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Gender aspects in kidney transplantation.



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LIST OF PAPERS

I. Øien CM, Reisæter AV, Leivestad T, Pfeffer P, Fauchald P, Os I.

Gender imbalance among donors in living kidney transplantation: the Norwegian experience.

Nephrol Dial Transplant 2005; 20: 783-9.

II. Øien CM, Reisæter AV, Leivestad T, Dekker FW, Line PD, Os I.

Living donor kidney transplantation: the effects of donor age and gender on short- and long-term outcomes

Transplantation 2007; 83: 600-6.

III. Øien CM, Reisæter AV, Os I, Jardine A, Fellström B, Holdaas H.

Gender-associated risk factors for cardiac end points and total mortality after renal transplantation: post hoc analysis of the ALERT study.

Clin Transplant 2006; 20: 374-82.

ABBREVIATIONS

ALERT	the Assessment of LEscol in Renal Transplantation
ALG	antilymphocyte globulin
ATG	antithymocyte globulin
AZA	azathioprine
CHD	coronary heart disease
CKD	chronic kidney disease
CMV	cytomegalovirus
CVD	cardiovascular disease
DD	diseased donor
ESRD	end stage renal disease
GFR	glomerular filtration rate
HLA	human leukocyte antigen
HR	hazard ratio
IHD	ischemic heart disease
LD	living donor
OKT 3	murumonab-CD 3
RAS	rennin angiotensin system
RRT	renal replacement therapy
RR	relative risk

1. INTRODUCTION

For most patients with end stage kidney failure, kidney transplantation has the greatest potential for restoring a healthy and productive life. Furthermore, kidney transplantation offers a survival benefit compared to dialysis treatment in recipients of all ages (1). However, kidney transplant recipients still have a reduced life expectancy compared to the background population (2). The main problem today is the organ shortage. It is therefore important to identify risk factors for graft loss in order to increase the graft survival and decrease the need for re-transplants. The incidence of acute rejection and early graft failure has declined dramatically as a result of new immunosuppressive medications. When considering reasons for graft failures, patient death and cardiovascular disease in the late period after transplantation, a different set of risk factors apply. It is important to identify the panorama of different risk factors that operate at different time periods after transplantation in order to increase both graft and patient survival.

1.1 Epidemiology of ESRD.

During the last decades the availability of care for patients with ESRD has grown rapidly throughout the medical developed world. The number of patients receiving treatment for ESRD has increased steadily. Modalities for the management of ESRD population vary among countries. Even in the Nordic countries there are striking differences. In Norway 71.6 % of the ESRD patients had a kidney transplant in 2005, while the corresponding number in Sweden was 53% (3, 4).

The average age of the ESRD population increase each year world wide. Age has not been a factor for patient selection in Norway during the last two decades. In Europe the majority of the dialysis patients are men while there is only a small difference among the sexes in the USA (5). This difference may be due to different etiology of ESRD and demographic differences between the USA and Europe. In the USA diabetic nephropathy is the cause of ESRD in 40 % of the patients, while only 20 % of the patients in Norway have this diagnosis. In Norway hypertensive nephropathy was the most commonly

reported primary renal diagnosis in incident ESRD patients, constituting 32% of all new patients in 2005 (6).

1.2 Kidney transplantation.

Although sporadic attempts at kidney transplantation had been made throughout the first half of 20th century, the current era of transplantation was pioneered in Boston in 1954 with live donor transplantation between identical twins (7). In January 1959 the first successful kidney transplantation between non-identical twins was performed (8). The first attempts at immunosuppression used total body irradiation; azathioprine (AZA) was introduced in the early 1960s, and was soon routinely accompanied by prednisolone (9). The polyclonal antibody preparations antithymocyte globulin (ATG) and antilymphocyte globuline (ALG) became available in the mid 1970s (10). With azathioprine and prednisolone as the baseline regimen and ATG or ALG used for induction or for the treatment of steroid resistant rejection, the success rate of kidney transplantation was approximately 50% at 1 year and the mortality rate was 10% to 29%. The situation was transformed in the early 1980s with the introduction of cyclosporine (11). Because the results of kidney transplantations were poor, the dramatic benefit of cyclosporine was clearly evident. Short-term graft survival rates increased to more than 80% at 1 year. Since the mid 1980s cyclosporine based immunosuppression has been the most common regimen in use fig 1.

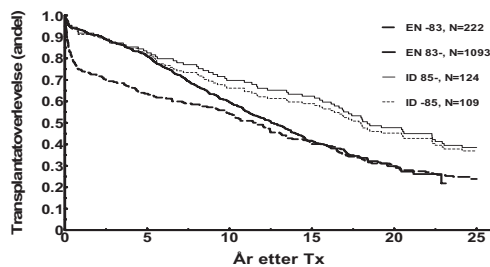


Fig. 1a Graft survival uncensored for death in living donor kidney transplantation according to identical or one haplo type mismatch before and after the introduction of cyclosporine in Norway.

Torbjørn Leivestad 2007

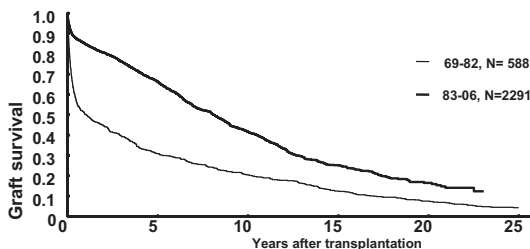


Fig 1b. Graft survival uncensored for death in deceased donor transplantation before and after the introduction of cyclosporine in Norway. *Torbjørn Leivestad 2007*

Although the benefits of cyclosporine were obvious, its capacity to produce both acute and chronic nephrotoxicity was soon recognized to be a major disadvantage (12). In 1985, OKT3, the first monoclonal antibody used in clinical medicine, was introduced based on its capacity to treat first acute rejection episodes, although the toxicity of the drug tended to restrict its use to episodes of rejection that were resistant to high dose steroids (13, 14). In some centers it was used as an induction agent (15). With these medications- cyclosporine, azathioprine, corticosteroids and the antibody preparations- the transplant community entered the 1990s, achieving success rates of up to 90% in many centers and minimal mortality. Tacrolimus was introduced as an alternative to cyclosporine (16). Mycophenolate mofetil was found to be more effective than azathioprine by virtue of its capacity to reduce the incidence of acute rejection episodes (17). In 1999 sirolimus was added to the immunosuppressive menu, and studies are in progress to evaluate several new chemical and biologic agents (18). Also contributing to the improved graft and patient survival was diagnosis and treatment of infections such as CMV as well as the increased focus on cardiovascular disease in the transplant population with improved blood pressure control and treatment of hyperlipidemia (19, 20).

