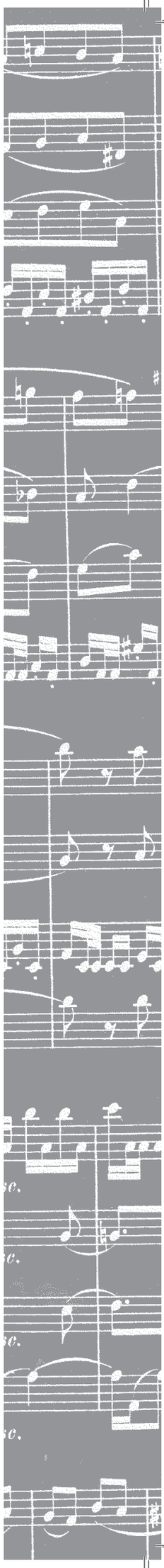
PEDIATRIC KIDNEY TRANSPLANTATION IN THE NETHERLANDS

collaborative studies

NIERTRANSPLANTATIES BIJ KINDEREN IN NEDERLAND

uitkomsten van gezamenlijk onderzoek



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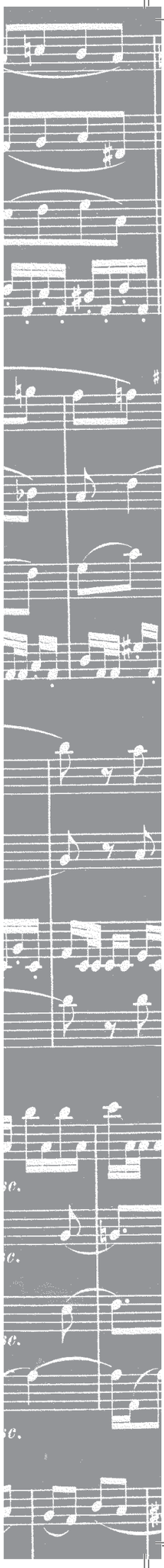
Contents

CHAPTER 1

BACKGROUND: AN OVERVIEW OF THE CURRENT STATE OF AFFAIRS



Introduction

The first successful kidney transplantation dates back to the year 1954. A healthy adult donated a kidney to his identical twin brother with life-threatening kidney disease. No immunosuppressive medication was used. The recipient lived for nine more years, got married, had children, and had a job to his liking.[1] This event raised the hopes of all patients with end stage kidney disease. It took another five years before the first kidney transplantation in a child succeeded, with a kidney donated by his identical twin.[2]

Transplantation is the ultimate modality of renal replacement therapy for end stage renal failure in children. It replaces most of the lost functions of the native kidneys, in contrast to any form of dialysis. Transplantation may restore kidney function within normal ranges, whereas with dialysis no more than 10% of normal clearance is reached. Moreover, dialysis is associated with high morbidity and even mortality. Chronic dialysis in children is therefore not considered as a permanent solution, but as a bridge to transplantation. In the Netherlands kidney transplantation in children is an accepted and feasible option since 1973.

In our country the procedure is thought to be feasible in children with a minimum age of 3 years, or a minimum body weight of 12 kg. Either a pediatric or a vascular surgeon performs the transplantation, in some centers together with a pediatric urologist. The grafted kidney is usually placed in the iliac fossa; in case of a small child receiving an adult kidney, the abdomen may be the graft site. The renal blood vessels are anastomosed with the recipient's external or commune iliac vessels, or, in case of intra-abdominal placement, with the aorta and inferior caval vein. The donor ureter is implanted in the recipient's bladder. In most centers a temporary intra-ureteral splint is placed to enable the urine to drain without resistance from the transplanted kidney.

Table 1. Differences between pediatric and adult kidney transplantation (adapted from Ettenger '92 [3])

1	Different spectrum of primary kidney disease
2	Different pharmacokinetics of immunosuppressive agents
3	Higher risk of vascular thrombosis in children
4	Longer anastomosis times with resulting delayed graft function in children
5	Immunologic responsiveness different in young children
6	Diagnosis of acute rejection more difficult in disproportionate graft size
7	Non-compliance with medication, especially in adolescents
8	More recurrence of original disease
9	Impaired growth before as well as after transplantation
10	Negative effect of renal failure on children's neurodevelopment
11	Children more often naïve to viral infections (CMV, EBV)

Pediatric kidney transplantation differs from adult transplantation in a number of aspects, as summarized in Table 1. Many of these factors will be discussed below.

Graft survival in children used to be lower than in adults, but recent outcomes tend to converge. A recent report of the OPTN/SRTR [a] showed a 5 year deceased donor graft survival rate of 73% both in recipients aged 6 – 10 years and recipients aged 35 – 49 years.[4,5]

Short-term risks of graft loss include acute rejection and thrombosis. Grafts surviving the first year may fail due to chronic allograft nephropathy, a gradual, multifactorial decline of kidney function.

Once it comes to transplantation, a child may already have a wide range of comorbid conditions, e.g. of skeleton, heart and blood vessels. Transplantation will bring improvement, but nevertheless is a source of comorbidity of its own, for example through the toxic side effects of immunosuppressive medication. The sad truth therefore is that patients do not stop being patients. They get saddled with inevitable rules of life, first and foremost taking medication: immunosuppressive, antihypertensive.
In spite of all this, renal transplantation is the optimal way to replace kidney function. Its ultimate goal is to achieve a higher quality and quantity of life of renal failure patients. Reaching this goal in children requires ongoing, scrupulous attention to medical and non-medical aspects, notably compliance with medication, psychosocial development, and education.

End stage renal failure in numbers

The incidence of end stage renal disease in children younger than 16 years in the Netherlands has remained rather stable between 1990 and 2006, fluctuating between 25 and 30 new patients per year (Stichting Renine, www.renine.nl). Until recently this figure equalled the annual number of children receiving a kidney transplantation, which implies that the number of children on dialysis remained fairly stable. Since 1972, approximately 800 children have received transplants in the four centers for pediatric kidney transplantation in the Netherlands. The proportion of children with end stage renal disease is very low relative to the incidence in the total population, which over this period surged from 1000 to 1800 patients per year. It is mainly the older age groups that explain this increase. The actual annual number of kidney transplantations in the Netherlands is below 1000, allowing the waiting list to grow. The number of patients enlisted for a deceased donor kidney at Eurotransplant increased from 2,000 in the year 1980 to 12,000 in 1998, and did not change significantly since then (www.eurotransplant.nl). Proportions of living donor transplantation in the entire Eurotransplant cohort increased from 9% in 1990 to 40% in 2007.

[a] United States Organ Procurement and Transplantation Network / Scientific Registry of Transplant Recipients

Primary kidney disease (Table 2)
The spectrum of diseases that lead to end stage renal failure in children differs from that in adults. Congenital structural abnormalities of the urinary tract – including obstructive uropathy (posterior urethral valves), refluxnephropathy, and renal dysplasia – form the larger part. Some of these urological disorders are associated with bladder dysfunction. Such dysfunction may seriously complicate the outcome of renal transplantation. Focal segmental glomerulosclerosis and hemolytic uremic syndrome are difficult to manage, not only before, but also after transplantation. These diseases show high recurrence rates and may lead to graft loss (see below).

In contrast to adults, polycystic kidney disease in children is mainly of the autosomal recessive type, associated with end stage renal failure within the first decade of life, often complicated by liver disease. Metabolic diseases like cystinosis and oxalosis are both systemic diseases characterized by enzyme defects.
Diabetic nephropathy, of growing influence in adult chronic kidney disease, is of minor importance in children.

Table 2. Etiology of end stage kidney disease in the NAPRTCS [b] registry (adapted from [6])

		%
Aplasia/hypoplasia/dysplasia	1432	15.9
Obstructive uropathy	1424	15.8
Focal segmental glomerulosclerosis	1049	11.7
Reflux nephropathy	466	5.2
Chronic glomerulonephritis	307	3.4
Polycystic disease	262	2.9
Nephronophthisis/medullary cystic disease	249	2.8
Hemolytic uremic syndrome	244	2.7
Membranoproliferative glomerulonephritis	241	2.7
Prune belly syndrome	239	2.7
Congenital nephrotic syndrome	230	2.6
IgA nephritis / Henoch-Schonlein nephritis	223	2.5
Familial nephritis	200	2.2
Cystinosis	185	2.1
Idiopathic crescentic glomerulonephritis	166	1.8
Pyelo/interstitial nephritis	164	1.8
SLE nephritis	141	1.6
Renal infarct	127	1.4
Denys-Drash syndrome	49	0.5
Wegener's granulomatosis	48	0.5
Wilms' tumor	47	0.5
Oxalosis	45	0.5
Membranous nephropathy	41	0.5
Other systemic immunologic diseases	32	0.4
Sickle-cell nephropathy	15	0.2
Diabetic glomerulonephritis	10	0.1
Other	806	9.0
Unknown	548	6.1
Total	8990	100.0

[b] North American Pediatric Renal Trials and Collaborative Studies

Patient survival

In 1998 the expected life span of pediatric dialysis patients in the USA was 40 to 60 years shorter than of an age- and race-matched healthy population. For transplanted patients it was 20 to 25 years shorter.[7] Data on transplantation in childhood are limited. In the beginning of this century, Groothoff et al. performed extensive studies on outcome of a Dutch cohort of 250 young adult patients who had started renal replacement therapy in childhood (LERIC, or Late Effects of Renal Insufficiency in Children). The mortality rate of these patients was alarming: 25% had died before the age of 30.[8] In this study and a similar one in Germany, 41 and 50% of deaths, respectively, were ascribed to cardiovascular or cerebrovascular events.[8,9]

Infections formed the second most frequent cause of death. Mortality was associated with hypertension and with prolonged dialysis prior to transplantation. Fortunately, the last decade has seen a considerable rise in life expectancy of children with end stage renal disease, thanks to better dialysis therapy, immunosuppressive therapy, and treatment of infectious complications.[8,10]

Graft survival

Primarily thanks to the expansive development of immunosuppressive agents, short-term graft survival has improved considerably over recent years. Longer term graft survival, however, appears to stay behind. Few studies extend 5 years' follow up, and some of these are based upon extrapolation of short-term data. In a study of 2004 in adults transplanted between 1988 and 1995 Meier-Kriesche et al demonstrated only marginal progress in long term deceased donor graft survival.[11] Though the 1 year graft survival rate nicely improved by 7% over this period, the 8 year graft survival rate had increased only from 66% to 70%. In children, with exclusion of the youngest recipients and donors, the 1 year graft survival rate improved significantly as well: from 88% in the 1994-1998 cohort registered for the UNOS[c] to 95% in the 1999-2002 cohort.[12] Limited to the first 5 years post-transplant the NAPRTCS data indicate a similar improvement: the 5 year graft survival rate of all deceased donor grafts registered since 1987 is 65%; that for grafts transplanted during the last decade is 77%.[6] Longer term graft survival has not been published by NAPRTCS. Nevertheless, in a large single center in the USA, 10 year graft survival in children who received deceased donor grafts, also had hardly improved over the first 30 years of pediatric kidney transplantation: from 51% in the 1970s to 55% in the 1980s and 1990s, confirming the findings of Meier-Kriesche.[10]

Graft survival in children is related to recipient age, and is worst in adolescents: at 5 years post-transplant it is 72% in the 1 to 5 year olds, 77% in the 6 to 10 year olds, but no more than 60% in the 11-17 year old recipients, according to recent data of OPTN/SRTR.[13] These differences appear to be based on long-term events, rather than events during the first year after transplantation. In addition to recipient age, other risk factors for graft loss are poor HLA matching, a prior transplant, multiple blood transfusions, prior dialysis, prolonged cold ischemia time, and African-American race.[6]

[c] United Network for Organ Sharing

Improved short-term graft survival has come at a price. Expansion and intensification of immunosuppressive therapy went hand in hand with higher incidence of infections – notably caused by cytomegalovirus, Epstein-Barr-virus and BK-polyomavirus –, long term toxicity of the immunosuppressive agents, and more malignancies, to name a few. Therefore current strategies on immunosuppression focus on minimization of these complications, by avoidance or withdrawal protocols.

Pre-transplant factors: donor source and duration of dialysis

A range of factors contribute to successful transplantation, including recipient factors (e.g. primary kidney disease, age, panel reactive antibodies, modality and duration of dialysis), donor factors (living *vs.* deceased, heart beating *vs.* non heart beating, age, cause of death) and factors relating to the transplantation procedure itself (ischemia times, HLA-matching, surgical complications). Here we comment on some of these.

Donor source

A kidney may be retrieved from a deceased or from a living donor. In the Netherlands and in the United Kingdom at present approximately 30 to 35% of pediatric transplantations concern a living related donation. [this thesis,15] In North America, this percentage is higher, 50-60%. [6,14]

Living kidney donation in pediatric transplantation usually involves one of the parents. In recent years, grandparents have been added to this source of kidney donors. In most countries siblings cannot decide for organ donation until they have reached adulthood. Short-term survival of living donor grafts is superior to that of grafts from deceased donors, persistent for the first 5 years. The difference seems to decrease afterwards: graft survival at 5 years it is 80-85% and 65-66%, and at 10 years 54 and 51% for living and deceased donors, respectively, in pediatric data from Great Britain and North America.[15,16]

Allocation of deceased donor organs within the region of Germany, Austria, the Benelux, Slovenia and Croatia is entrusted to Eurotransplant. Because longer dialysis period interferes with children's growth and neuropsychological development, and leads to less school attendance with permanent gaps in knowledge, most countries give children priority on the waiting list. Since the waiting lists grow, the average waiting time for children also increases. The Eurotransplant allocation system ETKAS, implemented in 1996, favors children on the waiting list for a deceased donor graft.[17] Since the introduction of this system, the waiting time for children averages 1.5 to 2 years in our experience, and is shorter than that for adults. Waiting times for young children enlisted exclusively for a pediatric kidney donor, are even longer.

Size discrepancy between donor and recipient

An issue of controversy in kidney transplantation is the question whether pediatric patients should be given pediatric donor kidneys preferentially.[18,19] Adult kidneys in young children have some drawbacks.

First, as children have lower blood pressure, the discrepant size of the kidney carries the risk of hypoperfusion of the graft, which is associated with potential nephron loss.

Second, the large organ heavily taxes the child's circulation, with acute cardiovascular risks. In addition, the functional overcapacity of an adult kidney might mask complications such as acute rejections, reflected by delayed rise of plasma creatinin values.
Several studies argue in favor of transplantation of pediatric donor kidneys to pediatric recipients. Of children receiving a deceased pediatric donor kidney the absolute glomerular filtration rate (GFR) gradually rose throughout the first four years after transplantation, reflecting the progressive use of reserve capacity.[20,21] Increase in GFR in pediatric donor kidneys was paralleled by sustained growth of the grafts.[22] In contrast, the absolute GFR of pediatric recipients of adult grafts, living as well as deceased, did not increase, and therefore, the relative GFR declined.
Nevertheless, graft survival of kidneys from donors younger than 10 years transplanted into young recipients is lower than that of all other combinations of age categories, as established by NAPRTCS and UNOS registries.[19,23] The data of the Eurotransplant registry, collected for the study described in chapter 8, show a similar trend (data not published).

Therefore, NAPRTCS advocates to transplant adult living donor kidney grafts, even into the youngest children and claims excellent results. In North America recipients aged 1 to 5 years have become the age group with the best graft survival after 5 years, according to the OPTN/SCTR registry.[5]

Two of the four centers for pediatric kidney transplantation in the Netherlands provide recipients below the age of 6 years only with pediatric donor kidneys. This policy is based on the lack of experience with the complicated surgery and anesthesia of adult kidney grafts in infants.

Therefore, these children are dependent on deceased pediatric donors. In the Eurotransplant region, the number of pediatric donors is decreasing annually. Until recently, children did not get priority for pediatric donor kidneys. France and, since a few years the USA as well, offer organs of donors younger than 30 and 35 years respectively, preferentially to pediatric candidates.[24,25] Only patients with 0 HLA mismatches and those who are highly sensitized have higher priority. The waiting time for children in these countries is less than 6 months. Eurotransplant also recognized the benefit of allocating pediatric kidneys to pediatric recipients and recently launched a program called 'Young for young', advocating organs of donors younger than 10 years to be given to recipients younger than 6 years, given full HLA-DR matching. Nine months later no transplantations have been realized in this program yet.

Strategies to expand the donor pool

A major issue in transplantation practice is the falling number of deceased donors over the years in combination with increasing numbers of patients in need for a transplantation. Road traffic in the Eurotransplant region has become safer, which has changed donor profiles. While 20 years ago the typical donor would have died from traumatic cerebral injury, nowadays the cause of death more often is intracranial hemorrhage. Due to this shift in cause of death, in the Eurotransplant region mean donor age has risen from 30 years to 50 years. Graft survival is negatively correlated with adult donor age; therefore, the shift in age has repercussions for graft survival.[26]

In the Netherlands a national registry of potential organ donors was instituted in 2001. Registration is on a voluntary, 'informed consent', basis. Surprisingly, since then fewer effective organ donations have been effectuated, and waiting time for candidates on the waiting list increased. Especially the large pool of unregistered people yielded less donors. Countries like Belgium, Austria, and Spain, which have the 'presumed consent' system, which implies that someone who dies is a donor unless he registered as non-donor, yield more donors resulting in shorter waiting times than in the Netherlands.

The scarcity of organs calls for widening of the donor criteria. One approach that raised the number of potential donors is a gradual rise in donor upper age limit. Another option would be more active stimulation of living donation, for pediatric as well as adult recipients.

In addition to heart beating donors, organs from non heart beating donors are increasingly being used in the Netherlands, despite the higher risk of delayed graft function and even primary non-function.[27] Numbers of non heart beating donation have largely increased in recent years, however at the expense of heart beating, brain dead donation.

Given the donor shortage, even ABO incompatible donor-recipient combinations are considered for transplantation nowadays, provided specific precautions be taken. The use of immunoadsorption has yielded reasonable results.[28] Small-scale experience within the pediatric age group has recently been reported from the United States.[6]

Many centers discard organs from donors under the age of 5 years. However, the use of these kidneys *en bloc* may expand the donor pool, especially for older children or adults. The outcome is better than that of single kidneys of these young donors in adult recipients, according to two reports from large United States registries, and is similar to that of 'ideal' donors.[29,30] Still, this procedure carries higher risk of surgical complications and early graft loss due to thrombosis. Once the graft survives the first three months, long term graft survival may be similar to that of live adult transplantation.[29,31] The glomerular filtration rate may increase over the first post transplant year.[31] We reported good results with *en bloc* transplantations in pediatric recipients as well.[32]

A new program for the exchange of living donor kidneys was introduced in 2004 in our country, the 'cross-over' program. In short, of two incompatible donor-recipient

pairs the donor kidney from one pair is transplanted to the recipient of the other pair, and vice versa, given a negative cross match.[33] This new system already effectuated 100 extra living organ donations so far, with good results.

Pre-emptive transplantation

One of the determinants of transplantation success is duration of the dialysis episode prior to the transplantation. Shorter dialysis leads to better transplantation results. In adults, this was shown by comparing kidney pairs transplanted into recipient pairs of whom one had dialyzed maximally 6 months and the other at least 2 years.[34] This would seem to imply that pre-emptive transplantation, without prior dialysis, gives even better results. Indeed a NAPRTCS survey reported that LD graft survival in pre-emptively transplanted or short-time dialyzed children was by far superior to that in the longer dialyzed recipients.[35] This effect could not be demonstrated for deceased donor transplantations. Adolescents, who show the worst graft survival, may benefit most from pre-emptive transplantation, as shown from Australian data.[36] The reason for better graft survival is not evident. One could speculate that pre-emptively transplanted patients will be fitter at the time of transplantation than those who have undergone prolonged dialysis. Also, the healthier cardiovascular system of such recipients has been suggested to adapt more properly to the circulatory demands of the new organ. Pre-emptively transplanted patients more often have immediate graft function than dialyzed patients.[35]

In Europe, rates of pre-emptively performed pediatric kidney transplantations vary with country and donor source: Scandinavian countries, with high numbers of living donors, report high figures, in contrast to Greece, Belgium, and the Netherlands.[37]

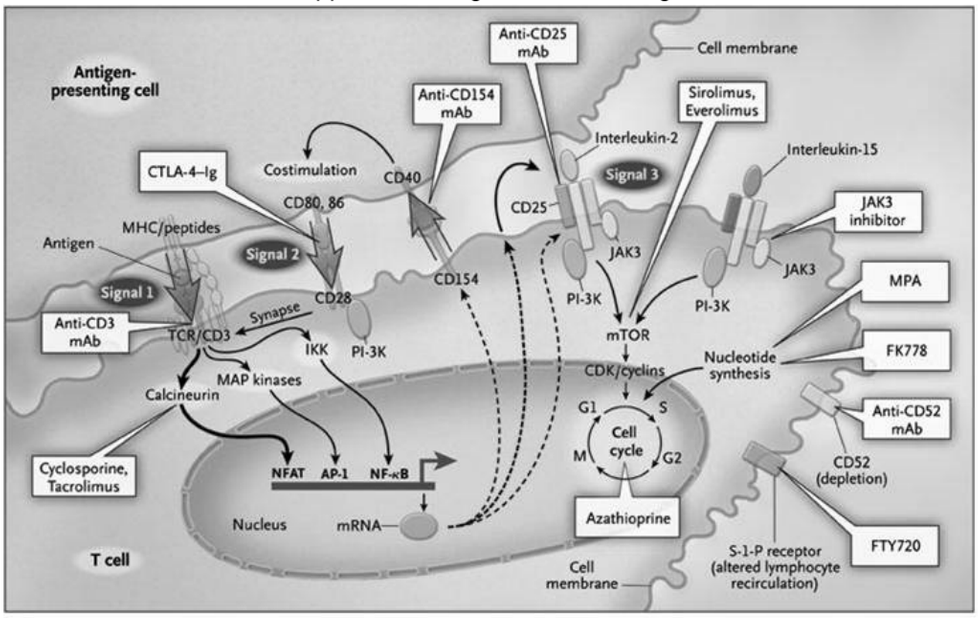
Immunologic processes in kidney transplantation

A grafted organ elicits an immune response to the foreign antigens in the recipient, which may result in rejection of the organ. Therefore, the central issue in organ transplantation remains suppression of allograft rejection, or, better still, induction of tolerance.[38] Human Leukocyte Antigens (HLA) play a major role in the immune responses that cause rejection of transplanted organs. They are controlled by highly polymorphic genes, encoding groups of antigens in two categories, class-I (HLA-A, -B, and -C) and class-II (HLA-DR and -DQ). The spectrum of expressed HLA antigens is specific for each individual. These antigens are expressed on the membranes of allogen presenting cells and are instrumental in the generation of humoral and cellular immune responses.[39] Avoiding HLA mismatches between donor and recipient of an organ transplantation appears to be relevant to its success.

In allograft rejection naïve T cells and memory T cells are stimulated by antigen presenting cells (Figure 1). Naïve T cells are stimulated optimally by dendritic cells in secondary lymphoid tissue; antigen experienced cells also by other cell types, like endothelial cells of the graft. Three signals from outside the T-cells are necessary to initiate and continue the alloimune response.

Only one of these is antigen specific, i.e. signal 1: the antigen-presenting cell presents an antigen in connection with HLA-antigen, to engage with a T cell via the T cell receptor. This binding has no effect until it is parallelled by a costimulatory signal from the antigen presenting cell to the T cell (signal 2). Signals 1 and 2 together activate three pathways in the T cell, i.e. the calcineurin pathway, the mitogen-activated protein kinase pathway, and the protein kinase C- nuclear factor κB pathway. These pathways trigger the transcription factors nuclear factor of activated T-cells (NFAT), activating protein 1, and NF- κB.
This will lead to the expression of numerous molecules, including several interleukins, that result in a cascade of reactions. Interleukin-2 and -15, extracellularly, bind to the membrane-bound IL-2 receptor (CD25) of activated T-cells, forming signal 3, leading to activation of mTOR ('mammalian target of rapamycin') and phosphoinositide-3-kinase, with subsequent initiation of the cell cycle. This results in T cell proliferation and differentiation. In lymfoid tissue B cells are activated as well by their antigen receptors, to produce alloantibodies against donor HLA antigens. [38]

Figure 1. Sites of action of immunosuppressive drugs in the three-signal model



From: P. Halloran, NEJM 2004 [38]

Lymphocytes require synthesis of purine- and pyrimidine nucleotides for replication, regulated by inosine monophosphate dehydrogenase (IMPDH) and dihydro-orotate dehydrogenase (DHODH), respectively. Effector T-cells infiltrate the graft and create typical rejection lesions such as tubulitis, and, in more advanced rejection, endothelial arteritis.

Immunosuppressive therapy
Immunosuppressive therapy aims to prevent or treat acute rejection episodes of the allograft. Its mechanisms of action are depletion of the responsible cells, diverting lymphocyte traffic, or interfering with lymphocyte response pathways.[38]

During the past decades the effectiveness of immunosuppressive medication has increased dramatically, both in numbers of available drugs and in their effectiveness.[40] The first immunosuppressive agents were azathioprine and corticosteroids. At that time 5 year kidney graft survival was 30-50%. The introduction of cyclosporine in the 1980s was rewarded with a fall in the incidence of acute rejections and large improvement of graft survival [41]. Since then, many immunosuppressive agents have been developed, the most widely spread of which are mycophenolic acid and tacrolimus.
Polyclonal and monoclonal immunoglobulins found their way into induction therapy and anti-rejection therapy. Immunosuppressive prophylaxis usually consists of several immunosuppressive agents simultaneously. Currently, frequently used combinations are prednisolone, a calcineurin inhibitor and mycophenolate mofetil, sometimes in combination with an induction agent. The ultimate goal of research in immunosuppression is induction of complete or 'prope' tolerance to the organ, necessitating treatment with only a minimal amount of immunosuppression.[42]

Immunosuppressive drugs in general have three different pharmacological effects: 1. prevention and suppression of allograft rejection, 2. undesired immunosuppression leading to (opportunistic) infections and malignancies, and 3. nonimmune toxicity to other tissues.

Table 3. Classification of immunosuppressive agents (from P.Halloran, 2004 [38])

- Small molecular drugs
  - Corticosteroids
  - Immunophilin-binding drugs
    - Calcineurin inhibitors
      - Cyclophilin-binding drugs: cyclosporine
      - FKBP12-binding drugs: tacrolimus, modified-release tacrolimus
    - Target-of-rapamycin inhibitors: sirolimus, everolimus
  - Inhibitors of nucleotide synthesis
    - Purine synthesis (IMPDH) inhibitors
      - Mycophenolate mofetil
      - Enteric-coated mycophenolic acid
      - Mizoribine (only used in Japan)
    - Pyrimidine synthesis (DHODH) inhibitors
      - Leflunomide
      - FK778
  - Antimetabolites: azathioprine
  - Sphingosine-1-phosphate-receptor antagonists: FTY720
- Protein drugs
  - Cell depleting antibodies (T-cells and/or B-cells)
    - Polyclonal antibody: horse or rabbit antithymoglobulin
    - Mouse monoclonal anti CD3 antibody (muromonab-CD3)
    - Humanized monoclonal anti-CD52 antibody: alemtuzumab
    - B-cell-depleting monoclonal anti-CD20 antibody: rituximab
  - Nondepleting antibodies and fusion proteins
    - Humanized or chimeric monoclonal anti-CD25 antibody (daclizumab, basiliximab)
    - Fusion protein with natural binding properties: CTLA-4-Ig
  - Intravenous immune globulin

Description of immunosuppressive agents currently in use in pediatric kidney transplantation (Table 3)

Corticosteroids

The effects of glucocorticoids on immunoresponsive cells are complex and not completely understood. After entering the immunoresponsive cell and binding to intracellular glucocorticoid receptors, steroids are phosphorylated and transported to the nucleus, where they activate or repress various genes. This results in either a release or a decrease in a vast number of interleukins and growth factors, ultimately resulting in dramatic immunosuppression.[43]

The toxic side effects of glucocorticoids are as diverse as its immunosuppressive actions: hypertension, overweight, cushingoid appearance, acne vulgaris, osteopenia, psychosis, behavioral disturbances and sleeping disorders, cataracts, and gastric ulceration. Growth failure is one of the most impressive side effects in children. A possible remedy is decreasing the dosage or bringing down the frequency of administration from daily to alternate daily.

Calcineurin inhibitors

The benefits of calcineurin inhibitors of reducing acute rejection and better short-term graft survival are counteracted by a wide range of toxic effects.

Cyclosporine acts by binding to cyclophilin; the complex inhibits calcineurin.[44] By blocking calcineurin, cyclosporine ultimately inhibits the production of interleukin 2. The drug's effects are proportional to degree of exposure to the drug, which makes monitoring of blood levels essential.

Adverse effects of cyclosporine depend on degree of exposure as well, and include nephrotoxicity, neurotoxic effects like tremor and convulsions, hypertension, hyperlipidemia, hypomagnesemia, cosmetic side effects including hirsutism and gingiva hyperplasia. Furthermore, hemolytic uremic syndrome and post-transplant diabetes mellitus may occur.

Cyclosporine is metabolized by CYP3A4 and CYP3A5, and therefore interacts with other drugs that need these enzymes for metabolism.[45]

Pharmacokinetics: Serum trough levels are the easiest way to assess the drug's pharmacokinetics and adjust the dosage accordingly. These levels do not correlate well, however, with total exposure and bioavailability over time. The level at 2 hours after ingestion, or levels at several time points, correlate better with the area under the concentration-time curve.[46, 47]

Pediatric aspects: clearance of cyclosporine in young children is faster than in adults. Thrice daily administration therefore may be better than twice daily in this group of patients. This has been confirmed by studying the AUC.[48, 49] Neoral® (cyclosporine) capsules are larger than the small pills of Prograft® and are smelly. These aspects, together with the cosmetic side effects of cyclosporine may lead to non-compliance, especially for adolescent girls.

Tacrolimus inhibits calcineurin after binding to another immunophilin: FKBP12. In adults as well as children the use of tacrolimus has gradually taken over that of cyclosporine.

