholesterol levels, whereas organs from female donors ran slightly higher risks for failure.
After the first year, transplantation period barely influenced the risk of graft failure ($p=0.0445$). The number of previous transplants influenced the graft failure risk ($p=0.0333$).
In the Cox proportional hazards analysis the proteinuric population turned out to have a higher **risk of death**. Both as a continuous and as a categorical variable, proteinuria showed an independent influence on the risk of patient death. There was no interaction with any of the other variables in either of the analyses (table 3). The different patient survival lines for proteinuria are shown in figure 4.

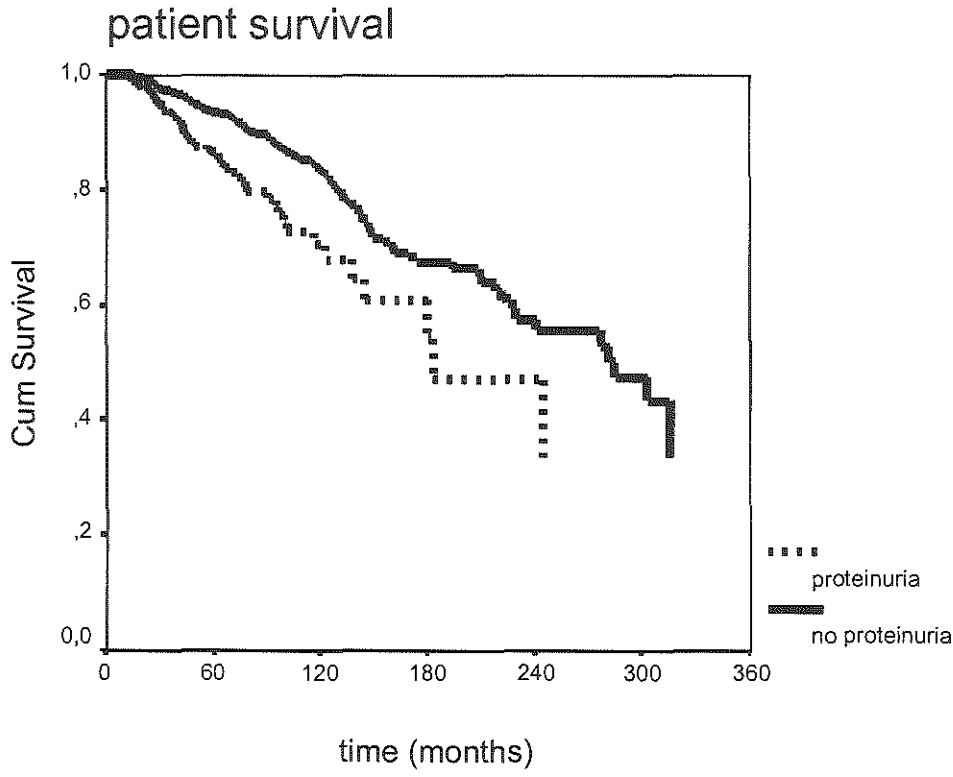

***Figure 4**: Patient survival curve obtained by the Cox analysis.*

The influence of increasing amounts of proteinuria on the death risk is shown in figure 5.

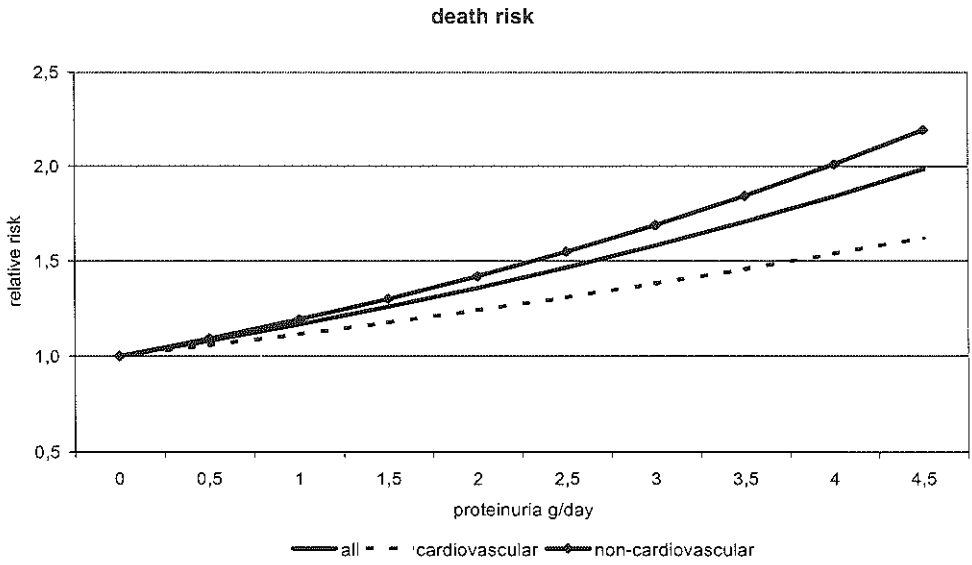

Figure 5:
Calculated relative risk of death with increasing amounts of proteinuria. The reference value is 0 g/day. The three curves can not be mutually compared because the absolute risk per death cause is unknown.

The other variables with an independent influence on the risk of patient death were recipient race (p= 0.0077) and original disease (p= 0.0023). Both diabetes mellitus and hypertension increased the RR for patient death significantly. The influence of transplantation period on the death risk was also significant although rather unconvincing (p=0.0453).
When the analysis of patient death was limited to cardiovascular death, an increased risk was found for both proteinuria as a categorical variable (p<0.0001, RR=2.27) and for proteinuria as a continuous variable (p=0.0474, Exp(B)=1.11, figure 5) . The risk of non-cardiovascular death was also significantly influenced by proteinuria, both as a categorical and as a continuous variable (p=0.0253, RR=1.81 resp. p=0.0021, Exp(B)=1.19, figure 5). There was no interaction with either of the other variables. Cardiovascular death occurred in 63% of the patients that died with a functioning graft, the remaining 37% had a non-cardiovascular

death cause. Serum cholesterol significantly increased the risk of cardiovascular death (p=0.0185) but not of non-cardiovascular death. The same holds true for diabetes or hypertension as original disease.

Discussion:

Proteinuria is known to be negatively associated with renal function in both the native and the transplanted kidney. In this study the influence of proteinuria was analysed in two ways: as a continuous and as a categorical variable. In a former study we found a significant influence of proteinuria as a categorical variable on the failure risk after transplantation (23). We wondered whether this influence was dependent on the amount of proteinuria or on its mere presence.

Although proteinuria as a categorical variable did not interact with any of the other variables, proteinuria as a continuous variable did interact with original disease in the analysis regarding the graft failure risk. Congenital or hereditary disease was chosen as the reference category, which means that its graft failure risk at the reference value zero for proteinuria is settled to 1. The risk associated with the amount of proteinuria is different for the various original diseases. E.g. the risk of patients with systemic diseases, and to a lesser degree hypertension and glomerulonephritis, is higher and increases more steeply with increasing amounts of proteinuria than in other original diseases (figure 2). With increasing amounts of proteinuria there is no significant increase of the graft failure risk of patients with diabetes (compared to congenital and hereditary diseases). This can be explained by their disproportionally high competing death risk (table 3). Although there is no interaction between proteinuria and original disease when death is the endpoint of observation, the exponential model implies that the higher risk of death in diabetics should be multiplied with the risk of proteinuria when present, resulting in an extraordinarily high death risk in this subgroup. In other words, as is known from the non-transplanted diabetic population with proteinuria, many subjects will die before renal failure develops (24).

Proteinuria, both when analysed as a categorical or as a continuous variable, had a negative influence on the death risk (figure 5). There was no interaction with any of the other variables. Both the cardiovascular and the non-cardiovascular death risks were significantly higher in the proteinuric population whether it was introduced as a categorical or as a continuous variable. Although the magnitude of the risks is comparable, the influence of proteinuria as a continuous variable on the cardiovascular death risk is hardly significant. It appears that the mere presence of proteinuria is associated with the cardiovascular death risk, whereas the quantity

seems less important. The non-cardiovascular death risk is influenced primarily by increasing amounts of proteinuria (and probably the sort of protein lost) and less by its mere presence.

In accordance with the findings in our whole population (including the first year failures) increasing recipient age is associated with a decreasing graft failure rate (25). However, contrary to the results in our whole population, increasing donor age is associated with a decreasing graft failure risk when the first year failures are excluded. The prognosis of old donor organs that survive the first year is even better than that of young donor organs. Possibly a lower immunogenicity of elderly organs plays a role. Another explanation may be that damage to elderly organs (e.g. by rejection) results more often in failure within the first year because of a lower reserve capacity compared to young donor organs.
A higher systolic blood pressure significantly increased the graft failure rate but not the death risk (figure 3). It is surprising that the diastolic blood pressure did not influence any risk. An explanation might be that a high diastolic blood pressure more often triggers treatment than a high systolic blood pressure, resulting in a smaller blood pressure range in the diastolic, compared to the systolic pressure. The mere use of anti-hypertensive medication did not influence the risk of graft failure.
For our whole population, including the failures of the first year, we showed that with passing time (influence of transplantation year) the results of renal transplantation improved significantly (25). However, one year after transplantation both graft failure and death risk seem not to have taken advantage of the improvements of medical care. One reason may be that selection criteria for both donor and recipient became less stringent in time. Although the short-term results of these "higher risk" donors and recipients may be adequate, the long-term results may be hampered. Another reason may be that ailments and diseases specific for the long-term follow-up of renal transplant patients have been treated insufficiently.