1.3 Kidney transplantation in Norway.

The first five kidney transplantations in the Nordic countries were performed at Rikshospitalet in Oslo by Leif Efskind and his team. The first Norwegian patient was transplanted in 1956 (21). However, the patient died one month after the transplantation of a heart attack during another surgery. The next 4 patients died of acute rejection episodes of the graft or septicemia between 14 and 40 days post-transplantation. In 1963 the first successful kidney transplantation in Norway was performed at Ullevål Hospital. The operation was performed in collaboration between Ole Jakob Malm and a surgeon from Boston, R.E. Wilson, who brought with him AZA (21). From 1983 all solid organ transplantations in Norway have been performed at Rikshospitalet. Since 1984, also unrelated donors have been used. Acceptance criteria for kidney transplantation have been wide and strict age limits have never been applied. This is illustrated in Fig 2 where the age of first transplant recipients since 1969 is shown..

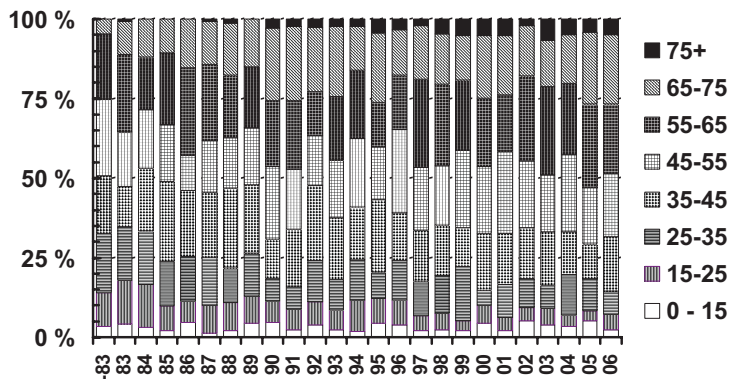


Fig. 2 Recipient age at first time renal transplantation 1969-82 and annually 1983-2006 in Norway.

Torbjørn Leivestad 01.07

Furthermore, there has been a tradition to encourage living donor transplantation that has resulted in a stable rate of living donor transplantation for over 30 years (22). This is different from other countries where there has been an increase in the rate of living donor transplantation during the last 10 years (23). Donor demographics are also different even

in the Nordic countries as shown in fig 3.

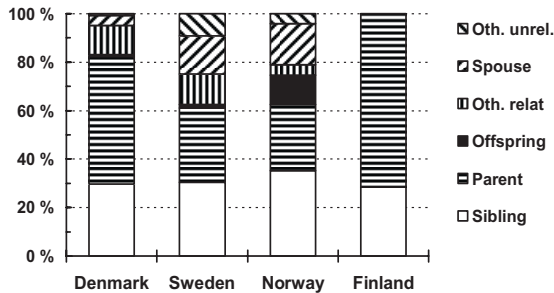


Fig 3. Relationship between donor and recipient in different Scandinavian countries from 1995 to 2004
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Demographic data on all patients that receive a renal transplant in Norway are transferred to the Norwegian Renal Registry.

1.4 Gender aspects in ESRD

Male gender is associated with a more rapid progression of renal injury in non-diabetic kidney disease (24). In Norway 64.5% of the patients receiving renal replacement therapy in 2005 were men (6). Potential mechanisms for gender-related protection in women include differences in renal structure including glomerular number and size, renal hemodynamics, and different effects of estrogen or androgen on the synthesis and release of vasoactive substances, growth factors and cytokines. In a study of potential kidney donors where glomerular filtration rate (GFR) was measured, the age dependent decline in kidney function between 20 and 50 years of age was more pronounced in men than in women (25). It was speculated that pre-menopausal females was protected by estrogens, however, this was not specifically investigated.

The role of sex hormones in modulating the activity of several regulatory systems, including the rennin-angiotensin system (RAS), has been suggested as an explanation for the slower progression of non-diabetic renal disease in women. Clinical support for this hypothesis is provided by the REIN study, which reported that women with proteinuric

renal diseases exhibited greater reductions in proteinuria and better renal outcomes in response to ACEIs than did men, despite similar blood pressure control (26). Furthermore, the etiology of ESRD may differ in men and women. There are reports of more men with chronic glomerulonephritis while there is a predominance of women with tubulo-interstitial disease (27, 28). The importance of these differences is not explored in the literature.

1.5 Acute rejection episodes.

Acute renal allograft rejection is defined as an immunologic process resulting in a deterioration in allograft function that is associated with specific pathologic changes. These changes have been standardized by the Banff criteria (29). The allograft biopsy remains the gold standard for confirming the diagnosis of acute rejection.