A meta-analysis of 30 trials comparing tacrolimus and cyclosporine showed a higher efficacy of tacrolimus in preventing acute rejection, and improved graft survival of tacrolimus treated patients.[50] In children co-treated with azathioprine and corticosteroids, tacrolimus yielded fewer acute rejections, and superior graft survival and glomerular filtration rate at four years post-transplant as compared with cyclosporine.[51, 52]
Regrettably, tacrolimus has serious toxic effects: hypomagnesemia, neurological and gastrointestinal side-effects are more pronounced than with cyclosporine.[50] Nephrotoxicity is similar. *De novo* diabetes mellitus occurs more often as well, especially if tacrolimus is combined with higher doses of glucocorticoids.[53]
In recent years, however, with further refinement of tacrolimus-based regimens the incidence of diabetes has declined.[54] Tacrolimus does not have the cosmetic side-effects of cyclosporine, and also hyperlipidemia and hypertension are usually milder.

mTOR inhibitors
Both sirolimus and everolimus bind to the same immunophilin as tacrolimus, FKBP12. This complex blocks the effect of the 3rd signal. By inhibiting the target of rapamycin (mTOR) it prevents the IL-2 IL-2-receptor complex to activate the cell cycle. Favorable properties of this drug include the lack of nephrotoxicity,[55] and the potentially beneficial role in the prevention of malignancies.[56] mTOR inhibitors have been evaluated in different primary immunosuppressive algorithms, either as replacement for calcineurin inhibitors or anti-metabolites, or in combination with calcineurin inhibitors at low or high doses.
In children sirolimus is especially used to reduce side effects of calcineurin inhibitors. mTOR inhibitors generally favor surrogate endpoints for graft survival, e.g. glomerular filtration rate, but have a deleterious effect on some surrogate endpoints for patient outcome, like bone marrow depression and lipid disturbance.[57] The mTOR inhibitors have a wide range of toxic effects: hyperlipidemia, thrombocytopenia, mouth ulcers, skin lesions, aggravation of proteinuria. Their use shortly after transplantation is relatively contraindicated due to delay of wound healing and increased occurrence of lymphoceles.[58] In addition, sirolimus used in adult males has been reported to result in decreased testosterone levels, disruption of spermatogenesis and deleterious effects on the testis.[59] This effect of sirolimus has not been investigated in children yet, but may be even more important in this age group.

Mycophenolate mofetil (MMF)
Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid. It acts as an inhibitor of inosin monophosphate dehydrogenase, a key enzyme in purine synthesis in lymphocytes.[60] It was developed in the nineties. In combination with corticosteroids and cyclosporine, it led to lower incidences of acute rejections as compared with placebo or azathioprine,[61,62] which was confirmed by a meta-analysis of trials.[63] It has achieved widespread use, due to both its immunosuppressive action and its mild toxicity profile. Side effects affect the gastrointestinal tract (mainly diarrhea) and bone marrow (anemia, leukopenia). In addition, MMF may increase the incidence of viral infections.

The dosage of MMF in children is derived from that in adults and based on body surface area, 600 mg/m^2 twice daily.[64] MMF dosage is dependent on co-medication: in combination with tacrolimus the dose should be lower than in combination with cyclosporine.[65-67] With tapering of steroids, the availability of mycophenolic acid increases and the dosage should be adapted.[68] Young children need higher relative dosage of MMF than older children.[69] All these interactions stress the value of therapeutic drug monitoring.[70]
As an alternative for MMF, enteric coated capsules of mycophenolic acid are available. The gastrointestinal side effects of these tablets might be lower. Children seldom have trouble swallowing the large tablets of Cellcept®.

Azathioprine
After corticosteroids, azathioprine was the first immunosuppressive drug used during the first 20 years of transplantation. Azathioprine acts by converting 6-mercaptopurine, which leads to interference with DNA synthesis. Its immunosuppressive effect in transplant patients is inferior when compared to that of calcineurin inhibitors or MMF. Toxic effects are of a gastrointestinal (nausea) or bone marrow suppressive (anemia, leukopenia) nature.

IL2-receptor antagonists
Both daclizumab and basiliximab block the IL-2 receptors expressed on activated T-cells. Its addition to a combination of cyclosporine, azathioprine and prednisolone, or of tacrolimus and steroids, respectively, reduces the incidence of acute rejections in both adults and children.[71,72] Basiliximab has no proven beneficial effect on patient or graft survival. Its administration carries low risk, and is associated with fewer adverse events than T-cell depleting agents, like anti-thymocyte globulin (ATG). In recipients retransplanted after graft loss, however, life-threatening reactions were reported following re-exposure to basiliximab.(see [73])

Alemtuzumab
A recently introduced immunosuppressive agent, although not yet in Dutch pediatric kidney transplantation, is alemtuzumab. Alemtuzumab is a humanized monoclonal antibody against CD52, present on all B- and T-cells, monocytes, macrophages and natural killer cells. Applied in adults as induction therapy, it causes cell lysis and prolonged depletion of all affected cells. Though alemtuzumab was thought to play a role in the development of tolerance, recent studies contradict this.[74]

Minimization of immunosuppressive therapy
Confronted with the toxicity of the powerful immunosuppressive therapies of recent years, clinicians now attempt to minimize or even completely avoid administration of these drugs, immediately or later after transplantation, yet without compromising their immunosuppressive effects. The most important toxic effects to be avoided are cardiovascular toxicity (steroids and calcineurin inhibitors), nephrotoxicity (calcineurin inhibitors), growth inhibition, overweight, osteopenia and avascular bone necrosis (steroids), and cosmetic side effects that may lead to diminished compliance (steroids and calcineurin inhibitors).

Minimizing protocols therefore focus on eventual withdrawal of corticosteroids and calcineurin inhibitors in particular. Ideally, safety of this strategy should be guaranteed by careful monitoring of immunologic reactivity during tapering off. Recent studies have shown that this is well feasible.[75]
Broyer reported the favorable effect on children's growth of reducing the cumulative steroid dosage, i.e. by switching from a daily to an alternate daily schedule.[76] Such switch in adults, or to half the daily dose of prednisolone, resulted in a clinically significant reduction of overweight, blood pressure, antihypertensive medication, and HbA1c. No further benefit was registered after complete withdrawal.[77] The drawback of an alternate daily schedule is the risk of accidental non-compliance. The only randomized study of complete steroid withdrawal in children was prematurely stopped due to too many adverse events of the used immunosuppressive regimen (basiliximab, calcineurin-inhibitor, sirolimus and steroids), not because of steroid withdrawal itself. (see [78]) A meta-analysis of steroid withdrawal studies in adults showed a higher risk of graft loss; however, no such difference was detected in the subset of trials containing MMF.[79,80] In children even complete avoidance has been possible in the presence of extended daclizumab induction, tacrolimus and MMF.[81] A prospective, randomized multicenter steroid-based versus steroid-free trial in pediatric kidney transplantation is currently underway in the United States.[78]
Minimizing exposure to calcineurin inhibitors aims to reduce the toxic effects. A meta-analysis of numerous relatively small, randomized, controlled trials – designed to evaluate the replacement of cyclosporine by azathioprine or the withdrawal of cyclosporine from an azathioprine containing regimen – concluded that elective CsA withdrawal increases the risk of acute rejection by about 11%, but does not reduce graft survival.[79] Replacement of a calcineurin inhibitor by sirolimus immediately after transplantation has to be weighed against the considerable toxicity profile of sirolimus at this stage. In the long term, however, a combination of sirolimus and mycophenolate mofetil may exert a beneficial effect on GFR, graft survival, blood pressure and malignancies.[82,83] It would be advisable, however, to first evaluate the effects of sirolimus on male fertility, especially important in youth.

Factors threatening the survival of the grafted kidney

Renovascular thrombosis

Unfortunately, transplanted kidneys may be lost within days due to thrombosis of the anastomosed blood vessels. Some predisposing factors are specifically present in pediatric recipients. For example, transplant surgery can be complicated by small diameters of blood vessels of recipient or donor and by calibre differences between donor and recipient vessels. Moreover, when a large adult kidney is transplanted into a young child, blood pressure and blood flow will be considerably lower than what that kidney was used to. These factors in combination with the usual post-surgery hypercoagulable state predisposes particularly small children to thrombosis of the graft. In adults the reported incidence is 2%, in children most reported percentages are higher, i.e. 2 - 10%.[6,23,84,85]

Early graft loss due to thrombosis is associated with previous treatment with peritoneal dialysis, with prolonged cold ischemia time, and donor age below 6 years.[23,84,86,87] It occurs more often in deceased donor grafts than in living donor grafts, and more in retransplants than in first transplants.[84,88] Recently, the use of IL-2 receptor antagonists has been reported to decrease the risk of renal allograft thrombosis.[89]

Apart from the usual post-surgical hypercoagulable state, thrombosis may originate from inherited or acquired thrombophilia. Patients with ESRD have greater risk of thrombophilia due to higher levels of homocystein and clotting factors VIII and IX as compared with the normal population.

Also, within the framework of their primary kidney disease they may have acquired anti-phospholipid antibodies or lupus anti-coagulans, a strong risk factor for arterial and venous thrombosis. Furthermore, relatively high incidences of inherited causes of thrombophilia have been reported in patients with ESRD: 13% in adults and 27% in a relatively small population of pediatric patients.[90,91] The causes in question were deficiency of protein C, S and antithrombin III, factor V Leiden mutation, and prothombin gene G20210 polymorphism.[92] Patients with thrombophilia may lose their grafts in the first weeks due to thrombosis of the anastomosed blood vessels, but also long term graft survival can be hampered by sequential infarction of smaller artery branches.[92] Cyclosporine and corticosteroids both have procoagulable properties, but clinical trials evaluating their effects on thrombosis have yielded conflicting results.[93-95]

Different forms of prophylactic therapy to prevent thrombosis have been reported as successful: low dose aspirin in adults,[96] low dose heparin or low molecular weight heparin in children,[97,98] and in patients with a persisting risk of thrombosis heparin followed by aspirin during at least 12 months.[91] Nevertheless, others reported lack of any impact of low dose heparin on the incidence of graft loss by thrombosis.[85] Any benefit of treatment with (low molecular weight) heparin shortly after surgery must be balanced against the risk of bleeding, which may necessitate blood transfusions or surgical re-exploration.

Delayed graft function

Delayed development of graft function (DGF) has a detrimental effect on long term graft function and graft survival in both children and adults.[99-102] It is largely caused by ischemic injury to the allograft in the period without blood circulation. Of these, the first warm ischemic period is the most harmful followed by the cold ischemia time, when the organ is perfused with protective cold preservation solution and stored on ice. In contrast to heart beating donation, non heart beating donation procedures are associated with a considerable first warm ischemia time, between cardiac arrest of the donor and perfusion of the organs. Moreover, the circulation of the donor will not be optimal prior to cardiac arrest. Non heart beating donor grafts, therefore, more often show DGF than do heart beating donor grafts.

On the other hand, organs from heart beating donors may suffer from ischemic injury as well, as a result of circulatory and hormonal disturbances and inflammatory processes in the donor.[12] Organ quality could be improved by extended intensive care treatment of the donor, i.e. insulin therapy for strict glycemic control, adequate inotropic support, and possibly anti-inflammatory and cytoprotective treatments. Finally, at transplantation, reperfusion of the ischemically damaged organ activates a sequence of events that sustain renal injury, e.g. by the induction of reactive oxygen species.[103] Preservation fluids aim to limit ischemia and reperfusion damage to the graft.[104] In Dutch pediatric kidney transplantation practice mannitol is given immediately before removing the vessel clamps, based on its combined properties as a diuretic and an oxygen radical scavenger.

Both in adults and children, DGF is associated with prolonged cold ischemia time.[101,102] Other predictors in adults are higher donor age and higher donor creatinine levels; in North American children HLA-DR incompatibility and African-American race.[101,102] Evidently, grafts from living donors carry lower risk of DGF than do grafts from deceased donors, since the living donor is in good health at the time of donation, and the cold ischemia time is much shorter. In a NAPRTCS report the incidence of DGF in pediatric recipients was 6% for living donor grafts and 19% for deceased donor grafts.[101]

Cyclosporine, with its vasoconstrictive action, may aggravate DGF. Attempts have been made, therefore, to postpone start of cyclosporine and replace it by a form of immunoglobulin induction. The findings of several investigators were not unanimous.[105] A sequential therapy with ATG as induction therapy successfully prevented DGF in children.[106,107]

Acute rejection

The immune reaction against the foreign cells of an allograft can result in an acute rejection. Clinically, this presents as elevation of serum creatinine level, sometimes in combination with hypertension, fever, and haematuria and proteinuria. The diagnostic golden standard is kidney biopsy. The degree of acute rejection has been standardized in consensus meetings in Banff, defined as Banff criteria.[108]

It has been suggested that children experience more allograft rejections than adult patients. Incidences of rejections may have been overestimated, however, by inclusion of rejections not proven by renal biopsy. Another explanation for the observed higher frequency of rejections may be the often relatively late introduction of new immunosuppressive drugs in pediatric programs. On the other hand, the diagnosis of acute rejection may be delayed in small children with relatively large grafts due to considerable functional reserve capacity of the donor kidneys. Only few pediatric transplant centers perform protocol biopsies, either for suspected rejections or as surveillance biopsies at fixed time points during the first year after transplantation. Consequently, to date there is still insufficient evidence to ascertain whether young age really is a risk factor for allograft rejection.

The incidence of acute rejection episodes gradually declined since the expansion of the arsenal of immunosuppressive drugs: cyclosporine in the 1980s, mycophenolate mofetil, tacrolimus and interleukin-2 receptor antagonists in the 1990s; viz. from 69% in 1988 to 16% in 2004 as reported by the NAPRTCS.[6] Noteworthily, the more recent data are flattered by the high (50%) percentage of living donor transplantations, which are associated with a lower incidence of acute rejections.

Acute rejection remains one of the main determinants of long term graft survival. Survival tends to be better in patients free from acute rejection episodes. Chronic allograft nephropathy, the most frequent cause of kidney allograft loss, remains to be associated with previous acute rejection episodes in children, particularly if these occur late in follow-up, or are repetitive.[109]

Chronic allograft nephropathy

Chronic allograft nephropathy is defined as a state of impaired renal allograft function at least three months post-transplant, independent of acute rejection, acute drug toxicity, and recurrent or de novo specific disease entities, and with typical features on biopsy, i.e. fibrotic changes of vascular endothelium, renal tubules, interstitium and glomeruli.[110] It is the major cause of failure of kidney transplants after the first year. Sadly, its incidence has hardly declined, despite decreased incidence of acute rejection as a result of improved immunosuppression.

This kind of transplant nephropathy is predicted by many factors. These include donor factors (aging, hypertension, brain death with hypocirculation), immunologic factors (HLA-matching, panel reactive antibodies, host responsiveness, effectiveness of and adherence to immunosuppressive therapy) and exogenous stressors on the graft (hypertension, donor-recipient size-disparity, hyperlipidemia, drug toxicity, and infectious agents).[110] The pathogenesis is only partly understood. It is thought that endothelial activation in response to one or more of the aforementioned factors stimulates leukocyte activation and recruitment. These leukocytes activate effector cells, resulting in the secretion of an excessive amount of abnormal extracellular matrix, leading to fibrosis.[111] Chronic allograft nephropathy resembles an accelerated normal aging process of the kidney.[110]

Non-compliance

Transplantations in adolescents have the lowest long term graft survival of all pediatric age groups. Specific properties of this age group include hormonal and behavioral changes of puberty. To the best of our knowledge, the effects of hormonal changes on graft survival have never been studied. The natural zest for independency of the adolescent would naturally seem to conflict with the prospect of lifelong dependency on and pampering by the medical system. Transplantation brings along a therapeutic regimen of continuous drug therapy, including drugs for immunosuppression, prophylactic antibiotics, and often for the treatment of hypertension and other comorbid diseases as well. And then, the youngsters are instructed to avoid risk factors for cardiovascular disease and cancer (sun protection, not smoking).

Non-compliance has been defined as failure or refusal to conform and adapt one's actions to a rule, or to another person's wishes.[112] For young children it will be less of a problem, because living to the rules is primarily dependent on the parents. Parents generally are concerned for their children and are anxious to comply with doctors' orders. Adolescents, however, are different. For several reasons they find it hard to adhere to the prescriptions. They like to see immediate results of what they are doing, and are not well able to conceptualize future consequences of present actions. Furthermore, they abhor the visible side effects of especially cyclosporine (hirsutism, gingiva hyperplasia, hand tremor) and corticosteroids (moon face, acne vulgaris, binge eating, overweight). In addition, they hate the smell of certain pills, or find them difficult to swallow. Finally, and not least importantly, they cannot face being different from their peers. All these are good reasons for some of the adolescents to revolt to the medication, overtly or silently.[113]

Measuring the extent of non-compliance is virtually impossible. Often non-compliance will out when trough concentrations of cyclosporine appear to be highly variable, unexpectedly low, or consistently lower when measured in the outpatient clinic than during admissions. Sometimes the clue might be provided by the pharmacy registry or more high-tech by electronic monitoring devices in the lids of the medication jars.[114]

Some adolescents will be open about non-compliance and others will not, often dependent on the person who puts the question to them. A nurse or a social worker may be more capable than a physician of convincing them that the rules will work out well in the long run. Better compliance might be achieved e.g. through discussion groups with fellow patients coached by a social worker or psychologist.[115]

A notorious moment to quit complying is the transition from pediatric to adult care.[116,117] It is important therefore to prepare the patients for this event, and the medical and social teams at both the institutions of pediatric and adult care should be able to provide a high level of special support focused on this issue.

Complications related to the primary kidney disease

A few diseases leading to end stage kidney disease may harm the allograft as well.

Dysfunction of the lower urinary tract

Several urologic abnormalities are associated with dysfunction of the lower urinary tract. These include posterior urethral valves, prune belly syndrome, meningomyelocele, sinus urogenitalis and cloacal malformation. In those cases the graft may be connected to a malfunctioning bladder, which raises the risk of urinary drainage problems and urinary tract infections. Nonetheless, long term graft survival in these cases is similar to that in patients with normal bladder function [118-120].

Pre-transplantation urodynamic studies should ensure adequate management of the lower urinary tract. Augmentation cystoplasty before as well as after transplantation is reported to be without major complications.[121]

Focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is one of the frequently recurring diseases, especially if the original disease set on before the age of 6 years, and progressed rapidly to end stage disease. FSGS accounts for 11% of pediatric patients on renal replacement therapy. The recurrence rate is up to 50%.[122] Recurrence usually presents as proteinuria, and usually within 2 weeks from transplantation. Nephrectomy of the native kidneys has been recommended to detect proteinuria originating from the graft. Nephrotic range proteinuria within 2 weeks after transplantation is associated with a 50% risk of graft loss. Therapies such as plasma-exchange and high dosed CsA have been successful in some cases to save the graft.[123-125] Pre-emptive plasmapheresis for 2 to 8 sessions, starting immediately following transplantation, has been reported to reduce recurrence in children and high-risk adult patients.[123,126] The use of a living donor kidney allows the start of plasmapheresis already during the preceding week. Prospective studies are required to delineate the optimal approach to prevent and/or treat the recurrence of FSGS.

Hemolytic uremic syndrome

Atypical, diarrhea negative hemolytic uremic syndrome (HUS) has been reported to recur in up to 50% of pediatric cases after transplantation, especially if related to factor H deficiency.[122,127] Some cases can be treated successfully with plasmapheresis, but HUS will recur again in many cases after plasmapheresis tapering off.[128,129]

IgA nephropathy

Recurrence of IgA nephropathy, in terms of mesangial IgA deposits and hypercellularity, can be demonstrated in up to 50 - 60% of patients, though not always clinically significant. Graft loss due to recurrent disease has been reported in 1.3 - 16% of patients with IgA nephropathy.[130]

Membranoproliferative glomerulonephritis

Membranoproliferative glomerulonephritis types 1 and 2 both recur frequently in kidney allografts, but only rarely are associated with graft loss.

Primary hyperoxaluria type 1

Primary hyperoxaluria type 1, resulting from the absent or dysfunctional hepatic enzyme alanine glyoxylate aminotransferase, is associated with renal failure on account of the deposition of calcium oxalate crystals. After transplantation the circulating oxalate crystals may continue to deposit in the kidney allograft, leading to graft failure. Intensive haemofiltration and/or haemodialysis, before and after transplantation, has improved graft survival.[131] In some patients this metabolic disorder responds well to treatment with high dose vitamin B6.

Patients who do not respond to vitamin B6 can only be treated with liver transplantation to replace the missing enzyme.

Finnish type of congenital nephrotic syndrome and Alport syndrome
In the Finnish type of congenital nephrotic syndrome and Alport syndrome another phenomenon may occur after transplantation. Both diseases are associated with the hereditary absence of a structural component in the glomerulus: lack of nephrin between the epithelial cells in the former disease, and lack of a component of the collagen of the glomerular basement membrane in the latter. The transplanted kidney will possess these components, and in some cases the recipient develops antibodies against the foreign components. This may result in a recurrent nephrotic syndrome in the congenital nephrotic syndrome, and in a form of anti-glomerular basement syndrome in Alport syndrome.[132]

Extrarenal comorbidity

The child with chronic kidney disease is at risk for extrarenal complications, including cardiovascular and bone disease, and growth retardation. Some of these may be overcome when renal function is restored following transplantation. Other complications may aggravate as side effect of medication such as corticosteroids. Some comorbid conditions, such as infections and malignancies, directly result from the immunosuppressive medication.

Cardiovascular changes
Cardiovascular death is by far the most frequent cause of death in patients who started renal replacement therapy in childhood. Cardiovascular morbidity therefore has to be prevented or counteracted in an early phase of chronic renal failure.
Insight into the pathophysiologic mechanisms leading to cardiovascular damage in children with renal failure gradually has grown during recent years.

Cardiac morbidity in pediatric dialysis patients is different from that in adults. Arrhythmia, cardiomyopathy, valvular disease, and cardiac arrest prevail, whereas ischemic heart disease is rare in this age group.[133] Risk factors that make children with renal disease prone to cardiovascular disease include hypertension, dyslipidemia, anemia, disturbed calcium-phosphate metabolism, malnutrition and hyperhomocysteinemia.[134,135] Hypertension occurs in a large proportion of patients with chronic renal failure, and its prevalence is generally not lower after transplantation. It is a toxic side effect of several immunosuppressive agents, including corticosteroids, and calcineurin inhibitors. Furthermore, two processes have a detrimental effect on structure and function of the blood vessels. First, in the phase of chronic renal failure, high serum phosphate levels and hyperparathyroidism, the treatment of which may cause hypercalcemia, result in an increased calcium phosphate product. Calcium phosphate, subsequently, can precipitate in the medial wall of the blood vessels resulting in stiffening and thickening of the arterial walls; arteriosclerosis.[134,136]

This process may be aggravated by a low serum level of the potent inhibitor of serum calcium-phosphate complex formation fetuin-A, which may occur in combination with inflammation and malnutrition in patients with CRF.[137] Second, blood vessels may be harmed by atherosclerotic plaques. Dyslipidemia and endothelial dysfunction are two of the main factors contributing to atherosclerosis, and were demonstrated in both children and adults on dialysis as well as after transplantation.[138]

These degenerative changes in the vessel walls, together with the frequently occurring volume overload and metabolic disturbances in the dialysis phase, may lead to cardiac hypertrophia and replacement of the myocardium by fibrous tissue.[136] The increase in mass and the change in composition of the ventricle wall may cause disturbances of the heart rhythm and a fall in compliance of the ventricle wall, resulting in functional changes. Diastolic function of the heart usually deteriorates first: early diastolic filling of the ventricle is injured followed by the late diastolic filling by atrial contraction. Later the systolic function is at risk as well. Both diastolic and systolic dysfunction lead to congestive heart disease.[136] Left ventricular hypertrophy and cardiac dysfunction have their onset early in chronic renal disease, and are progressive throughout the dialysis period.[139]

After transplantation certain risk factors will disappear. The calcium phosphate metabolism usually normalizes, but other factors may now exert their influence, such as hypertension and toxicity of immunosuppressive drugs. Left ventricular hypertrophy decreases, but does not disappear: many pediatric patients still show left ventricular hypertrophy after transplantation.[134,140]

Growth retardation

Growth retardation is a major concern in children with chronic renal insufficiency. It originates from metabolic disturbances, malnutrition and dysfunction of the growth hormone axis. Even despite the use of growth hormone before transplantation to increase final height of growth retarded children with renal failure, around 60% of young adults with chronic renal disease since childhood had an adult height below -2 standard deviation score.[143,144] After transplantation some catch-up growth may occur, dependent on the child's age and body height at transplantation, graft function, and dose and frequency of corticosteroids.[141,142]

One way to improve longitudinal growth after transplantation is by reducing the frequency of administration of corticosteroids from once daily to alternate daily.[76,145] Prolonging of treatment with growth hormone is another option. Haffner et al studied patients with chronic kidney disease, including renal replacement therapy, who were treated with growth hormone for an average of 5 years. Their standard deviation score for body height increased from -3.2 to -1.7 at final height, as opposed to a decrease from -1.4 to -2.1 in patients who did not use growth hormone.[146] Controlled studies of growth hormone treatment after transplantation similarly reported significantly improved growth in pre-pubertal and pubertal children.

These studies showed that growth hormone treatment does not affect renal function nor increases occurrence of acute rejections, though earlier reports suggested this.[147-149] Currently in the Netherlands growth hormone therapy is reimbursed only for children with chronic renal failure and a GFR of less than 50 ml/min.1.73m^2. Mean GFR one year post-transplant is 60 ml/min.1.73m^2. This implies that many of these children have to wait for their GFR to decrease before treatment can be continued.

Bone disease

As in adults, chronic renal failure in childhood is often accompanied with renal osteodystrophy, a type of bone disease associated with osteopenia and osteomalacia, caused by disturbances in the calcium phosphate metabolism and hyperparathyroidism. These factors are especially hard to control in young children who have a relatively high intake of protein and phosphate. Prevention of renal osteodystrophy by means of dietary measures, phosphate binders and vitamin D analogues is feasable in many, but not all children. After successful transplantation this form of bone disease may improve, but a new kind of bone disorder may arise. In the first place corticosteroid therapy is associated with osteopenia, which may be monitored using dual energy X-ray absorptiometry (DEXA).[150,151] However, DEXA results need to be corrected for bone age and pubertal stage, since transplanted children are often growth retarded and do not fit well in the normal values for age and sex.[152] Secondly, avascular necrosis of the femoral head and condyles, and of the talus, may develop as a serious complication after transplantation. This may hardly be reversible and may well lead to crippling of the patient. It is associated with cumulative corticosteroid dose. In a recent cross-sectional study in Finland 3.6% of pediatric solid organ recipients had signs of osteonecrosis of the hip. All patients were older than 12 years at the time of diagnosis.[153] In the LERIC study, 13% of patients with renal replacement therapy since childhood had experienced avascular bone necrosis, and 18% mentioned disabling bone disease in young adulthood.[144] Metabolic bone disease after transplantation is a solid argument to search for long term immunosuppressive regimens with only low steroids dosage.

Infection

Apart from preventing rejection of the allograft, immunosuppression suppresses the healthy response to infective agents as well, with more risk of serious infections. Infections in the first month after transplantation are generally caused by bacteria, and are associated with surgery, indwelling drains and catheters. The next five months notably bring viral diseases. Cytomegalovirus (CMV), Epstein Barr virus (EBV), varicella zoster virus (VZV, or chicken pox) and polyomaviruses then are the most threatening to immunosuppressed children.[154] Furthermore, human herpes viruses 6 and 8 are associated with acute rejection and Kaposi sarcoma, respectively. In contrast to adults, children often still are naïve for some of these viral infections at the time of transplantation. It is therefore important that vaccinations are started already prior to transplantation, all the more because vaccination during immunosuppressive therapy is less effective. Unfortunately, vaccines for CMV and EBV are not available yet.

Finally, immunosuppressed patients are at risk of opportunistic infections, e.g. by Pneumocystis pneumoniae, toxoplasma, and certain fungi. Antibiotic prophylaxis with trimetoprim - sulfamethoxazol during the first three months could prevent most urinary tract infections, as well as some of the above mentioned opportunistic infections.[155]

CMV

CMV disease in a transplant patient is most commonly associated with fever, weakness and depression of bone marrow, and may include end-organ disease as well: nephritis, pneumonitis, colitis, esophagitis, hepatitis, retinitis and encephalopathy. CMV infection can also induce allograft rejection. The combination of CMV seropositive donors with CMV seronegative recipients has been reported to give a 4 times higher incidence of CMV disease, a non-significant increase in acute rejection incidence, and more than 20% increase in graft loss as compared with seronegative donors.[156] Not only overt, but also subclinical CMV disease may be deleterious to the graft.[157]

CMV may also increase susceptibility to co-infection with other viruses, like EBV. CMV disease is treated with gancyclovir.[154] In kidney transplant patients resistance of CMV to gancyclovir is rare. Prophylactic treatment during the first three or six months after transplantation is usually instituted when either the donor or the recipient, or both, are seropositive at transplantation.

EBV

EBV infection due to transplantation of EBV-positive grafts into EBV-seronegative children is associated with post-transplant lymfoproliferative disease (PTLD). Infection or PTLD may present as unexplained fever, a mononucleosis-like syndrome, gastrointestinal bleeding, abdominal-mass lesions, central nervous system disease, but also as infiltrative disease of the allograft.[154] Regular EBV-PCR surveillance and carefully monitored reduction of immunosuppression remain the mainstay of treatment. This may be followed by the administration of rituximab, and in case of atypical lymphoma, of chemotherapy. Antiviral prophylaxis may provide some protection against EBV disease as well as CMV disease.

VZV

The course of a primary VZV disease may be violent and atypical.[158] Some patients have even died from disseminated VZV infection. Renal transplant patients who are seronegative for VZV antibodies, should receive zoster immunoglobulin if they have had contact with a child with chicken pox. Preferably, seronegative patients should be vaccinated before transplantation.

BK-polyomavirus

BK-polyomavirus may cause nephropathy with the risk of graft loss or ureteral obstruction.[154] Histologic evidence of nephropathy precedes renal allograft dysfunction in more than 50% of cases.[155]

It has been suggested to test all kidney transplant patients every three months for viruria by cytology (decoy-cells) or quantitative PCR. If viremia measured by PCR exceeds 10,000 copies per ml, a renal biopsy can confirm the diagnosis of BK-nephropathy. Unfortunately, no effective antiviral treatment is available for BK disease. Less immunosuppression is currently the only chance of recovery, but consequently increases the risk of acute rejection and graft loss.

Malignancies
Malignancy is a major cause of morbidity and mortality in adult renal transplant patients. In long term follow-up studies incidences range between 2 and 20%.[159-161] Non-melanoma skin cancer is the most frequent malignancy in adults. Incidence of malignancies in children are still fairly low (1 - 4%), but have increased parallel to expansion of immunosuppressive therapy.[162,163] In a 1994 report from the Cincinnati Transplant Tumor Registry, 53% of post renal transplant tumors occurring during childhood were lymphomas, followed by 19% skin and lip carcinomas. Ninety-eight percent of lymphomas were non-Hodgkin lymphoma, of which 90% of B-cell type, especially those occurring in the first year post-transplant.[164] Most lymphomas occurred in adolescents, with a mean of 3 years post-transplant. Lymphomas are strongly associated with EBV infection (see above).