The fact that in the macro albuminuric range the risk of proteinuria is linearly dependent upon its quantity suggests that extrapolation of this relationship to the micro albuminuric range is permitted.
There is an overwhelming amount of literature on the association between proteinuria and cardiovascular risk factors or an increased cardiovascular death risk in non-transplanted diabetics. In some studies a comparison is made with the general population (9,10,11,14,16,17). As there is an increased transcapillary

escape rate of albumin in microalbuminuric type 1 and 2 diabetic patients, the most plausible explanation is that (micro-) albuminuria is a manifestation of widespread (micro-) vascular disease (26,27). Treatment with ACE inhibition not only reduces albuminuria and blood pressure but also the cardiovascular death risk (11).

The first time an association was found between proteinuria and both cardiovascular and non-cardiovascular death in the general population was in the Framingham study (8). Since then the influence of proteinuria on the cardiovascular disease or death risk in the general population was studied and confirmed by many others (10,12-20). The association between proteinuria and cardiovascular risk factors was also shown (28-34). Only a few authors studied and found the influence of proteinuria on the non-cardiovascular death risk (8,10,13). In the general population the positive correlation between the transcapillary albumin excretion rate and proteinuria found in diabetes was confirmed (35). This is in agreement with the hypothesis that proteinuria is an expression of generalised (cardio-)vascular disease.

In essential hypertension this association between proteinuria and cardiovascular disease or death has also been confirmed (21,22,36), as well as the association between proteinuria and cardiovascular risk factors (37-39). Adequate treatment of hypertension may cause micro-albuminuria to disappear (36).

In patients with their own kidneys in situ a relationship between generalised or systemic disease and proteinuria is acceptable since the kidneys have been exposed to the system from the start of the ailment. However, how can proteinuria of a graft be associated with death of the recipient? Usually, proteinuria is supposed to be a primarily renal phenomenon, e.g. (chronic) rejection or recurrence of original disease. However, many non-renal diseases or pathologic situations are associated with proteinuria in the non-transplanted population. Most of them are described in the non-renal literature. Seriously ill patients with traumas, burns, surgery or infections are reported to have transient proteinuria (40-42). During exacerbations of inflammatory bowel disease or rheumatoid arthritis proteinuria flares up and disappears again when in remission (43,44). A significant correlation was found between the acute phase reactant C reactive protein and both clinical disease activity and microalbuminuria in patients with inflammatory bowel disease (44). Proteinuria, often heavy, is an infrequent but well recognised complication of many different malignancies and the presence may be associated with a substantially reduced survival time (45). Microalbuminuria can be found within hours after a myocardial infarction and is proportional to its size (46). It disappears again when the patient recovers (46). In patients with known

cardiovascular disease the presence of proteinuria is shown to have a negative influence on the cardiac prognosis (47-49). After positive exercise EKG testing and after exercise testing in patients with claudicatio intermittens proteinuria can be found (50,51). In patients with peripheral vascular disease the degree of microalbuminuria is proportional to the severity of the disease and can be attenuated by revascularisation (52). In vascular disease ischemia-reperfusion probably elicits a systemic response in which inflammatory mediators activate neutrophils, leading to endothelial damage and increased vascular permeability (53). These observations suggest that proteinuria non-specifically reflects a wide variety of severe diseases.

As described before an intimate relationship has been found between low-level proteinuria and vascular permeability in studies where the transcapillary albumin excretion is tested. The postulated mechanism of albumin leakage is that there is increased microvascular permeability secondary to increased circulation of inflammatory mediators, such as cytokines (54).

In the individual patient with a renal transplant we are faced with the problem to make a distinction between "renal" proteinuria on the one hand and proteinuria as a symptom of widespread (vascular) disease and "acute phase" proteinuria on the other. Besides, both causes of proteinuria could be present at the same time, especially when proteinuria is caused by widespread atherosclerotic disease, involving the kidney graft.

The main reason for graft loss in our population was chronic rejection; the main death cause was cardiovascular disease. A high incidence of cardiovascular disease is a well-known complication in the renal transplant population (55). On histological grounds it is highly possible that atherosclerotic phenomena play pivotal roles in the origin of chronic rejection (56).

We found that proteinuria was an independent predictor of graft failure and patient death. In our proteinuric population, both the cardiovascular and non-cardiovascular death risks were significantly increased compared to the non-proteinuric population. In a former study we found that serum cholesterol is associated with an increased risk of patient death and of graft loss (23). One way to improve the results of transplantation after the first year is to reduce the amount of proteinuria and to decrease blood pressure and serum cholesterol levels.

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CHAPTER 7

Cold ischemia time influences the short-term, donor serum creatinine the long-term renal graft failure risk.

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Submitted

Abstract

Introduction: The results of renal transplantation are dependent on many variables. In order to simplify the decision process related to a kidney offer we wondered which variables had the most important influence on the graft failure risk.

Methods: All patients transplanted between January 1981 and July 2000 were included in the analysis (n=1124, 2.6% missing values). The variables included were: donor and recipient age and gender, recipient original disease, race, donor origin, current smoking, cardiovascular disease, body weight, peak and current PRA, number of preceding transplants and type and duration of renal replacement therapy and time since failure native kidneys. Also, HLA identity or not, 1st and 2nd warm and cold ischemia times, left or right kidney and fossa, donor kidney anatomy, donor serum creatinine and proteinuria, and transplantation year were included.

Results: In a multivariable model cold ischemia time and its time-dependent variable significantly influenced the graft failure risk censored for death ($p<0.0001$) independently of any of the other risk factors. The influence primarily affected the risk in the first week after transplantation thereafter it gradually disappeared during the first year after transplantation. Donor serum creatinine also significantly influenced the graft failure risk in a time-dependent manner ($p<0.0001$). The risk of a high donor serum creatinine is already enlarged in the immediate post-operative phase and increases thereafter, the curve is closely related to the degree of the elevation. The other variables with a significant influence on the graft failure rate were in sequence of decreasing significance: Recipient age, donor gender, donor age, HLA identity, transplantation year, preceding transplantations, donor origin and peak PRA.

Conclusion: Donor serum creatinine and cold ischemia time are very important time-dependent variables independently influencing the risk of graft failure censored for death. The best strategy to improve the results of postmortal transplantations is to decrease the cold ischemia time and to allocate kidneys from donors with an elevated serum creatinine to low risk recipients.

Introduction

Physicians working in the renal transplant field are often confronted with kidney offers with doubtful data. A lot of work has been done to recognise unfavourable data as influential variables but their cohesion and the importance of their influence in the whole constellation of variables is not clear. Should we choose for a better HLA match or for a shorter cold ischemia time? How important is donor proteinuria? What is the impact of an elevated donor serum creatinine in a donor with a fair renal function beforehand? We carried out a multivariable analysis

including all known primary variables to reveal the importance of their influence on the graft failure risk censored for death.

Materials and Methods

Patients:

Between January 1981 and July 2000 1124 kidney-only-transplantations were carried out at the University Hospital Dijkzigt in Rotterdam. There were 243 living donor and 881 postmortal donor transplantations. Living donor transplantations were included because they represent the "better part" of the spectrum of results. Including them enlarges the chance to identify those variables most responsible for the extremes in the results. Observation was until June 2001, all patients having at least 1 year of follow-up. One or more variables were missing in 29 patients (2.6%).

Transplantation related variables regarding donor, recipient or both were gathered (table 1).

Recipient race was subdivided in white and non-white. Current smoking was scored as any smoking at the moment of transplantation. Cardiovascular disease was defined as demonstrated cardiovascular, peripheral vascular or cerebrovascular disease. Time on renal replacement therapy (RRT) is the time spent on haemodialysis or CAPD. Donor serum creatinine is the last known value. The donor centre qualitatively determined donor proteinuria. A normal kidney anatomy is defined as one renal artery and no cysts. HLA identity is dichotomised: yes or no. As we were primarily interested in avoidable risks, only primary variables were included. In this way all post-transplant or secondary variables, that most probably find their origin in primary variables, are left out of this study (acute rejection, delayed graft function, etc.).