In the 1980s, at least one acute rejection episode occurred in 50 to 60% of renal allograft recipients (30). In Norway acute rejection episodes occurred in 48 - 82% according to HLA-DR mismatch during the early 1990s (31). With newer immunosuppressive regimens many centers are achieving acute rejection rates below 15%, and only 13% of kidney transplant recipients in 2003 required therapy for acute rejection in the USA (32)

It has been reported that early acute rejection episodes (occurring within 60 days of engraftment) have a major effect on allograft survival (33). According to these reports kidneys that recover function still have a 10% decrease in one-year survival when compared to rejection free kidneys (30). This is the reason why early acute rejection episodes have been used as surrogate endpoint for future graft loss in many studies. However, despite decreasing rates of acute rejection episodes, and improved 1 yr graft survival, an overall lack of improvement in long-term allograft survival is reported in recent publications (34, 35). Although the underlying reasons are unclear, this may be related to a higher proportion of acute rejection episodes that fail to recover to previous baseline function (35). In addition the use of potent induction agents may prevent acute rejection in predisposed recipients. Still, these patients may develop subclinical rejections

resulting in significant tubulo-interstitial damage to the transplanted kidney with reduced graft survival as the result (36, 37). It follows that, an acute rejection episode is not a satisfactory surrogate end-point for graft survival today.

1.6 Graft survival

Graft survival is one of the most important measures of success in kidney transplantation. However, since one-year graft survival rates generally increased to 90% in 1996, it has been difficult to use short term graft survival as a sensitive measure of progress in transplantation (35, 38). As the recipient population has aged by nearly 10 years over the past decade, the prospects for long term survival are hampered by age related problems. The recipient age obviously has a very clear effect on causes of graft loss. Fewer than 20% of grafts in patients over the age 60 yr are lost because of acute or chronic rejection while 50 % of the graft losses are due to patient death (39, 40). Donor age is also an important risk factor for reduced graft survival. In response to organ donor shortage, there has been a broadening of the age limits traditionally applied to organ donors. This has resulted in an increase in donor age over the years in both deceased and living donor transplantation (6)

In general, living donor grafts are superior to deceased donor grafts (2, 32). This benefit applies across all degrees of HLA mismatching (41). The better outcomes reflect several factors: healthy living donors, avoidance of ischemia-reperfusion injury, high nephron mass and probably the effect of shorter waiting time for the recipient. Excellent results are now being demonstrated with living unrelated kidney transplantation where HLA matching is not optimum (42).

1.7 Cardiovascular disease and mortality after transplantation.

Renal transplant recipients may develop a variety of complications related to the allograft, the immunosuppressive therapy, progression of pre-existing diseases, and aging with the appearance of new diseases. Allograft failure is usually defined either by death

or by a patient's need to undertake new treatment for ESRD (i.e., chronic dialysis or retransplantation). To improve graft survival approaches to prevent death and graft failure must be undertaken.

Death with graft function accounts for 40% to 50% of all graft losses (43). The three most commonly defined causes of death in the late post transplant period are cardiovascular disease, infection and malignancy (44). Studies of renal transplant recipients in the late 1980s and early 1990s showed that ischemic heart disease alone caused as much as 53% of the deaths with a functioning graft in Scandinavian transplant recipients(45). However, the relative risk of CHD has progressively decreased since the 1990s along with a 50% reduction in post-myocardial infarction mortality. This improvement has occurred despite the increase in renal transplant surgery in older patients.

To understand how to prevent post transplant CVD deaths and complications, it is crucial to define the etiological risk factors. Identifying risk factors is important for two reasons. Some risk factors can be modified, and for some of these, there is strong evidence from studies that intervention improves survival (20). It is also important, however, to identify risk factors that cannot be modified because these risk factors help to identify high-risk patients who can be targeted for screening, as well as for treatment of modifiable risk factors after transplantation. Transplantation confers additional risks for CHD because key immunosuppressive medications can cause hypertension, hyperlipidemia, impaired glucose tolerance and allograft dysfunction. Consequently, coronary risk factors specific for transplantation, such as the use of steroids, calcineurin inhibitors and post-transplant diabetes mellitus, may be in transition to becoming modifiable risk factors.

2. AIMS OF THE STUDIES

The aims of this thesis were to evaluate gender related issues in renal transplantation.

More specifically the primary purposes were to assess:

- Whether there is a predominance of female-to-male donations among first time living donor kidney transplantation in Norway.
- The effect of donor gender and age on outcomes after a first time living donor renal transplantation.
- Gender differences in cardiovascular events and total mortality in recipients of a kidney transplant.

3. SUBJECTS AND METHODS

3.1 Study population

The Norwegian Renal Registry

National data on renal replacement therapy has been collected within the Renal Association since 1980. In 1994 the Norwegian Renal Registry was formally constituted as collaboration between The Norwegian Renal Association and Rikshospitalet University Hospital, with the latter as the formal owner. The Registry has obtained concession from the National Data Inspectorate. The study protocols of paper I and II have been approved by National and Regional Committees for Research Ethics in Norway.

The data base contains donor variables: age, gender and relationship to the recipient, recipient variables: age, gender, original disease, time spent on dialysis, time spent on the waiting list, panel reactive antibodies, transplant factors: human leukocyte antigen [HLA] –A, -B, and –DR mismatches, and post transplantation features including immunosuppressive regimen, rejection history, patient survival, graft survival and serum creatinine values. Serum creatinine values, causes of death or graft failure have been reported yearly throughout the whole study period.

In paper I all first time LD transplantations performed between 1984 and 2002 are included in the study. In paper II data on first time LD transplantation performed between January 1, 1994 and December 31, 2004 in recipients and donors above 18 years of age were analyzed. Only one patient was lost to follow up in this cohort due to emigration. This patient was not included in the analysis.