In young Dutch adults on renal replacement therapy since childhood an overall 8.4% incidence of malignancies has been reported. Fifty-nine percent of these malignancies were skin cancers, and 23% non-Hodgkin lymphoma.[165] The probability of developing a malignancy within 25 years after starting renal replacement therapy in childhood was estimated to be 17%.[165]

Organisation of pediatric kidney transplantation in the Netherlands

Renal replacement therapy in children in the Netherlands – both dialysis and kidney transplantation – is limited on a statutory basis to four institutions, in Amsterdam (Emma Children's Hospital / Academic Medical Center), Utrecht (Wilhelmina Children's Hospital / University Medical Center Utrecht), Nijmegen (University Medical Center St. Radboud), and Rotterdam (Erasmus MC / Sophia Children's Hospital). The yearly number of kidney transplantations is stable, i.e. between 25 and 30, distributed over the four centers. Due to the small numbers of cases per institution, single center evaluations and randomized controlled trials performed are hardly feasible within a reasonable time frame. Therefore, in 1997 the pediatric kidney transplant departments of the four Dutch centers set up a collaboration with that of the University Hospital Antwerp, Belgium. During the past ten years I had the privilege to coordinate our collaborative efforts. We had frequent contacts, and met formally at least four times per year. Consensus protocols were defined and clinical data were pooled. The collaboration served three goals. It allowed us to evaluate the results of these protocols, to perform prospective clinical trials, and to exchange individual experiences in the day to day care for renal transplant patients.

The ultimate goal of these efforts obviously was to improve the prospects of children with end stage renal disease.

Since 1998 the centers use a shared immunosuppressive regimen. It has been agreed to adjust the immunosuppressive therapy after every transplanted 100 patients, considering the results of the latter regimen and recent publications. The results of the first two cohorts are documented in this thesis. In addition we reached consensus on protocols for CMV prophylaxis and thrombosis prophylaxis. Currently we are working on consensus protocols for perioperative fluid management and for control of hypertension during follow up. Application of such standardized protocols is thought to enable further detailed assessment of transplant results in our pooled population.

The collaborative database was developed in cooperation with the Dutch Organ Transplant Registry (NOTR). It contains the medical data of all pediatric kidney transplantations performed since the start of the first immunosuppressive protocol, 1 January 1998. A research nurse regularly visits the different institutions and retrieves data from medical charts and digital databases. Data include detailed clinical data and medical events, like acute rejections, infections and other complications, growth, medication, education, and social environment. In addition, laboratory data are recorded. The Dutch Kidney Foundation supported the database financially during the first years, and since then Roche Nederland and Novartis Pharma Nederland have kindly provided financial sponsorship. Without this support, we would not have been able to set up and maintain the database, the core of the collaborative studies.

It is my conviction that the improved outcome of our patients – as shown by fewer acute rejections and improved graft survival – is largely due to our joined efforts in developing new treatment strategies. The benefits of this cooperation are reflected in the research outcomes described in this thesis.

Outline of the thesis

This thesis is a compilation of joint studies in pediatric kidney transplantation in and around the Netherlands. Most were within the framework of the Netherlands - Antwerp collaboration, one at Eurotransplant level, and one at interdepartmental level within one institution.

Part I describes the two retrospective studies of transplantations in Dutch children that formed the point of departure for the collaboration between the pediatric kidney transplantation centers in the Netherlands and Antwerp.
Chapter 2 reviews the short and intermediate term results of the transplantations performed between 1985 and 1995, the decade preceding the start of shared protocols. By then, every institution used a different protocol, the results of which did not allow firm conclusions due to relatively small numbers of cases.
Chapter 3 deals with the clinical evaluation of Dutch young adults after they had undergone kidney transplantation in childhood, both retrospectively and cross-sectionally. It forms part of the extensive investigations on Late Effects of Renal Insufficiency in Childhood, by Jaap Groothoff and coworkers.

Part II reports the effect of different immunosuppressive protocols on outcome, both retrospectively and prospectively. These studies were conducted within the framework of the Netherlands - Antwerp collaboration.
Chapter 4 compares the outcome of one shared immunosuppressive regimen, consisting of corticosteroids, cyclosporine and mycophenolate mofetil, without induction therapy, with that of the historic group described in Chapter 2.
Chapter 5 compares the outcome of a modification of the initial immunosuppressive regimen with that of the cohort of Chapter 4. This modification aimed to reduce the incidences of delayed graft function and acute rejection episodes, and comprised sequential quadruple therapy starting with an IL-2 receptor antagonist, corticosteroids and mycophenolate mofetil; cyclosporine was added only after graft function was established.
Chapter 6 describes a randomized controlled trial in which dual immunosuppressive therapy with MMF and prednisolone was compared with cyclosporine and prednisolone therapy. This trial started at the end of the first year post-transplant, and had 2 years of follow-up. The objective of this study was to reduce long term toxicity of cyclosporine.

Part III studies the influences of pre-transplant factors on the outcome.
Chapter 7 is a retrospective report from the Eurotransplant community describing the effect of avoiding dialysis treatment prior to transplantation on transplantation outcome.

Part IV deals with comorbid conditions in pediatric kidney transplantation.
Chapter 8 reports five cases with a rare pulmonary complication after kidney or liver transplantation, i.e. bronchiectasis.
Chapter 9 brings the results of the evaluation of respiratory complaints and pulmonary function in children with a functioning kidney transplant. This study was initiated after we became aware of bronchiectasis in some of our patients.
Chapter 10 reports the prevalence in a single center of diastolic dysfunction of the left ventricular of the heart in children with a functioning kidney transplant.

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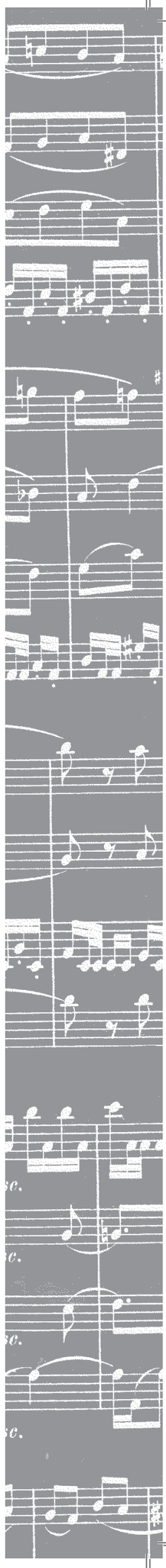
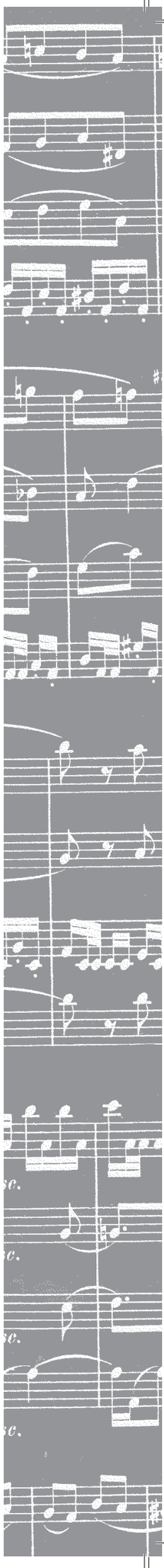
PART I

Pediatric kidney transplantation in the Netherlands: outcome of earlier cohorts



CHAPTER 2

PEDIATRIC RENAL TRANSPLANTATIONS IN THE NETHERLANDS



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Abstract

Introduction
In the Netherlands, pediatric kidney transplantation programs are available in four centers. We retrospectively analyzed the results obtained over the past decade.

Methods
Between 1985 and 1995 231 patients (139 boys) received 269 transplants, including 61 repeat. The recipients were aged 1.9 - 21.8 years (mean 10.9), the donors 0.3 - 63.3 years (median 11.4, mean 19.7). Immunosuppression consisted of corticosteroids, cyclosporine A and azathioprine, in various combinations and dosages.

Results
The patient survival during follow up was 97%. The overall graft survival was 73% at 1 year and 60% at 5 years after transplantation. Major causes of graft loss were acute rejection (21%), thrombosis (12%), and chronic rejection (28%). Acute rejection episodes were noted in 74% of all grafts. First acute rejection episodes had a moderate predictive value for graft loss (relative risk (RR), compared to rejection free grafts, 5.9). First rejection episodes occurring later than 3 months after transplantation were considerably more predictive (RR 18.3) than early ones. Grafts from living related donors (n=35) yielded a superior 5 year graft survival (77%) and remained free of rejection more often than grafts from adult cadaveric donors (43% vs 25%). The results of pre-emptive transplants were excellent (n=13, 5 year survival 100%). Repeat transplants had the same results as primary transplants. Recipients younger than 4 years showed a poor 5 year graft survival of 38% (n=13). Single kidney grafts from donors younger than 4 years (n=35) had a 5 year graft survival of 44%. In contrast, kidneys from these young donors did well if transplanted en bloc (n=10, 5 year graft survival 89%).

Conclusion
These overall results are in line with those of others. The results may be improved by expansion of immunosuppressive therapy in the first year and by thrombosis prophylaxis in high-risk patient-donor combinations. Better results may be expected from more extensive use of living related donations, pre-emptive transplantations and en bloc transplantation instead of single kidneys of young donors.

Introduction

Renal transplantation is a firmly established mode of renal replacement therapy in children. Since its introduction in the early 1970s the success rate gradually increased.[1, 2] The development of new immunosuppressive drugs has contributed to the prevention of acute rejection and therefore to better graft survival. In the first half of the 1980s cyclosporine A was introduced and took a central place in immunosuppressive therapy. Recently, new drugs have been introduced, such as mycophenolate mofetil [3, 4] and tacrolimus. [5]
In the Netherlands the first kidney transplantation in a child was performed in 1973. There are four pediatric renal transplantation centers. All four started their programs before 1977. The annual number of transplantations stabilized from 1980 onwards to between 25 and 30, distributed over the four centers. The centers used to work independently, and each developed an immunosuppressive regimen of its own. The limited number of patients per center impeded the development of experience with a certain immunosuppressive protocol and the possibility to evaluate it. Therefore, recently the four centers decided to join forces and to design a shared immunosuppressive regimen, as well as a single database for the clinical information of all children transplanted in the Netherlands. The current retrospective analysis provided the basis for this Dutch consensus protocol.

Patients and methods

Clinical data were collected from patient records by two medical students (M.B. and M.D.), on specially designed data forms. Included were all renal transplantations performed in the four pediatric transplantation centers in the Netherlands between 1985 and 1995. From 1985 on, all centers used prednisone and cyclosporin as the pivot of immunosuppressive therapy. Acute rejection was defined as a full course of anti-rejection therapy, consisting of methylprednisolone in pulse therapy, high dosed prednisone, antithymocyte globulin (ATG) or OKT3. Graft failure was defined as the start of any other form of renal replacement therapy, or a glomerular filtration rate (GFR) of less than 10 mL/min/1,73m^2. Death with functioning graft was considered as graft failure.

Statistics

Actuarial patient and graft survival were calculated according to the Kaplan-Meier method. Comparisons between curves were performed with the log rank test. In survival studies with multiple subgroups, figures were cut off at 6 years because of the small numbers of patients beyond this time point. Relative risks (RR) of graft failure were calculated with Cox proportional hazards regression model, adjusted for selected parameters. To investigate whether the risk of graft failure is affected by the occurrence of a rejection episode and the timing of the first rejection episode, Cox regression with time dependent co-variates was employed. Means were compared using Student's two-sample *t*-test or Wilcoxon's two-sample test where appropriate. In all statistical tests, a *p*-value below 0.05 was considered significant.

Results

Patient and graft characteristics (Tables 1 and 2)
In the 10 year period 269 kidney transplantations were performed in 231 patients (139 boys). The age at transplantation ranged from 1.9 to 21.8 years, with a mean of 10.9 and a median of 11.3. In 208 cases, this was the first transplantation, in 53 a second and in 8 a third.
The primary renal diseases leading to end-stage renal failure are shown in Table 1.
The largest group of patients (n=81) had a urological malformation or dysfunction. Of these patients 62 (77%) were boys, explaining the preponderance of boys in the whole population. Differentiation within the group of urological diagnoses was difficult: it was not always clear whether dysplastic kidneys or vesico-ureteral reflux were associated with urethral valves or not.
The modes of renal replacement therapy (RRT) at the time of transplantation included chronic intermittent hemodialysis (n=144), continuous ambulatory or cycling peritoneal dialysis (n=102) and a graft with residual function (n=8). Thirteen patients (5%) did not receive RRT before transplantation and thus were transplanted pre-emptively.
In 35 cases (13%) a living related donor (LRD) procedure was performed. In 33 cases, the donor was a parent and in two an adult brother. Seven of the 35 LRD cases concerned a repeat transplantation.

Table 1. Primary renal disease

	n	%
Patients	231	100
Glomerular disease	62	27
FSGS	29	
MPGN	9	
IgA nephropathy	4	
RPGN	7	
Alport syndrome	3	
Other/not specified	10	
Congenital nephrotic syndrome	13	6
Congenital structural abnormalities	81	35
Tubulointerstitial nephritis, not specified	3	1
Cystic diseases	19	8
Metabolic diseases	7	3
Vascular and systemic diseases	25	11
HUS	17	
Renal malignancy	5	2
Other / unknown	15	7
Missing	1	0

FSGS, focal segmental glomerulosclerosis;
MPGN, membranoproliferative glomerulonephritis;
RPGN, rapidly progressive, or crescentic, glomerulonephritis;
HUS, hemolytic uremic syndrome

The kidneys from cadaveric donors (n=234; 87%) were HLA-matched and obtained in the framework of the Eurotransplant Organ Exchange Organisation. The donor age varied from 0.3 years to 63.3 years. The mean donor age was 19.7 years, median 11.4. Of 10 cadaveric donors, all younger than 4 years, the kidneys were transplanted *en bloc*. The cold ischemia time of the cadaveric kidneys ranged from 6 to 46 hours, with a mean and a median of 29 hours. The number of HLA-B/DR-mismatches of cadaveric donors are shown in Table 2.

Table 2. Transplantation characteristics

		n	%
Transplantations		269	100
Rankorder	1st	208	77
	repeat	61	23
Recipient age (yrs)	0 - 2	1	0
	2 - 4	12	5
	4 - 9	88	33
	9 - 18	151	56
	> 18	17	6
Donor age (yrs)	0 - 4	45	17
	4 - 9	65	24
	9 - 18	52	19
	18 - 40	54	20
	40 - 50	38	14
	> 50	14	5
	missing	1	0
Donor source	CAD	234	87
	LRD	35	13
RRT prior to this transplantation	hemodialysis	144	54
	peritoneal dialysis	102	38
	transplantation	8	3
	none	13	5
	missing	2	1
PRA pre-transplantation (%)	0 - 10	210	78
	10 - 40	28	10
	> 40	20	7
	missing	11	4
HLA-B/DR-mismatches (CAD)		(234)	(100)
	0	30	13
	1 - 2	183	78
	3 - 4	20	9
	missing	1	0
Cold ischemia time (CAD)		(234)	(100)
	< 24 hr	49	21
	24 - 36 hr	159	68
	> 48 hr	0	0
	missing	26	11

CAD=cadaveric
LRD=living related donor
RRT=renal replacement therapy
PRA=panel reactive antibodies

Perioperative management
All grafts were implanted in the iliac fossa with the donor vessels connected to the external or common iliac vessels of the recipient. In two centers the ureter was implanted in the bladder with an anti-reflux procedure, in two centers without. The peri-operative fluid administration was generous in all centers. Prophylactic heparin was not routinely administered throughout the observation period.

Immunosuppressive therapy (Table 3)
During the observation period all centers prescribed corticosteroids, azathioprine and cyclosporine A. No ATG or OKT3 induction therapy was used, except for nine patients treated in one center. All centers used high-dose corticosteroids starting preoperatively and rapidly tapered in the first 6 to 12 weeks to 0.25 mg/kg/day. Prednisone was given daily during the first postoperative months in all centers. A proportion of the patients were subsequently switched to an alternate-day schedule. This switch was made routinely at 3 months in one center, and at a later stage in others. At 3 years after transplantation overall half the patients received prednisone on alternate days. The mean daily dosage of prednisone was equal when given once daily or on alternate days.
The time-point at which cyclosporine A was started, and the dosage, varied among the centers and within the observation period. In one center, cyclosporine A was only started when the creatinine level was below 100 µmol/L. In the absence of cyclosporine A, azathioprine was administered.
Maintenance immunosuppressive therapy consisted of prednisone and cyclosporine A for the majority of patients. The percentage of patients treated with triple therapy (prednisone, azathioprine, cyclosporine) increased through the years. It was prescribed either from transplantation onwards as standard therapy, or, at a later stage, when cyclosporine A toxicity was suspected and the cyclosporine A dosage needed to be lowered. In this situation in some cases prednisone / cyclosporine A was converted to prednisone / azathioprine.

Table 3. Immunosuppressive regimens throughout the years

	1985 – 1990 (n=130)		1990 – 1995 (n=139)	
Years after transplantation	1	4	1	4
Transplants at risk:	88 (100)[a]	68 (100)	99 (100)	51 (100)
Pred/AZA	47 (53)	34 (50)	10 (10)	7 (14)
Pred/CsA	34 (39)	16 (24)	46 (47)	14 (27)
Pred/CsA/AZA	5 (6)	17 (25)	43 (43)	29 (57)
Other/missing	2 (2)	1 (1)	0	1 (2)

[a] number of grafts (percentage)
Pred= prednisone; AZA= azathioprine; CsA= cyclosporine A

Patient survival

Eight patients died with a functioning graft. Causes of death included a cerebrovascular accident (n=3), infection (sepsis, n=1, and toxoplasmosis, n=1), myocardial infarction (n=1) and sudden death, most likely from cardiovascular origin (n=1). In one case the cause of death remained unknown. The mean age at transplantation for the deceased patients (9.1 years) was comparable to that of the surviving patients. The majority of the deaths (n=6) occurred during the first 6 months after transplantation.

Graft survival

The overall graft survival rate at 1 year was 73%, at 3 years 67%, and at 5 years 60%. The slight difference in results of the cohort transplanted between 1990 and 1995 compared to that transplanted between 1985 and 1990 (5 year survival of 64% vs. 56%) was not statistically significant.

In the investigated period 107 of the 269 grafts failed. Table 4 shows the causes of the graft failure according to survival time. Graft loss within the first month after transplantation occurred in 14% of all transplants and was largely due to acute allograft rejection and vascular thrombosis. Graft failure after the first months was mainly due to chronic rejection.

Thrombosis was responsible for 12% of all failures. The lower age of the donors of these thrombosed grafts was the only factor associated with a higher risk of thrombosis (median 7.1 vs. 10.1 years for the grafts failed for another reason, p=0.06). In contrast to the suggestion by Van Lieburg et al.,[6] patients with dys- and hypoplastic kidneys were not over-represented in the group with vascular thrombosis compared to the total population (36 vs. 32%).

Table 4. Causes of graft failure according to time (months) after transplantation

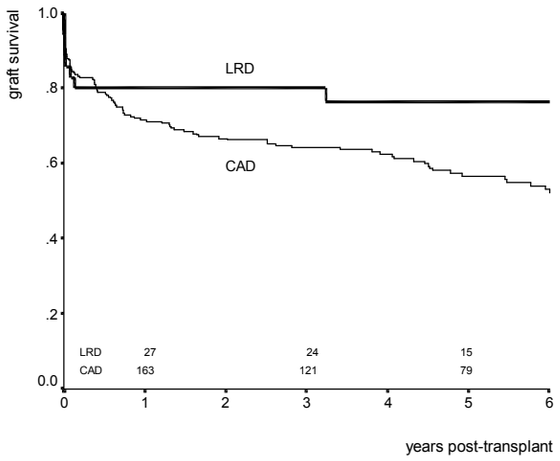
	0 - 1	**1 - 6**	**6 - 12**	**12 - 60**	**> 60**	***Total 0 - 60***
Grafts at risk (n)	*269*	*231*	*209*	*188*	*87*	*269*
Acute rejection	12	5	4	0	0	*21*
Thrombosis	12	1	0	0	0	*13*
Primary non function	5	0	0	0	0	*5*
Death	3	3	1	1	0	*8*
Recurrence	2	0	3	3	0	*8*
Infection	1	0	0	0	0	*1*
Chronic rejection	0	3	7	18	2	*30*
Other/missing	3	6	2	7	3	*21*
Grafts failed (n)	*38*	*18*	*17*	*29*	*5*	*107*

Living related donor transplants (LRD) (Figure 1)

In the group with LRD transplants (n=35), early failures occurred with approximately the same frequency as in the group with cadaveric (CAD) kidneys. Two of these early failures (n=7) were due to accelerated acute rejection. Once the first month after transplantation had passed, only one graft was lost, after 39 months, because of recurrence of the original disease.

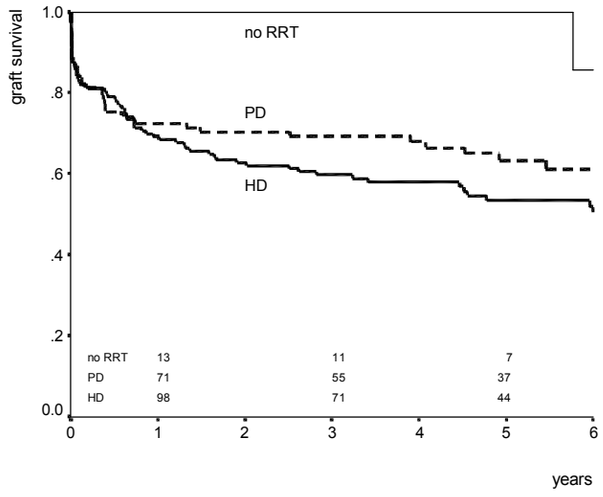
Comparing the results of LRD grafts that survived the first month after transplantation with those of CAD grafts from adult donors that survived this period (n=72), the difference in graft survival was strongly in favor of the LRD (p<0.01). The age at transplantation and the number of HLA-mismatches did not differ significantly between recipients of LRD and CAD kidneys. Of the LRD grafts 43% did not show any rejection episode, compared to 25% of the grafts from adult cadaveric donors (not significant).

Figure 1. Cumulative graft survival of living related vs. cadaveric kidneys



LRD= living related, CAD= cadaveric, (n=234), (p=0.07).
The remaining number of patients at risk is indicated at the bottom of the figure

Figure 2. Cumulative graft survival according to prior RRT (p<0.05)



Thirteen patients did not receive any prior RRT, 102 were treated with peritoneal dialysis and 144 with haemodialysis.
Eight patients received a second graft without dialysis after failure of the first graft and are not included here. Two patients' dialysis mode was missing. The remaining number of patients at risk are indicated at the bottom of the figure

Prior renal replacement therapy (Figure 2)
Transplantation prior to any mode of RRT was performed in 13 patients. These grafts did considerably better than grafts in dialyzed children (n=246) (p<0.05). The 5 year patient and graft survival was 100%. Within this pre-emptively transplanted group of patients, a relative overrepresentation of LRD transplants (31% vs. 12% in the group with prior dialysis) was noted as well as of an original disease of congenital structural abnormalities (62% vs. 35%).

However, when compared to a dialyzed control group matched for age, donor source and primary renal disease (n=28), the 5 year survival was significantly better (100% vs. 63%, $p<0.05$). The pre-emptive and post-dialysis groups were not different regarding donor age, panel reactive antibodies (PRA), number of mismatches, cold ischemia in the CAD kidneys, or number of rejection episodes. The slight difference in graft survival between patients on hemodialysis and those on peritoneal dialysis was not statistically significant.

Retransplantation

Comparing the results of repeat CAD transplantations (n=54) with first CAD transplantations (n=180), we noticed a similar 5 year graft survival (53% vs. 58%, not significant). The repeat grafts had better matching (mean number of HLA–B/DR mismatches 1.2 vs. 1.6 in the first CAD grafts, $p<0.05$), with higher antibodies prior to transplantation (mean 26% vs. 5%, $p<0.001$).

The recipients as well as the donors were slightly older (mean recipient age 12.1 vs. 10.4 years, $p<0.01$, median donor age 16.9 vs. 10.0 years). The repeat grafts showed fewer rejection episodes (0.84 vs. 1.1 episodes per graft in the 1st year, $p<0.05$), which may be explained by the better matching, the older age of donors and recipients, and the negative cross-matching despite circulating PRA.

When correcting for the above-mentioned factors by Cox proportional hazards model, still no significant difference was seen between the survival of first and repeat transplantations.

Young recipients (< 4 years)

Although the graft survival in recipients younger than 4 years (54% at 1 year, 38% at 5 years after transplantation) was worse than in older patients, the difference was not statistically significant in this small group of patients (n=13). They received kidneys of younger donors (median age 4.3 vs. 12.2, $p<0.01$). Young patients tended to have more acute rejection episodes than older recipients. Of the 13 recipients aged < 4 years, 2 did not have any rejection episode (15%), compared to 29% of older recipients ($p<0.05$).

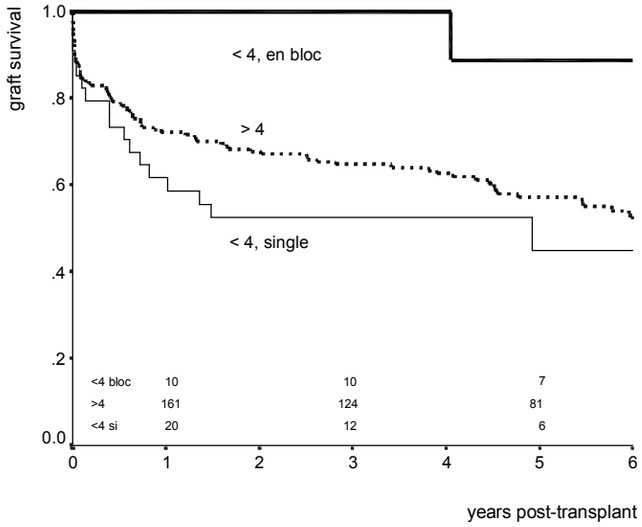
Young donors (< 4 years)

Forty-five patients received a kidney from a donor younger than 4 years. The donor age varied between 1.6 and 4 years, with the exception of one very young single kidney donor aged 0.3 year. The recipients of these young kidneys were slightly younger than the overall group: mean age 8.5 vs. 10.9 years.

Causes of graft failure in this group included thrombosis (n=3, 16% of all failures in this group), acute rejection (n=3, 16%), and chronic rejection (n=5, 26%). The survival of the grafts from young donors was equal to that from older CAD donors (69% at 1 year, 54% at 5 years after transplantation). However, when within the young donor group a distinction was made between *en bloc* transplanted kidneys (n=10) and single kidneys (n=35), the graft survival was significantly different: the 5 year survival was 89% vs. 44% (Figure 3, $p<0.05$).

The recipients of single and of *en bloc* kidneys did not differ in age, original disease, number of mismatches, PRA, or cold ischemia time. The function of surviving grafts at 1 year after transplantation was significantly better for *en bloc* grafts (n=9) than for single kidney grafts (n=16): the estimated GFR[7] amounted to 87 vs 64 mL/min/1.73m^2, p<0.05.
Young donor kidneys tended to induce more rejection episodes than older ones: a rejection-free follow up was observed in 20% of grafts from donors younger than 4 years, 26% of grafts from donors 4 to 10 years old and 32% of grafts from donors older than 10 years (n.s.).

Figure 3. Cumulative cadaveric graft survival according to donor age (p<0.05)



The group of grafts of donors younger than 4 years (n=45) are divided in single (n=35) and en bloc (n=10) transplanted kidneys.
The older group counted 189 grafts.
The remaining number of patients at risk are indicated at the bottom of the figure

Acute rejections

A biopsy confirmed the diagnosis of acute rejection in 47% of all episodes.
The occurrence of any acute rejection episode affected the graft survival substantially (RR 5.9, p<0.001). With an increasing number of rejections per patient the long-term outcome became worse. Chronic rejection was the main cause of graft loss in the long term, and occurred in 30 cases. Looking only at the grafts that did not fail by other causes than chronic rejection (n=192), the difference in graft survival between the groups without any acute rejection episode (5 year survival 100%), with one (90%) and with more than one episode (72%) was highly significant (p<0.001).
In the majority of cases the first acute rejection episode occurred within the first 3 months after transplantation (Table 5). The small number of grafts with first rejection episodes occurring later than 3 months after transplantation (31 out of 189 episodes) had a significantly worse prognosis than early rejections, compared to the grafts without any rejection episode.

Table 5. Relative risk of graft failure according to timing of first rejection episode

	timing of 1st acute rejection episode	n[a] (%)	RR of graft failure [b]	p
days after transpl.	0 - 14	102 (54)	5.1	< 0.001
	14 - 30	22 (12)		
months after transpl.	1 - 3	34 (18)	3.6	< 0.01
	3 - 6	12 (6)	18.3	< 0.001
	6 - 12	9 (5)		
	> 12	10 (5)	12.7	< 0.001

[a] Of 9 rejection episodes the date of occurrence was missing.

[b] relative risk (RR) of graft failure compared to grafts without any acute rejection

Malignancies

In our series one case of malignancy was registered. It concerns a Kaposi sarcoma in a patient treated with chemotherapy for a bilateral Wilms tumor, who had therapy-resistant rejection episodes in the 4 months she had her graft. After graftectomy and cessation of the immunosuppressive medication the Kaposi sarcoma vanished.

Discussion

The results of renal transplantations in Dutch children over the past decade were similar to those reported for North American[8] and European children[1], when comparing the CAD grafts. We noticed a slight statistically not significant improvement over time. Our multi-center graft survival was comparable to that reported a decade earlier from a retrospective analysis in one of the centers.[9] The causes of graft failure in our population were distributed similarly as in the most recent report of the North American database.[8] Early graft loss was predominantly caused by thrombosis (12%) and acute rejection (20%). We will discuss the major complications that determine graft loss and highlight the potential benefits of LRD transplantation, of pre-emptive transplantation and of transplanting young donor kidneys *en bloc*.

Thrombosis

In our population, the only factor significantly associated with a high risk of thrombosis was young age of the donor. In contrast to other reports, no correlation could be found between the incidence of thrombosis and prolonged cold ischemia time, low recipient age, or a primary renal disease with high urine production at the time of transplantation.[6, 10, 11] The most evident factors increasing the risk of thrombosis may be summarized as those circumstances where blood flow problems are to be expected, as in young donors and small recipients, or in donor kidneys with multiple arteries. Thrombosis may be prevented in these high-risk situations by the administration of low molecular weight heparin during 2 - 3 weeks post-transplant.[12]

Acute rejection episodes

A large proportion of grafts was lost directly due to acute rejections. It has been suggested that acute rejection episodes in young children may lead to graft failure more often than in older ones. According to the North-American data on CAD grafts, 21% of all rejection episodes in children 0-1 years of age ended in graft loss, 15% in children aged 2-5 years, and 7-8% in older children.[13] The number of young children in our population was too small to influence the failure rate by acute rejection of our population as a whole. Apart from this direct effect, our results confirm the data of others, that acute rejections substantially increase the risk of graft failure in the longer term, especially by chronic rejection.[14, 15] Important determinants of this risk appear to be the number of the rejection episodes per patient, and the timing of the first rejection. Although low in number, first rejections occurring more than 3 months after transplantation have a significantly worse prognosis. These observations parallel those of others.[16, 17, 18] Whether this graft loss long after a late acute rejection is related to a relatively low immunosuppressive effect of the drug regimen and/or to compliance problems cannot be concluded from our data.