***Table 1**: Transplantation characteristics.*

number	1124
Recipient age (mean±sd)	44.8±13.5
Male recipients (%)	58.5
Recipient race (% white)	80.1
Recipient weight (kg)	68.9±14.0
Current smoking (%)	38.4
Cardiovascular disease (%)	11.4
Peak PRA (mean±sd)	27.6±30.0
Current PRA (mean±sd)	10.9±21.0
First transplant (%)	79.4
No preceding RRT (%)	9.7
Time since failure native kidneys (months)	41.2±50.9
Time on RRT before present transplantation (months)	30.3±31.4
Donor age (mean±sd)	39.8±16.3
Male donors (%)	58.9
Donor creatinine (umol/l)	86.4±36.7
Donor proteinuria (%)	19.1
Normal anatomy kidney (%)	78.7
Left kidney (%)	45.6
Left fossa (%)	47.4
First warm ischemia time (min)	1.4±3.5
Second warm ischemia time (min)	30.5±11.0
Cold ischemia time (min)	1295±718
HLA identical (%)	16.0
Living donors (%)	21.6

Immunosuppression:
All patients were treated with a double immunosuppressive regime including prednisone. ALG is not part of our preventive therapy. Initially Azathioprine (AZA) was given as primary immunosuppressive therapy in a dose of 2-3 mg/kg body weight. From July 1983 onwards Cyclosporine (CsA) was used as primary immunosuppressant and was dosed on the basis of 12-hours trough levels. Steroids were started on the day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily 6 months after transplantation. Throughout the study period patients were included in several immunosuppressive treatment protocols. Although some of these treatments resulted in a significant reduction in the number of acute rejection episodes, none of them resulted in a significant increase in one-year graft or patient survival.
Statistical analysis:
"Graft survival" was censored for death. "Patient survival" was censored for graft failure; death was scored until three months after graft failure. There were no exclusions for technical or non immunologic failures. Potential associations with survival were analysed by means of the Cox proportional hazards regression model. Apart from the 24 variables mentioned in table 1, transplantation year and native kidney disease were candidate explanatory variables (26 variables). As the number of variables was large compared to the number of events (n=362) a univariable analysis was carried out on all variables separately. The nine variables with the highest p-values ($p>0.23$) were excluded and the model was run with the 17 others. Variables were selected by backward elimination using likelihood ratio tests. When no more variables met criteria for exclusion, the 9 variables that were excluded beforehand were included simultaneously in the model and were tested again using backward elimination.
In order to investigate the time dependency of the effect of all remaining variables we performed an analysis according to Smits et all (1). At first an attempt was made to estimate and test time-dependency through one coefficient for the interaction between the linear predictor as a whole and the logarithm of time (one mutual time interaction term). Next a comparison was made with a model in which each explanatory variable had its own interaction with the logarithm of time, irrespective of its coefficient in the linear predictor of main effects (a time-interaction term for each variable in the model). In case the latter model would appear to be superior in terms of goodness-of-fit, backward elimination would be

used to try to take out time-dependencies in this latter model so as to obtain the final model.
Because the influence of AZA and CsA on survival appeared not to satisfy the proportional hazards assumption, we chose to stratify for immunosuppressive medication. The analyses were performed with SPSS for Windows version 9.0. P-values ≤ 0.05 were considered significant.

Results:
Transplantation characteristics are shown in table 1. Donor serum creatinine ranged between 38 and 190 μmol/L in 95% of cases. The cold ischemia time ranged between 109 and 2505 minutes in 95% of cases.
There were 362 failures and 244 deaths (including death within 3 months after graft failure) in the period studied. The proportional hazards assumption was tested first for all variables through one coefficient representing the interaction of the linear predictor as a whole with the logarithm of time. This estimated single coefficient was not significantly different from zero (p=0.6974). Moreover, a model with each explanatory variable having its own interaction with the logarithm of time irrespective of its main effect in the linear predictor, fitted significantly better to the data (p=0.008 likelihood ratio test). After backward elimination of the time interaction terms the only variables for which the interaction with the logarithm of time remained significant were cold ischemia time (p=0.0126) and donor serum creatinine (p=0.0393). This final model was not a significantly worse fit to the data than the model with all time interactions (p=0.099 likelihood ratio test). The significance of cold ischemia time together with its time interaction term was smaller than 0.0001 (table 2). The significance of donor creatinine together with its time-interaction term was also smaller than 0.0001 (table 2).

Table 2: *Results of the Cox proportional Hazards Analysis.*

	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death			
Cold ischemia time (hours)	1.0102	0.9939-1.0267	<0.0001
Cold ischemia time*ln(time) time in months	0.9999	0.9999-1.0000	
Donor creatinine (umol/L)	1.0035	1.0005-1.0065	<0.0001
Donor creatinine*ln(time) time in months	1.0009	1.0000-1.0017	
Recipient age (years)	0.9829	0.9744-0.9914	0.0001
Donor gender (female)	1.3985	1.1220-1.7433	0.0028
Donor age (years)	0.9764	0.9496-1.0041	0.0030
Donor age squared (years)	1.0004	1.0001-1.0008	
HLA matching (non-identical)	1.6262	1.1433-2.3131	0.0068
Number of previous transplants			0.0106
one	1.4637	1.1291-1.8973	0.0040
more			ns
Transplantation year (per year)	0.9653	0.9383-0.9931	0.0147
Donor origin (postmortal)	1.7523	1.0548-2.9111	0.0303
Peak PRA	1.0037	1.0003-1.0072	0.0338
Patient failure			
Recipient age (per year)	1.0750	1.0595-1.0907	<0.0001
Current smoking	1.7260	1.3062-2.2807	0.0006
Recipient race			0.0050
White	1.000		
Non-white	1.6554	1.1643-2.3537	
Cardiovascular disease	1.4455	1.0326-2.0236	0.0318

Interaction was tested between all possible combinations of variables with a significant influence on the graft failure risk. There were no interactions between any of them, which means that the influence of all variables is independent of that of any of the other variables.
As estimated through the model, figure 1 shows that the RR of graft failure censored for death increases significantly with the increasing cold ischemia time.

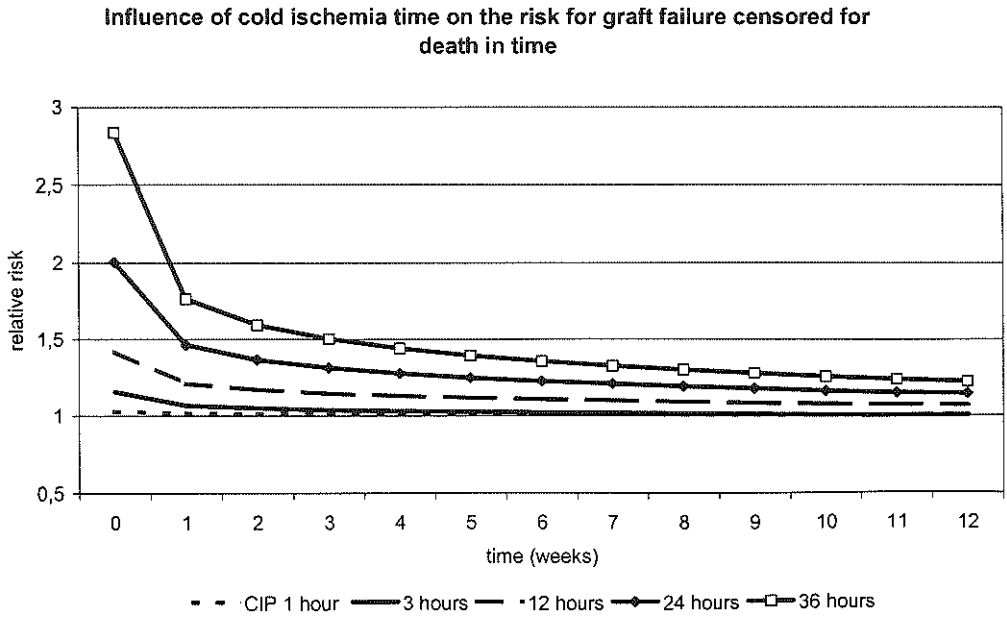

Figure 1: *The time-dependent influence of increasing cold ischemia time on the calculated RR for graft failure censored for death in the first three months after transplantation (reference cold ischemia time is 0 hours).*

The cold ischemia time of zero hours was used as the reference. The risk is largest in the first week after transplantation but remains slightly elevated for a relatively long time after that. At one year after transplantation the RR nears one. This risk is independent of any of the other risk factors, as there was no interaction with any of them. Figure 2 shows the time dependent influence of donor serum creatinine on the risk of graft failure censored for death. For this graph a donor serum creatinine of 80

µmol/L was chosen as the reference category. It is clear that an elevated donor serum creatinine is associated with a slightly enlarged risk in the directly post-operative phase and increases thereafter. The curve of the increase is closely related to the degree of the elevation of donor serum creatinine.

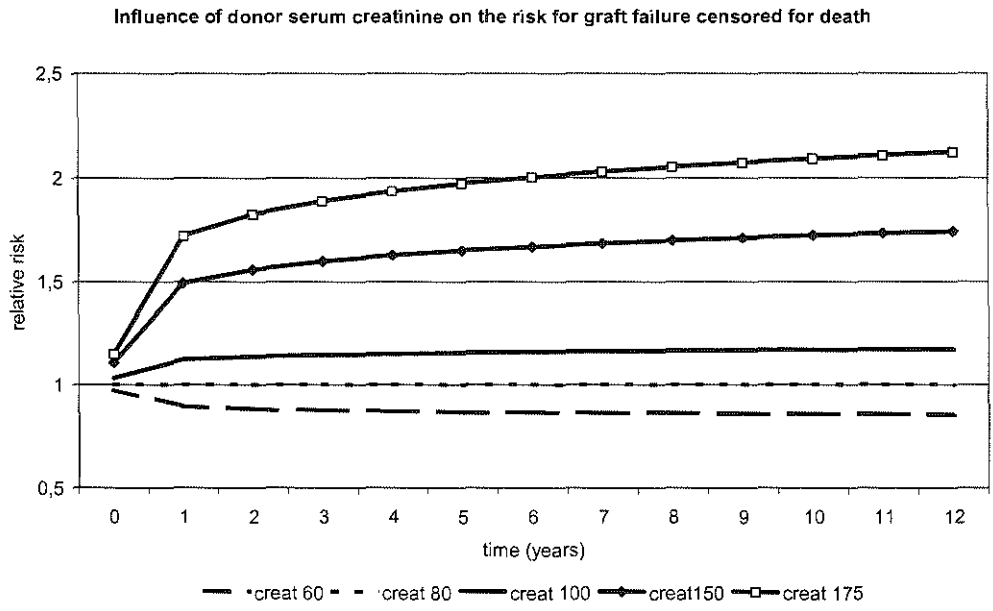

***Figure 2**: The time-dependent influence of increasing donor serum creatinine on the calculated RR for graft failure censored for death in the first year after transplantation (reference is 80µmol/L).*

The other variables with a significant influence on the graft failure risk are also shown in table 2.