ALERT study

The Assessment of LEscol in Renal Transplantation (ALERT) study recruited 2102 renal transplant recipients from nephrology and transplant clinics in Belgium, Denmark,

Finland Germany, Norway, Sweden, Switzerland, the UK and Canada. This is the first large-scale clinical trial to address the cardiovascular complications of renal transplantation. Men and women aged 30-75 years who had received renal or combined renal pancreas transplants more than 6 months before randomization and who had stable graft function were recruited. Patients were included from June 1996 until the end of October 1997. The median time since transplantation was 4.5 yr, and the median follow-up time was 5.4 yr. All patients were receiving immunosuppressive therapy with cyclosporine and had total cholesterol concentrations of 4.0-9.0 mmol/L. Patients with a history of myocardial infarction more than 6 months before randomization could be enrolled if their total cholesterol concentration was 4.0-7.0 mmol/L. Patients were excluded if they were on statin therapy, had familial hypercholesterolaemia or had experienced an acute rejection episode in the 3 months before randomization. In addition, patients with a predicted life expectancy of less than 1 yr were excluded. Only patients in the placebo arm of the study (n=1052 patients) was evaluated in paper III. This was considered to be the best way to evaluate the impact of different cardiac risk factors over time in a statin naïve population. The predefined end points used in this study were cardiac death or definite non-fatal myocardial infarction verified by hospital records and total mortality. Electrocardiographic changes were classified according to the Minnesota code. The study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and was in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the ethics committees in each participating country approved the trial (20).

3.2 Statistical analyses

Continuous data are expressed as mean \pm 1 standard deviation (SD) and proportions were expressed as percent. Quartiles are given when the distribution is skewed.

Independent Samples T-test and Chi-square statistics- were used to examine for baseline differences between two means or proportions in all papers. All tests are 2-sided, and a significance level of 5% was used. All analyses were performed with SPSS 12.0.

Survival analyses

Survival analysis is concerned with studying the time between entry to a study and a subsequent event. Censored survival times occur if the event of interest does not occur for a patient during the study period. When examining graft survival after kidney transplantation, graft survival censored for death and graft survival uncensored for death are most frequently used. When graft survival is censored for death only graft failures are considered. When graft survival uncensored for death is evaluated graft losses and patient death with a functioning graft are evaluated. In the relatively old kidney transplant population in Norway, recipient age gives a large contribution to the result when graft survival uncensored for death is utilized.

Kaplan Meier analyses

The Kaplan Meier is a univariate analysis that produces a plot of the survival curve for each group of interest. There are, however, two important methodological limitations. First, only the influence of categorical variables can be estimated because the analysis cannot deal with continuous variables. Secondly, the importance of a specific variable cannot be tested if adjustment for the whole set of other relevant variables is required. Moreover, this non-parametric analysis can only test global differences in survival curves between groups. On the other hand, the main advantage with Kaplan Meier plots is that the readers are used to this way of presenting the data and it shows clearly the time to the event.

Cox analysis

The Cox proportional hazard model is a robust mathematical model where a number of independent continuous and categorical variables on survival can be studied simultaneously. 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 assumption has been tested in the three papers in a log-minus-log survival plot. 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, the maximum number of variables that can be included in the analysis equals 10% or the square root of the number

of events. Although the number of variables is limited it is important to include those variables that are supposed to influence the outcome. It is important to be aware of that multivariable analysis can never adjust for unknown or unmeasured confounders. There are several methods for variable selection depending on which type of model that is constructed.

In an explanatory model, the goal is to correctly characterize the relationship of each predictor to the outcome variable. For that purpose, the identities of the variables in the model are critical, and one has to take great care in choosing which variables to include and in what mathematical form. Using statistical significance levels in the univariate analysis to choose potential confounders to include in this kind of model is not a very good idea. That is because the amount of confounding depends on two associations, the relation between the potential confounder and the exposure and the relation between the potential confounder and the outcome. The coefficient that is tested for significance in a stepwise algorithm evaluates only the relation between the potential confounder and the outcome, but it ignores the relation between the potential confounder and exposure. This method can thus only include variables that are not confounding. It can also omit variables that are confounding, but for which the relation with the outcome is not statistically significant. This kind of multivariate Cox model was constructed in paper II.

Predictive models try to predict outcomes for patients with particular characteristics. This kind of model was constructed in paper III where p values in the univariate analysis was a selection criterion. Even if a prediction model is reliable, it may not be useful in clinical practice for several reasons. It may require clinicians to have certain laboratory results that may not be available, or it may have been developed and validated on patients different from those seen in clinical practice.

Interaction

An interaction occurs when the impact of a risk factor on outcome is changed by the value of a third variable. Interaction is sometimes referred to as effect modification, since the effect of the risk factor on outcome is modified by another variable. In extreme cases,

an interaction may completely reverse the relationship between the risk factor and the outcome. This would occur when the risk factor increased the likelihood of outcome at one value of the interaction variable but decreased the likelihood of outcome at a different value of the interaction variable. Paper II a sex difference in susceptibility to age as a risk factor in donor kidneys on graft loss was explored by testing for biological interaction according to Rothman (46).

Gender

Several questions regarding the impact of donor and recipient gender on CHD, patient and graft survival after kidney transplantation arise. In paper III male and female transplant recipients have been evaluated separately. The reason for this is that women and men differ biologically, and these differences can, in general, affect risk factors and outcomes. When gender only is included in a multivariate model, the sex related differences in degree of exposure of different risk factors are lost.

4 RESULTS

4.1 Paper I

Gender imbalance among donors in living kidney transplantation: the Norwegian experience.

Gender demographics and outcomes of first time living donor transplantations in Norway performed in the period 1985-2002 were assessed in this paper.

Of 1319 first time kidney transplantations performed in the study period, females constituted the majority of the donors (57.8%; $p < 0.001$) and men the majority of the recipients (62.7%; $p < 0.001$). The donors were related in 88.3% of the population. Of the 220 unrelated donors, 90.5 % were spouses and 9.5 % friends or family members by law. Siblings constituted the largest group of LRD, and there was no significant difference between the frequency of donation between brothers and sisters. The second largest group of donors was parents donating to their children. Mothers were more often donors than fathers ($p < 0.001$). This gender difference was, however, only apparent when the recipients were ≥ 30 years old. The proportion of child-to-parent donation was 10.6% of all LD transplantations, and there was no gender difference among the donors ($p = 0.69$). Of the spousal donors the majority was females (65.8%).