Living related donations

The long-term survival of LRD grafts in our series is good. This is consistent with the results of others.[8, 19] The direct post-operative period, however, was less favorable, resulting in the loss of one patient and 20% of the grafts. Two losses by accelerated acute rejection might have been prevented by applying a donor specific transfusion prior to transplantation and cross-matching. Another explanation could be that the relatively low number of LRD transplantations was performed in a negatively selected patient population. It is likely that, in some of the patients, dialysis problems and other medical and social complications contributed to the decision to plan an LRD in order to shorten the waiting time.

The low proportion of LRD transplantations in our series is in contrast to the general policy in other parts of the world.[8, 19] Historically it reflects a low need for LRD grafts in our country, since sufficient CAD grafts were available from the service of Eurotransplant. The waiting time for CAD kidneys was relatively short and the cold ischemia time acceptable. Reluctance towards the use of LRD grafts was based on the awareness of the impact on the psychosocial environment of the patient and his family, on the physical risks for the donor, as well as on the notion that potential family donors might be saved as a “backup” for later. Recent evidence regarding LRD transplants, however, indicates that the physical risks are relatively low,[20] the psychosocial circumstances may even be positively affected[21] and the “backup” parents may be lost as a potential donor as a result of antibody formation following a preceding transplantation. The waiting time is shorter, the time of transplantation can be chosen and the outcome is better. Based on these considerations we now encourage LRD transplantations more.

Young donors

Transplantation of single kidneys from donors younger than 4 years yielded poor results, confirming the data of several other groups[22, 23] but contradicting a recent single center report.[24] In particular, the use of kidneys from young donors in young recipients accumulates risk factors for graft failure[22] and should therefore be avoided. If the small kidney is severely affected by long warm ischemia caused by the technical difficulty of the anastomoses, or acute rejection, the tissue reserve is limited. Hyperfiltration damage and chronic rejection may follow. The favorable circumstance of mass equivalence between recipient and donor appears not to counterbalance these increased risks.

On the other hand, good long-term results have been reported for young donor kidneys into adult recipients. Single kidneys from donors aged 1 year grafted into adult recipients yielded acceptable results for both graft survival and GFR.[25] Early acute rejections threatened the survival of the graft however, resulting in the need for aggressive immunosuppressive induction and rejection therapy.

In another study comparing transplant results of donors younger than 7 years with adult donors, it was found that grafts from young donors did equally well in terms of graft survival, GFR, incidence of acute rejections and incidence of proteinuria.[26] Therefore, the risk for chronic rejection, reflected by proteinuria, seems not to be increased, and the growth in volume seen in these kidneys seems to reflect accelerated normal maturation growth, as has been demonstrated before.[27, 28]

In contrast to the findings with single kidneys, our results of *en bloc* kidneys of the youngest donors were good.

These data expand on an earlier report by one of the centers.[29] Two factors may contribute to this favorable outcome. The wider caliber of the blood vessels leads to both safer anastomoses and better flow properties of the arterial bed. In addition, the double transplanted renal mass implies a doubling of the functional capacity and potentially less damage from compensatory hyperfiltration.[30] *En bloc* kidneys are also suitable for adults.[31-34] An obvious drawback of the *en bloc* use of young donor kidneys is that fewer patients are being helped. However, in the Eurotransplant area many centers (49 out of 68) do not accept kidneys from these very young donors at all, neither single nor *en bloc* (De Meester, personal communication).

Although our overall results with young donors are disappointing as in other studies, we are encouraged by the results of *en bloc* transplanted kidneys. If these findings are substantiated, then this approach may become worthwhile in these times of organ scarcity.

Pre-emptive transplantations

Pre-emptive transplantations were remarkably successful in our series. Some authors have suggested that pre-emptive transplantations will do worse than post-dialysis transplantations due to an assumed immunologically protective effect of uremia and the greater motivation to take medication in the post-dialysis group.[35] However, favorable results similar to ours were reported by other authors.[1, 36]

Several groups found an equal success rate for pre-emptive and post-dialysis transplantations, both in adults[37-39] and children.[35, 40, 41, 42] Schurman and McEnergy, on the other hand, noted an adverse effect of pre-emptive transplantations in children receiving CAD grafts, in contrast to LRD grafts.[43] LRD graft were used in a high proportion of all described pre-emptively transplanted patients. In addition to this favorable graft survival, transplantation before the need of dialysis has a number of other potential advantages. The better physical condition of the patient when compared to most dialyzed adult patients has been associated with quicker recovery from surgery[38] and with a better cardiovascular patient survival.[44] An enormous advantage obviously is the avoidance of the burden of dialysis and its complications. This will undoubtedly lead to better conditions for growth and psychosocial development and ultimately to a better quality of life for these children. In addition to these advantages, two potential drawbacks of pre-emptive transplantations need to be addressed. First, in many cases the timing of transplantation is not easy. Renal failure due to congenital disorders of the renal architecture tends to progress very slowly, implying a considerable risk that transplantation is carried out years before actually needed. Second, the compliance to drug therapy after transplantation might be better for children with dialysis experience. Compliance is well recognized as a major determinant of long-term outcome of renal transplantations in children.[45]

In conclusion, our results are in line with those of others. The success rate may be enhanced by decreasing the incidence of acute rejection and thrombosis. Prevention of acute rejection is expected to postpone extinction of the graft by chronic rejection as well.

The centers now share a new and potentially more effective immunosuppressive regimen, as well as default thrombosis prophylaxis in high-risk patients. In most centers LRD transplantations are promoted more actively, as is pre-emptive grafting. In this way, we hope to improve the graft survival and quality of life of our patients without considerable increase of the incidence of infections and malignancies.

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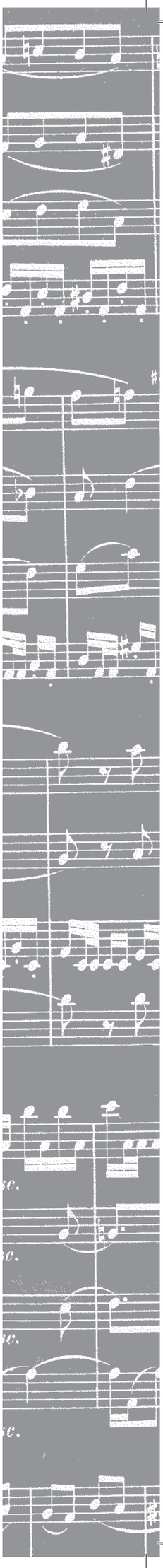
CHAPTER 3

LONG TERM FOLLOW UP OF RENAL TRANSPLANTATION IN CHILDREN: A DUTCH COHORT STUDY



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Abstract

Background
Few data exist on long-term morbidity, overall survival and graft survival of pediatric renal transplantation.

Methods
The authors performed a long-term cohort study in all Dutch patients, born before 1979, with onset of end-stage renal disease (ESRD) between 1972 and 1992 at age 0 to 15 years. Data on graft survival and determinants of outcome were obtained by reviewing all medical charts. The health status was assessed by cross-sectional examination of surviving patients.

Results
Three hundred ninety-seven transplantations were performed in 231 of all 249 patients, of whom 25 died with a functioning graft. Cardiovascular disease was the most prominent cause of death. Graft survival estimates for all transplantations were 59.2%, 45.3%, 35.4%, and 30.3% at 5, 10, 15 and 20 years, respectively. In comparison with azathioprine, cyclosporine as immunosuppressant was associated with increased graft survival in retransplantations but not in first transplantations. Cross-sectional examination was performed on 110 patients. In 44 patients, the most recent graft survival exceeded 15 years. Comorbidity was found in 40% of all patients; motor, hearing or visual disabilities in 19%. Bone disease, headaches, itching and tremors were the most reported disabling problems. Cyclosporine use was associated with hypertension and a history of epilepsy. Compared to all age-matched Dutch inhabitants, the educational attainment was low, and unemployment and parental dependency were high.

Conclusions
The authors' results emphasize the need for reducing cardiovascular disease and metabolic bone disease in pediatric ESRD, a policy towards less toxic antirejection therapy, a more strict treatment of hypertension and more attention for schooling and social development towards independency.

Introduction

Over the past 20 years, transplantation has become routine treatment for children with end-stage renal disease. To increase the rate of graft survival, more potent immunosuppressive regimens have been introduced over the past decade. However, concern has risen regarding the side effects of these new immunosuppressive drugs. In the discussion about the optimal immunosuppressive regimen in children undergoing transplantation, there is need for more information about the long-term effects of the earliest immunosuppressive treatment with respect to graft survival as well as comorbidity. To date, only few data exist on these long-term outcomes. Our purpose was to investigate the long-term overall survival, graft survival, and morbidity, and the effect of introduction of cyclosporine as immunosuppressive therapy on these outcomes. For this purpose, we used data from the Late Effects of Renal Insufficiency in Children (LERIC) study, a national Dutch long-term follow-up study that aimed to evaluate late physical, social, and psychological effects of renal insufficiency in children.

Patients and methods

Study design

The study was designed as a cohort study and consisted of a cross-sectional part and a retrospective part. The aim of the cross-sectional study was to establish the current health status of the patients. The aim of the retrospective part of the study was to evaluate the influence of a set of predefined determinants on outcome parameters. The study covered the total period of renal replacement therapy (RRT) for each patient. The end of the study was marked by the day of last chart review for potential non-participants in the cross-sectional study and the day of the cross-sectional examination for participants. The medical ethical committees of all participating centers approved the study.

Formation of the cohort

The LERIC cohort comprises all Dutch patients who had started chronic RRT at age 0 to 15 years between 1972 and 1992, and who were born before 1979. Patients in whom renal function recovered within 4 months after commencing dialysis were excluded. Patients who underwent transplantation pre-emptively were included. Data on gender, date of birth, initiation of RRT, and date of death of all the patients who fulfilled the inclusion criteria were provided by the National Dutch Registry of patients on RRT (RENINE, Rotterdam, The Netherlands). RENINE, founded in 1985, is the Dutch source of the European Dialysis and Transplantation Association. The completeness approaches 100% as registration is compulsory for reimbursement of RRT. We checked the accuracy of data on these patients by comparing RENINE data with the databases of all four Dutch centers for pediatric dialysis and kidney transplantation and with the databases of all centers for adult dialysis and transplantation. The cohort formation has previously been described in detail.[1]

Data collection

Between November 1998 and August 2000, members of the LERIC-team visited 37 hospitals in The Netherlands. They collected information about the period, duration and onset of renal replacement therapy, the total number and duration of dialysis and transplant periods; and all immunosuppressive drugs that were used. The date of graft failure was defined as the day of onset of chronic dialysis or a next transplantation after the particular transplantation. An analysis was made of the effect of the introduction of cyclosporine on the long-term graft survival by comparing two groups of transplantations: one characterized by an initial immunosuppressive regimen consisting of azathioprine and prednisone (the AZA group), and one characterized by an initial immunosuppressive regimen consisting of cyclosporine, prednisone with or without azathioprine (the CsA group). Immunosuppressive regimens, in which cyclosporine was introduced within 1 month after transplantation, were included in the CsA group. Transplantations with initial immunosuppressive regimens containing other drugs, such as mycophenolate mofetil, tacrolimus, antithymocyte globulin, cyclophosphamide or OKT3, were excluded from this analysis. All medical charts of all patients, participants as well as non-participants in the cross-sectional study, were reviewed. Emigrated patients were located and medical information was obtained from their current physician.

All patients who were alive were invited for examination in our hospital. Data were gathered on health, current medication, current renal function and social status, by using questionnaires, reviewing medical charts, and performing a physical examination. Data on renal function and comorbidity were gathered from the medical charts. A systolic blood pressure above140 mmHg was considered to be systolic hypertension; a diastolic blood pressure above 90 mmHg was considered to be diastolic hypertension. The glomerular filtration fraction (GFR) was estimated on basis of the serum-creatinine, using the Cockroft–Gault formula.[2] Comorbidity was considered to be present in the event of the occurrence of one or more of the clinical diseases as defined by Davies et al. (i.e. malignancy, clinical apparent ischemic heart disease, peripheral vascular disease, clinical apparent left ventricular dysfunction, diabetes mellitus, systemic collagen vascular disease, cerebrovascular disease, chronic obstructive airway disease, or other significant abnormality).[3] Disabilities were considered present in case of severe deafness, blindness or disability resulting from motor function disorders. For quality of life assessment, the RAND-36 Health Survey was used;[4] data were compared to data derived from a Dutch age-matched control group.[5] Data on social status included employment, educational attainment and residence.

Statistical analysis

We used Kaplan-Meier analysis to calculate graft survival probabilities. Survival curves were compared using the log-rank test. Logistic regression analysis was used to establish associations between comorbidity and disabilities on one side and patient characteristics (i.e. GFR, gender, duration of dialysis, duration of renal replacement therapy, and duration of transplantation) on the other side.

We used the Student *t* test to analyze the effect of cyclosporine therapy on mean blood pressure. Chi square test was used to analyze the association between cyclosporine therapy and binominal dichotomous outcomes.
We performed a stratified analysis for linear trend in proportions (EpiInfo, Centers for Disease Control and Prevention, Atlanta, GA) to analyze the difference in educational attainment and the general Dutch population. SPSS 10.0.07 was used for all other statistical calculations.

Results

Patient characteristics

The LERIC cohort consisted of 249 subjects. Of these, 62 had died at time of investigation, leaving 187 alive. One patient died 4 months after the cross-sectional investigation and is included in the analysis of mortality. For 82 patients (33%), the follow up was more than 20 years. At the end of the study no patients were lost to follow up.
The mean total duration of RRT was 15.5 years (range 0.3-28), the mean total duration of transplantation was 11.3 years (range 0-29.9), the mean total duration of hemodialysis 3.3 years (range 0-25.6), and the mean total duration of peritoneal dialysis 0.8 years (range 0-14.7). Patients changed from therapy modality (i.e. dialysis or functioning graft) from one to nine times during the study period. Of all 187 living patients, 140 participated in the cross-sectional part of the study, of whom 110 had a functioning graft at time of investigation. The main characteristics of the total cohort and the participants in the cross-sectional study are given in Table 1.

Table 1. Patient characteristics of the LERIC cohort

	LERIC cohort		Participants cross-sectional study with a functioning graft	
Number of patients (male/female)	249	(136/113)	110	(54/56)
Primary disease (n)				
Glomerulopathy %	90	(36.1)	40	(36.4)
Obstructive uropathy %	70	(28.1)	32	(29.1)
Congenital renal malformation %[a]	44	(17.7)	22	(20)
Hemolytic Uremic Syndrome %	18	(7.2)	9	(8.2)
Metabolic disease[b]	8	(3.2)	0	
SLE %	5	(2.0)	2	(1.8)
Other %	14	(5.6)	0	
Mean follow up time from start of RRT, yrs (range)	15.5	(0.2-30.0)	18.2	(6.2-30.0)
Mean age at start of RRT, yrs (range)	10.6	(1.9-14.9)	11.0	(1.9-14.9)
Mean age at first transplantation, yrs (range)	12.6	(3.9-27.0)	12.6	(6.6-27.0)
Mean age of all patients alive at the end of the study, yrs (range)	29.5	(20.7-1.7)	29.3	(20.7-41.7)
Number of deaths	63			
Number of patients ever transplanted	231			
Number of patients never transplanted	18			
Number of pre-emptive transplantations	7			

[a] dysplasia (15), nephronophtisis (22), autosomal recessive polycystic kidney disease (3), Alport's syndrome (1), congenital nephrotic syndrome (2), prune belly syndrome (1)
[b] cystinosis (5), oxalosis (3)

Transplantations

A total of 397 transplantations were performed in 231 out of 249 patients, 45 of which were from a family-related donor and 352 from a cadaveric donor. Only seven patients underwent transplantation pre-emptively.

The number of transplantations per patient was 1 in 112 patients, 2 in 79 patients, 3 in 33 patients and 4 in 7 patients. Living related donation (LRD) transplantations were performed significantly more often as retransplantation than as primary transplantation (17% [29 of 166] vs 7% [16 of 231], p=0.001). In 206 (52.3%) of all 397 transplantations, only azathioprine and prednisone were used as initial anti-rejection regimen (AZA-group).

In 160 transplantations (40.7%) the combination of cyclosporine and prednisone with or without azathioprine was used as anti-rejection therapy (CsA-group) (Table 2).

Table 2. Initial Immunosuppressive regimens all transplantations

Regimens	All Tx (n=397)	First Tx (n=231)	Second Tx (n=119)	Third Tx (n=40)	Fourth Tx (n=7)
AZA/p	206	161	41	3	1
CsA/p	90	33	43	14	0
CsA/AZA/p	70	23	29	15	3
ATG/OKT3+	14	8	3	3	3
MMF/CsA/p	10	0	2	5	7
Other	7	6	1	0	1

Tx = transplantation; AZA/p =azathioprine & predniso(lo)ne; CsA/p = cyclosporine & predniso(lo)ne; CsA/AZA/p = cyclosporine & azathioprine & predniso(lo)ne; ATG/OKT3 + = ATG or OKT3 & other drugs; other = cyclophosphamide/azathioprine/prednisone, or radiotherapy/azathioprine/prednisone

Graft survival

Graft survival estimates of all transplantations were 59.9%, 46.2%, 36.1%, and 30.9% at 5, 10, 15 and 20 years, respectively. The survival of all first transplantations of the AZA group and the CsA group were similar. The survival of all retransplantations was significantly higher in the CsA group than in the AZA group (p=0.016; Figure 1 & Table 3).

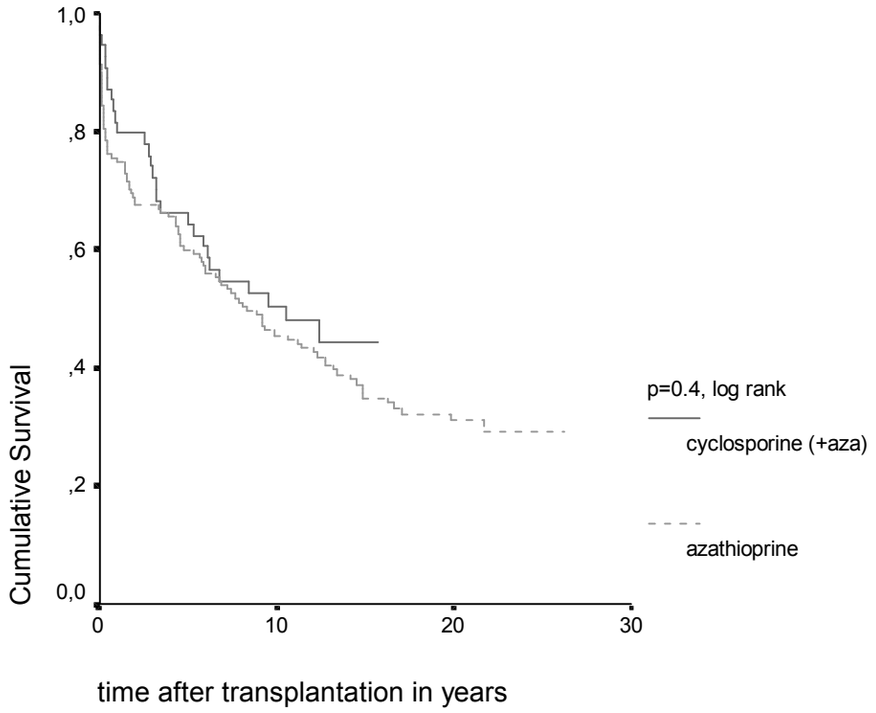
Graft survival estimates for all family-related transplantations (n=45) were significantly higher than for all post-mortem transplantations: 77.7%, 74.5%, 65.7% and 49.3% at 5, 10, 15 and 20 years, respectively, versus 58.0%, 43.3%, 33.3% and 28.9% (p=0.003) (Figure 2). Overall, 219 of 397 transplantations were complicated by a complete graft failure. In 29.7% this was caused by acute rejection, in 52.2% by chronic graft nephropathy, and in 9.1% by thrombosis (Table 4). We found no influence on the cause of the graft failure of the period of transplantation, the immunosuppressive regimen (AZA group vs. CsA group), or the consecutive number of transplantation within one patient.

Mortality

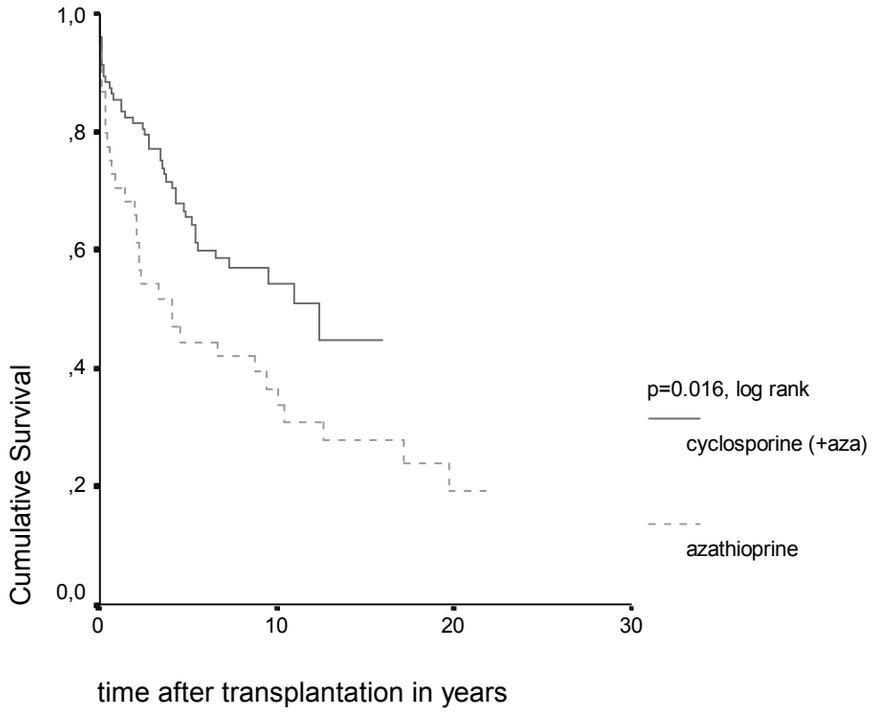
Of the 63 patients who died, 25 died with a functioning renal graft. Of these 25 patients, 7 (28%) died within 4 months after transplantation and 13 (52%) died within one year, at a mean age of 18.0 years (range 4.6-33.5 years).

Figure 1. Kaplan-Meier survival estimates of all first transplantations (1a) and all retransplantations (1b) with either azathioprine and prednisone (=azathioprine, dotted line), or cyclosporine, (azathioprine) and prednisone (=cyclosporine) as initial immunosuppressants

1a



1b



From the onset of transplantation, patient survival was 91%, 86%, 83% and 78% at 5, 10, 15 and 20 years respectively (Figure 3). Causes of death of the 25 patients who died with a functioning graft were the following: cerebral bleeding (n=4), congestive heart failure (n=4), non-Hodgkin lymphoma (n=3), Pneumocystis carinii infection (n=2), cytomegalovirus infection (n=2), refusal of further treatment at the time of graft failure (n=2), myocardial infarction (n=1), septicemia (n=1), dengue (n=1), fibrosarcoma (n=1), ruptured aortic aneurysm (n=1), gastro-intestinal bleeding (n=1), acute rejection complicated by cardiac arrest (n=1) and unknown cause (n=1). In the first year after transplantation, infectious disease was the most common cause of death (38.5%). Mortality and causes of death in the LERIC cohort are described in detail elsewhere.[1]

Table 3. Transplant survival

	Total no	5 yr survival	10 yr survival	15 yr survival	20 yr survival
All TX [a]	397	60%	46%	36%	31%
AZA	206	57%	43%	33%	28%
Censored	*72(35%)*	*14 (5%)*	*19 (9%)*	*30 (14%)*	*42 (20%)*
CsA	160	66%	56%	48%	
Censored	*90 (56%)*	*24 (15%)*	*51 (32%)*	*83 (52%)*	
First Tx [a]	231	61%	46%	36%	32%
AZA	161	60%	46%	35%	31%
Censored	*59 (36%)*	*11 (6%)*	*13 (8%)*	*22 (8%)*	*32 (20%)*
CsA	56	66%	51%	44%	
Censored	*28 (50%)*	*4 (7%)*	*8 (14%)*	*23 (41%)*	
Secound-Fourth TX [a]	166	59%	48%	39%	27%
AZA	45	44%	37%	28%	19%
Censored	*13 (29%)*	*3 (7%)*	*5 (11%)*	*8 (18%)*	*10 (22%)*
CsA	104	65%	54%	45%	
Censored	*62 (60%)*	*20 (19%)*	*43 (41%)*	*50 (58%)*	

Tx = transplantation; AZA = azathioprine & prednisone; CsA = cyclosporine, (azathioprine) & prednisone; [a] including those on other immunosuppressive regimens

Health state at cross-sectional examination

Patient characteristics

Of all 187 patients who were alive, 140 participated in the cross-sectional study (75%). Of these 140, 110 patients, 54 male and 56 female, were living with a functioning graft. Previously, we have shown that the clinical characteristics of participants and non-participants in the cross-sectional study were similar.[6] The mean age of the cross-sectionally investigated patients was 29.3 years (range 20.7-41.7). The mean duration of the last transplant period was 12.3 years (range 0.3-26.3, median 11.7) (Table 1). In 44 patients, the survival of the latest graft exceeded 15 years. In 7 patients, the GFR was less than 25 mL/min/1.73m² at the time of investigation. On 106 of 110 patients, data on health and social status could be obtained. Cyclosporine was used by 38 (34.5%) of all patients at time of investigation; 35 patients (31.8%) had never used cyclosporine. Tacrolimus was used by 7 (6.4%) patients. Anti-hypertensive medication was used by 64 (58.2%) patients; 24 (21.8%) used angiotensin-converting enzyme inhibitors, 31 (28.2%) calcium-antagonists, 39 (35.5%) beta-blockers and 7 (6.4%) diuretics.

Figure 2. Kaplan-Meier survival estimates of all family related (n=45) and all post-mortem transplantations (n=352)

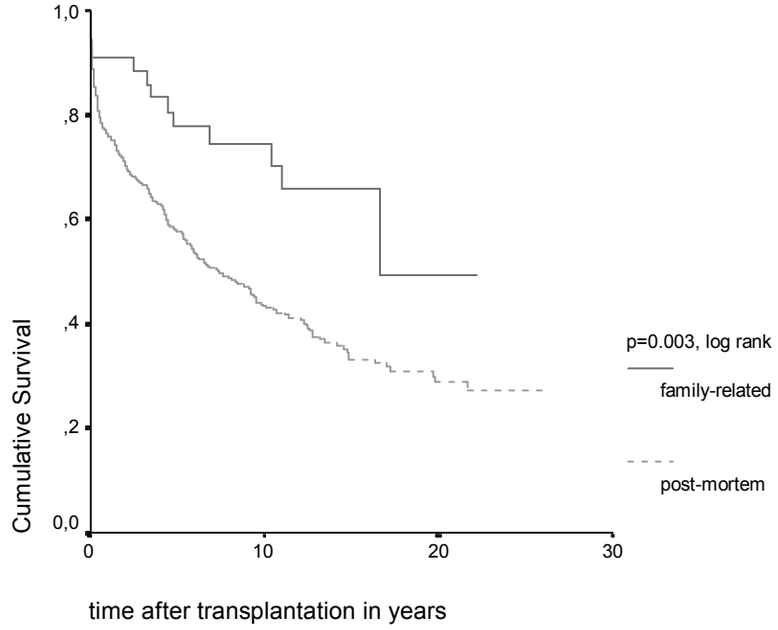


Figure 3. Kaplan-Meier patient survival estimates of all transplanted patients

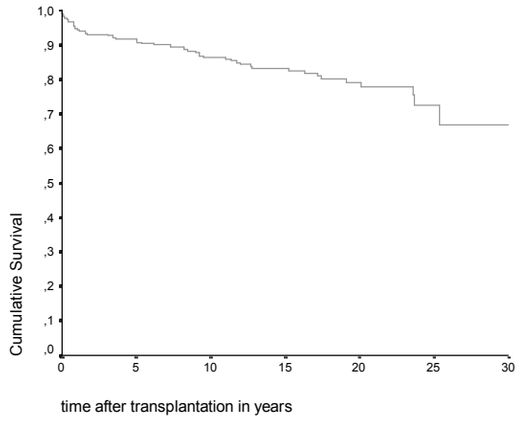


Table 4. Diagnosis of graft failure of all transplantations, and of all transplantations with initial azathioprine/prednisone therapy (AZA Tx) and of those with cyclosporine / (azathioprine) / prednisone (CsA Tx)

	all Tx		all AZA Tx		all CsA Tx	
	n	(%)	n	(%)	n	(%)
No failure	178	(44.8)	72	(35.0)	90	(56.3)
Acute rejection	65	(16.4)	35	(17.0)	21	(13.1)
Chronic allograft nephropathy	115	(29.0)	69	(33.5)	41	(25.6)
Thrombosis	20	(5.0)	15	(7.3)	4	(2.5)
Renal artery stenosis	3	(0.8)	2	(1.0)	1	(0.6)
Urethral obstruction/Obstructive uropathy	2	(0.5)	2	(1.0)	0	
Primary disease	9	(2.3)	7	(3.4)	2	(1.3)
Unknown	2	(0.5)	2	(1.0)	0	
Bleeding	2	(0.5)	1	(0.5)	1	(0.6)
Reduction immunosuppressive therapy because of a lymphoma	1	(0.3)	1	(0.5)	0	
Total	397[a]		206		160	

Tx = transplantation;[a] 31 transplantations with other immunosuppressive regimens than the combination of azathioprine/prednisone or cyclosporine /prednisone/(azathioprine)

Table 5. Health status in patients with a functioning graft (n=106)

General condition:	
Comorbidity	40.0%
Motor, visual or auditive disabilities	19.1%
General Health Perception	
>50% healthy, no disabilities	58.7%
>50% healthy, "disabled"	29.3%
< 50% healthy	11.9%
Fatigue:	
Never or sometimes	65.1%
Often	11.9%
Daily	22.9%
Limitation of daily activities	27.3%
Inactivity	37.3%
Bone disorders	34.5%
Disabling bone disorders	17.3%
Aseptic bone necrosis	11.8%
Height <-2SD	60.9%
Skin disorders:	
Itching	27.3%
Warts	59.1%
Fertility women (n=56):	
Amenorrhoea	5.6%
Ever been pregnant	16.4%
Offspring	16.4%
Infertility established	3.6%
Cardiovascular symptoms:	
Exercise intolerance	18.2%
Ankle edema	14.5%
Angina pectoris	8.2%
Intermittent claudication	7.3%
Neurological symptoms:	
Severe headache	49.5%
Once a month	32.1%
Weekly	17.4%
Sensory disorders	16.4%
Epilepsy	8.2%
Dysbalance	11.8%
Paresis	15.5%
Restless legs	21.8%
Tremors	38.2%
Fertility men (n=54)	
No erection	7.1%
No ejaculation	17.8%
Offspring	16.1%
Infertility established	5.4%
Quality of life[a]	
Physical Component Summary	47.5 (10.1)[b]
Mental Component Summary	49.9 (9.1)

[a] RAND-36 questionnaire, scores for general population of Physical Component Summary and Mental Component summary both 50 with SD 10; [b] significantly lower than found in the general population (p=0.05); mean score general population for both Physical and Mental Component summary is 50.