One "study" variable was composed where patients treated according to immunosuppressive study protocol could be compared to patients treated according to standard regime. With this variable the different protocols could be compared and a comparison could be made with the standard regime in the mutual controls and patients not included in studies. In a separate Cox proportional

hazards analysis this variable was included together with all other variables with a significant influence (table 2). This "study" variable did not have a significant influence on the graft failure risk (data not shown). This means that none of the patient groups that were treated according to a study protocol had a survival significantly different from the population treated according to standard regime.
The variables with a significant influence on the death risk (patient failure) are shown in table 2. The most important are recipient age and current smoking. As could be expected, the RR for death increases with increasing recipient age and this is contrary to the influence of recipient age on the graft failure risk censored for death where an increasing age is associated with a decreasing risk (table 2: Exp(B)=0.9829).

Discussion:
In the Cox proportional hazards analysis the influences of all variables are weighed and the influence of one variable can be expressed after adjustment for the influences of all other variables in the analysis. We found that the RR for graft failure censored for death was significantly influenced by cold ischemia time and by donor serum creatinine and their time-interaction variables. There was no interaction between them or between them and any of the other variables in table 2. This means for example that an increased donor creatinine in kidneys from donors with different ages represents comparable damage. It also means that the deleterious effect of increased cold ischemia times affects living and postmortal donor kidneys to the same extent.
Figure 1 shows that the longer the cold ischemia time, the higher the graft failure risk. These risks are highest in the directly post-operative phase and decrease in time to approach each other very slowly. One year after transplantation the difference in risks has disappeared. This means that the risk of a long cold ischemia time predominantly concerns the immediate post-operative phase while a small deleterious effect remains over a longer period after transplantation. The main part of the effect is exerted in the first weeks after transplantation. The influence of increased cold ischemia time on graft function is studied in a few univariable (2,3) and multivariable analyses (1, 4-12). A significant effect on graft function or on the graft failure risk was shown by some of them (1, 3, 4, 8-11). Only Smits et all studied time dependency of the cold ischemia time and they concluded that there was a permanent detrimental effect of cold ischemia time on the risk of over-all graft failure (no time dependency). Although we applied their method to our data we did find a time interaction of cold ischemia time. The difference was

possibly caused by the fact that in their study both graft failure and death were used as end points of observation (over-all graft failure) whereas we only studied graft failure risk (censored for death).
The most recent donor serum creatinine was introduced in the study (not necessarily the worse or the best ever found). The reason was that we wondered about the importance of an elevated serum creatinine in a (postmortal) donor with a fair renal function beforehand. Acceptance of a donor kidney offer with a high serum creatinine was only then considered when other donor factors were acceptable and a creatinine value in the normal range had preceded recently. A very strong influence of donor serum creatinine and its time interaction term on the risk of graft failure censored for death was found. A significant effect was also present when donor serum creatinine was introduced as a categorical variable (<80 versus >80 μmol/L; p=0.0055) but the fit of the model was better when creatinine was introduced as a continuous variable. An elevated donor serum creatinine is associated with an elevated risk dependent on the degree of the impairment of renal function (figure 2). This risk increases in time, in coherence with the degree of the elevation of donor serum creatinine. Most probably an elevation of a previously normal donor serum creatinine in the phase before death does mean irreversible damage and a reduced renal functional reserve that will be reflected after transplantation in impaired renal function and increased graft loss. Many studies have been performed on the effect of histological findings in donor renal biopsies on recipient renal function (13-20). There are few studies on the effect of donor serum creatinine, whereas the latter is more available. In univariable analyses, it has been shown that donor creatinine clearance is associated with recipient graft function in the high-risk donor population (13, 21,22).
Recipient and donor age were introduced as continuous variables (table 1). Both are known to influence the graft failure risk and that was confirmed in this analysis (23, 24). The RR for graft failure decreases linearly with increasing recipient age (figure not shown). The influence of donor age on the RR for graft failure follows a J-shaped curve with the lowest risk at a donor age of 30 years (figure not shown). The RR for graft failure on receiving a female donor kidney is 1.4 compared to a male donor kidney (p=0.0028, table 1). The RR for graft failure of a HLA non-identical graft is 1.62 compared to the receipt of an HLA-identical kidney (p=0.0068, table 1). Although the significance is not extremely high, the fact that transplantation year still influences the graft failure rate suggests that influential variables are still left out of our model (table 2: p=0.0147). Most probably they are

partly represented by "measurable" variables as donor events before and during procurement. Another part may be caused by "un-measurable" influences as "improvements in medicine".

In our study the results of living donor transplantations are superior to those of postmortal transplantations as has been shown by others (2, 25-31). The RR for graft failure of a postmortal donor kidney is 1.75 compared to that of a living donor kidney (p=0.0303, table 1). The significance, but not the influence, of donor origin increased considerably when the cold ischemia time was left out of the model (data not shown). Obviously the dichotomy in cold ischemia time explains to a great extent the difference in risk between the living and postmortal donor population. Omission of none of the other variables led to such a change in significance or influence of donor origin.

Peak PRA, and not current PRA influences the graft failure risk. Peak PRA has been associated with an increased risk of graft failure in some studies (1, 3, 6, 9, 27, 32), while others could not confirm it (5,29). PRA was introduced as a continuous variable and the risk of graft failure increases linearly with increasing PRA (p=0.0338, table 1).

All variables that were introduced in the model (table 1) but are not shown in table 2 did not significantly influence the risk of graft failure censored for death. In agreement with 2 other multivariable analyses, no influence was found of the duration of renal replacement therapy before the present transplantation (6, 27). One study did find a significant effect but there was an extraordinarily high number of missing values in this study (33).

In conclusion: The one and most important avoidable risk factor for graft failure censored for death appears to be an increased cold ischemia time. Although aiming at HLA-identity is one of the variables known to lengthen the cold ischemia time, both have a significant and independent effect on the risk of graft failure (table 2). The best strategy would be to substantially reduce the cold ischemia time while continuing HLA-matching. If our aim is to approach the results of living donor transplantations, cold ischemia times of postmortal donor transplantations should come close to those of living donor transplantations. If it is not possible to shorten HLA typing and cross-matching, one possible measure could be to transplant high-risk kidneys locally, without allocation to un-sensitised patients, whereas low risk kidneys are allocated to highly sensitised patients. But also for these patients more speed is indicated as prolonged cold ischemia time has been shown to negate the beneficial effect of HLA matching (5, 34).

Although donor proteinuria does not seem to influence the graft failure risk, donor

serum creatinine does. While sophisticated studies have been performed to predict renal function of a kidney offered, the simple donor serum creatinine is shown to be a valuable tool. The appendage "high-risk" is provided to a donor with an elevated serum creatinine in a degree dependent manner. The Cox proportional hazards analysis does not provide us with "acceptable limits", but with relative risks that must be weighed in a given donor-recipient situation. This means that, in order to keep risks as low as possible, a kidney from a donor with an elevated serum creatinine should preferably not be given to a high risk recipient. And for this "high-risk" donor population cold ischemia time should be kept as short as possible.

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CHAPTER 8

The superior results of living donor renal transplantation are not completely due to selection or short cold ischemia time.

A single centre, multivariable analysis

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Transplantation: accepted for publication

Abstract

Introduction: The results of living donor renal transplantations (LD) are better than those of postmortal donor transplantations (PMD). In order to investigate whether this can be explained by a more favourable patient selection procedure in the LD population we performed a Cox proportional Hazards analysis including variables with a known, important influence on graft survival.

Methods: All patients transplanted between January 1981 and July 2000 were included in the analysis (n=1124, 2.6% missing values). There were 243 LD (including 30 non-related) and 881 PMD transplantations. The other variables included were: donor and recipient age and gender, recipient original disease, race, current smoking, cardiovascular disease, body weight, peak and current PRA, number of preceding transplants and type and duration of renal replacement therapy, time since failure of native kidneys. The number of HLA identical combinations, 1st and 2nd warm and cold ischemia periods (CIP), left or right kidney and fossa, donor kidney anatomy, donor serum creatinine and proteinuria, and transplantation year were also included.

Results: In a multivariable model donor origin (PMD versus LD) significantly influenced the graft failure risk censored for death independently of any of the other risk factors (p=0.0303, RR=1.75). There was no time interaction. When, in the same model the variable cold ischemia time was excluded the significance of the influence of donor origin on the graft failure risk increased considerably, whereas the magnitude of the influence was comparable (p=0.0004, RR=1.92). The influence of all other variables on the graft failure risk was unaffected when cold ischemia period was excluded. Exclusion of none of the other variables resulted in a comparable effect. Donor origin did not influence the death risk.