Females received a kidney from male and female donors with the same frequency (248 vs. 244; $p = 0.86$). In opposite sex pairs the female to male donations were as expected based on the incidence of ESRD and gender make up in the general population. However, the female to female donation rate was higher than expected and the male to male donation rate lower than expected.

There was no difference in the number of early acute rejections according to donor sex. Donor sex did not affect graft survival uncensored for death ($p = 0.75$). Donor age had a substantial impact on serum creatinine values after transplantation.

4.2 Paper II

Living donor kidney transplantation: The effects of donor age and gender on short- and long-term outcomes.

The aim of this paper was to assess the influence of donor age and gender on short-term, graft survival, < 5 years after transplantation, long-term graft survival beyond 5 yr after transplantation and acute rejection episodes in first time LD transplantation.

In this study 739 first time LD transplantations in recipients above 18 years performed between January 1, 1994 and December 31, 2004 were evaluated. The recipients were followed until graft loss, death or last recorded status up to June 30, 2005. Only early acute rejection episodes occurring within 3 months after transplantation were included in the analysis. There were 71 graft losses during the study period and 74 patients died with functioning grafts. In the donor population 346 (46.8%) were above 50 years and donor age remained fairly constant throughout the study.

In the Cox multivariate model of risk factors for acute rejection episodes recipient age ≥ 50 years decreased the risk of experiencing an acute rejection episode (HR 0.69; 95% CI 0.55-0.87). Donor age ≥ 65 years (HR 1.57; 95% CI 1.09-2.27) and number of HLA DR mismatches were predictors for acute rejection episodes.

In the multivariate analysis of risk factors for death censored graft survival donor age ≥ 65 years was a risk factor in all time periods after transplantation. During the first 5 years after transplantation, short term follow-up, a steroid resistant rejection episode was an additional risk factor (HR 3.96; 95% CI 1.46-10.75).

Long term follow-up, more than 5 years after transplantation, male donor gender was the only additional risk factor for graft loss (HR 3.58; 95% CI 1.57-8.17). A sex difference in susceptibility to age as a risk factor in donor kidneys on graft loss was explored by testing for biological interaction. No interaction between donor sex and age was found.

4.3 Paper III

Gender-associated risk factors for cardiac end points and total mortality after renal transplantation: post hoc analysis of the ALERT study.

The aim of the present article was to explore whether renal transplantation restores the gender-dependent cardiac protection in women. The distribution of risk factors and their impact on cardiac outcome and total mortality in men and women were also evaluated.

This post hoc analysis of pre-defined end points in the placebo group (n=1052) of the ALERT study provided an opportunity to evaluate the impact of the different risk factors over time in statin-naïve renal transplant recipients. The mean age was 50.1±11.1 years and 65.3% of the study population was males. At baseline HDL cholesterol levels were higher in women than men (1.48±0.002, p<0.0001). No differences in LDL cholesterol levels or triglyceride levels were observed. Furthermore, more men than females had ST-T abnormalities in ECG at baseline (147 (21.4%) vs. 52 (14.2%), p=0.0046). There was also more men than women who had left ventricular hypertrophy at baseline (121 (17.6%) vs. 35 (9.6%), p=0.0005).

A total of 104 patients experienced a definite non-fatal MI or cardiac death, and 138 patients died of any cause. There was no gender difference in the occurrence of any of these end points.

In the multivariate analysis for the cardiac end-point, previous coronary heart diseases (HR 3.21; 95% CI 1.55-6.45), diabetes (HR 1.96; 95% CI 1.01-3.83), treatment for rejection (HR 2.32; 95% CI 1.22-4.42) and serum triglycerides (HR 1.29; 95% CI 1.07-1.56) were predictors in men. In women the LDL/HDL ratio (HR 1.70; 95% CI 1.30-2.23) was the only significant risk factor.

A slightly different risk factor pattern appeared in the Cox multivariate analysis for total mortality. Diabetes (HR 1.96; 95% CI 1.01-5.50), ECG abnormalities (HR 1.15; 95% CI 1.10-1.20), plasma triglycerides (HR 1.28; 95% CI 1.05-1.57), serum creatinine (HR

1.09; 95% CI 1.05-1.13) and age at baseline (HR 1.09; 95% CI 1.05-1.13) predicted total mortality in men, while ECG abnormalities (HR 3.41; 95% CI 1.42-8.24), age at baseline (HR 1.07; 95% CI 1.01-1.13) and LDL/HDL ratio (HR 1.32; 95% CI 1.01-1.74) were predictors in women.

5. DISCUSSION

5.1 Methodological considerations.

When renal transplantation was a new treatment method for ESRD, graft failures, patient deaths and cardiac events were more prevalent and successes could be directly derived from facts and events. Results have improved dramatically over the last decades and many factors have seemed to be involved in these continuously improving results. The multitude of risk factors present makes it difficult to ascertain the individual contribution of each factor. Therefore multivariable analysis is needed because most outcomes have multiple causes, and both prognosis and the etiology are usually influenced by a large number of factors. Identification of risk factors through observational studies has been particularly important because it is not possible to randomize people to many of the conditions that cause inferior outcomes after a transplantation. Causality, however, is established on the basis of biological plausibility and rigorous study designs. Although the result of an epidemiological study may reflect the effect of an exposure on the development of disease, it is also possible that the findings may have an alternative explanation. Our results should therefore be evaluated in this context. An overall goal of an epidemiological study is accuracy in estimation. To achieve this, the study should be designed and conducted with the aim of reducing random and systematic errors (46).

Precision (lack of random error)

The primary way to increase precision in an epidemiological study is by increasing the size of the study. Random error is the variability in the data that we cannot readily explain. The degree to which chance may account for the results can be evaluated by tests of statistical significance. The P-value is defined as the probability that an effect could have occurred by chance alone. It is a statistic that can be viewed as a measure of the compatibility between the data and the null hypothesis. A more informative measure of the role of chance is the confidence interval (CI), which is an expression of the amount of random error in the estimate. A wide CI indicates low precision and increase the probability that some of the results may be due to chance.