Health state

Of all patients, 60% reported a good overall health state. Comorbidity was reported in 40%, skeletal disabilities, impaired vision or hearing were reported in 19% (Table 5). Clinical signs of bone disease (i.e. chronic joint and bone pain, disabling malformations, pathological fractures, aseptic bone necrosis), severe headaches, severe itching and tremors were the most frequently occurring disabling problems. Systolic and diastolic hypertensions at cross-sectional examination were found in 31% and 22%, respectively. The mean educational attainment of all patients was significantly lower than that of the general Dutch population (Chi-square for linear trend p<0.001). Also, compared to the age-matched general Dutch population, significantly more patients lived without a partner (58.8% versus 29.8%; Table 6).

Table 6. Social status (n=106)

	Patients	Dutch population[a]
Educational attainment		
Low vocational training	57.0%	27.2%[b]
Intermediate vocational training	30.8%	47.0%
High vocational training	12.1%	25.9%
Unemployment[c]	25.5%	11.1%[d]
Domestication		
Living alone	29.4%	20.7%
With partner	38.5%	64.8%
With parents	29.4%	12.7%
Institution	2.7%	5.4%

[a] Dutch population 20-44 years old: N =5.975.843; [b]Chi-square for linear trend: p<0.001; [c] students excluded; [d] 381.900 people Unemployment Benefit (Werkloosheid Wet) or on Employment Unfitness Benefit (Wet Arbeids Ongeschiktheid) (6.39%) & 283.260 people on Employment Unfitness Benefit (4.74%). Data from Central Bureau for National Statistics, 1999 (Centraal Bureau voor de Statistiek: www.cbs.nl).

Associations between disease characteristics, medication and outcome

Overall comorbidity was associated with a low actual GFR and male gender (adjusted odds ratios 14.1 (p=0.02) and 2.5 (p=0.03), respectively). We found no association between the appearance of disabilities and patient characteristics. Being on cyclosporine at time of investigation was associated with a higher mean systolic blood pressure (135±17.9 mmHg vs. 128±19.0 mmHg; p=0.05), severe warts (p=0.02), hypertrichosis (p<0.001), complaints of general fatigue (p=0.03) and a history of epilepsy (p=0.05). Of all patients on cyclosporine therapy, 75% used anti-hypertensive drugs, compared to 50% of those without cyclosporine (p=0.01). We found no associations between other outcome measurements and the use of cyclosporine.

Discussion

This is the first study with detailed information reporting on pediatric kidney transplantations with a follow up in nearly one third of more than 15 years. In Europe, only a few reports on the long term follow up of the early kidney transplantations in children have been published over the past decade.[7-9]

The large North American databases on kidney transplantations (the United Network for Organ Sharing and the North American Pediatric Renal Transplant Cooperative Study) started to collect their data in 1987. Thus, their follow-up is shorter than in the current cohort, and their patients have been treated with more modern immunosuppressive therapy.[10-12]

Graft survival: the effect of cyclosporine therapy

The overall 20 year graft survival of 31% that we found in our cohort is in accordance with the report of a single European center.[9] The fact that no patient of our historical cohort was lost to follow-up made it possible to compare the effect of the introduction of cyclosporine as immunosuppressive therapy on graft survival in the long run. As expected, we found that cyclosporine therapy was associated with a higher mean graft survival than azathioprine. However, to our surprise, this only counted for retransplantations. We found no beneficial effect of cyclosporine on graft survival in all first transplantations. Moreover, the number of graft losses resulting from acute rejection was not different in the AZA group compared to that in the CsA group. These are important observations, because azathioprine has clear advantages above cyclosporine. The cross-sectional part of the study showed that survivors on cyclosporine suffered from more side effects than those on azathioprine (i.e. a higher mean blood pressure and more cosmetic and neurological problems). Nevertheless, the apparent better results of the cyclosporine group with respect to graft survival in the retransplantations could be biased as a result of the strategy of some Dutch centers in the late 1980s. Their protocols prescribed a conversion from azathioprine to cyclosporine only after a decrease in serum creatinine below a certain value (i.e. 100 μmol/L) in most centers. The same policy is described by Chavers et al.[8] Because some patients never reached this value and remained on azathioprine, this protocol led to a selection of grafts with worse function compared to the AZA group in the current study. The fact that cyclosporine was found to be beneficial only in retransplantations suggests that its additional immunosuppressive action only overrules its nephrotoxicity with respect to graft function in the more immunogenic circumstances that obviously come with retransplantations. During the first years after the introduction of cyclosporine in renal transplantation, most regimens prescribed very high dosages of the drug, inducing severe renal toxicity. Chavers et al made the same observations with respect to the effect of cyclosporine on first and repeat transplantations.[8]

Post-mortem versus living related transplantations

Compared to reports of the North American Pediatric Renal Transplant Cooperative Study database, the proportion of living related transplantations was relatively small.[12] Between 1970 and 1983, only 7 LRD transplantations in children have been performed in the Netherlands. This low figure could partly be explained by the excellent allocation system in Europe of Eurotransplant, which has resulted in a relatively short waiting time for postmortem grafts. In the Netherlands, this situation has induced a policy to postpone LRD kidneys to a later stage, in which finding a suitable cadaveric donor can be expected to be more difficult.

The far more favorable survival of LRD grafts as compared with cadaveric grafts that we found has been confirmed by others. However, in The Netherlands, the proportion of LRD transplantations has remained low until the late 1990s. Lately, the scarcity of cadaveric organs and the changed attitude of the teams towards LRD transplantations have pushed its proportion up to 30%.[13] Our findings underline the need for a further increase in LRD transplantations.

Outcome

Cardiovascular disease was the most common cause of death, a remarkable feature considering the young age at which our patients died. Previously, we reported on cardiac and vascular disease in the LERIC cohort.[6,14] We found a high incidence of left ventricular hypertrophy, calcification of the aortic valve, and on average an increased arterial wall stiffness of the carotid wall.

Strikingly, we found these abnormalities to the same extent in transplanted patients with good renal function, as in patients on dialysis at the time of investigation. Hypertension was strongly associated with most of these cardiovascular abnormalities, all of which have proved to be mortality risk factors.[15]

Health status at cross-sectional investigation

Although most patients appeared to be in good health at cross-sectional investigation, still 40% of all patients suffered from concomitant diseases and nearly 20% had disabilities. Skeletal, neurological, and skin disorders were most frequently reported. In an earlier report of the European Dialysis and Transplant Association database concerning young adult transplanted patients with pediatric end-stage renal disease, 20% of the patients had one disability and 11% more than one.[16] Like in our cohort, musculoskeletal disorders were most frequently reported (15%), followed by vision (8%) and the hearing (8%) problems. In their long-term follow-up study on adult kidney transplant recipients, Lee et al described a high prevalence of skin, skeletal, and cardiovascular comorbidity in the 20 patients surviving at least 25 years after transplantation.[17] Our data are in line with these observations. Whereas cardiovascular disease is the most prominent life-threatening problem, bone disease is the most disabling problem in young transplanted patients with pediatric ESRD.

Quality of life and social outcome

The subjective health perception of our patients was very good, despite the disabilities and comorbidity. Other authors also found a favorable quality of life in these patients.[17-19] It seems that graft recipients do not feel that their health status interferes with their social life and that they often understate the degree of their disabilities in questionnaires.[20] At the same time however, life career milestones of social independency and development (i.e. living without parents and having a partner) were significantly different from the normal population. Also, we found involuntary unemployment in our patients to be more than twice as high as in the age-matched population. The fact that some American reports have shown a more favorable outcome with respect to employment could reflect the difference in social security of both societies.[17-18]

Other European studies are in line with our data, confirming the relatively high social dependency and low grade of employment as compared to with healthy peers or patients with another chronic disease, such as diabetes mellitus.[16,20,21] Our finding emphasizes the need for a psychosocial approach in children with ESRD that will stimulate them to become a socially well-adapted and independent individual in adulthood.

Suggestions for improvement of therapy
Although efforts to reduce the incidence of graft failure should be continued, more attention should be given to the prevention and reversal of cardiovascular, skeletal, and neurological comorbidity. Replacing cyclosporine by less vascular toxic medication and a more strict anti-hypertensive treatment are mandatory to reduce cardiovascular disease. The beneficial role of statins in young renal transplant recipients in this respect needs to be investigated. Last but not least, attention should be paid to the psychosocial development of children with ESRD, especially with respect to schooling and development towards social independency.

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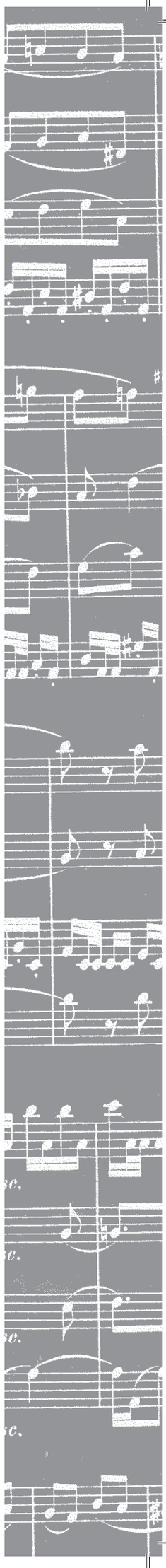
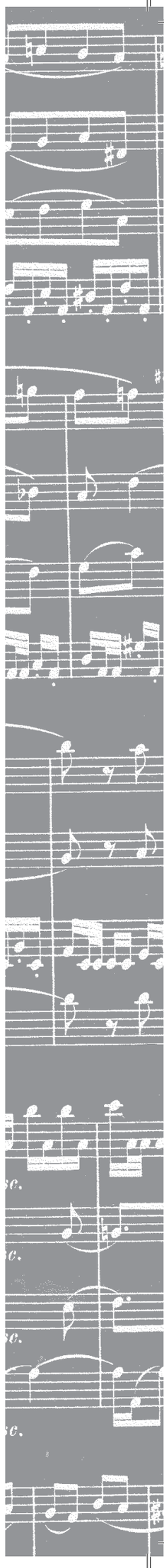
PART II

Studies on immunosuppressive therapy in pediatric kidney transplantation



CHAPTER 4

IMPROVED OUTCOME OF PEDIATRIC KIDNEY TRANSPLANTATIONS IN THE NETHERLANDS – EFFECT OF THE INTRODUCTION OF MYCOPHENOLATE MOFETIL?



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Abstract

Background

Collaboration of the Dutch centers for kidney transplantation in children started in 1997 with a shared immunosuppressive protocol, aimed at improving graft survival by diminishing the incidence of acute rejections. This study compares the results of transplantations in these patients to those in a historical reference group.

Methods

Ninety-six consecutive patients receiving a first kidney transplant were treated with an immunosuppressive regimen consisting of mycophenolate mofetil, cyclosporine and corticosteroids. The results were compared to those of historic controls (first transplants between 1985 and 1995, n=207), treated with different combinations of corticosteroids, cyclosporine A and/or azathioprine. Cytomegalovirus prophylaxis was prescribed to high-risk patients in the study group, and only a small proportion of the reference group.

Results

The graft survival at 1 year improved significantly: 92% in the study group, vs 73% in the reference group ($p<0.001$). In the study group 63% of patients remained rejection-free during the first year; in the reference group 28% ($p<0.001$). After statistical adjustment of differences in baseline data, as cold ischemia time, the proportion of LRD, pre-emptive transplantation, and young donors, the difference between study and reference group in graft survival (RR 0.33, $p=0.003$) and incidence of acute rejection (RR 0.37, $p<0.001$), as the only factor, remained statistically significant, indicating the effect of the immunosuppressive therapy. In the first year 1 case of malignancy occurred in each group. CMV disease occurred less frequently in the study group (11%) than in the reference group (26%, $p=0.02$). As a new complication in 4 patients bronchiectasis was diagnosed.

Conclusion

A new consensus protocol, including the introduction of mycophenolate mofetil, considerably improved the outcome of pediatric kidney transplantation in the Netherlands, measured as reduction of the incidence of acute rejection and improved graft survival.

Introduction

In 1997 the Dutch centers for pediatric kidney transplantation together with the one in Antwerp, Belgium, started sharing treatment and research protocols. The first project was a shared immunosuppressive regimen, primarily aimed at ameliorating long-term graft survival by diminishing the incidence of acute rejection episodes (AREs) in the first year.

This aim originated from results of transplantation performed in the Netherlands between 1985 and 1995, which were recently reviewed.[1] In this cohort, which included 13% living related (LRD) transplantation, the one-year graft survival was 73%. Similar cohorts of children in other countries showed higher one-year graft survival rates: in post-mortally donated (PMD) grafts 79% in Great Britain[2], 78% in Scandinavia[3], and 81% in North America[4]; in LRD grafts 88% in Scandinavia[3] and 91% in North America[4]. In the Dutch cohort 22% of the graft losses in the first year was due to acute rejection, equivalent to 7% of all transplants. During subsequent years graft failure was primarily caused by chronic allograft nephropathy.[1] In the course of time acute rejection has become less important as a cause of direct graft loss. Chronic allograft nephropathy, however, has acquired a leading role in the loss of grafts in the long run.[4, 5]

To improve graft survival, the development of chronic allograft nephropathy should therefore be prevented. Several authors showed that acute rejection is the major risk factor for chronic allograft nephropathy: without a previous acute rejection episode the incidence of chronic allograft nephropathy is very low. One rejection episode contributes to chronic allograft nephropathy, but multiple rejection episodes carry a higher relative risk.[6-9] Late occurrence of the first rejection episode (later than 6 months) is also an independent risk factor for chronic allograft nephropathy.[7-9] No fewer than 63% of North American pediatric patients with a PMD graft still have one or more acute rejection episodes within 4 years after transplantation,[10] this proportion is 51% in patients with a LRD graft.[11]

Having to design an immunosuppressive regimen for all Dutch centers, we aimed at reducing the graft losses caused by acute rejection and chronic allograft nephropathy by way of diminishing the incidence of acute rejection. In adult kidney transplantation, adding mycophenolate mofetil (MMF) to the immunosuppressive regimen consisting of corticosteroids and cyclosporine (CsA) significantly reduced the incidence of acute rejection.[12-14] This is why we decided on a combination of MMF, CsA and corticosteroids as immunosuppressive treatment of our pediatric kidney transplant patients during the first year after transplantation. In order to be able to assess the efficacy and safety of this regimen, we agreed upon a standard protocol for additional treatment variables. Here we present the results of this new protocol in the first cohort of pediatric transplant patients, using the 1985-1995 cohort as a reference group.

Patients and methods

Patients

All patients receiving a kidney transplant in the Netherlands and Antwerp, Belgium were treated with the same immunosuppressive protocols from October 1997. Until December 2001, 121 transplants had been performed; data were collected for at least one year after transplantation. The results were compared to those from transplants performed in the Netherlands between 1985 and 1995, the retrospective evaluation of which has been published earlier.[1] To enable comparison, only first transplants were analyzed.

Immunosuppressive regimen

The immunosuppressive regimen consisted of three drugs: corticosteroids, CsA and MMF. Of each drug a loading dose was given 1 to 6 hours pre-operatively: of methylprednisolone 300 mg/m^2 intravenously, of CsA 10 mg/kg orally, and of MMF 600 mg/m^2 orally. Post-transplant (methyl)prednisolone was prescribed at 40 mg/m^2 twice daily, with weekly tapering off the dosage to 7.5 mg/m^2, once daily, at week 6. Six months post-transplant it was further decreased to 5 mg/m^2. From 6 hours after recirculation CsA was administered intravenously for 24 hours in a dosage of 3 mg/kg/24 hr, followed by oral administration titrated towards a trough level of 150-250 mg/L during the first 6 weeks, of 150-200 mg/L during the second 6 weeks, and 100-150 mg/L thereafter. MMF was administered as soon as oral intake was possible, in a dosage of 600 mg/m^2 twice daily. Triple therapy was scheduled to be continued during the first year.

In the reference group patients had been treated with various combinations of corticosteroids, CsA and azathioprine (AZA). In both groups no induction therapy with ATG or monoclonals had been applied.

Acute rejection

An acute rejection was defined as a sudden increase of serum creatinine to more than 115% of the previous values with no other identifiable cause, followed by the prescription of a full course of anti-rejection treatment. In the study group anti-rejection treatment consisted of one or two courses of intravenous methylprednisolone. If this treatment failed, a course of rabbit ATG was instituted. Prior to ATG treatment a kidney biopsy was taken. In the reference group the choice of antirejection treatment had been determined by hospital policy. Complete recovery was defined as a creatinine level of <115% of the pre-rejection value 2 weeks after treatment.

Chronic allograft nephropathy

Chronic allograft nephropathy was defined as slowly progressive renal failure with no other identifiable cause, at least 3 months after transplantation. Renal biopsies were performed in some, but not all cases of chronic allograft nephropathy.

Delayed graft function
Delayed graft function was defined as the need of dialysis treatment in the first week after transplantation.

Graft survival
Start of any form of chronic dialysis or a new transplant or death with a functioning graft was considered as graft loss.

Thrombosis prophylaxis
All patients in the study group with a high risk of vascular thrombosis of the graft – recipients younger than 6 years of age, donors younger than 10 years, multiple renal arteries or veins to the graft, or a history of thrombosis – received anti-thrombotic therapy during 2 weeks, either low dose heparin or low molecular weight heparin. Data on prophylaxis in the reference group were not available.

CMV prophylaxis
In the study group all CMV seropositive patients, and seronegative patients with a seropositive donor (D+R-, D+R+, D-R+) received prophylaxis against CMV during the first 3 months after transplantation, consisting of (val)acyclovir, or gancyclovir in high-risk patients, in two centers in combination with hyperimmune globulin every other week. In the reference group (for the CMV data restricted to the patients transplanted between 1990 and 1995) only one center prescribed hyperimmune globulin only to CMV seronegative recipients with seropositive donors (D+R-).

CMV disease
CMV disease was defined as CMV-infection (PCR in plasma or pp65 in leukocytes positive) together with clinical symptoms, with admission to the hospital for treatment with i.v. gancyclovir.

Statistics
Primary endpoints were the incidence of acute rejection during the first year and the graft survival during the first 2 years. The difference between Kaplan Meier survival curves, used for patient and graft survival, and time to first acute rejection, was tested for its statistical significance with a log rank test. Differences in other parameters were tested with the following tests: chi-square, or Fisher's exact test if appropriate, for categorical variables, Student's *t*-test for continuous variables. Cox' regression analysis was used to adjust the comparison between study and reference group for possibly confounding factors.

Results

Between 1997 and 2001, 121 kidney transplants were performed in 117 patients, with a minimum follow-up of 12 months. Ninety-six patients received a first graft. The reference group consisted of 269 transplantations in 231 patients, of which 207 were a first graft. Baseline demographic and clinical data of the patients are shown in Table 1.

Table 1. Baseline demographic and clinical characteristics

	Triple therapy n (%)	Reference group n (%)	p
Transplantations	96 (100)	207 (100)	
Primary kidney disease			ns
congenital struct. abnorm.	45 (47)	74 (36)	
glomerulonephritis	15 (16)	54 (26)	
cystic disease	9 (9)	18 (9)	
HUS	4 (4)	12 (6)	
TIN/Alport/Goodpasture	7 (7)	7 (3)	
cystinosis/oxalosis	4 (4)	7 (3)	
cong. nephrotic syndr.	5 (5)	10 (5)	
miscellaneous	4 (4)	14 (7)	
unknown	3 (3)	11 (5)	
Recipient age (yr)			ns
< 6	16 (17)	41 (20)	
6 - 10	23 (24)	56 (27)	
10 - 14	33 (34)	67 (32)	
> 14	24 (25)	43 (21)	
Donor age (yr)			<0.001
0 - 10	8 (8)	104 (50)	
10 - 20	15 (16)	30 (15)	
20 - 45	42 (44)	50 (24)	
> 45	30 (32)	22 (11)	
missing	1	1	
Donor source			0.001
PMD	68 (71)	179 (86)	
LRD	28 (29)	28 (14)	
Renal replacement therapy prior to this transplantation			<0.001
haemodialysis	43 (46)	96 (47)	
peritoneal dialysis	31 (33)	97 (47)	
none	20 (21)	13 (6)	
missing	2	1	
PRA pre-transplantation			ns
0 - 10	90 (99)	179 (89)	
10 - 40	1 (1)	17 (8)	
> 40	0 (0)	6 (3)	
missing	5	5	
HLA-mismatches (only PMD transplantation)			ns
0	6 (9)	13 (7)	
1 - 2	34 (52)	74 (41)	
3 - 4	26 (39)	88 (49)	
5 - 6	0 (0)	3 (2)	
missing	2	1	
Cold ischemia time (only PMD transplantation)			<0.001
< 24 hr	39 (67)	58 (32)	
24 - 36 hr	18 (31)	89 (50)	
> 36 hr	1 (2)	32 (18)	
missing	10		

HUS = hemolytic uremic syndrome, TIN=tubulo-interstitial nephropathy, PRA=panel reactive antibodies, PMD=postmortal donation

Differences include the increased proportion of grafts from living related donors and a higher percentage of pre-emptive transplants. Of the pre-emptive transplants, 5 out of 20 in the study group were from PMD, as compared to 9 out of 13 in the reference group (p=0.012). The proportion of young donors was lower in the study group. The cold ischaemia time in the study group was lower than that in the reference group.

Patient survival

After one year survival in the study group is 100%, in the reference group 96% (p=0.07). In the reference group 7 patients died within the first year, due to cerebrovascular accident (3), cardiac death (2), toxoplasmosis (1), unknown cause (1).[1]

Graft survival

Mean duration of follow-up is 20 months (range 0-61) in the study group and 45 months (range 0-137) in the reference group. The graft survival rate for both LRD and PMD transplants is presented in Figure 1.

The one-year graft survival of the study group is 92% overall, i.e. 96% for LRD and 90% for PMD. In the reference group these values are 73%, 78% and 72%, respectively (p<0.0001 for all transplants; p=0.08 for LRD; p=0.0003 for PMD). The proportion of grafts lost due to acute rejection in the first year was 2.1% in the study group and 7.2% in the reference group (p=0.044) (Table 2).

Table 2. Causes of graft loss in the 1st year after transplantation. 'Other' causes include in the reference group: death with functioning graft (6), primary non function (4), recurrence of primary renal disease (4), surgical problem (1) and miscellaneous (9); in the study group miscellaneous (2)

	Triple therapy		*Reference group*		*p*
	n	(%)	n	(%)	
Number of transplants	96	(100)	207	(100)	
Acute rejection	2	(2.1)	15	(7.2)	0.044
Chronic rejection	1	(1.0)	6	(2.8)	n.s.
Thrombosis	2	(2.1)	12	(5.8)	n.s.
Other	2	(2.1)	24	(11.5)	0.006
Total	7	(7.3)	56	(27.1)	<0.001

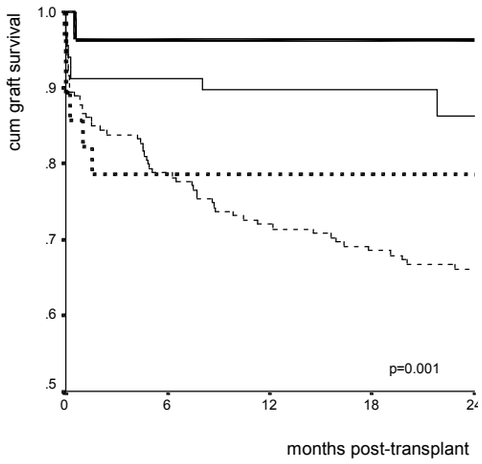
Chronic allograft nephropathy caused graft loss during the first 2 years after transplantation in 2.1% in the study group vs 5.8% in the reference group (n.s.). Thrombosis caused graft loss in 2.1% in the study group and 5.3% in the reference group (n.s.). Graft failure was not observed in any of the 32 pre-emptive transplants.

In Cox regression analysis we adjusted for the possible confounding effect of the following factors: pre-emptive vs post-dialysis transplantation, donor source, primary kidney disease, donor and recipient age at transplantation. The result was an adjusted RR for graft loss for study vs reference group of 0.33 (p=0.003). Adding CMV disease to the list of confounding factors, selecting the transplants that survived 3 months, did not change the results for the other variables: the influence of CMV disease on graft survival was not statistically significant, and the difference between study and reference group remained statistically significant (RR=0.22, p=0.028).

Acute rejections

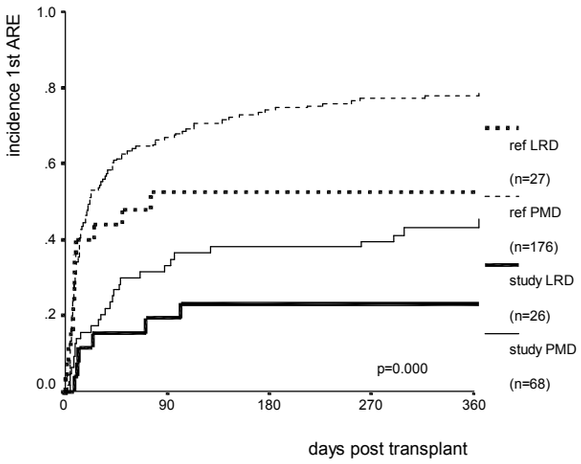
During the first year 46 acute rejection episodes occurred in the study group, amounting to 0.5 episodes per patient; 235 in the reference group, 1.1 episodes per patient (p<0.001). About 50% in both groups were proven by biopsy. Figure 2 shows the time to first ARE in the study and the reference group, classified by donor source.

Figure 1.



Cumulative graft survival during the first 2 years after transplantation for study group and reference group, both divided in LRD and PMD transplantations

Figure 2.



Cumulative incidence of first acute rejection episode in the first year after transplantation for study group and reference group, both divided in LRD and PMD transplantations

In the study group 58 patients (63%) remained rejection-free during the first year, in LRD 77%, PMD 59%. This was significantly better than in the reference group: 56 patients (28%) remained rejection-free, in LRD 52%, PMD 26% (p<0.001). The incidence of steroid-resistant acute rejection was 16% in the study group, compared to 30% in the reference group (p=0.055). The trend towards a higher degree of complete recovery in the study group was not significant (Table 3).
After correction for possibly confounding factors (pre-emptive versus post-dialysis transplantation, donor source, primary kidney disease, donor and recipient age at transplantation) in Cox regression analysis the adjusted relative risk for an ARE for study versus reference group was 0.37 (p<0.001).

Delayed graft function

In both the study group and the reference group delayed graft function occurred in 17% of cases. The one-year graft survival rate after delayed graft function is significantly lower than after immediate function, in the study group 79% vs 99% (p=0.001), in the reference group 50% vs. 76% (p<0.001).

Graft function

The average GFR, calculated according to Schwartz et al,[15] modified by Morris et al,[16] of the functioning grafts at the end of the first year was 63 mL/min.1.73m^2 in both groups. In the study group patients who had experienced at least one acute rejection episode, had a GFR of 52 mL/min.1.73m^2 at the end of the first year; patients without a rejection episode had a GFR of 69 mL/min.1.73m^2 (p<0.001). In the reference group these values were 58 and 75 mL/min.1.73m^2 respectively (p<0.001).

Table 3. Acute rejection episodes (ARE) within 1st year

	Triple therapy		Reference group		
	n	(%)	n	(%)	*p*
Number of transplants	96	(100)	207	(100)	
Number of AREs per patient					
0	58	(63)	56	(28)	<0.001
1	24	(26)	77	(39)	
2	8	(9)	40	(20)	
3 or more	2	(2)	25	(13)	
Missing	4		9	9	
Total of ARE in 1st year (n)	46	(100)	235	(100)	
Biopsy proven AR	21	(46)	118	(50)	ns
Mean nr of ARE per patient	0.5		1.1		<0.001
Recovery					
Complete[a]	29	(69)	120	(62)	ns
Partial[b]	11	(11)	63	(32)	
Graft loss	2	(5)	15	(8)	
Missing	4		37		
Steroid sensitivity of ARE (1st treatment methylprednisolone)					
Number with data	45	(100)	171	(100)	
Sensitive	38	(84)	120	(70)	
Resistant	7	(16)	51	(30)	0.055

[a]Complete recovery is defined as serumcreatinine value 2 weeks after end of treatment of less than 115% of pre-rejection value
[b]Partial recovery as more than 115%

Use of antihypertensive medication

In the study group 50 out of 89 (61%) patients did not use any antihypertensive drug at the end of the first year, in the reference group 51 out of 144 (35%, p=0.002).

Adverse events

In both groups one case of malignancy was registered during the first 2 years, i.e. in the study group a case of lymphoproliferative disease, associated with primary EBV-infection, and in the reference group a HHV8-related Kaposi sarcoma (n.s.). CMV disease with hospitalisation for intravenous treatment with gancyclovir occurred in 9 out of 81 patients in the study group (11%) and in 20 out of 78 cases in the reference group (26%, Table 4).

The data were incomplete in 15 patients of the study group, and in 21 of the reference group, these patients were not included in this analysis. The two groups showed similar distribution of serologic CMV status of donor (D) and recipient (R) at transplantation. In the study group 5 patients with a CMV status D+R- (n=26) developed CMV disease (19%), 1 with D-R+ (10%), and 3 with D-R- (8%). In the reference group the distribution was as follows: 10 cases with D+R- (45%), 3 with D+R+ (38%), 3 with D-R+ (33%) and 4 with D-R- (10%). The differences between the groups are significant (p=0.017). Five of the 6 patients with CMV disease in the study group who had received prophylaxis, developed the disease within the first 3 months. In 4 patients in the study group a new complication was described: persistent respiratory complaints, with bronchiectasis on high resolution CT-scan.[17]

Table 4. Symptomatic CMV disease during the first year after transplantation, according to CMV serology at transplantation (cases with incomplete data in the study group n=15, in the reference group n=21, have been left out)

	Study group			Reference group (1990-1995)			
CMV-status	n	Disease	No disease	n	Disease	No disease	
D+R-	26	5 (19)[a]	21	22	10 (45)	12	p = 0.05
D+R+	9	0 (0)	9	8	3 (38)	5	p = 0.02
D-R+	10	1 (10)	9	9	3 (33)	6	
D-R-	36	3 (8)	33	39	4 (10)	35	
Total	81	9 (11)	72	78	20 (26)	58	p = 0.02

[a] numbers (percentages)

Change of therapy

At the end of the first year data on immunosuppressive therapy were available in 80 of 89 patients in the study group. Sixty five of these 80 patients (81%) were still on triple therapy, 55 on the initial Pred/CsA/MMF, in 10 patients the CsA was switched to tacrolimus (TCL). In 9 patients the MMF had been stopped: 8 of them were treated with Pred/CsA, 1 with Pred/TCL. Reasons for the interruption of MMF were side effects (diarrhea), CMV disease, lymphoproliferative disease or inclusion in another study. Six patients were treated with Pred/MMF, because they had entered into another study 6 months after transplantation. Switching from CsA to TCL was precipitated because of the cosmetic side effects of CsA.