Conclusion: The superior results of LD versus PMD transplantations can be partly explained by the dichotomy in the cold ischemia period in these populations (selection). However, after adjustment for CIP the influence of donor origin still remained significant, independent of any of the variables introduced. This superiority is therefore possibly caused by factors inherent to the transplanted organ itself e.g. absence of brain death and of cardiovascular instability of the donor before nephrectomy.

Introduction

The dire need of kidneys available for transplantation led to increasing numbers of living (un-) related donor transplantations. In univariable analyses living donor kidney transplantations were shown to have better results than those of postmortal donors (1-5).

Figure 1 shows the results of a univariable analysis regarding over-all graft survival (un-censored for death) of LD versus PMD transplantations in our centre.

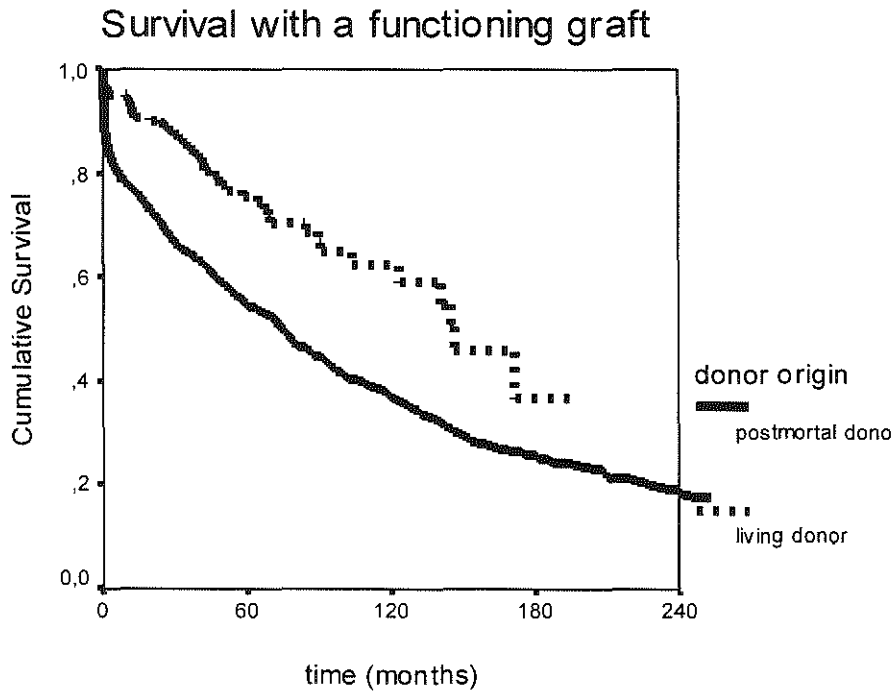

Figure 1:

Kaplan Meier curve comparing survival after a LD versus a PMD transplantation.

At 5 years the over-all graft survival is 75% in the population that received an organ from a living donor versus 58% in those who had a postmortal donor. We wondered whether this difference could be attributed completely to organ origin or whether other differences between the LD and the PMD populations would play a role. In other words: can we explain the difference in graft survival between the two populations on the basis of differences in prevalence of variables with a known influence on graft survival? We performed a multivariable analysis in order to correct for other variables with a known important influence on the graft failure risk censored for death.

Materials and Methods

Patients:

The first living (un) related renal transplant performed at the University Hospital Rotterdam "Dijkzigt" was in 1981. Between January 1981 and July 2000 1124 kidney-transplantations were performed. The analysis was done in June 2001, all patients having at least 1 year of follow-up. One or more variables were missing in 29 patients (2.6%).

Immunosuppression:

Initially Azathioprine (AZA) was given as primary immunosuppressive therapy in a dose of 2-3 mg/kg body weight. From July 1983 onwards Cyclosporine (CsA) was used as primary immunosuppressant and was dosed on the basis of 12-hours trough levels. Steroids were started on the day of transplantation and were gradually tapered to a maintenance dose of 10 mg of prednisone daily 6 months after transplantation. Throughout the study period patients were included in several immunosuppressive treatment protocols. Although some of these treatments resulted in a significant reduction in the number of acute rejection episodes, none of them resulted in a significant increase in one-year graft or patient survival.

Statistical analysis:

Two separate analyses were done: in the first "graft survival censored for death" was the end-point of observation. In the second "patient survival" was censored for graft failure; observation included three months after graft failure. There were no exclusions for technical or non immunologic failures. Potential associations with survival were analysed by means of the Cox proportional hazards regression model. Apart from donor origin (LD or PMD) the 23 variables mentioned in table 1 and transplantation year and native kidney disease were candidate explanatory variables (26 variables).

Table 1: *Transplantation characteristics.*

	All	LD	PMD	p
number	1124	243	881	
Recipient age (mean±sd)	44.8±13.5	39.5±13.7	46.3±13.0	<0,0001
Male recipients (%)	58.5	58.4	58.5	ns
Recipient race (% white)	80.1	81.9	79.6	ns
Recipient weight (kg)	68.9±14.0	70.0	68.6	ns
Current smoking (%)	38.4	39.9	38.0	ns
Cardiovascular disease (%)	11.4	10.3	11.7	ns
Peak PRA (mean±sd)	27.6±30.0	18.9±25.8	30.0±30.7	<0,0001
Current PRA (mean±sd)	10.9±21.0	8.4±19.1	11.6±21.5	0.035
First transplant (%)	79.4	87.7	77.2	0.001
No preceding RRT (%)	9.7	24.7	5.6	<0,0001
Time since failure native kidneys (months)	41.2±50.9	28.1±51.3	44.8±50.1	<0,0001
Time on RRT before present transplantation (months)	30.3±31.4	16.6±23.8	34.0±32.2	<0,0001
Donor age (mean±sd)	39.8±16.3	45.6±12.7	38.2±16.8	<0,0001
Male donors (%)	58.9	49.4	61.5	<0,0001
Donor creatinine (umol/l)	86.4±36.7	74.2±14.9	89.9±40.1	<0,0001
Donor proteinuria (%)	19.1	0	25.8	<0,0001
Normal anatomy kidney (%)	78.7	84.4	77.2	0.013
Left kidney (%)	45.6	48.1	44.9	ns
Left fossa (%)	47.4	48.6	47.1	ns
First warm ischemia period (min)	1.4±3.5	2.7±4.2	1.1±3.2	<0,0001
Second warm ischemia period (min)	30.5±11.0	28.4±10.4	31.1±11.1	<0,0001
Cold ischemia period (min)	1295±718	147±45	1614±436	<0,0001
HLA identical (%)	16.0	23.0	14.1	0.001

As the number of variables was large compared to the number of events (n=362) a univariable analysis was done on all variables separately. The nine variables with the highest p-values (p>0.23) were excluded and the model was run with the 17 others. Variables were selected by backward elimination using likelihood ratio tests. When no more variables met criteria for exclusion the 9 variables that were excluded beforehand were included simultaneously in the model and were tested again using backward elimination.

Linearity of the influence of all variables was checked by introduction of high order variables (e.g. squares). In order to investigate the time dependency of the effect of all remaining variables on the risk of graft failure censored for death, we performed an analysis according to Smits et all (6). At first an attempt was made to estimate and test time-dependency through one coefficient for the interaction between the linear predictor as a whole and the logarithm of time (one mutual time interaction term). Next a comparison was made with a model in which each explanatory variable had its own interaction with the logarithm of time, irrespective of its coefficient in the linear predictor of main effects. In case the latter model would appear to be superior in terms of goodness-of-fit, backward elimination would be used to try to take out time-dependencies in this latter model so as to obtain the final model.
Because the influence of AZA and CsA on survival appeared not to satisfy the proportional hazards assumption, we chose to stratify for immunosuppressive medication. The analyses were performed with SPSS for Windows version 9.0. p-values ≤ 0.05 were considered significant.

Results:
Similar to the trend observed in many centres world wide, in our centre we also see an increasing number of LD whilst the number of PMD transplantations tends to decrease. In the last 5 years the percentage of LD transplantations represents more than one third of the total number. Most living donors were sibs, who relatively more often donated to brothers than to sisters. Parent donors take the second place, while partner donation is growing rapidly during the last 2 years.
The LD and PMD recipient populations differed in many respects. Transplantation characteristics are shown in table 1. Recipients were significantly younger and donors were significantly older in the LD population compared to the PMD population. Peak and current PRA were lower in the population that received a LD

organ. Recipients of a LD organ waited shorter for the present transplantation and haemodialysis was less often used. This is because many more patients received a LD organ without preceding renal replacement therapy. First warm ischemia time was significantly longer in the LD population because of the introduction of the laparoscopic donor nephrectomy in 1999. As could be expected, cold ischemia time was significantly shorter in the LD population. Donor serum creatinine was significantly lower in the LD population. Although the mean number of HLA mismatches was not different between the groups, there were significantly more HLA identical transplantations in the LD population. Besides, there were more first transplants, more female donors and anatomy was more often normal in the LD population (table 1). The prevalence of the various native kidney diseases was not different between the populations.

There were 362 failures and 244 deaths in the period studied. The proportional hazards assumption was tested first for all variables through one coefficient representing the interaction of the linear predictor as a whole with the logarithm of time. This estimated single coefficient was not significantly different from zero ($p=0.6974$). Moreover, a model with each explanatory variable having its own interaction with the logarithm of time, irrespective of its main effect in the linear predictor, fitted significantly better to the data ($p=0.008$ likelihood ratio test). After backward elimination of the time interaction terms, the only variables for which the interaction with the logarithm of time remained significant were cold ischemia time ($p=0.0126$) and donor serum creatinine ($p=0.0393$). This final model was not a significantly worse fit to the data than the model with all time interactions ($p=0.099$ likelihood ratio test).