Validity (lack of systematic error)

The validity is usually divided into two parts: Internal and external. Internal validity is defined as the degree to which the results of an observation are representative for the particular group of people being studied. External validity or generalization is the extent to which the results of a study apply to people outside the population being studied.

Internal validity

A study can be biased because of the way in which the subjects have been selected, the way the study variables are measured, or some confounding factor that is not completely controlled. These errors remain even in an infinitely large study and are also called systematic errors.

a. Selection bias

Selection bias in a study stems from the procedures used to select subjects and from factors that influence the study participation. Although one might expect minimal sampling error when using the Norwegian Renal Registry because it is a national based registry following the patients for the rest of their lives, it should be standard to treat all epidemiological studies as having sampling errors. When looking at the ALERT trial it should be recognized that the overall statistical power of the study was low. The size of the ALERT study was based on registry data from the Scandinavian countries, which estimated a primary endpoint rate of 5% per year. The cardiovascular risk and cardiovascular event rate among the recruited study population was low. In a post hoc analysis, based on a 17% reduction in the chosen primary endpoint, it was estimated that 6800 renal transplant recipients followed for 5 years would be required to provide 80% power and $\alpha=0.05$ two-tailed. This study is, however, the biggest randomized trial performed in renal transplant recipients.

b. Information bias

Information or observation bias includes any systematic error in the measurement on exposure or outcome. Misclassification of subjects for either exposure or disease can be differential or non differential. These terms refer to the mechanism for misclassification. Misclassification of exposure is non differential if it is unrelated to the occurrence or presence of disease and differential if the misclassification of exposure is different for those with and without disease. Similarly, misclassification of disease is non differential if it is unrelated to exposure, otherwise it is differential. Non differential misclassification between two exposure categories will in general make the effect estimates for those two categories converge toward one another.

The high numbers of cardiovascular events in renal transplant recipients reported to registries are differential misclassifications. It has been much easier to report a cardiovascular event in a kidney transplant recipient because it is a well known fact that cardiovascular disease and mortality is much higher in this group than in the general population. This problem is avoided in randomized clinical trials where a committee adjudicates the events. An independent critical events committee of two nephrologists and two cardiologists reviewed all end points for adjudication in the ALERT trial. Therefore, the patients who get the diagnosis of a MI are correctly diagnosed in paper 3. The parameters in paper I and II are not likely to be affected by differential misclassification.

c. Confounding

Confounding occurs when the apparent association between a risk factor and an outcome is affected by the relationship of a third variable to the risk factor and to the outcome. For a variable to be a confounder, the variable must be associated with the risk factor and causally related to the outcome.

The effect of confounders may be adjusted for by multivariable analysis or stratification. Stratification works well when there are only two or three confounders. However, when there are many potential confounders, stratifying for all of them will create literally hundreds of groups. In this thesis we have used proportional hazard (Cox) regression. A major advantage of proportional hazard analysis is that it includes persons with varying lengths of follow-up. Unmeasured factors that could explain the result should always be

considered. In our papers we have no information on life style related factors as alcohol consumption, physical activity and dietary nutrition intakes in either the registry data or the ALERT placebo group.

External validity

For a result from a randomized controlled study or epidemiological study to be clinically useful, it must be relevant to a definable group of patients. Lack of external validity is the most frequent criticism by clinicians of studies, systematic reviews and guidelines, and is one explanation for the widespread under use in routine practice of many treatments that have been shown to be beneficial in trials and that are recommended in guidelines.

Another problem for the external validity of a study is an inadequate duration of treatment and/or follow-up. Furthermore, the external validity of a trial also depends on whether the outcomes are clinically relevant. There are many examples of treatments that have had a major beneficial effect on a surrogate outcome, which had previously been shown to be correlated with a relevant clinical outcome in observational studies, but where the treatments have proved ineffective or harmful in subsequent large randomized controlled trials that used these same clinical outcomes (47, 48).

Regarding the gender demographics of Norwegian living donors given in paper I the external validity is high as there are no missing data on this issue in the registry. The outcomes after transplantation, early acute rejection episodes, graft loss censored for death and uncensored for death that are reported in paper I and II are not surrogate endpoints and all have been reported to the registry. In paper III, however, the results may be applicable only to long time survivors after a renal transplantation with a good graft function.

5.2 Importance of results

Donor epidemiology (paper I)

Our main result in this article is that although the majority of living kidney donors in Norway is women, there has been no predominance of female to male donations in

Norway during the last two decades. It has been reported from other centers that women donate to men and children to a greater degree than men do (49, 50). This has been explained by women having greater obligations to their families as well as economical reasons. Albeit the majority of the donors are women, and no difference in female to male donations was found, the overall higher number of female donors was caused by a higher female to female donation rate than expected. In a recent Scandinavian study it was found that there were only minor differences in attitudes regarding kidney donation between men and women (51). Thus the rather small gender difference could be due to LD kidney donation having been strongly advocated as a treatment option for ESRD in Norway for over 30 years.

A rather surprising finding was that fathers were as likely as mothers to donate to younger children. This is in contradiction to reports from the USA where mothers were especially prone to donate to younger children and this was explained by the strong emotional bondage between mother and child (49). The result is especially stunning as the system for reimbursement of lost income is not optimal in Norway. Westlie et al. have reported that 21% of Norwegian donors experienced an economic loss (22). One might be tempted to speculate that the care of small children is equally shared among mothers and fathers and this may override the disadvantage of poor reimbursement.

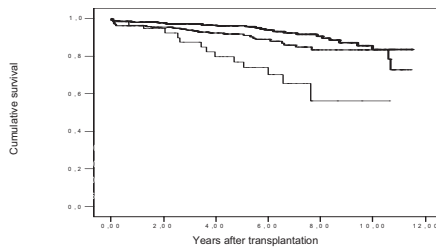
Our finding that the frequency of wife to husband transplantations mirrors the incidence of ESRD in men in Norway differs from reports from other countries. In a report from Canada 90% of the spousal transplantations were from wife to husband (52). Furthermore, in opposite sex pairs in living related transplantation the observed donation rate was similar to what could be expected based on the gender composition in the general population and in the incidence of ESRD.