In the reference group at the end of the first year 28% of patients were on Pred/CsA/AZA, 29% on Pred/AZA, 43% on Pred/CsA.

Discussion

In this study the one-year graft survival rate showed a spectacular, highly significant improvement: from 73% in the historical reference group to 92% in the more recent study group with a new immunosuppressive protocol, consisting of corticosteroids, CsA and MMF. The differences were recognizable both in LRD and in PMD transplants. In a comparable German study with Pred/CsA/MMF in pediatric kidney transplantation the 3 year graft survival in the study group was 98%, compared to 80% in the historical reference group.[18]

In the pooled placebo- and AZA controlled studies in adults on the same triple therapy the one-year graft survival improved only slightly and not significantly, from 88% to 90%.[14] The difference between the 2 pediatric studies and the adult studies may reflect the difference between children and adults, but also between the use of a historical and a randomized control group.
As was our primary objective, the harm done to the grafts by ARE was evidently less in the study group than in the reference group, measured in several ways. First, the incidence of ARE strongly diminished. The lower incidence of CMV disease may have contributed to this effect.[19] Second, the graft losses that were the direct consequence of ARE, decreased. Third, the ARE episodes tended to be less aggressive in the MMF group, as suggested by a two-fold reduction of the frequency of steroid resistance. No significant improvement in reversibility is shown. The German Pediatric Renal Transplantation Study Group reported a similar incidence of ARE at 6 months posttransplant, i.e. 28% of patients treated with Pred/CsA/MMF, compared to 59% in the historical reference group on Pred/CsA/AZA.[20] In the large placebo and azathioprine controlled studies on MMF in adults the incidence of ARE during the first 6 months significantly decreased from 31-46% to 13-20%.[12,13]

However, as mentioned above, part of the improvement may be the result of the use of a historical control group. Primarily the new immunosuppressive protocol was developed not as a study protocol, but rather as a shared treatment protocol, of which the results would be compared to those of transplantations in the past years. But, the values of several baseline parameters differed between the study and the reference group. Therefore, the recent improvement will reflect contributions of a number of factors. First, there was a general trend over time towards a higher proportion of LRD transplants. As LRD grafts obviously yield better graft survival than PMD grafts, both were analyzed separately. Second, partly due to the higher LRD/PMD ratio the proportion of pre-emptive transplants was higher in the study group, because an LRD transplantation can be arranged more easily. The graft survival of pre-emptive transplants was excellent. Third, in recent years the Dutch centers used fewer kidneys from pediatric donors, especially from those under 5 years old. Inquiry with Eurotransplant revealed a strongly decreased contribution of children in the donor pool over the studied period (G. Persijn, pers. communication). Apart from the smaller pediatric donor supply the use of small pediatric kidneys for pediatric recipients may be restricted by publications reporting the increased risk of combining young donors with young recipients.[4] Fourth, the cold ischemia time fortunately became shorter over time, reflecting more efficient logistics of organ exchange. And finally, the incidence of CMV disease decreased as a result of the simultaneous introduction of a shared protocol for the prophylaxis of CMV. However, after statistical correction for all the above mentioned factors by Cox' regression analysis, the results of the study group still remained significantly better than those of the reference group, indicating the influence of the introduction of MMF. A part of the improvement in graft survival in the earlier mentioned German pediatric study[18] may be due to the same change in baseline data over time as we saw, e.g. cold ischemia time, since the German centers obtain their post-mortal donor kidneys from the same source as we do, Eurotransplant.

Our results do not show an improvement in kidney function in the study group compared to the reference group, in contrast to the German study group data which report better GFR values in the study group than in the reference group. Taking the GFR as a surrogate marker for graft survival, the increased number of recipients who remained rejection-free, and the finding that rejection-free patients had a better GFR, support the hypothesis that this regimen will benefit the long-term graft survival.

One of the major side effects of many immunosuppressive drugs is high blood pressure. The introduction of MMF led to the use of less antihypertensive therapy. The same trend was reported by the German study.[18]

More potent immunosuppression could lead to a higher incidence of malignancies and of (opportunistic) infections. We chose to compare the incidence of malignancies and CMV disease as indicators of overimmunosuppression. Fortunately the incidence of malignancies was fairly small. In both study and reference group only one case of malignancy occurred, both virus-associated, each within the first six months after transplantation. To detect differences in incidence a larger population and a long term follow up is required. In the NAPRTCS database the reported incidence of malignancies in pediatric kidney transplantation is 1.5%.[4] In the adult MMF studies at 3 years post-transplant an incidence of lymphoproliferative disease of 1.2% after 3 years was reported in the MMF group (2 g daily), compared to 0.6% in the control group, of skin cancer of 11.1% in the MMF group and 13.6% in the control group.[21] These figures suggest that following the introduction of MMF the incidence of malignancies did not increase.

The finding that the proportion of transplanted children with CMV disease in our study (18%) is much higher than that reported in adult patients, e.g. from the USRDS between 1994 and 1998 (2.2%),[22] relates to the larger proportion of D+R- patients in the pediatric age group. A high incidence of CMV disease in MMF treated children has been reported in an early uncontrolled study, creating fear of an increase of CMV disease caused by MMF.[23] In the above mentioned USRDS report, among the factors independently associated with a higher risk of CMV-disease, maintenance therapy with MMF was one with a slightly elevated relative risk (RR 1.75).[22] In this study by far the most important factor was the D+R- status (RR 5.19), followed by D+R+ (RR 2.04). In the adult MMF studies no significant increase in CMV disease in MMF patients was shown compared to the control groups: 3.6% vs 2.4%,[12] 7.0% vs 6.8%.[13] In the German pediatric study 16% of the patients who used MMF, showed tissue-invasive CMV disease.[20] Whether or not CMV-prophylaxis was prescribed, is not mentioned. Remarkably, in our study the incidence of CMV-disease in the study group was significantly lower (11%) than in the reference group (26%). For a large part this may be explained by the fact that CMV prophylaxis was given to all patients in the MMF group with CMV status D+R+, D+R-, and D-R+, in contrast to therapy with only hyperimmunoglobulin in a small selection of patients in the reference group.

Withholding CMV prophylaxis to D+R- graft recipients treated with Pred/CsA/MMF has been reported to result in a high incidence of CMV disease, compared to patients treated with Pred/CsA.[24] Our results confirm the efficacy of CMV prophylaxis in the Pred/CsA/MMF group.

Conclusion

In our study group treated with corticosteroids, cyclosporine and mycophenolate mofetil, compared to a historical reference group treated with different combinations of corticosteroids, cyclosporine and azathioprine, in both LRD and PMD grafts the incidence of ARE has decreased, and the short term survival of pediatric kidney transplantation improved considerably. Also the graft loss due to ARE has been reduced. Apart from the effect of the immunosuppressive regimen the change in baseline data and the introduction of CMV prophylaxis may have contributed in this improvement, though not significant in Cox' regression analysis. The finding that patients without ARE have better GFR than those who have ARE, together with the increased proportion of patients who remain rejection-free, suggests improvement of the graft survival in the long run. Whether the diminished incidence of ARE effectively will lead to the reduction of chronic allograft nephropathy can only be determined during long-term follow up.

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CHAPTER 5

INDUCTION WITH BASILIXIMAB ALLOWS DELAYED INTRODUCTION OF CYCLOSPORINE IN PEDIATRIC KIDNEY TRANSPLANTATION

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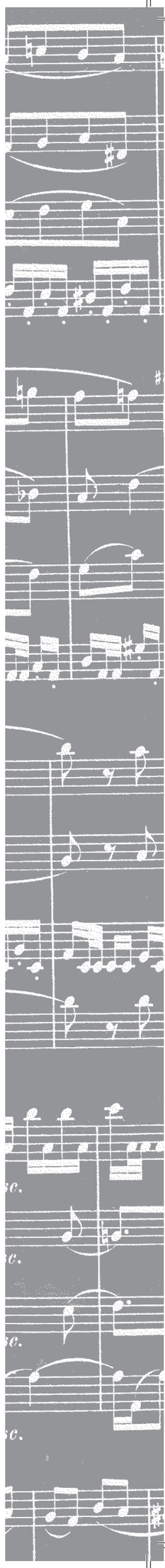


Abstract

Background

Delayed graft function and acute rejections adversely affect the long-term survival of kidney transplantation. In order to decrease the incidences of these phenomena we changed the initial immunosuppressive protocol in pediatric kidney transplantation in the Netherlands.

Methods

We compared a 4 year cohort (n=123) treated with basiliximab and delayed onset cyclosporine (CsA), to the preceding cohort (n=110) in which CsA was started already pre-operatively. Both cohorts were treated with mycophenolate mofetil (MMF) and corticosteroids as well. All consecutive transplantations were included.

Results

The incidence of delayed graft function did not significantly differ between the cohorts (10% and 13%, in basiliximab and control group). Significantly fewer patients in the basiliximab group had acute rejection episodes (20% vs. 36% in control group, p=0.007). The mean estimated glomerular filtration rate at 1 year and graft survival at 2 years post-transplant did not differ between groups (62 vs. 64 ml/min.1.73m^2, and 89% vs. 92% respectively).

Conclusion

Adding basiliximab to triple initial immunosuppressive therapy (corticosteroids, CsA and MMF) while delaying the onset of CsA did not reduce the incidence of delayed graft function in pediatric kidney transplantation. Yet, fewer acute rejections were noted. However, long-term favorable effects could not be detected in this study.

Introduction

Delayed development of graft function (DGF) has adverse long-term effects, both in children and adults,[1-3] and increases the risk of acute rejections.[4] The incidence of DGF is approximately 30% in adult kidney transplantation and has been relatively stable over recent years.[5] In pediatric transplantation, its incidence is 15-20%, and higher in deceased than in living donated transplantations.[3, 6] In the Netherlands, this incidence has not changed significantly between 1985 and 2000.[6] The use of cyclosporine (CsA) has been shown to prolong delayed graft function, in adults as well as in young children.[7-9]

The incidence of DGF could therefore perhaps be reduced by delaying exposure of the graft to CsA. This nephrotoxic agent has a vasoconstrictive effect in the grafted kidney, thereby aggravating the ischemic injury of the transplantation procedure. This strategy proved beneficial in an adult population at high risk for DGF treated with steroids, MMF and basiliximab.[10, 11] Basiliximab, an interleukin-2 (IL-2) receptor antagonist, allowed delayed introduction of CsA, a suppressor of IL2 synthesis. It is a chimeric mouse-human monoclonal antibody to the IL-2 alpha receptor on the surface of activated T-lymphocytes. The addition of IL-2 receptor antagonists to double (CsA and corticosteroids) or triple (CsA, azathioprine and corticosteroids) immunosuppressive therapy has led to significantly fewer acute rejection episodes.[12-14]

The early post-transplant period may additionally be complicated by acute rejection episodes.[15] In the Netherlands 37% of children with a kidney transplant treated with triple immunosuppressive therapy – CsA, mycophenolate mofetil, and corticosteroids – experienced at least one acute rejection episode.[6]

Five years ago we added basiliximab to our initial triple immunosuppressive regimen, aiming to start CsA only when graft function was established. We hypothesized that this would decrease the incidence of DGF without increasing the risk of acute rejection episodes in the first year. Ultimately this might improve long-term graft function, assessed in terms of glomerular filtration rate (GFR) at various time points.

Methods

Design

This was a retrospective multicenter open-label cohort study conducted in five institutions for pediatric kidney transplantation, i.e. four in the Netherlands and one in Belgium, comparing two regimens for initial immunosuppression in consecutive cohorts.

Patients

Enrolled were all consecutive patients receiving a kidney transplant in the participating centers between 01-01-1998 and 01-01-2006, with a follow-up of at least 1 year and ending at the transfer to the adult care.

The primary endpoint of the study was the incidence of DGF. Secondary endpoints were the incidence of acute rejection episodes, the estimated GFR (eGFR) at 3 months and 1 to 3 years post-transplant, and graft survival.

Immunosuppression

The participating centers shared the same initial immunosuppressive regimen for pediatric recipients of a kidney transplant. The first cohort (control group), transplanted between 01-01-1998 and 01-01-2002, received corticosteroids, CsA and mycophenolate mofetil (MMF). Preoperative loading doses were: 300 mg/m^2 methylprednisolone intravenously, 10 mg/kg CsA orally, 600 mg/m^2 MMF orally. Post-operatively they received 40 mg/m^2 (methyl)prednisolone twice daily. Thereafter, the dose was tapered to 7.5 mg/m^2, once daily, at week 6, and 5 mg/m^2 at 6 months post-transplant. From 6 h after revascularisation CsA was administered intravenously for 24 h in a dosage of 3 mg/kg/24 h, followed by an oral dose adjusted to a trough level of 150–250 mg/L during the first 6 weeks, of 150–200 mg/L during the second 6 weeks, and of 100–150 mg/L thereafter. A dose of 600 mg/m^2 MMF was administered twice daily as soon as oral intake was possible.

From 2002 onwards patients were treated with a revised protocol (second cohort, basiliximab group). This involved administration of two doses of basiliximab, the first 1-6 hours preoperatively, the second on the fourth day post-transplant. The dose was 10 mg in children weighing less than 35 kg, and 20 mg in children weighing more. CsA was introduced after the creatinine level reached 50% of the pre-transplant and pre-dialysis value. The initial dose was 4 mg/kg twice daily, adjusted to a trough level of 150-200 mcg/l, after 6 weeks adjusted to 100-150 mcg/l. Treatment with corticosteroids and MMF was according to the earlier protocol. If DGF occurred, tapering of steroid dosage was postponed and CsA was withheld.

In both cohorts triple therapy was scheduled to be continued at least during the first year.

Definitions

DGF was assessed with two methods. The need for dialysis therapy in the first week after transplantation, with subsequent recovery of kidney function, was a dichotomous criterion for DGF. In order to construct a time-dependent variable, the time to reach 50% of the pre-transplant plasma creatinine value (creat t½) was calculated in periods of 24 hours post-transplant.

Acute rejection: a sudden rise in creatinine to more than 115% of the previous stable value with no other identifiable cause, followed by the prescription of a full course of anti-rejection treatment consisting of methylprednisolone IV 10-20 mg/kg, 3 to 5 times on consecutive or alternate days. Taking a biopsy in case of a suspected acute rejection episode was left to the discretion of the local physician. However, in case of a steroid resistant acute rejection, a graft biopsy was taken per protocol and a 10-14 days course of rabbit ATG was instituted.

Glomerular filtration rate (eGFR) was estimated according to the Schwartz formula, modified according to local laboratory practices.[16, 17]

Graft loss
Graft loss start of any form of chronic dialysis, a new transplant, or death with a functioning graft.

Hypertension
Hypertension: scored with a scale assessing two parameters: a. systolic or diastolic blood pressure below (score 0) or above (score 1) the 95th percentile for age and sex;[18] and b. number of antihypertensive and diuretic drugs (no drugs = 0, 1 drug = 1, 2 or more drugs = 2). Scores could thus range from 0 to 3, from no hypertension to serious hypertension.

Other treatment aspects
Thrombosis prophylaxis: All patients with a high risk of vascular thrombosis of the graft – i.e. recipients younger than 6 yr of age, donors younger than 10 yr, multiple renal arteries or veins to the graft, or a history of thrombosis – received antithrombotic therapy during 2 weeks. This consisted of either low-dosed heparin or low-molecular weight heparin.
CMV prophylaxis: seronegative recipients with a seropositive donor (D+R-) and CMV seropositive recipients (D+R+, D-R+) received prophylaxis against CMV infection during the first 3 months after transplantation. Seropositive patients received valacyclovir; seronegative patients with seropositive donors valgancyclovir. In the first cohort two centers used acyclovir and megalotect as CMV-prophylaxis instead.

Statistical analysis
The data were analysed on an intention-to-treat-basis. Differences between Kaplan–Meier survival curves, used for patient and graft survival, time to first acute rejection and creat t½, were tested for statistical significance with the log rank test. Differences in other parameters were tested with either chi-square for categorical variables, Student's *t*-test for continuous variables with a normal distribution or Mann Whitney test for continuous variables without a normal distribution. Cox regression analysis was used to adjust the comparison between cohorts for possible confounding factors. Repeated measurements ANOVA by SAS statistical software was used to detect differences in GFR over time between the groups. For the multivariate analyses potential independent predictors of outcome were identified by univariate analysis. Variables correlated to the outcome variable of interest with a significance level $p<0.10$ were included in the multivariate model and the factor of primary interest, cohort, was forced into the model. Two-sided *p*-values less than 0.05 were considered to indicate statistical significance.

Results

Included in the study were 215 patients, who received 233 kidneys, 123 in the control and 110 in the basiliximab group. The baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics

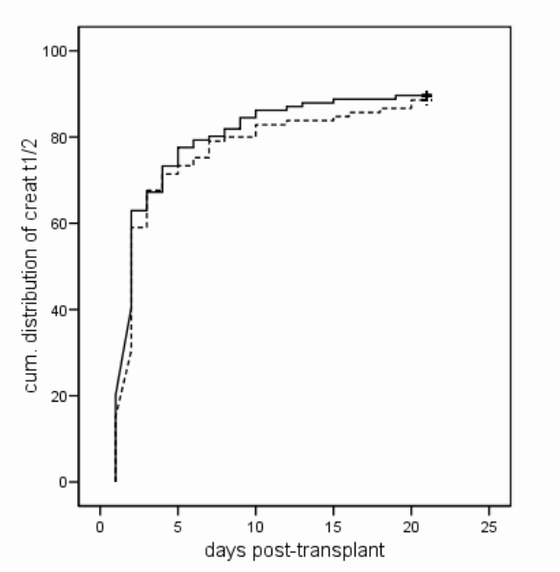
	control n = 123	available n	basiliximab n = 110	available n	*p*
Age at transplantation [a]	11.6 (3.4-18.0)	123	12.6 (2.7-18.1)	109	ns
Age at start RRT [a]	9.5 (0.02-17.9)	116	10.9 (0.04-17.6)	99	ns
Recipient gender (female (%))	52 (42%)	123	45 (41%)	110	ns
Primary kidney disease [c]		119		107	
Congenital structural abnormalities	63 (53%)		51 (48%)		
Acquired disease	33 (28%)		37 (35%)		
Other	19 (16%)		14 (13%)		
Unknown	4 (3%)		5 (5%)		ns
First transplant [d]	95 (79%)	121	92 (84%)	109	ns
No previous RRT	22 (18%)	123	25 (23%)	109	ns
Type of previous dialysis (PD/HD/both/none)	36/35/14/22	107	40/21/11/25	97	ns
Duration previous RRT (yr) [a]	1.2 (0.0 – 12.8)	120	1.0 (0.0 – 16.4)	102	ns
Donor source living [e]	33 (27%)	123	38 (35%)	110	ns
Mismatches HLA - DR [b]	0.6 ± 0.6	102	0.6 ± 0.5	101	ns
Mismatches HLA - A, B, DR [b]	2.2 ± 1.2	102	2.2 ± 1.2	101	ns
Donor age (only DD) [a]	39 (2-62)	88	41 (3-63)	71	ns
Cold ischemia time (only DD) [b]	21 ± 7	69	19 ± 5	64	ns
Donor/recipient CMV serological status		111		91	
Positive/negative	31 (28%)		33 (36%)		
Positive/positive	10 (9%)		15 (17%)		
Negative/positive	19 (17%)		15 (17%)		
Negative/negative	51 (46%)		28 (31%)		ns

Data shown as: [a] median (range), [b] mean ± SD, other parameters n (%)
[c] Congenital nephrotic syndrome is included in acquired disease
[d] Of subsequent transplants, 3 in the control group and 0 in the basiliximab group were third
[e] Of the DD grafts 1 in the control and 2 in the basiliximab group were from non heart beating donors
Abbreviations: DD deceased donor, PRA panel reactive antigens, RRT renal replacement therapy

No relevant differences between the groups were detected. The basiliximab group tended to contain more CMV positive donors.

DGF, defined as the need for dialysis in the first week after transplantation, occurred in 16 patients of the control group (13%) and 11 of the basiliximab group (10%) (n.s.). The cohorts did not significantly differ in creat t½ either (Figure 1, n.s.). Cold ischemia time, donor source, recipient age group and pre-transplant dialysis were significantly associated with a longer creat t½, using univariate analysis (Table 2a). Type of dialysis before transplantation (PD vs. HD), donor age (< 10 vs. > 10 years) and serologic CMV status of donor and recipient (D+R- vs. other) were not significantly associated with creat t½. In Cox regression analysis, recipient age (older than 6 compared to younger) and donor source (DD compared to LD) were the major independent predictors of DGF.

Figure 1. Half life of pre-transplant plasma creatinine after transplantation

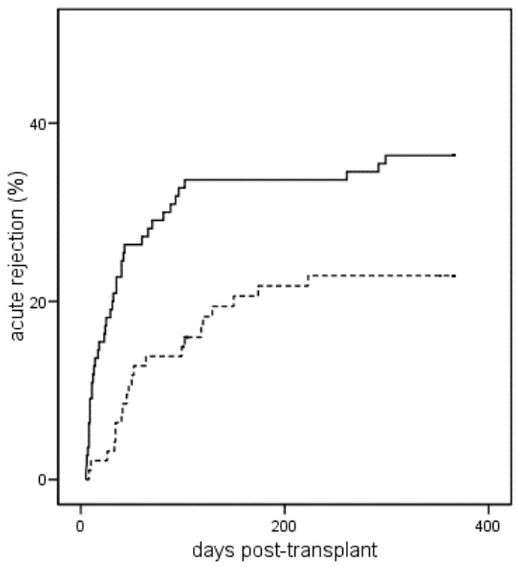


Control group solid line, basiliximab group dotted line

Acute rejection

As indicated in Figure 2, at the end of the first year after transplantation 36% of patients in the control group had experienced at least one acute rejection episode, compared to 20% of patients in the basiliximab group (p=0.007). Two patients in the control group, and one in the basiliximab group, lost their graft due to an acute rejection (n.s.).

Figure 2. Incidence of acute rejections during the first year after transplantation



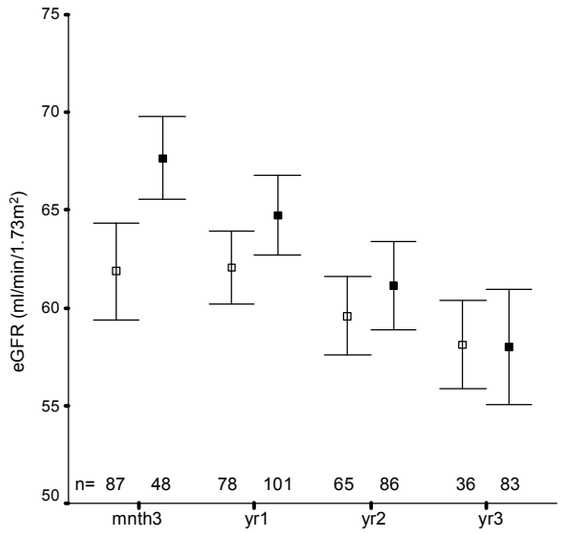
Control group solid line, basiliximab group dotted line
p-value = 0.007

Univariate as well as multivariate analysis by Cox regression (Table 2b) identified cohort and donor source to be factors independently predicting the occurrence of acute rejections. Recipient age, donor age, transplantation pre-emptively or following dialysis, DR-mismatches and previous PRA did not show a significant association with the incidence of acute rejections. Cox regression showed no significant differences between the 5 centers. Also the difference between cohorts did not differ between centers. Grafts with a creat t½ < 3 days and a survival of more than 14 days remained rejection free during the first year more often than transplants with a longer creat t½ (77% vs. 62%, p=0.043). Of the suspected acute rejection episodes, 16% were steroid resistant in both the control group and basiliximab group. Forty-one per cent of the acute rejection episodes in the control group and 52% in the basiliximab group were biopsy-proven (n.s.).

eGFR values at several time points are shown in Figure 3. Repeated measurements ANOVA demonstrated a significant decrease over time in both groups (p=0.004). The difference between the groups was not significant, neither regarding the mean level at each time point nor the mean decrease over time. At one year post-transplant the eGFR was 62 in the control and 64 ml/min.1.73m^2 in the basiliximab group. Predictors of eGFR were analysed in 197 transplants that were followed at least 1 year (107 control and 90 basiliximab patients) (Table 2c). Creat t½, incidence of acute rejection and recipient age were significant determinants of eGFR at 1 year (p<0.05). The factor cohort was not predictive. After DGF a mean one-year eGFR of 47 ml/min.1.73m^2 was seen, compared to 65 in patients with immediate function (p<0.001). Likewise, eGFR was lower in patients with acute rejections in the first year: 51 vs. 67 ml/min.1.73m^2 in patients without acute rejections (p<0.001).

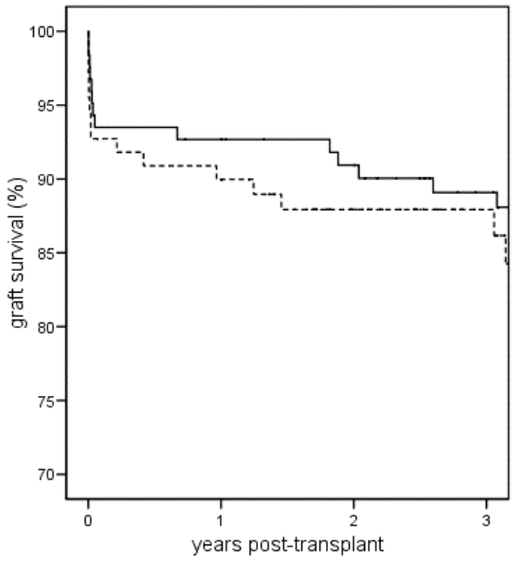
Graft survival at one year, censored for death, was similar: 92% in the control group, and 89% in the basiliximab group (n.s., Figure 4).

Figure 3. Estimated GFR (mean ± SEM) along time, at 3 months, 1, 2 and 3 years post-transplant, by study group (solid squares – control; open squares – basiliximab)



Graft survival at 3 years was 88% and 87% respectively. Thrombosis of the renal vessels caused graft loss in the first year in four cases (3.3%) in the control group, and in six cases (5.5%) in the basiliximab group (n.s.). Grafts with a creat t½ of less than 3 days that survived at least 14 days, had a significantly better two-year graft survival than grafts with a longer creat t½: 99% and 87%, respectively (p<0.001). The absence or presence of an acute rejection episode in the first year had a similar effect: 98% vs. 91%, p=0.004. Regarding patient survival: four patients died, i.e. one in the control group, due to a cerebrovascular accident, and three in the basiliximab group, 1 due to a cerebrovascular accident, one to an infectious cause and one with an uncertain cause of death.

Figure 4: Graft survival, censored for death



Control group solid line, basiliximab group dotted line

Comorbidity

CMV disease occurred in 11 patients in the first year post-transplant, three in the control and eight in the basiliximab group (p=0.046). None of these were associated with acute rejection or graft loss. Four cases of malignancy were registered (1.7%), all within the first two years after transplantation: three in the control group and one in the basiliximab group. All four concerned lymphoproliferative disease.

Other treatment aspects did not change evidently over the years. The immunosuppressive regimens used after the initial treatment did not differ significantly between the two groups. Within the cohorts, however, the regimens gradually changed over time, as delineated in Table 3. After the first year, double therapy replaced triple therapy to become the most prevalent form. The proportion of patients using tacrolimus instead of cyclosporine increased. Between 3 months and 2 years after transplantation the dose of prednisolone decreased from 8.3 ± 2.9 to 5.4 ± 2.3 mg/m^2 body surface area in the control group, and from 8.1 ± 4.1 to 4.8 ± 2.6 mg/m^2 in the basiliximab group (n.s. between the groups). The hypertension score changed over time in the control group from 2.0 to 1.7 between 3 months and 2 years, in the basiliximab group from 1.9 to 1.5 (n.s. between the groups).

Table 2. Univariate and multivariate analysis

A Creatinine t 1/2	univariate analysis		multivariate analysis		
Factor	% of patients with creat t½ 3 days or longer[a]	*p*	hazard ratio	confidence interval	*p*
Cohort (control vs. basiliximab)	37 vs 41	0.432	1.3	1.0 to 1.8	0.088
Donor source (LD vs. DD)	15 vs 50	<0.001	1.8	1.2 to 2.6	0.002
Recipient age (< 6 vs. > 6 yr)	29 vs 41	0.04	1.5	1.0 to 2.3	0.041
Cold ischemia time (< 19 vs. > 19 hr)	26 vs 61	<0.001	1.4	1.0 to 2.1	0.079
Previous dialysis (yes vs. no)	41 vs 30	0.051	---	---	---

B Occurrence of acute rejection					
Factor	% of patients rejection-free at 1 yr[b]	*p*	hazard ratio	confidence interval	*p*
Cohort (control vs. basiliximab)	64 vs 79	0.012	1.7	1.0 to 3.0	0.038
Donor source (LD vs. DD)	81 vs 66	0.036	0.5	0.3 to 1.0	0.031
Recipient age (< 6 vs. > 6 yr)	83 vs 69	0.144	0.4	0.2 to 1.0	0.05
Donor age (< 10 vs. older)	73 vs 70	0.831	---	---	---
Previous dialysis (yes vs. no)	71 vs 80	0.187	---	---	---
Ranking order (1st transplant vs. later)	74 vs 66	0.585	---	---	---
Creat t½ (< 3 days vs. longer)	82 vs 72	0.178	---	---	---

C eGFR at 1 year post-transplant					
Factor	mean per group (ml/min.1.73m^2)	*p*	regression coefficient	confidence interval	*p*
Cohort (Control vs. basiliximab)	62 vs 64	0.383	1.0	-7.9 to 2.6	0.316
Acute rejection (vs no rejection)	50 vs 66	<0.001	---	---	---
Creatinine half-life (< 3 d vs longer)	68 vs 53	< 0.001	22.9	8.4 to 20.1	< 0.001
Recipient age (< 6 vs > 6 yr)	61 vs 70	0.016	4.4	0.4 to 14.6	0.038
Donor source (LD vs DD)	66 vs 61	0.097	0.2	-4.6 to 7.4	0.654
Ranking order (1 vs > 1)	64 vs 58	0.128	---	---	---

[a] Kaplan-Meier estimates of percentage of patients not having reached half of pre-transpl. creatinine before 3 days

[b] Kaplan-Meier estimates of percentage of patients with no acute rejection within the 1st year

Table 3. Immunosuppressive regimens over time after transplantation

	M3		Y1		Y2	
	control	basiliximab	control	basiliximab	control	basiliximab
pred-CsA-MMF	99 (93%)	76 (84%)	81 (76%)	60 (71%)	27 (28%)	12 (18%)
pred-TCL-MMF	3 (3%)	4 (4%)	9 (8%)	9 (11%)	10 (10%)	5 (8%)
pred-CsA	3 (3%)	3 (3%)	7 (7%)	7 (8%)	30 (31%)	24 (36%)
pred-MMF	1 (1%)	4 (4%)	7 (7%)	3 (4%)	20 (21%)	14 (21%)
pred-TCL	0 (0%)	4 (4%)	3 (3%)	4 (5%)	8 (8%)	10 (15%)
other	0 (0%)	0 (0%)	0 (0%)	1 (1%)	2 (2%)	2 (3%)
n	106	91	107	84	97	67

Discussion

In this retrospective cohort study we examined the influence of the addition of basiliximab and the delayed introduction of CsA on delayed graft function and acute rejections in pediatric kidney transplantation. We also assessed the effects of this strategy on longer-term graft function and survival. The modified immunosuppressive treatment did not significantly decrease creat t½ nor the need for post-transplant dialysis, two markers for delayed graft function. In the first cohort, 13% of patients needed dialysis post-transplant, a figure lower than expected, based upon earlier results.[6] In view of its confidence limits - 8 to 21% -, however, this low incidence does not conflict with the previously reported incidence of 19%.[19] The decline to 10% in the second cohort could not reach statistical significance in this study. A possible drawback of defining DGF as need of dialysis in the first week post-transplant is the rather crude discriminatory capacity, since the graft function development may be well slowed down without dialysis being necessary. Furthermore, individual doctors will assess the need for dialysis differently, and patients may be dialyzed for other reasons than lack of graft function, e.g. in hyperoxaluria. By measuring the half life of the pre-transplant creatinine value, we tried to find a more objective criterion. Still, time to reach 50% of baseline creatinine value did not differ between the two cohorts. Previous studies on effects of the addition of basiliximab on DGF incidence are not equivocal.[14, 20, 21] In a study including adults with long ischemia time, older donors and/or recipients, and a lower degree of HLA-matching, the trend towards a positive effect appeared to be more outspoken in high risk patients.[11] A possibly favorable effect of sequential immunosuppression was found in a small study with very young recipients, although the agent was ATG rather than an IL2-receptor antagonist.[9] All in all, our findings and those from the literature do not support delayed introduction of CsA – with basiliximab added to cover the immunosuppressive gap in the first days after transplantation – to decrease the incidence of DGF in a non-selected pediatric renal recipient population.