Donor origin significantly influenced the graft failure risk censored for death, without interaction with any of the other variables or time. When the same model was run without cold ischemia time the same variables turned out to influence the risk of graft failure with comparable significance and magnitude in both models (table 2). However, the influence of donor origin became more significant with a comparable magnitude (table 2). Exclusion of none of the other variables resulted in such a large shift in significance of the influence of donor origin (data not shown). The other variables with a significant influence on the graft failure risk are shown in table 2. In a separate analysis donor origin was found not to influence the death risk.

***Table 2**: Results of the Cox Proportional Hazards Analysis*

	Model with cold ischemia time			Model without cold ischemia time		
	Exp(B)	95%CI for Exp(B)	p	Exp(B)	95%CI for Exp(B)	p
Graft failure censored for death						
Cold ischemia period (hours)	1.0102	0.9939-1.0267	<0.0001			
Cold ischemia period*ln(time) time in months	0.9999	0.9999-1.0000				
Donor creatinine	1.0035	1.0005-1.0065	<0.0001	1.0039	1.0009-1.0068	<0.0001
Donor creatinine*ln(time) time in months	1.0009	1.0000-1.0017		1.0007	0.9999-1.0016	
Recipient age (years)	0.9829	0.9744-0.9914	0.0001	0.9829	0.9745-0.9914	0.0001
Donor gender (female)	1.3985	1.1220-1.7433	0.0028	1.4029	1.1260-1.7478	0.0025
Donor age (years)	0.9764	0.9496-1.0041	0.0030	0.9751	0.9483-1.0027	0.0029
Donor age squared (years)	1.0004	1.0001-1.0008		1.0005	1.0001-1.0008	
HLA matching (non-identical)	1.6262	1.1433-2.3131	0.0068	1.5992	1.1249-2.2734	0.0089
Number of previous transplants			0.0106			0.0272
one	1.4637	1.1291-1.8973	0.0040	1.4468	1.1166-1.8745	0.0052
more			ns			ns
Transplantation year (years)	0.9653	0.9383-0.9931	0.0147	0.9612	0.9352-0.9879	0.0046
Donor origin (cadaveric)	**1.7523**	**1.0548-2.9111**	**0.0303**	**1.9157**	**1.3359-2.7472**	**0.0004**
Peak PRA	1.0037	1.0003-1.0072	0.0338	1.0039	1.0004-1.0073	0.0288

Discussion:

There are very few studies comparing the influence of donor origin (LD or PMD) on the risk of graft failure in a multivariable analysis (7-9). Two of them are multi-centre studies with large numbers of patients (7,8), the third is a small single centre study (9). All of them studied the graft failure risk uncensored for death and showed a better prognosis for the LD population. This means that death was included in the analysis as an end point of observation. Including death as an end point of observation will influence and obscure the results, as the risk factors that determine death are unlikely to be the same as those that determine graft failure (10). Even worse, a variable that favourably affects the risk of graft failure may have a negative effect on the risk of death. This has been shown in the present and other studies for recipient age; increasing recipient age is associated with increasing risk of death but with a decreasing risk of graft failure censored for death (11-13). Cold ischemia time was included in all three studies; the effect on the risk was not mentioned (7), not significant (8) respectively significant (9).

The population studied in the present analysis consisted of patients that received a LD or a PMD transplantation. Sibs and parents represent the vast majority of donors but the number of partners is increasing. Table 1 shows the characteristics and it is striking that there are remarkable differences between the two populations concerning variables that are known from other studies to influence graft survival. In fact, the differences between the populations could explain the differences in graft survival between the LD and PMD populations. In the LD population we relatively more often see a lower number of previous transplants, lower peak and current PRA, shorter cold ischemia period, lower donor serum creatinine, shorter period of renal replacement therapy before the present transplantation, normal anatomy of the kidney graft and more HLA identical combinations. These have all been associated with a better prognosis (5,7-9,13-23). On the other hand, lower recipient and higher donor age are associated with a worse prognosis concerning graft survival and they also dominate in the LD population (11,12,24). In the Cox proportional hazards analysis all these influences are weighed and the influence of one variable can be expressed after adjustment for the influences of all other variables in the analysis. We found that the RR for graft failure censored for death was significantly influenced by donor origin, independent of any of the other variables (table 2, p=0.0303). However, after exclusion of cold ischemia time and its time-dependent effect the influence of donor origin is much more significant

(p=0.0004, RR=1.92) while the influence of the remaining variables is unaffected (table 2). In the complete model no interaction was found between cold ischemia period (and its time-interaction) and donor origin. There is a dichotomy in cold ischemia time on the basis of donor origin as 95% of the recipients of a LD organ have cold ischemia times between 77 and 255 minutes, whereas recipients of a PMD organ have cold ischemia times between 821 and 2536 minutes. All this suggests that the superior results after living donor transplantation are partly, but not completely, due to the shorter cold ischemia periods in this population. An increased sharing of unknown immunologic variables (e.g. minor antigens) in the LD population, that beneficially influences graft survival cannot be excluded. The rest of the difference in graft survival between LD and PMD populations is inherent to the quality of the donor organ itself. Possibly donor related factors preceding and during nephrectomy, e.g. brain death, use of vasopressants, cardiovascular instability and differences in anaesthesics during LD and PMD kidney procurement could explain this difference (25-29).

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CHAPTER 9

Summary and conclusions

Summary
In this thesis we describe our multivariable analyses that enabled us to define the variables with the most important influence on the risk of graft failure (censored for death) and of death (censored for graft failure) after renal transplantation in our population.

Chapter 1 is a general introduction on the multitude of variables supposed to influence the results of renal transplantation.

In **chapter 2** the methods for survival analysis are described and compared. It is described, substantiated and concluded that univariable analysis, as the Kaplan Meier method, is obsolete in the renal transplant population which is highly complex with a multitude of factors influencing the risks. Furthermore, it is concluded that in this situation the use of the Cox proportional hazards is the most appropriate analysis.

In **chapter 3** we describe the results of a Cox proportional hazards analysis including race as a variable. The end-point of the analysis is over-all graft failure. In spite of the small number of patients included in the analysis, we unambiguously showed that in our centre race does not have a significant influence on the risk of graft failure (uncensored).

In **chapter 4** we describe the results of our study on the influence of both donor and recipient age. It is concluded that increasing recipient age is associated with a decreased risk of graft failure censored for death but as could be expected, with an increased risk of death. Age was introduced as a continuous variable and the graft failure risk censored for death decreased linearly with increasing recipient age. The death risk increased linearly with increasing recipient age. There are no break points in the risks at certain ages because they gradually increase with age; there

are no absolute age-limits. The risk of over-all graft failure is a composition of the risks of death and of graft failure censored for death and increases with increasing recipient age. This means that although the elderly less often lose the graft, they die sooner. Over all, this leads to less functioning time per graft in the elderly.
The association between donor age and the graft failure risk (censored for death) follows a J-shaped curve. Both extremely young and elderly donor organs are associated with an increased risk of graft failure censored for death and of over-all graft failure. There is a gradual increase in the risk from 30 years onwards and a break point is missing. As could be expected, the death risk is not influenced by donor age.
An important reason to put these risks into perspective is the fact that the influence of the period of transplantation on all risks mentioned is considerable too; the risk decreased in time. This means that a difference in risks between the old and young in a given period may disappear when representatives from different periods are compared.

Cardiovascular disease is the main cause of death in the renal transplant population. Chronic allograft nephropathy is the main cause of graft failure after the first year and histologically this entity shows close resemblance to atherosclerotic disease. We wondered about the role of cholesterol in these processes.
In **chapter 5** the influence of serum cholesterol at one year after transplantation on the risk of failure is studied. It is shown that an elevated serum cholesterol level seriously influenced the risk of graft failure censored for death. The increase in risk is largest for the patients with a fair renal function and decreases with increasing serum creatinine levels. The influence of elevated serum cholesterol on the death risk is age dependent: the risk is highest for the young and decreases with increasing recipient age. The risk of over-all graft failure is a composition of both previously described risks. It is concluded that the young recipient runs the largest risks.
It is discussed that a decision matrix for treatment of patients with elevated serum cholesterol levels should not include age as increasing age is associated with an increased death risk independent of anything else. More interesting is the question as to whether and how much an elevated serum cholesterol level adds to this risk. It is shown that, in the renal transplant population, the addition of the influence of an elevated serum cholesterol level to the death risk is much larger in the young compared to the elderly.

Proteinuria after renal transplantation is an ominous sign as it is associated with loss of graft function. In **chapter 6** we describe our Cox proportional hazards analysis determining the importance of proteinuria in the scope of other important variables influencing the risk of failure. We found that proteinuria (both as a continuous variable and as a categorical variable) significantly influenced the risk of graft failure censored for death. When proteinuria was introduced as a continuous variable the graft failure risk turned out to be different for categories of original diseases. Populations with glomerulonephritis, hypertension and systemic diseases as their original disease had an increased risk of graft failure censored for death when proteinuria was present, other populations did not have an increased risk under the same circumstances. We also found that systolic, but not diastolic, blood pressure significantly influenced the risk of graft failure censored for death.
Proteinuria both as a categorical and as a continuous variable significantly influenced the risk of death. It is remarkable that both the cardiovascular and the non-cardiovascular death risks are increased when proteinuria is present.
It is discussed that proteinuria may be an expression of renal disease, but that this may also be a marker of widespread (vascular) disease. This places proteinuria in a larger scope of diseases, underlining the necessity of intense treatment of cardiovascular risk factors as hypertension, hypercholesterolaemia and diabetes. It also reinforces the preference for treatment with ACE-inhibitors or angiotensin II antagonists in this population.