Short and long term outcomes after living donor kidney transplantation (paper II)

Our main result in paper II was that a donor age ≥ 65 year was a risk factor for graft loss in recipients with more than 3 months graft survival. In deceased donor transplantation

donor age ≥ 50 years has been associated with reduced graft survival in the recipient (53). In Norway there has never been a strict age limit for donors or recipients. In the study period 46.8% of our donors were above 50 years. It is therefore a study population where evaluation of outcomes after kidney transplantations with donors above 50 years can be done. Fig 4 shows death censored graft survival in recipients of living donors in 3 age categories.

Fig 4. Death censored graft survival according to donor age in paper II.



No at risk:	0	1	2	3	4	5	6	7	8	9	10
20-49.9 yr	392	359	318	274	225	189	148	120	87	65	42
50-64.9 yr	288	256	223	193	157	128	103	75	53	41	23
≥65 yr	56	53	43	35	31	27	20	10	4	2	1

Our result is supported by the findings in a paper by Gill et al where the risk of graft loss with living donors 55-64 years was similar to that with deceased donors <55 years. Their conclusion was that outcomes are excellent with living donors <65 years (54). The implication of our results is that although graft survival with LD >65 yr is inferior to younger donors; this source of donors should still be exploited. As shown in our study, older donors can be successfully used for older recipients. Already an old for old program exists in DD transplantation. Perhaps this strategy should be utilized further in living donor transplantation programs.

We found that a donor age ≥ 65 years is a risk factor for an early acute rejection episode in LD transplantation, while it has been observed that donor age above 50 yr is a risk factor for an early acute rejection episode in recipients of kidneys from deceased donors (53). Our result in living donor transplantation represents a 15-year shift in donor age compared with deceased donor transplantation regarding the risk of early acute rejection

episodes. How much impact an early acute rejection episode has on later graft loss today is not established. Our finding that only a steroid resistant rejection episode was a risk factor for early graft loss is in accordance with other recent reports. Meier Kriesche et al showed that early rejection episodes where creatinine was normalized back to baseline levels was no predictor for later graft failure (35). In our study a steroid resistant rejection episode was no longer a risk factor for graft loss 5 years after transplantation. This may not be a surprise as other factors may be stronger predictors by then (55). These findings are in disagreement with the use of acute rejection episodes as a surrogate endpoint. Donor age, however, should be used as one factor in the total risk score for graft loss as well as when adjustment of immunosuppressive medication is considered.

A rather surprising finding in our study was that male donor gender was a risk factor for graft loss for the whole period as well as for long term graft survival beyond 5 years. There are few publications on long term results in LD transplantation and especially with a gender perspective. Earlier studies have reported poorer overall graft survival in females donating to males, and this was explained by the theory of nephron underdosing (56, 57). In an autopsy study, however, there was no gender difference in the number of glomeruli (58). Individuals with larger kidneys had more glomeruli and older individuals had fewer glomeruli. Furthermore, the increased kidney weight seen in men was solely dependent on greater body surface area (58). These results have been supported in a study where kidneys from living donors were weighed after the donor nephrectomy. The mass of kidneys from men and women were not statistically different. Furthermore, no difference in graft survival until 3 years after transplantation by donor and recipient gender were found in this study (59). Before the introduction of cyclosporine, no difference in graft survival between male and female donor kidneys was reported (60).

To evaluate whether our result was due to a biological interaction between donor age and sex, an interaction analysis was performed Table 1.

Table 1.

Incidence of graft loss and excess RRs because of biological interaction (RERI) between donor age and sex.

DONOR	GRAFT LOSS Whole period (n=739)	RR (95% CI)	RERI (95% CI)
Female <50 yr	0.86/100 pt yr	1.00	
Male<50 yr	1.75/100 pt yr	2.03 (0.91-4.60)	
Female>50 yr	2.35/100 pt yr	2.73 (1.29-5.92)	
Male >50 yr	3.46/100 pt yr	4.02 (1.86-8.96)	
			0.26 (-2.10-2.62)

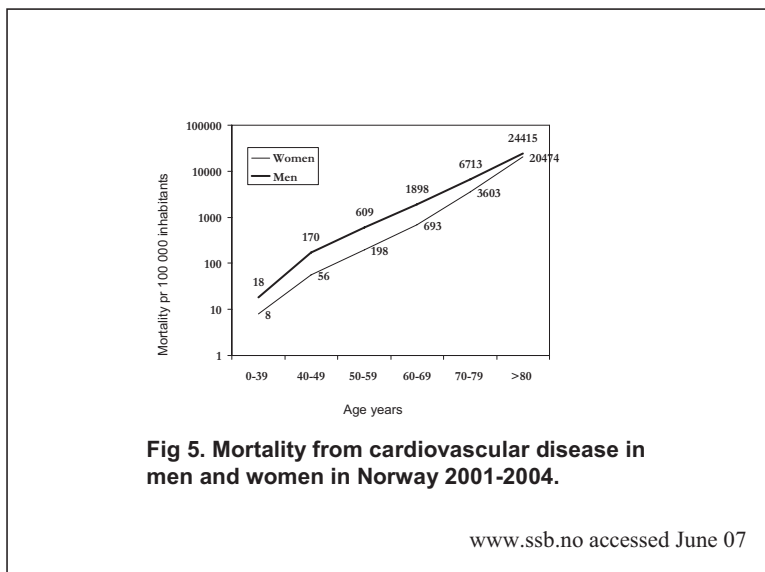
NOTE. RERI=0 means no interaction. RERI=0.5 means that because of interaction between the 2 risk factors, the RR is 0.5 greater than expected based on the addition of the 2 risk factors.