The change in immunosuppression resulted in fewer acute rejection episodes, with an incidence comparable to that achieved by the introduction of IL2 receptor antagonists with concurrent CsA therapy in adults.[12-14] In contrast, a large multinational pediatric study comparing a tacrolimus-based regimen with and without basiliximab found no difference in incidences of acute rejection.[22]

Another study introduced IL-2 receptor antagonist with low dosed CsA (trough level 50-100 mcg/l) at transplantation, together with MMF and steroids. This strategy did not result in fewer acute rejection episodes at 6 months as compared with standard CsA dosage therapy without the IL2 receptor antagonist.[23] It would seem, therefore, that adding an IL-2 receptor antagonist is most effective in studies using a control group receiving less immunosuppression: double instead of triple therapy, and cyclosporine instead of tacrolimus.

Creatinine level at 1 year post-transplant in adults has been associated with long term graft survival.[1] As Filler and colleagues suggested, the eGFR calculated as a function of body height and serum creatinine, could be used as surrogate marker for long term graft survival in children.[24] Despite fewer acute rejection episodes in the first year in the basiliximab group, the change in initial immunosuppressive therapy was not beneficial in terms of estimated GFR over the first 3 years. In line with our findings, Ekberg and colleagues reported that low dose CsA combined with an IL2-receptor antagonist gave no better graft function at one year after transplantation than did standard CsA dose without an IL-2 receptor antagonist. But, as mentioned above, these authors also found no difference in the incidence of acute rejections.[23]

CMV disease was more frequent in the basiliximab cohort, but still, this incidence was low. This cohort tended towards a higher proportion of CMV D+R- combination compared to the control cohort. Schnitzler and colleagues found a 4 times higher incidence of CMV disease, a non-significant increase in acute rejection incidence, but more than 20% increase in graft loss for the seronegative recipient receiving a kidney from a seropositive donor as compared with a seronegative donor.[25]

A limitation of a retrospective cohort study is the possibility that changes in other aspects of care than the ones studied underlie differences between cohorts. In the present study, we could point at the (non-significant) higher rate of biopsies to prove acute rejection in the later cohort – suggesting that physicians over time have become less reluctant to perform biopsies. Judging from the higher proportion of biopsy proven acute rejection episodes, the diagnosis of acute rejection tends to be stricter in the second cohort, thus attenuating the found difference in incidences of acute rejection in the two cohorts.
As far as other treatment schedules are concerned, the antihypertensive and maintenance immunosuppressive treatment remained similar in both cohorts.

In conclusion, the addition of basiliximab to triple initial immunosuppressive prophylaxis, in combination with delayed start of CsA, reduced the incidence of acute rejections, but not that of delayed graft function in this unselected population of children with a kidney transplant. Effects on graft function and survival at one year post-transplant could not be detected.

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CHAPTER 6

MAINTENANCE IMMUNOSUPPRESSION WITH MYCOPHENOLATE MOFETIL AND CORTICOSTEROIDS IN PEDIATRIC KIDNEY TRANSPLANTATION: TEMPORARY BENEFIT BUT NOT WITHOUT RISK

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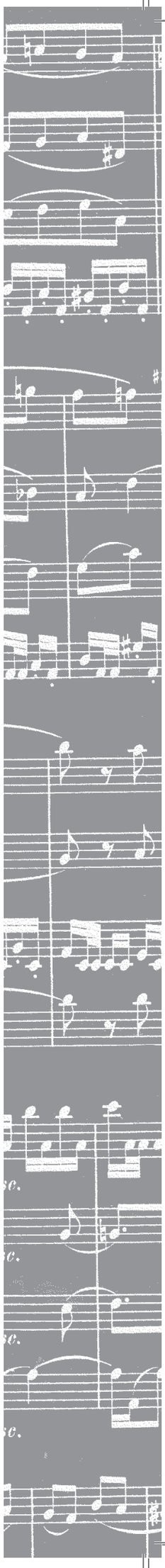


Abstract

Background

Aiming at reducing cyclosporine toxicity, we investigated safety and efficacy of mycophenolate mofetil (MMF) as an immunosuppressive drug in pediatric kidney transplantation compared to cyclosporine, both in combination with corticosteroids.

Methods

One year after kidney transplantation, children on triple immunosuppression, having experienced no more than one, steroid-sensitive, acute rejection episode, were randomized to withdrawal of either cyclosporine (CsA) or MMF, and were followed for 2 years.

Results

In each group, 2 patients had an acute rejection episode during withdrawal. Treatment failure occurred in 3 of 21 MMF and 5 of 23 CsA patients. Final analysis was for 18 patients in either group. A larger than 10 mL/min.1.73m^2 decrease in glomerular filtration rate was seen in more patients on CsA than on MMF (73 vs. 29%, p=0.019). No differences in blood pressure or nightly drop of blood pressure were noted. Hypercholesterolism improved in the MMF (-16%), but not the CsA group (+5%, p<0.05), over the first, but not over both study years. Differences in triglycerid levels between groups were not shown. At study end, MMF patients tended to have lower hemoglobin levels than patients on CsA. Two MMF patients experienced a first acute rejection episode during the second study year, resulting in chronic transplant glomerulopathy with graft loss in one and deterioration of kidney function in the other.

Conclusion

In pediatric kidney transplantation, maintenance immunosuppression with MMF together with corticosteroids has short-term benefits for kidney function and lipid pattern compared to CsA, but is not without risk of complications.

Cochrane trial registration number ISRCTN89278733
The Dutch Kidney Foundation funded the study

Introduction

Mycophenolate mofetil (MMF) has gained a place in the immunosuppressive treatment of kidney transplantation, either added to a regimen of corticosteroids and cyclosporine (CsA), or replacing azathioprine in the triple regimen corticosteroids, CsA and azathioprine. Its introduction has reduced the incidence of acute rejection episodes, both in adults[1,2] and in children.[3] In addition, it considerably improved short-term graft survival in children in the Netherlands.[4] MMF, like azathioprine (AZA), inhibits lymphoproliferation. Reported side-effects are gastrointestinal discomfort, diarrhea, and bone-marrow depression; it has no known negative effects on blood vessels or lipids. In contrast, CsA negatively affects the cardiovascular system and the kidneys: it causes vasoconstriction and increases of blood pressure, nephrotoxicity, and corroborates the dyslipidemia caused by corticosteroids.[5] Furthermore its use produces cosmetic changes, which may induce non-compliance, especially in adolescent girls. We felt it would be better, therefore, to restrict CsA treatment to the first year after transplantation. In the present prospective, open-label, study we investigated the safety and efficacy of MMF as an immunosuppressive drug in the maintenance phase of pediatric kidney transplantation as compared to CsA, both in combination with corticosteroids.

Patients and methods

This prospective, open-label, clinical trial was conducted in the 5 centers of the Dutch pediatric kidney transplantation group and was approved by the medical ethical committees of the participating hospitals. Since 1998 all patients receiving a kidney transplant in one of these centers have been treated with initial immunosuppressive triple therapy consisting of corticosteroids, CsA and MMF. In detail: (methyl)prednisolone (pre-operatively 300 mg/m^2, first week after transplant 40 mg/m^2 b.i.d., weekly dose-reduction to 7.5 mg/m^2 once daily at 6 weeks, and 5 mg/m^2 6 months after transplant); CsA (trough level in the first 6 weeks of 200-250 mcg/l, 150-200 mcg/l at 6 weeks and 100-150 mcg/l 3 months after transplant) and MMF (600 mg/m^2 b.i.d.). In 2001 the protocol was modified by adding basiliximab to the triple therapy on day 0 and day 4, while CsA was not given until the graft had started to function.

Eligible for the study were patients who at one year after transplant had not experienced more than one, steroid-sensitive, acute rejection episode, received triple immunosuppression as per protocol and showed stable kidney function. All participating patients and/or their parents gave informed consent. After enrolment they were randomized to the MMF group or the CsA group. Randomization was conducted by randomly permuted blocks stratified by center. The third drug was tapered off over 3 months and then discontinued. During these 3 months the dose of prednisolone was doubled. The dosage of MMF remained unchanged; that of CsA was adjusted to a trough level of 100-150 mcg/l. All patients were followed for at least 2 years.

Primary endpoints were change of GFR, number of acute rejection episodes (ARE), serumlipids, blood pressure and number of antihypertensive drugs. Secondary endpoints were patient and graft survival, change of hemoglobin level and the incidence of malignancies. Time-points of measurement were before withdrawal of MMF or CsA, and at 1 and 2 years after randomization. The blood tests were performed in a fasting state: creatinine, hemoglobin level, concentrations of total cholesterol, HDL- and LDL-cholesterol, and homocystein. However, LDL-cholesterol was measured in only few patients. Therefore, the concentration of the lower density lipoprotein bound cholesterol was also estimated by subtracting HDL-cholesterol from total cholesterol. The glomerular filtration rate (GFR) was measured using either the inulin single injection[6] or the inulin urine collection technique, according to local hospital policy. With survival analysis differences between the groups were studied in number of patients surviving without a decrease in GFR of more than 10 ml/min.1.73m^2. For this analysis GFR values were used estimated by a modified calculation according to Schwartz,[7,8] by which method complete data could be obtained. The 24 hour ambulatory blood pressure was measured yearly. Incidences of acute rejection episodes and numbers of antihypertensive drugs were registered.

Hypertension

High blood pressure was treated according to local policy, with ACE-inhibitors, Ca-entry blockers, beta-blockade, and/or diuretics. The mean daytime blood pressure of the 24 hour ambulatory recording was used for analysis. If missing (6 measurements in the MMF group, 5 in the CsA group), this was filled in by the mean of triple office measured blood pressures. Hypertension scores were arrived at by adding values of two parameters: mean daytime systolic or diastolic blood pressure below (score 0) or above (score 1) the 95th percentile for age,[9] and number of antihypertensive and diuretic drugs (no drugs = 0, 1 drug = 1, 2 or more drugs = 2). Scores could thus range from 0 to 3. Differences between mean day and night blood pressure were calculated.

Definitions

Graft failure was defined as the start of any form of chronic dialysis, repeat kidney transplant, or death with functioning graft. Acute rejection was defined as the prescription of a course of anti-rejection therapy, consisting of methylprednisolone, or ATG. Graft biopsies were taken only in case of steroid resistent rejection. Treatment failure was defined as the replacement of the intended immunosuppressive drug, or addition of another one.

Design and statistics

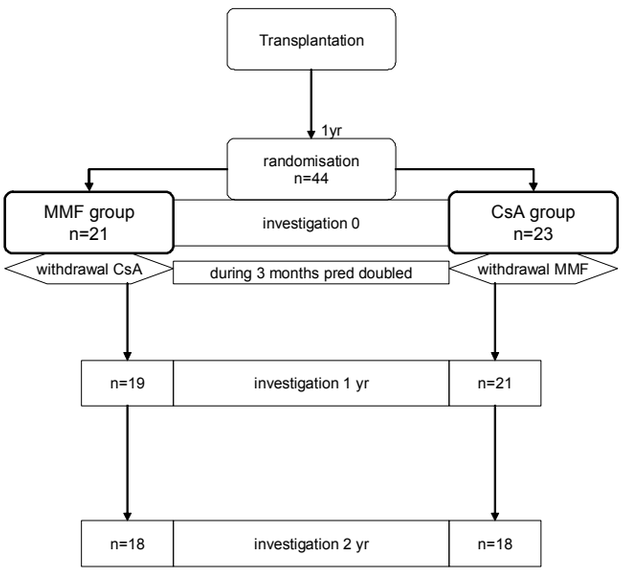
The study began 12 months after transplantation. To detect a difference in GFR of 15% at a power of 80% 50 patients were needed, calculated according to a historic population.[10] To measure the net effect of the treatment with pred/MMF compared to pred/CsA, patients in whom treatment failed were excluded from the final analysis. The reason for treatment failure is described in the Results section. Changes of variables were calculated as percentage of the value at start of the study, except for the blood pressure score which was measured as absolute difference.

Differences between the groups were tested with Pearson's chi-square for categorical variables, Student's *t*-test for continuous variables, and Wilcoxon-Gehan test in the survival analysis. A *p*-value below 0.05 was considered statistically significant.

Results

Forty-four patients were included (Figure 1). Randomization resulted in 21 patients in the MMF and 23 in the CsA group. Only one patient, in the CsA group, had experienced an acute rejection episode in the first year after transplantation. Treatment failure resulted in exclusion from analysis in three patients in the MMF group, two in the first study year, one in the second year, all caused by acute rejection. Four patients in the CsA group were excluded because of treatment failure, one in the first study year, because of acute rejection, and three in the second year because of cosmetic side-effects. One patient in this group withdrew from the study. Thus, 18 patients in the MMF group (86%) and 18 in the CsA group (78%) completed the study with the intended medication.

Figure 1.



Flow-chart of the study. One year after transplantation randomisation takes place, which is the starting point of the study, t=0. The numbers of available patients per group at yearly intervals are indicated

Acute rejections during withdrawal

Four patients, two of 21 MMF and two of 23 CsA patients, had acute rejection episodes during tapering off the other drug. In one of the MMF patients, this was caused by overt non-compliance with immunosuppressive therapy. All of these rejection episodes were steroid sensitive. They were reason to change immunosuppressive therapy for both MMF cases and one of the two CsA patients; these cases were excluded from further analysis (see in the section above).

Baseline data

Table 1 shows baseline data for the 18 remaining patients in each group. Boys were overrepresented in the CsA group; in association this group also showed a greater proportion of urologic primary kidney disease. Glomerular disease was evenly distributed between the groups. At start of the study, HDL- and LDL-cholesterol, homocystein and hemoglobin levels were within normal limits. Cholesterol was slightly above normal in the MMF group; triglycerid level was above normal in both groups. At start of the study no statistically significant differences between the groups were noted in mean age, glomerular filtration rate, blood pressure and difference in blood pressure between day and night, and hemoglobin level, nor in the distribution of donor source. Cholesterol and LDL-cholesterol levels were different, with higher values in the MMF group. Dosage of MMF was significantly higher in the MMF group than in the CsA group, dosage of prednisolone and CsA were similar.

Table 1. Baseline data

	MMF group n=18	CsA group n=18	normal
Age at start study, yr	11.9	10.9	
GFR (measured)	73.2	69.1	80 – 120 mL/min.1.73m^2
GFR (estimated)	72.1	67.4	
Hypertension score	1.4	0.9	0.0
MAP day-night	7.7	8.6	10.0 mmHg
Cholesterol	5.8	5.0	3.0 - 5.5 mmol/L
HDL-cholesterol	1.4	1.5	0.9 - 2.7mmol/L
LDL-cholesterol	4.2	2.7[a]	< 4.2 mmol/L
Triglycerids	2.0	1.7	0.4 - 1.6 mmol/L
Homocystein	14.9	16.7	6 – 18 mcmol/L
Hemoglobin	7.3	7.4	6.6 – 9 mmol/L
Primary kidney disease (n)			
Urologic/dysplasia	5	12	
Glomerular	6	6	
Metabolic	5	0	
Cystic	2	0[a]	
Gender (M/F)	8/10	14/4[a]	
Donor source (LD/DD)	7/11	8/10	
Dose prednisolone	7.5	7.6	mg/m^2.day
Dose MMF	1047	881[a]	mg/m^2.day
Dose CsA	5.6	5.9	mg/kg.day

The continuous variables are expressed as mean values
[a] $p < 0.05$

Dosage of immunosuppressive drugs

In the MMF group the dosage of MMF decreased from 1047 mg/m^2.day at start of the study to 989 at 12 months and 833 at 24 months. In the CsA group, the dosage of CsA decreased from 5.9 mg/kg.day to 4.9 and 4.7, with mean CsA trough levels of 129, 125, and 126 mg/L. The prednisolone doses gradually decreased in both groups: at start 7.5 and 7.6, at 12 months 5.6 and 4.8, and at 24 months 5.9 and 4.6 mg/m^2.day in the MMF and CsA group, respectively (n.s. at all time points).

Patient and graft survival

No patients died during follow-up. One graft in the MMF group failed at the end of the study due to chronic rejection. No malignancies were registered.

Glomerular filtration rate

Figures 2a en 2b show the individual GFR values measured by inulin clearance in patients with complete data at least at start and at the end of the first year of the study. Median values are 72, 76, and 72 at start of the study, 12 months and 24 months in the MMF group, and 69, 62, and 61 in the CsA group, respectively. Figure 3 illustrates the proportion of patients surviving without decrease of estimated GFR of more than 10 ml/min.1.73m^2. This was seen in 27% of CsA vs. 71% of MMF patients at 24 months after start of the study (p=0.0197).

Figure 2.
The individual GFR values with indication of the median GFR in patients with complete data at least at start and at the end of the first year of the study: (A) 72, 76, 72 ml/min.1.73m^2 at year 0, 1 and 2 in the MMF group, and (B) 69, 62, 61 ml/min 1.73 m^2 at year 0,1 and 2 in the CsA group

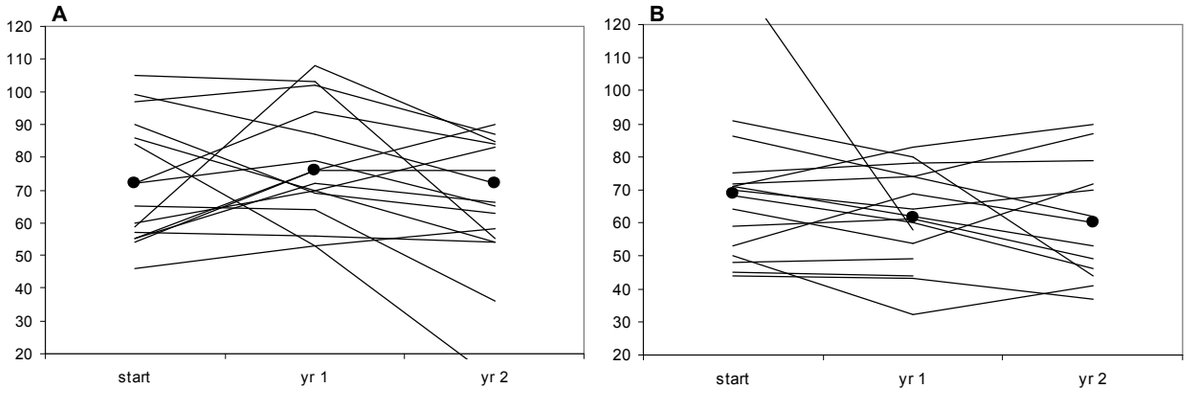
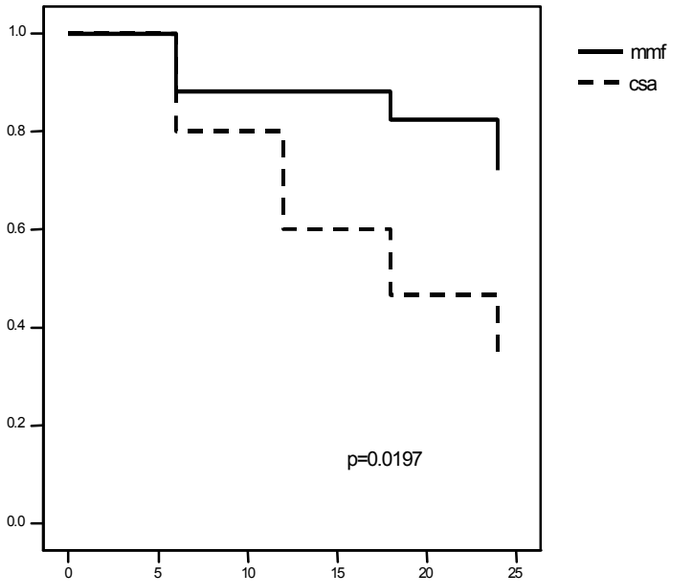


Figure 3.
Life table showing proportion of patients without decrease of GFR more than 10 ml/min.1.73m^2
On the horizontal axis the months post-transplant



Blood pressure

The mean blood pressure decreased by 9.7% in the MMF group over the first 12 months, compared to +0.8% in the CsA group (p=0.056); over 24 months these values were –6.2 and +2.8% (p=0.074). The change in blood pressure score did not differ significantly between the groups over the first 12 months (mean value in the MMF group –0.1 ± 0.2; CsA group +0.1 ± 0.3), nor over 24 months:–0.2 ± 0.3; and +0.1 ± 0.2, respectively. Two patients in the MMF, and six in the CsA group had a score 0 to begin with, and therefore could not improve. The proportion of patients with decreasing hypertension score tended to be greater in the MMF group, yet did not reach statistical significance: over 12 months 3/16 (21%) in the MMF group and 2/18 (11%) in the CsA group (n.s.), over 24 months 5/14 (36%) in the MMF group and 2/12 (17%) in the CsA group (n.s.). The mean nightly dip in blood pressure did not differ significantly between the groups at all time points: at start +5 in both groups, at 1 year +9 and +4 , and at 2 years +4 and +3 mmHg in the MMF and CsA group, respectively. The proportion of non-dipping patients (MAP day - night < 10 mmHg) at start of the study was 8/11 (73%) and 9/12 (75%), after 12 months 6/11 (55%) and 12/15 (80%) and at 24 months 9/11 (82%) in the MMF and 11/12 (92%) in the CsA group (at all time points n.s.).

Laboratory parameters

Table 2 shows the mean changes over the first 12 months and over the full 24 months of the study. Cholesterol levels, as shown in Figure 4, over the first 12 months significantly decreased in the MMF group (-16%), compared to a slight increase in the CsA group (+6%, p=0.002); over the full 24 months the difference did not reach statistical significance (-13 and +3%, respectively). However, LDL-cholesterol levels showed a similar pattern, for lower numbers of measurements however (n=7 and 8, respectively). The changes in non-HDL-bound cholesterol in the complete groups were in the same range. HDL-cholesterol levels decreased slightly in the MMF group and increased in the CsA group over 12 months, with less pronounced figures over the full 24 months. Triglycerid levels did not significantly differ between the groups. Remarkably, we noted widely ranging triglycerid levels in the MMF group, particularly over the full 24 months, resulting in large changes in mean values. Median levels, however, did not differ between the groups (at start 1.6 vs. 1.6, after 12 months 1.7 vs. 1.4 and at 24 months 1.9 vs. 1.4 mmol/L). Homocystein levels tended to decrease more in the MMF than in the CsA group (n.s.).

Mean hemoglobin level remained fairly constant for both groups and for both study periods, as shown in Table 2. At 12 months, 5/18 (28%) MMF and 1/18 (6%) CsA patients had a hemoglobin level below 6.6 mmol/L (p=0.05), at 24 months the difference was not significant (5/18 (28%) MMF vs. 2/17 CsA (12%), p=0.237). The absolute levels at 24 months levels were 6.8 ± 0.5 and 7.6 ± 0.3 mmol/L in the MMF and the CsA group, respectively (n.s.).

Adverse events

During the second year of the study, two patients in the MMF group had a first, biopsy-proven, acute rejection episode. For one of them this lead to graft loss due to chronic rejection 6 months later. The other patient had a C4d positive acute humoral rejection, which was treated with plasmapheresis and high-dose corticosteroids. It resulted in chronic proteinuria and deteriorated graft function.

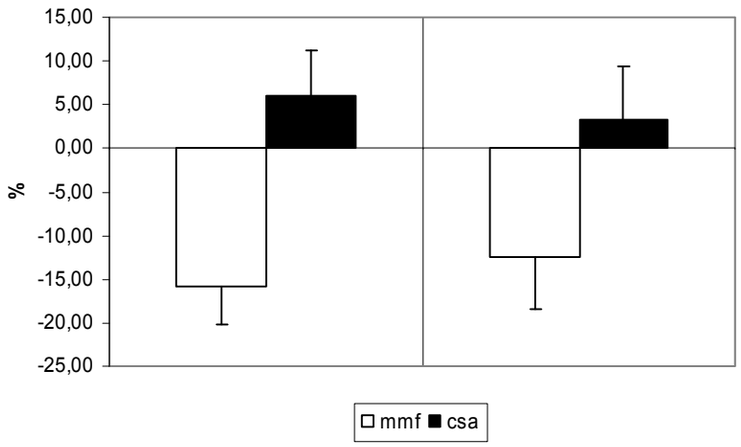
Table 2. Change in laboratory values over the first and both study years

	year 1			year 1+2		
	%(SEM)	n	*p* value	%(SEM)	n	*p* value
Cholesterol			0.002			0.065
MMF	-16(4)	16		-13(6)	16	
CsA	+6(5)	17		+3(6)	16	
LDL-cholesterol			0.045			0.650
MMF	-25(8)	7		-4(25)	6	
CsA	+7(10)	8		+12(24)	6	
Non-HDL-cholesterol[a]			0.013			0.379
MMF	-19(5)	13		-13(10)	15	
CsA	+3(7)	13		0(10)	11	
HDL-cholesterol			0.014			0.522
MMF	-7(6)	13		+5(8)	16	
CsA	+22(9)	14		+13(7)	13	
Triglycerids			0.166			0.185
MMF	-13(8)	15		+21(20)	16	
CsA	+8(12)	17		-5(6)	17	
Homocystein			0.146			0.462
MMF	-17(5)	14		-10(10)	12	
CsA	-5(6)	12		0(9)	10	
Hemoglobin			0.218			0.369
MMF	-2(3)	16		-5(6)	18	
CsA	+3(2)	18		+1(3)	17	

The values are given as mean change as percentage of original value; in brackets the standard error of the mean

[a] In all patients: calculated as cholesterol minus HDL-cholesterol

Figure 4.



Change in cholesterol levels (percentage of original value, ± SEM), left panel: change over the first year of the study, *p*=0.002; right panel over both study years, *p*=0.065

Discussion

This study was designed to test the safety and the efficacy of a dual immunosuppressive therapy combining MMF and corticosteroids in pediatric renal transplantation. Our results show improvement of GFR over the first year. This improvement suggests a reversibility of CsA toxicity, probably more related to its vasomotor side effects than to its potency to generate interstitial fibrosis. Adult studies also have shown better GFR, i.e. at 6 to 9 months after withdrawal of CsA from a triple regimen with MMF 3 to 6 months post-transplant.[11-13] In the second year a parallel decrease of GFR is demonstrated in both groups, finally resulting in better sustained GFR after 2 years in the MMF treated patients, as is illustrated by the survival analysis. Hollander et al. reported persistently better GFR with long-term use of AZA compared to CsA.[14] The second-year decrease of GFR in the MMF group in the present study may reflect less effective immunosuppression, with the occurrence of chronic rejection, as suggested by Smak Gregoor.[15]

Acute rejections

In each group two patients showed acute rejection during the withdrawal period (8-10%). Most studies in adults report higher incidences after withdrawal of CsA from triple therapy at 3 to 6 months post transplant.[11, 13, 15] In some of these studies withdrawal spanned a shorter period of time; for this reason, we opted for a 3-months period. Most reported rejection episodes were steroid sensitive and reversible. Hollander et al. reported better long-term graft survival in pred/AZA treated patients compared to patients on pred/CsA, despite a higher incidence of acute rejections during withdrawal.[14]

Nevertheless, the severe adverse events encountered in the MMF group in the present study, that is, a humoral rejection episode and graft loss six months after an acute rejection episode in the second study year, argue against the safety of prednisolone and MMF as maintenance immunosuppressive prophylaxis in children. Acute rejections in the third year after transplantation are rare, certainly in therapy-compliant 10-year-old girls like these. Similarly, Decloux et al. reported a late first acute rejection episode in 2 of 31 adult patients withdrawn from CsA and continuing on MMF.[16]

Another possible complication of long-term MMF treatment is the previously reported bronchiectasis.[17, 18]

Cardiovascular status

Mortality in pediatric kidney transplantation is largely the result of cardiovascular disease, in association with high blood pressure and dyslipidemia.[19-22] Cardiovascular disease must be restricted as much as possible by avoiding hypertension and dyslipidemia caused by drugs like CsA and prednisolone, and by keeping the GFR optimal. This study aimed at improving cardiovascular status by withdrawal of CsA. Dyslipidemia, especially total and LDL-bound cholesterol, in fact improved after withdrawal of CsA in the first study year. However, the beneficial effect subsided in the second year. None of the patients was treated with statins, as these are not generally applied in pediatric nephrology in the Netherlands.