A kidney offer is always accompanied by a huge quantity of information regarding donor, recipient or both. We wondered which variables are most important and should thus carry most weight in the decision to accept the given donor-recipient combination. In **chapter 7** we describe our study on the influence of a large number of variables attending a kidney offer. We found that the most important variables influencing the results of a renal transplantation are cold ischemia time and donor serum creatinine. Both variables are time-dependent which means that the influence varies in time. The influence of cold ischemia time on the risk of graft failure censored for death is largest in the directly post-operative phase and decreases in time. On the contrary, shortly after transplantation the influence of donor serum creatinine is small but it increases in time. It is concluded that cold ischemia time should be shortened. One measure could be to shorten the allocation process, another returning the addition "priority operation" to kidney transplantation. It is important that an elevated donor serum creatinine is recognised as a risk factor for renal transplantation.

The results of living donor renal transplantation are better than those of postmortal donor renal transplantation. In **chapter 8** we show that there are important differences in patient characteristics between the two populations. The prevalence of characteristics that are associated with a fair prognosis is over-represented in the living donor population, so that these differences by themselves might explain the superior results of graft survival. In a multivariable analysis we found that even after adjustment for all these variables the risk of graft failure censored for death is significantly lower in the living donor population. However, a large part of the difference in risk can be explained by the much shorter cold ischemia time in the living donor population. Omission of this variable from the model led to a major increase in significance of the influence of donor origin on the risk of graft failure censored for death. The magnitude of the risk was unchanged. Omission of none of the other variables led to such a shift in significance. The remaining part of the difference in graft survival between the two populations must be inherent to the quality of the donor organ itself

Conclusions

1. In this complex renal transplant population with so many factors influencing the risks, reliable results of survival analysis cannot be obtained with a univariable analysis. Use of a multivariable analysis is indispensable.
2. Graft failure risk censored for death decreases with increasing recipient age. It is not customary to tune our immunosuppressive regime to recipient age. This means that at this moment elderly recipients probably are over-immunosuppressed.
3. High donor age is associated with an increased risk of graft failure. However, considering the limited increase in relative risk, the mere presence of increased donor age is no contra-indication for renal transplantation.
4. Transplantation of elderly (high-risk) donor organs to elderly recipients adds an increased graft failure risk (censored for death) to their already enlarged death risk.
5. An increased serum cholesterol level primarily increases the graft failure risk in the population with a fair renal function. It primarily influences the death risk of the young renal transplant population. Although we did not show that treatment of elevated serum cholesterol levels reduces these risks, comparison with results in the non-transplanted population suggests the chances are high.

6. In addition to an increased risk of graft loss, proteinuria also causes an increased death risk. Both cardiovascular and non-cardiovascular death risks are increased. The results of long-term treatment with ACE-inhibitors or angiotensin II antagonists, on patient and graft survival in the renal transplant population are not yet known. In the meantime, this therapy is indicated in renal transplant patients with proteinuria.
7. As an increased systolic blood pressure negatively influences the risk of graft failure (censored for death), treatment should be initiated sooner and we should aim at lower values.
8. Donor serum creatinine significantly influences the graft failure risk. Organs from donors with an increased serum creatinine should be recognised as "high risk" kidneys.
9. An increase in cold ischemia time linearly increases the graft failure risk (censored for death). A substantially shorter cold ischemia time, in postmortal donor renal transplantation, should be a matter of major concern.
10. A substantial part of the superiority of the results of living donor renal transplantation can be explained by their shorter cold ischemia period. However, even after adjustment for cold ischemia time a difference in risk between the populations remains.

Concluding remarks

Multivariable analysis provides us with risk factors in a given population. Once known, an attempt can be made to influence some of these risk factors. Examples are treatment of hypercholesterolaemia, proteinuria and hypertension. Duration of cold ischemia time can be shortened and living donor renal transplantation can be stimulated. Donor serum creatinine cannot be changed, but it is conceivable that research in the pre-donation period of postmortal donors may lead to ameliorated donor management and eventually to lower creatinine values.

However, some risks, e.g. age, can not be influenced. Younger donor kidneys have better results but, as there is a shortage of donor organs, elderly donors cannot be excluded. Abiding by statistical arguments would mean that only young recipients would receive a graft and elderly patients would be left on the waiting list. In practice, individuals have equal chances to receive a kidney offer, independent of their age. This means that for the whole population, larger risks are run than should have been necessary on statistical arguments only. It is clear that this negatively influences the results of renal transplantation. However, our primary aim is the benefit of the individual patient, not just "getting the best statistical results".

CHAPTER 10

Samenvatting en conclusies

Samenvatting

In dit proefschrift beschrijven wij de multivariabele analyses waarmee wij, in de Rotterdamse populatie, de variabelen met de grootste invloed op het risico op falen na transplantatie, konden identificeren.

Hoofdstuk 1 is een algemene introductie waarin het grote aantal variabelen wordt besproken waarvan wordt verondersteld dat zij invloed uitoefenen op de overleving na niertransplantatie.

In **hoofdstuk 2** worden de verschillende overlevings analyses besproken en vergeleken. We beschrijven, beargumenteren en concluderen dat het gebruik van univariabele analyses, zoals de Kaplan Meier methode, obsoleet zijn in een complexe populatie met multifactoriëel bepaalde risico's als de niertransplantatie populatie. Wij concluderen dat in deze situatie de Cox proportional hazards analyse de aangewezen methode is.

In **hoofdstuk 3** beschrijven wij een Cox proportional hazards analyse waarin ras als variabele is opgenomen. Het eind-punt van de anlyse is over-all graft survival. Hoewel het aantal geïncludeerde patiënten in de studie klein was, kunnen we eenduidig concluderen dat in ons transplantatiecentrum ras geen significante invloed heeft op het risico op transplantaat verlies (niet gecensureerd voor overlijden).

In **hoofdstuk 4** beschrijven we de studie naar de invloed van donor en ontvanger leeftijd op het risico op falen. Toenemende ontvanger leeftijd bleek geassociëerd te zijn met een afgenomen risico op transplantaat verlies gecensureerd voor overlijden, maar zoals te verwachten met een toegenomen risico op overlijden. Leeftijd is geïntroduceerd als continue variabele en het risico op transplantaat falen, gecensureerd voor overlijden, neemt lineair af met toenemende ontvanger leeftijd. Het overlijdensrisico neemt lineair toe met toenemende ontvanger leeftijd. Vanwege het geleidelijke beloop van het risico met de leeftijd is er geen kritische

leeftijds grens aan te geven, waar boven transplanteren moet worden ontraden. Het risico op over-all transplantaat falen is een compositie van beide risico's en neemt toe met toenemende ontvanger leeftijd. Hoewel ouderen minder vaak het transplantaat verliezen, overlijden zij sneller en dit leidt uiteindelijk tot een kortere functionerende tijd per transplantaat bij ouderen.
De associatie tussen donor leeftijd en risico op transplantaat falen volgt een J-vormige curve. Zowel de organen van de jongste als die van de oudste donoren hebben een verhoogd risico op transplantaat verlies gecensureerd voor overlijden en voor over-all transplantaat verlies. Er is een geleidelijke toename van het risico op transplantaat verlies vanaf een donor leeftijd van 30 jaar. Ook hier zijn geen absolute leeftijds grenzen aan te geven. Het overlijdens risico van de ontvanger is niet geassocieerd met de donor leeftijd.
Een belangrijke relativerende factor is het feit dat de invloed van transplantatie jaar op alle genoemde risico's erg groot is; de risico's nemen af in de loop der tijd. Dit betekent dat het verschil in risico tussen een oude en jonge ontvanger binnen een bepaalde periode kan verdwijnen indien vertegenwoordigers van verschillende periodes worden vergeleken.

Cardiovasculair lijden is de belangrijkste oorzaak van overlijden in de niertransplantatie populatie. Chronische rejectie is de belangrijkste oorzaak van transplantaat verlies na het eerste jaar en deze entiteit vertoont grote histologische gelijkenis met atherosklerose. Wij vroegen ons af wat de rol van cholesterol in dit proces is. In **hoofdstuk 5** wordt de invloed bestudeerd van serum cholesterol op 1 jaar na transplantatie op het risico op transplantaat verlies. Een verhoogd serum cholesterol bleek een belangrijke invloed te hebben op het risico op transplantaat verlies gecensureerd voor overlijden. Het toegenomen risico is het grootst voor patiënten met een goede nierfunctie en neemt af met toenemende serum creatinine waarden. De invloed van een verhoogd serum cholesterol op het risico op overlijden is leeftijds afhankelijk: het risico is het hoogst voor de jongsten en neemt af met toenemende ontvanger leeftijd. Het risico op over-all transplantaat falen is een compositie van beide voorgaande risico's: de jongste ontvangers lopen de grootste risico's.
Besproken wordt dat in de in Nederland gebruikte "Cholesterol Consensus" de leeftijd ten onrechte mede bepaalt of al dan niet tot behandeling zal worden overgegaan. Hogere leeftijd veroorzaakt een verhoogd risico op overlijden, onafhankelijk van enige andere variabele. Belangrijker is de vraag hoeveel een verhoogd serum cholesterol bijdraagt aan dit risico. Wij hebben in de

niertransplantatie populatie aangetoond dat een verhoogd serum cholesterol bij jongeren een grote toename van het overlijdens risico geeft terwijl in de oudere populatie het additionele effect van een verhoogd serum cholesterol op het overlijdensrisico verwaarloosbaar is.