No interaction between donor gender and age was found in our study..

The implication of our study is that donor age, as a risk factor for acute rejections, should be considered in the choice of immunosuppressive regimen. Furthermore, donor gender should not be an important issue in donor selection.

Cardiovascular events and total mortality after renal transplantation (paper III)

The main result in this paper is that no difference in either the incidence or time to cardiac events and total mortality was observed in male and female transplant recipients. It is well known that women develop angina and MI later than men. This difference is confirmed in a Finnish report in the general population where the difference in CHD incidence and mortality in men and women was largest in persons below 50 years (61). Data from Norway is lacking, however there is a similar difference in cardiovascular mortality (Fig 5).



This is, however, not the case in dialysis patients where the female gender advantage is lost (62). In a Norwegian cross sectional study of renal transplant recipients multivariate analysis revealed that ischemic heart disease was independently related to male gender (63). This is in contrast to our result, the reason for this discrepancy is not known. In the follow-up study by the same authors, however, in which data was collected 5 yr after baseline, no difference between men and women regarding ischemic heart disease was found in the multivariate analysis (64). The fact that no difference in cardiac events and total mortality was observed in our study of a relatively low risk population of transplant recipients, suggests that the female gender advantage regarding CHD is not restored following a successful transplantation. When the study population was recruited between June 96 and October 97, patients who were considered to have a high CHD risk were already receiving lipid lowering therapy and were not eligible for the study. However, a relatively low risk transplant population is at a much higher CHD risk than the age matched general population. When compared to the general population, cardiovascular mortality in transplant recipients is increased by nearly 10-fold among patients with the

age range of 36 to 44 and at least doubled among those between the ages of 55 to 64 (65,62).

At baseline more men than women had ST-T abnormalities in the ECG. This is in contrast to findings in community based cohorts where women have significantly more ST changes and T wave abnormalities (66). This may indicate that only the healthiest women were randomized in our study. The prevalence of LVH at baseline was lower than reported by Midtvedt et al 1 year after transplantation where 45% of the recipients still had LVH. At baseline in their study 66% of the patients had LVH. Our results may imply that good blood pressure control after transplantation leads to regression of LVH in both men and women beyond 1 year after transplantation (67).

Baseline demographic data show that LDL cholesterol levels were the same in men and women who were 50 yr old. Women usually have lower levels at that age. In both sexes, the risk of CHD increases markedly with age. In most populations, serum cholesterol increases with age. In men, this increase usually levels off around the age of 45 to 50 years, whereas in women the increase continues sharply until the age of 60 to 65 years (68). The lack of difference in these subjects could be caused by immunosuppression, diabetes, reduced kidney function and history of CKD. One limitation is that we do not have any data on hormonal status in the women who participated. In the general population an early menopause is associated with increased ischemic heart disease mortality (69).

Regarding the impact of different lipid parameters on the cardiac end-point, a gender difference appeared in the multivariate analysis. In women the LDL/HDL cholesterol ratio was the only lipid parameter that remained a risk factor for the cardiac end-point. Our result is in accordance with a report from the Nurse's Health Study where lipid indexes, such as LDL/HDL cholesterol ratio, which reflects the proportion of atherogenic to antiatherogenic lipid fractions, was a powerful tool for predicting CVD among postmenopausal women (70). Furthermore, it has been shown that among women with the highest absolute risk of CAD, those aged >65 years, a low HDL cholesterol level was

the only significant lipid predictor of risk for CAD death in a meta analysis. A reduction in LDL cholesterol particle size in association with age, menopause and adiposity has been found in women. This may explain why high LDL cholesterol levels have been shown to affect women less in large epidemiological studies and the benefit of statins for the primary prevention of atherosclerotic CVD in women has not been established. However, secondary prevention trials support the efficacy of treating women who have established vascular disease with statin to lower LDL cholesterol levels. This may be due to the reduction in the LDL cholesterol particle size in these women. The average age of the women in our study is 50 years, but their risk factor profile is more like that of women in the general population above 65 years.

The main clinical implication of our study is therefore that women should receive CV prophylactic treatment at the same intensity as men.

5.3 Future research

Risk factors for cardiovascular incidents and mortality have not been analyzed in the Norwegian transplant recipients. These events are available in the registry and are very important for the patients and should be examined in the future. Furthermore, the use of different medications in men and women should be evaluated. In the placebo arm in the ALERT study gender related differences in the use of cardiovascular medication were observed. Whether this is the case in the Norwegian transplant population is not known nor if any differences in the use of medication have any implications on the outcome in the renal transplant recipients.

Graft survival after living donor transplantation has always been considered to be better than after deceased donor transplantation. Whether this still is the case today has not been elucidated in patients who have been transplanted more recently..

Investigation of how the outcome of an acute rejection episode influences graft survival should be explored in the Norwegian transplant population. It has been stated that in transplant recipients where the serum creatinine after the treatment for the acute rejection episode return to baseline, graft survival is equal to recipients who have not experienced an acute rejection episode.

6. CONCLUSIONS AND IMPLICATIONS

Paper #1

In opposite-sex pairs the female-to-male donation rate is similar to what could be expected based on the gender composition in the incidence in ESRD and gender make-up of the general population.

The implication of this result is that in order to maintain this situation, a continued work on attitudes towards donations in both men and women should be continued and the system for reimbursement should be improved.

Paper #2

In LD transplantation donor age up to 65 years provide excellent long term results. Female donor sex may convey superior long term graft survival compared to male donor sex.

Our findings support the use of all available donors as long as the medical criteria are met. An old for old program in living donor transplantation should be advocated.

Paper #3

No gender difference in cardiac events or total mortality was observed in this relatively low-risk population of renal transplant recipients suggesting that the female gender advantage regarding CHD and survival is not restored following transplantation.

The implication of this result is that great emphasis should be placed on prophylaxis for cardiovascular disease in renal transplant recipients irrespective of gender.

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