Proteïnurie na niertransplantatie is een omineus teken omdat het is geassocieerd met een verhoogd risico op verlies van nierfunctie. In **hoofdstuk 6** beschrijven wij de Cox proportional hazards analyse waarin het belang van de invloed van proteïnurie, in het kader van andere belangrijke variabelen, wordt bepaald. Wij vonden dat proteïnurie (als continue variabele en als categorische variabele) een significante invloed heeft op het risico op transplantaat verlies gecensureerd voor overlijden. Indien proteïnurie werd geïntroduceerd als continue variabele bleek het risico op transplantaat verlies gecensureerd voor overlijden verschillend te zijn voor verschillende categoriëen oorspronkelijke ziekten. Populaties met glomerulonephritis, hypertensie en systeem ziekten als oorspronkelijk lijden hadden een verhoogd risico op transplantaat verlies gecensureerd voor overlijden indien proteïnurie werd gevonden. De andere populaties hadden onder dezelfde omstandigheden geen verhoogd risico.
Wij vonden ook dat systolische bloeddruk (en niet diastolische bloeddruk) een significante invloed had op het risico op transplantaat verlies gecensureerd voor overlijden.
Proteïnurie, als categorische en als continue variabele bleek een significante invloed te hebben op het overlijdens risico. Het is opvallend dat zowel het cardiovasculaire alsook het niet-cardiovasculaire overlijdens risico zijn verhoogd indien proteïnurie aanwezig is.
Besproken wordt dat proteïnurie een uiting kan zijn van nierziekte, maar ook van gegeneraliseerd (vasculair) lijden. Dit plaatst proteïnurie in een groter kader van ziekten en onderstreept de noodzaak van adequate behandeling van cardiovasculaire risico factoren als hypertensie, hypercholesterolaemie en diabetes mellitus. Bovendien versterkt het de voorkeur voor behandeling met ACE–inhibitors of angiotensine II antagonisten in deze populatie.

Een nieraanbod gaat altijd gepaard met een grote hoeveelheid informatie betreffende donor, ontvanger of de combinatie. Wij vroegen ons af welke variabelen werkelijk van belang zijn en dus het zwaarst moeten wegen in de beslissing al dan niet tot acceptatie van de gegeven donor-ontvanger combinatie over te gaan. In **hoofdstuk 7** beschrijven wij onze studie naar de invloed van een

groot aantal variabelen die een nieraanbod vergezellen. Wij vonden dat koude ischemie tijd en donor serum creatinine de variabelen zijn met de grootste invloed op de resultaten van niertransplantatie. Beide variabelen zijn tijdsafhankelijk hetgeen betekent dat de invloed variëert in tijd. De invloed van koude ischemietijd op het risico op transplantaat verlies gecensureerd voor overlijden is het grootst in de direct postoperatieve fase en neemt af in tijd. De invloed van donor serum creatinine is daarentegen niet groot kort na transplantatie maar neemt toe in de tijd. De conclusie is dat geprobeerd moet worden de koude ischemie tijd zo kort mogelijk te houden, terwijl een hoog donor serum creatinine herkend moet worden als een risico factor. Mogelijke maatregelen zijn: verkorten van het allocatie proces en het teruggeven van de toevoeging "spoed" aan niertransplantatie operaties.

De resultaten van niertransplantatie met een levende donor zijn beter dan die van postmortale donornier transplantatie. In **hoofdstuk 8** laten wij zien dat er belangrijke verschillen zijn tussen de twee populaties. De prevalentie van patienten karakteristieken die zijn geassocieerd met een goede prognose zijn over-vertegenwoordigd in de populatie met een levende donor. Zelfs zodanig dat deze verschillen op zich de betere resultaten van levende donor nier transplantatie zouden kunnen verklaren. In een multivariabele analyse vonden wij dat, na correctie voor al deze variabelen, het risico op transplantaat verlies gecensureerd voor overlijden toch significant lager was in de populatie met een levende donor. Echter een groot deel van het verschil in risico kan worden verklaard door de veel kortere koude ischemie tijd in de populatie met de levende donor. Indien deze variabele uit het model werd gelaten trad een belangrijke toename op van de significantie van de invloed van donor oorsprong op het risico op transplantaat verlies gecensureerd voor overlijden. De grootte van de invloed was onveranderd. Verwijdering van geen van de andere variabelen leidde tot een dergelijke verschuiving in de significantie van donor oorsprong. De rest van het verschil in transplantaat overleving moet verklaard worden door verschil in kwaliteit van het donor orgaan zelf.

Conclusie

1. In een complexe populatie met multifactoriëel bepaalde risico's als de niertransplantatie populatie kan met univariabele survival analyse geen betrouwbaar resultaat verwacht worden. Toepassing van een multivariabele methode is geïndiceerd.
2. Het risico op transplantaat falen (gecensureerd voor overlijden) neemt af met toenemende ontvanger leeftijd. Het is niet gebruikelijk het immunosuppressief regime af te stemmen op de ontvanger leeftijd. Dit betekent dat op dit ogenblik ouderen waarschijnlijk teveel immunosuppressiva krijgen.
3. Hoge donor leeftijd is geassocieerd met een verhoogd risico op transplantaat falen. De toename van het relatieve risico met de leeftijd is niet extravagant en mede gezien het tekort aan donor organen is exclusie niet geïndiceerd.
4. Transplantatie van oudere (hoge risico) donor organen naar oudere ontvangers voegt een verhoogd risico op transplantaat falen toe aan het reeds verhoogde risico op overlijden.
5. Een verhoogde serum cholesterol spiegel vergroot met name het risico op transplantaat verlies in de populatie met een goede nierfunctie. Daarnaast beïnvloedt het vooral het overlijdensrisico van de populatie jonge niertransplantatie patiënten. Hoewel wij niet hebben aangetoond dat behandeling van een verhoogde serum cholesterol waarde deze risico's reduceert, doet vergelijking met resultaten in de niet-getransplanteerde populatie dit wel vermoeden.
6. Naast een verhoging van het risico op transplantaat verlies veroorzaakt proteïnurie een toegenomen overlijdensrisico. Zowel het cardiovasculaire als het niet-cardiovasculaire overlijdens risico zijn verhoogd. De resultaten van langdurige behandeling, met ACE–inhibitors of angiotensine II antagonisten, op de patiënt en transplantaat overleving na niertransplantatie zijn nog niet bekend. Vooralsnog lijkt deze therapie geïndiceerd teneinde te proberen de proteïnurie te beperken.
7. Een verhoogde systolische bloeddruk heeft een negatieve invloed op het risico op transplantaat verlies (gecensureerd voor overlijden). Snelle en adequate behandeling van hypertensie is dus geïndiceerd.
8. Een verhoogd donor serum creatinine heeft een negatieve invloed op het risico op transplantaat verlies. Nieren van donoren met een verhoogd serum creatinine moeten worden aangemerkt als hoog risico organen.

9. Een langere koude ischemie tijd is geassocieerd met een verhoogd risico op transplantaat verlies gecensureerd voor overlijden. Bij postmortale donor nier transplantaties moet worden gestreefd naar een substantiële verkorting van de koude ischemie tijd.
10. Een groot deel van de superieure resultaten van levende donor nier transplantaties kan worden verklaard door de kortere koude ischemie tijd. Echter, na correctie voor koude ischemie tijd blijft er een verschil in risico tussen de populatie met een postmortale en die met een levende donor nier.

Concluderende opmerkingen

Met behulp van multivariabele analyse kunnen risicofactoren in een populatie worden vastgesteld. Zodra deze bekend zijn kan een poging worden ondernomen deze risico factoren te beïnvloeden. Voorbeelden zijn: behandeling van hypercholesterolemie, proteïnurie en hypertensie. De duur van de koude ischemietijd kan worden verkort en donatie van nieren bij leven kan worden gestimuleerd. Donor serum creatinine kan niet worden beïnvloed, maar het is denkbaar dat onderzoek van de pre-donatie periode van postmortale donoren leidt tot een beter donor management en uiteindelijk lagere creatinine waarden.

Echter, sommige variabelen, zoals leeftijd, kunnen niet worden beïnvloed. De resultaten van jonge donor nieren zijn beter, maar gezien het tekort aan donor organen kunnen oudere donoren niet worden geëxcludeerd. Indien alleen statistische argumenten zouden worden gehanteerd, dan zouden uitsluitend jonge ontvangers een donor nier krijgen en ouderen zouden op de wachtlijst achterblijven. In de praktijk hebben individuen gelijke kansen op een donor nier, onafhankelijk van de leeftijd. Het is duidelijk dat dit een negatieve invloed heeft op de resultaten van niertransplantatie. Het past echter wel in ons primaire doel: het belang van de individuele patient, niet slechts "de beste resultaten".

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