A study in adults receiving pred/MMF for 2 years showed decreases in total cholesterol and in cholesterol/HDL ratio, notably in the short term, but not any more after 2 years.[15] Several studies on long-term treatment with pred/AZA in adults showed improved lipid pattern, better blood pressure control and lower incidence of cardiac death, compared to Pred/CsA treated patients.[14, 23] Homocystein levels as well showed a trend of improvement over the first study year. Lilien et al. reported elevated homocystein levels in children with end-stage renal failure and renal transplantation, and these were associated with GFR.[24] It is not clear yet whether homocystein level is independently associated with atherosclerosis.
In our study, blood pressure as expected improved after withdrawal of CsA, but only over the first 12 months, and improvement did not reach statistical significance. Studies in adults showed a slight short-term improvement in blood pressure after withdrawal of CsA,[12, 13] without taking antihypertensive treatment into account. The nocturnal decrease in blood pressure in our study did not change after withdrawal of CsA, confirming the findings of Schrama et al.[12] Then, we had expected a decreased nightly dip of blood pressure in patients treated with CsA, based on previously reported findings.[25] However, such an effect was not seen, neither at baseline nor at 12 and 24 months.

Side effects of MMF
Patients on MMF for one year before start of the study, generally did not show new side effects. Hemoglobin levels were slightly lower in the MMF group, with the difference more pronounced after 12 months than after 24 months. None of the patients participating in the study received erythropoetin.

Statistical power
For some parameters, for example blood pressure, only a trend of a change, without statistical significance, could be shown. The study finally may have become underpowered. We had aimed at 25 patients in each group, but failed to enroll these. One of the reasons was that many potential candidates switched from CsA to tacrolimus in the first year after transplantation. Then, we encountered protocol violation in more patients than expected. Because we had decided to analyze only the data of patients treated with MMF without a calcineurin inhibitor and patients treated with CsA without MMF to measure the differential effect of the drugs, patient numbers were smaller than planned.

Conclusion

In conclusion, our study shows that MMF and prednisolone as immunosuppressive prophylaxis in pediatric kidney transplantation temporarily improve kidney function and dyslipidemia, and therefore may possibly improve graft and patient survival in the long-term. However, in view of the two late acute rejection episodes observed in this study, and the previously reported cases of bronchiectasis in long-term treatment with MMF, we feel this regimen is not without risk of serious adverse advents.

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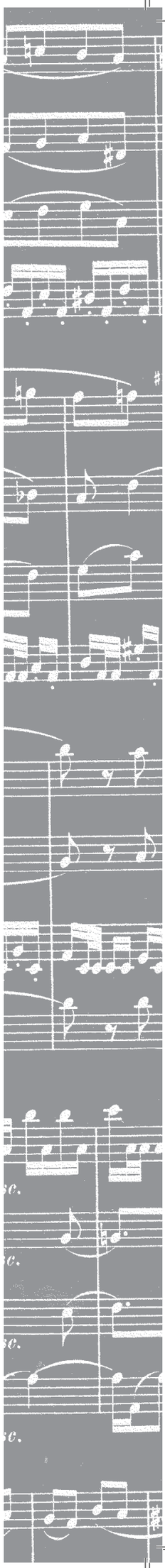
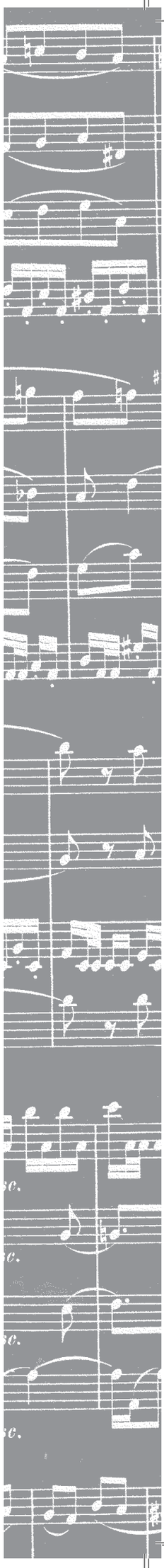
PART III

Study on improvement of outcome by shortening the dialysis period



CHAPTER 7

KIDNEY TRANSPLANTATION WITHOUT PRIOR DIALYSIS IN CHILDREN: THE EUROTRANSPLANT EXPERIENCE



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The Dutch Kidney Foundation supported the study financially.

Abstract

Background

Kidney transplantation without prior dialysis may prevent dialysis associated morbidity.

Methods

We analyzed the outcome of 1113 first kidney transplants in children performed between 1990 and 2000 in the Eurotransplant community.

Results

Enlistment for a deceased donor kidney before start of dialysis (127/895, 14%) made dialysis redundant in 55% of cases. Mean residual creatinine clearance at transplantation of these patients was 8 mL/min.1.73 m^2. Pre-emptive transplantations of deceased donor kidneys showed less acute rejections (52% vs. 37% rejection-free at 3 years, p=0.039), compared to transplantations following dialysis. The difference in graft survival between non-dialyzed and dialyzed patients (82% vs. 69% at 6 year) did not reach statistical significance (p=0.055). No differences were noted after living donor transplantation. Multivariate analysis showed that the period of transplantation was the strongest predictor of graft survival (p<0.001). Congenital structural abnormalities as primary kidney disease predominated in non-dialyzed patients compared to dialyzed patients (p<0.001); this factor did not influence graft survival.

Conclusion

Based on our conclusion that pre-emptive transplantation is at least as good as post-dialysis transplantation, as well as on quality of life arguments, we recommend to consider pre-emptive transplantation in children with end-stage renal failure.

Introduction

Dialysis is a well established treatment modality in children with end-stage renal disease (ESRD). However, based on both medical and quality of life arguments, its place is to bridge the time awaiting a renal transplantation. It is associated with cardiovascular damage, impaired cognitive development and retardation of growth.[1-4] Moreover, the quality of life during dialysis is poor. Transplantation without prior dialysis, or pre-emptive kidney transplantation (PKT), may therefore be preferable in children with ESRD.

However, for PKT to be advantageous, graft survival should at least equal that in dialyzed patients. Several publications on PKT with living donor (LD) allografts indeed report better graft survival, both in adults[5-9] and in children.[10,11] Others show equal results, in adults[12,13] and children.[14,15] Most publications on transplantations from deceased donors (DD) report equal graft survival in PKT, in adults[8,12,16,17] and children.[11,18] Just a few demonstrated better survival in PKT, predominantly in adults.[6,19] Two reports on European children with DD grafts could only suggest a favorable effect of PKT, using series that were too small to reach statistical significance.[20,21]

Here we report on a retrospective study to determine the outcome of PKT in a large pediatric cohort in the Eurotransplant community[d]. In addition we investigate the extent to which the current Eurotransplant allocation policy allows PKT, and the risk that children receiving a PKT were transplanted too early.

Patients and methods

Study design

A registry-wide retrospective study of all consecutive first kidney transplants performed in children younger than 16 years between January 1, 1990 and January 1, 2000 in the Eurotransplant region. Data were retrieved from the Eurotransplant database and the medical files at the 22 participating centers.

Definitions

Graft failure

The start of any form of chronic dialysis, transplantectomy, repeat kidney transplant, or death with functioning graft.

Acute rejection

The prescription of a course of antirejection therapy, consisting of methylprednisolone, ATG or OKT3.

[d] The Eurotransplant International Foundation is responsible for the mediation and allocation of all organs in Austria, Belgium, Germany, Luxemburg, the Netherlands and Slovenia (www.eurotransplant.nl).

Delayed graft function
The need of dialysis treatment in the first week after transplantation.
Creatinine clearance
Creatinine clearance at enlisting and at transplantation was estimated by a modified formula of Schwartz:[22] body height (cm) x 40 / serum creatinine (mcmol/L).
Hypertension
Hypertension scores 1, 3, and 5 years after transplantation were arrived at by adding scores on 2 parameters: systolic or diastolic blood pressure below (score 0) or above (score 1) the 95th percentile for age and gender,[23] and number of antihypertensive and diuretic drugs (no drugs = 0, 1 drug = 1, 2 or more drugs = 2). Scores could thus range from 0 to 3.

Statistics
The difference between Kaplan-Meier survival curves, used for patient and graft survival, and time to first acute rejection, was tested for its statistical significance with a log rank test. Differences in other parameters were tested with the chi-square test for categorical variables, Student's *t*-test for continuous variables. Cox regression analysis was used to adjust the comparison between study and reference groups for potentially confounding factors. A probability of type 1 error less than 0.05 was considered the threshold of statistical significance.

Results

The Eurotransplant database contained 1234 first transplants meeting the inclusion criteria. Data of 1113 of these were available for analysis, 895 (80%) from DD and 218 (20%) from LD. PKT were performed with 70 of DD grafts (8%), and 86 of LD grafts (39%). Mean as well as median follow-up time was 5.3 years (range 0–14.1). For analysis a maximal follow-up time of 6 years was set. The 22 participating centers contributed with a median number of 35 transplants (range 17 – 166).

Baseline characteristics
The PKT group had a relative over-representation of congenital structural abnormalities as primary kidney disease, in association with a higher residual diuresis. Stratified for donor source, no difference was seen in distribution of recipient and donor age, panel reactive antibodies, HLA-mismatches, cold ischemia time, and time window of transplantation. In the DD group PKT was associated with a shorter waiting time, as expected. The calculated creatinine clearance at enlisting and at transplantation were well below 10 mL/min.1.73m^2, and did not differ between the groups. Dialyzed patients with an LD graft had dialyzed shorter than those with a DD graft (Table 1).

Patient survival
The 6-year patient survival in DD patients was 96% for both the pre-emptive and post-dialysis group (n.s.), in LD patients 100% for the pre-emptive and 97% for the post-dialysis group (p=0.04).

Table 1. Baseline characteristics

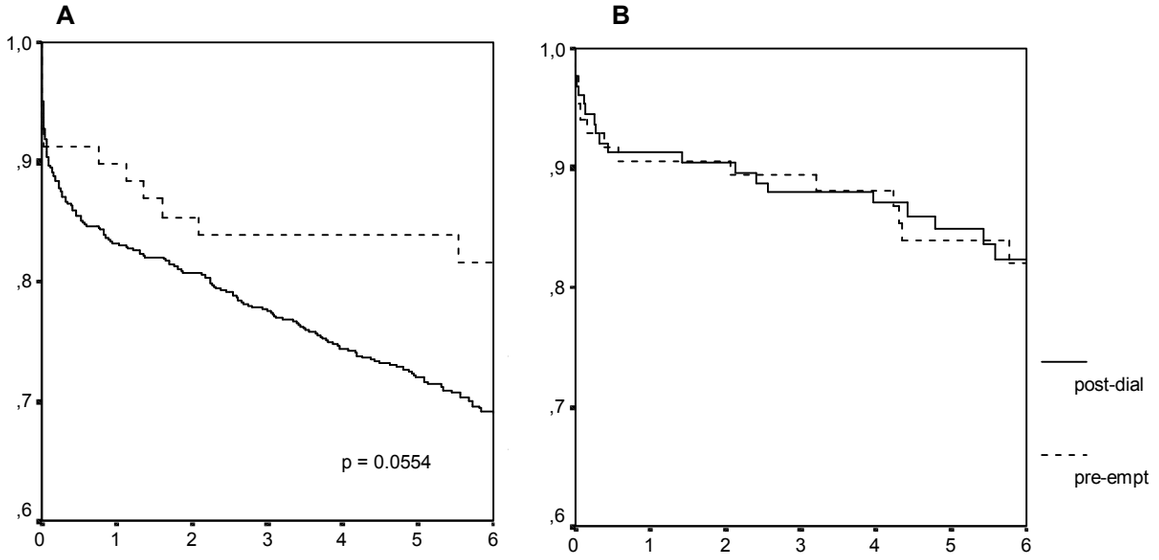
	LD			DD		
	pre-empt	post-dial		pre-empt	post-dial	
Number	86	132	*p*	70	825	*p*
Primary kidney disease [a]						
Structural	49 (57)	45 (36)		37 (54)	284 (35)	
Cystic	13 (15)	23 (19)		12 (17)	114 (14)	
Glomerular acquired	11 (13)	26 (21)		7 (10)	262 (33)	
Metabolic disease	6 (7)	7 (6)		5 (7)	43 (5)	
Cong. nephrotic syndrome	3 (3)	9 (7)		4 (6)	35 (4)	
Other	2 (2)	8 (7)		2 (3)	54 (7)	
Unknown	2 (2)	6 (5)	0.001	2 (3)	20 (2)	0.003
Recipient age at transplant (yr), mean	9.8	8.8	n.s.	9.2	9.9	n.s.
Panel reactive antibodies, recent (%)						
< 5	62 (90)	97 (87)		62 (91)	684 (84)	
5 – 85	7 (10)	14 (13)		5 (7)	125 (15)	
> 85	0 (0)	0 (0)	n.s.	1 (1)	8 (1)	n.s.
Missing	17	21		2	8	
Rest diuresis (ml per 24 hr)						
< 250	1 (2)	54 (52)		1 (2)	335 (47)	
250 - 1000	17 (27)	26 (25)		20 (33)	240 (34)	
> 1000	45 (71)	23 (22)	<0.001	40 (66)	132 (19)	<0.001
Missing	23	29		9	118	
Creat clearance at enlistment, mean				8.8	7.8	n.s.
Creat clearance at transplant, mean (mL/min.1,73m^2)	7.9	8.5	n.s.	8.4	8.4	n.s.
Donor age (yr)						
< 6	0	0		10 (14)	150 (18)	
6 – 20	0	0		26 (37)	276 (34)	
20 – 40	53 (62)	90 (70)		18 (26)	202 (25)	
> 40	33 (38)	39 (30)	n.s.	16 (23)	196 (24)	n.s.
Missing	0	3		0	1	
Duration dialysis (months), mean		14.7			18.7	0.006
< 6		51 (39)			130 (16)	
6 – 24		51 (39)			486 (59)	
> 24		28 (22)			205 (25)	<0.001
Missing		2			4	
Waiting time (months), from transplantable urgency						
0 – 6				50 (71)	439 (53)	
6 – 12				15 (21)	211 (26)	
> 12				5 (7)	171 (21)	0.001
Cold ischemia time (hrs), mean	3.6	3.1	n.s.	21.6	21.7	n.s.
HLA-A, -B, -DR mismatches (n), mean	2.3	2.1	n.s.	2.6	2.5	n.s.
Period of transplantation						
1990 - 1995	43 (50)	63 (48)		27 (39)	368 (45)	
1995 - 2000	43 (50)	69 (52)	n.s.	43 (61)	457 (55)	n.s.

Data are shown as number (percentage), or as mean value. Differences between pre-emptive and post-dialysis transplants are tested within the strata of donor source, with 1 exception: duration of dialysis was tested for the difference between LD and DD post-dialysis transplants. [a] Within the primary kidney diseases, differences are tested between congenital structural abonormalities and other diagnoses. Abbreviations: LD: graft from living donor; DD: graft from postmortal donor; pre-empt: pre-emptive; post-dial: post-dialysis; FSGS: focal segmental glomerulosclerosis; HUS: hemolytic uremic syndrome

Graft survival

Overall the 6-year graft survival (LD + DD) was better in the non-dialyzed than in the dialyzed patients (82% and 71%, respectively, *p*=0.008). Figures 1a and 1b show graft survival curves stratified for donor source. Only in the group of DD transplants PKT was found to be favorable, though not statistically significant (*p*=0.055). Figure 2 shows graft survival for patients with DD grafts grouped by duration of dialysis, demonstrating a better graft survival for PKT and transplants following less than 6 months of dialysis (*p*=0.035). Since patients with PKT had relatively more congenital structural abnormalities, we assessed the effect of primary kidney disease on graft survival in the DD dialyzed patients. This revealed a similar graft survival for structural abnormalities compared to other diagnoses (74% vs. 68% at 6 years, *p*=0.19).

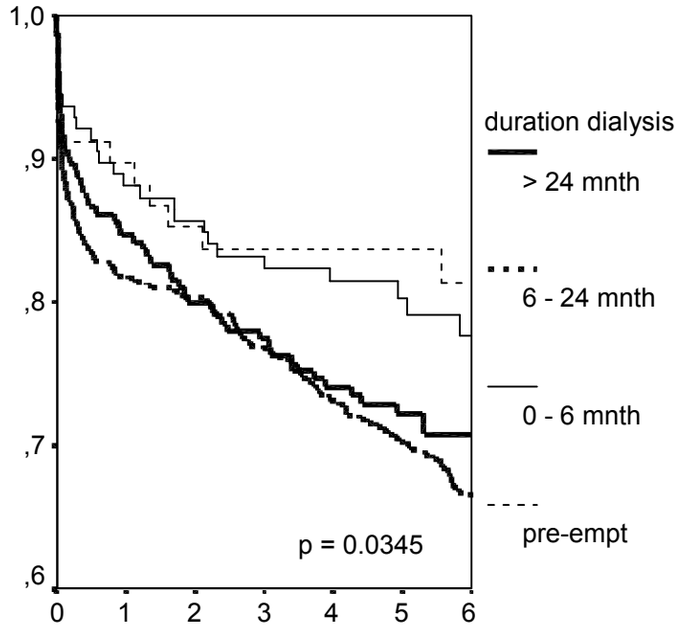
Figure 1. Six year cumulative graft survival of DD (Figure 1a) and LD kidney transplants (Figure 1b), comparing pre-emptive to post-dialysis transplants. On the horizontal axis the years post-transplant, on the vertical axis the cumulative graft survival



The time frame of transplantation had a distinct effect on DD graft survival: 6 year graft survival of dialyzed patients increased from 63% when performed between 1990 and 1995 to 78% between 1995 and 2000 (*p*<0.001); and remained unchanged in pre-emptively transplanted patients: 82 and 81%, respectively. This resulted in loss of the favorable effect of pre-emptive transplantation in the second part of the study-period. The mean duration of dialysis in both time periods was similar, 18.4 and 18.0 months, respectively.

Multivariate analysis (Table 2), stratified for donor source, revealed no significant association between the factor ‘duration of dialysis’ and graft survival (p=0.054). When this factor was replaced by ‘pre-emptive versus post-dialysis’ the significance level dropped (p=0.074). The factor strongest associated with graft survival was the time window.

Figure 2. Effect of duration of dialysis upon graft survival, in DD transplants. On the horizontal axis the years post-transplant, on the vertical axis the cumulative graft survival



Acute rejection episodes

In the DD, but not in the LD group, more patients remained rejection-free after PKT than after dialysis (52% vs. 37% at 3 years, p=0.039, Figure 3). In DD grafts the incidence of acute rejections correlated with duration of dialysis, with the lowest incidence (48%) in PKT patients and the highest (67%) in patients dialyzed more than 24 months (p=0.040).

Delayed graft function

The incidence of delayed graft function of DD grafts in dialyzed and non-dialyzed patients was 9% and 14%, respectively (p=0.27). Corresponding figures for the LD groups were 2.4% and 3.5% (n.s.).

Table 2. Multivariate analysis of the risk of graft failure within the first 6 years after transplantation

Factor	exp(B)	95% interval		*p*
		lower	upper	
Duration of dialysis				
0	1			
< 6 mnth	1.03	0.57	1.88	
6 - 24 mnth	1.64	0.99	2.72	
> 24 mnth	1.67	0.97	2.88	0.054
Period: 1995-2000 vs 1990-1995	0.59	0.43	0.76	< 0.001
HLA-mismatches	1.12	0.97	1.28	0.084
Age recipient	1.03	0.99	1.06	0.125
Primary kidney disease				
other diagnosis vs cong struct abnorm	1.20	0.89	1.60	0.255
Creatinine clearance at transplantation	1.00	0.98	1.02	0.714
Age donor	1.00	0.99	1.01	0.769

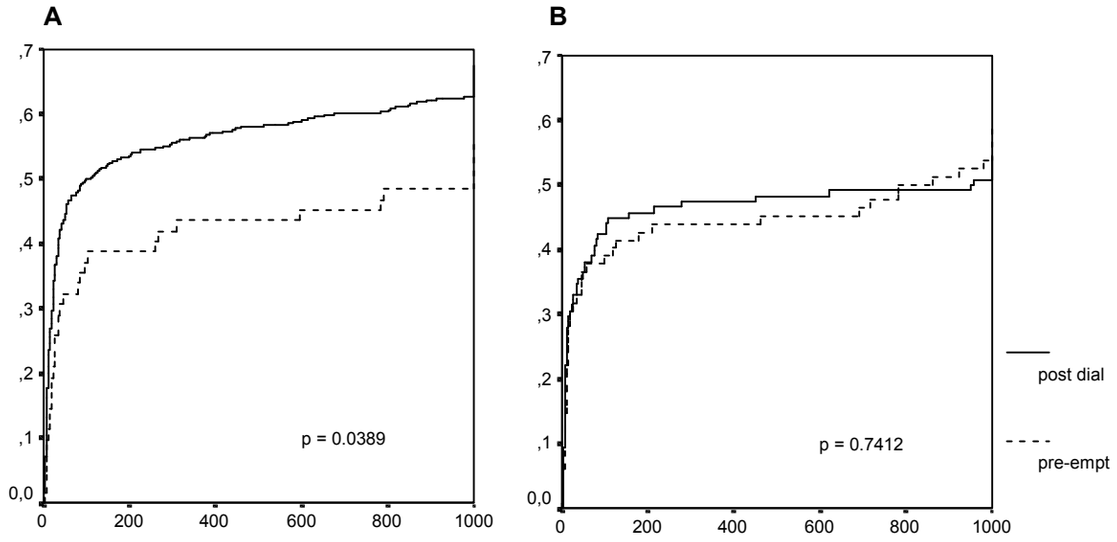
Immunosuppressive therapy

The immunosuppressive regimens at transplantation and 1 year later did not significantly differ between the groups. However, they showed a highly significant difference between time windows (p<0.001), with the appearance of 35% corticosteroids - cyclosporine - MMF as initial therapy in the later period, largely replacing corticosteroids – cyclosporine (- azathioprine).

Hypertension

In the DD group severe hypertension, defined as score 2 and 3, was found in PKT patients in 58%, 40% and 53%, at 1, 3 and 5 years post-transplant, and in dialyzed patients in 69%, 64% and 64% respectively (p-values 0.16 at 1 year, 0.016 at 3 years, and 0.12 at 5 years post-transplant). No differences were seen in the LD group.

Figure 3. The incidence of a first acute rejection episode, in DD (Figure 3a) and LD kidney transplants (Figure 3b). On the horizontal axis the days post-transplant, on the vertical axis the risk of a first acute rejection episode



Policy with regard to pre-emptive transplantation

Of the 895 DD patients, only 127 had been enlisted before start of dialysis (14%). Of these, 70 (55%) in fact were transplanted pre-emptively. Eight of the 22 centers did not perform pre-emptive DD transplants at all, the same number as in LD transplants.

Discussion

In this Eurotransplant study the survival of pre-emptively transplanted grafts was better than that following dialysis. This confirms our previous observations.[21,24] However, the favorable effect of PKT on graft survival appears to have vanished in the second half of the observed decade, when the outcome rates of pre-emptive and post-dialysis transplants were equivalent.

This resulted largely from an improved outcome of dialyzed patients, an improvement which has been attributed to the introduction of new immunosuppressive drugs in this period, as has been demonstrated for German as well as Dutch transplanted children.[25,26] Shorter duration of pre-transplant dialysis had a beneficial effect on the incidence of acute rejection episodes, as was previously shown by others.[15,18,27] Whether this contributes to better graft survival, could not be demonstrated with statistical significance in our study (p=0.035, univariate; p=0.055, multivariate analysis), in contrast to other investigators.[17,28]

Although in literature most data show an improved outcome of PKT in LD transplants, we did not see this in our study.[5-11,16] The shorter period of dialysis in our LD, compared to our DD recipients, may have contributed to this.

Pre-emptive transplantation will most likely improve long term mortality rates in the pediatric patient population. It is to be expected that a shorter exposure to ESRD hazards in combination with less hypertension[18] after transplantation will yield better life expectancy in the long run. Nevertheless, the mortality data in our study were hardly informative, maybe due to the relatively short observation period. Adults with pre-emptive transplantations show a lower mortality rate, especially from cardiovascular conditions, and less morbidity from hypertension and left ventricular hypertrophy.[8, 17, 28] As in adults, the primary cause of mortality in pediatric end-stage renal failure is cardiovascular, associated with hypertension.[2,29,30]

Congenital structural abnormalities are over-represented in pre-emptively transplanted children.[11] These patients are particularly suited to undergo transplantation without prior dialysis. Their renal failure usually progresses only slowly, giving time to find a donor. In our series, structural abnormalities of the urogenital tract did not affect graft survival, as confirmed by uni- and multivariate analysis.

In this retrospective study, we could not assess the effect of PKT on patients' quality of life. However, the literature is clear on this topic. End-stage renal disease in childhood is associated with retarded development of social as well as cognitive capacities.[4,31] Dialysis leads to prolonged parental dependency into adult age, unemployment and a lower level of education, relative to the duration of dialysis[1,12,32] The development of confidence and independency in adolescence is better in patients transplanted without prior dialysis.[33,34] Therefore, the prospect of a normal mental and physical development may be improved when dialysis is avoided or minimized.

The allocation system of Eurotransplant privileges children waiting for a kidney graft, resulting in shorter waiting times for children than for adults. This makes pediatric pre-emptive transplantation with DD grafts possible in certain cases. In our experience, half of the children listed before start of dialysis, could be transplanted without dialysis.

Currently only 14% of patients had been enlisted prior to dialysis. This number might at least double, considering the 35% of patients with slowly progressive disease based on congenital structural abnormalities. This expectation is confirmed by the experience in some centers, where already over one third of patients are enlisted prior to dialysis.

Conclusion

Graft survival in pre-emptive pediatric kidney transplantation in the Eurotransplant region currently at least equals that in dialyzed patients. In view of the reported benefits to quality of life, and possibly to the cardiovascular status in the long run, we recommend to promote pre-emptive enlistment and transplantation in children with ESRD. Eurotransplant's current allocation system allows to attain a larger proportion of pre-emptive DD transplants.

Acknowledgement

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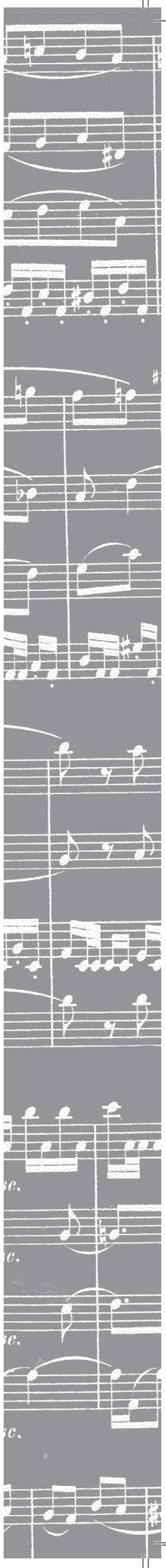
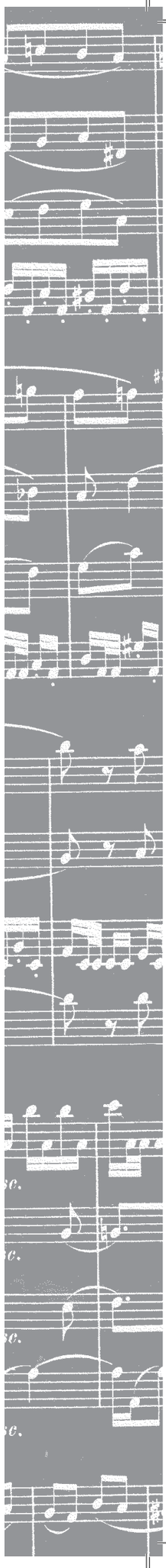
PART IV

Studies on complications in pediatric kidney transplantation



CHAPTER 8

BRONCHIECTASIS IN CHILDREN AFTER RENAL OR LIVER TRANSPLANTATION: A REPORT OF 5 CASES



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Abstract

Background

More effective immunosuppressive treatment in children following organ transplantation has significantly improved the survival of the grafts. Therefore, quality of life, long-term prognosis and adverse drug reactions have become more important. One of the main complications of immunosuppressive drugs is infections of the respiratory tract, but irreversible damage to the airways has not been described after renal or liver transplantation.

Patients

Five children following transplantation of kidney or liver were referred to the Pediatric Pulmonology department because of chronic respiratory complaints. Pulmonary function tests and high resolution computed tomographic (HRCT) scan were performed as routine patient care.

Results

Four children with a renal transplant and one with a liver transplant showed chronic bronchitis and moderate to severe airways obstruction. HRCT showed bronchiectasis in all of them.

Conclusion

We speculate that the immunosuppressive treatment (in)directly contributes to irreversible airway damage. We recommend including follow-up of lung function in the post transplantation protocol and considering bronchiectasis in case of respiratory symptoms, to try preventing further damage to the lung.

Introduction

Survival of transplanted organs and prognosis in general has improved considerably in children who receive an organ transplant.[1] The improved long-term survival is associated with more extensive and more potent immunosuppressive treatment. Therefore, the side effects of this treatment need more attention. The most relevant side effect of immunosuppressive agents is the increased susceptibility for (opportunistic) infections, the lung being one of the vulnerable sites.
Although not uncommon in developing countries, bronchiectasis is a rare disorder in children in the Western world; no recent data are available on the incidence.[2] Common causes of bronchiectasis are infections, and in developed countries, cystic fibrosis, immunodeficiencies and ciliary dyskinesia.[3] Obstructive lung disease and bronchiectasis have been reported after bone marrow transplantation, related to graft-versus-host disease (GVHD).[4,5] In pediatric heart transplantation. recurrent sinopulmonary infections and even bronchiectasis were described in children who had their transplant before the age of 4 years. It was speculated that impaired maturation of antipolysaccharide responses due to immunosuppression may be responsible for recurrent and damaging infections.[6] Bronchiectasis as a radiographical feature of bronchiolitis obliterans may be seen after lung transplantation as part of chronic lung allograft rejection.[7] However, no reports on bronchiectasis as a complication after liver or kidney transplantation are found in the literature. We describe four pediatric patients with a kidney transplant and one with a liver transplant, who developed chronic and progressive pulmonary symptoms, caused by bronchiectasis.

Patients

Characteristics of all 5 patients are summarized in Table 1.

Patient 1, a boy from a family with high socioeconomic status, received a cadaveric renal transplant at the age of 12.5 years because of congenital renal dysplasia with vesico-ureteral reflux. Chest X-ray at transplantation was normal. Immunosuppressive prophylaxis consisted of corticosteroids, cyclosporine A (CsA) and mycophenolate mofetil (MMF); no induction treatment was used. Three months after transplantation, the CsA levels were between 75 and 125 mg/L, the MMF levels between 1.3 and 7 mg/L. Prednisone doses was 7.5 mg, once daily (0.16 mg/kg). Acute rejection episodes did not occur. Three months after transplantation he had chickenpox, for which he was treated with intravenous acyclovir. One year after transplantation he was treated for a *Bordetella pertussis* infection (confirmed serologically), after which he recovered completely. Eighteen months later he was referred to the pediatric pulmonologist because of persistent productive coughing. He did not have any prior primary pulmonary complaints. Pulmonary function tests (PFT) revealed severe airway obstruction (Table 1). Sputum cultures grew *Pseudomonas aeruginosa* and high resolution computed tomography scan (HRCT) showed bronchiectasis in both lower lobes (Figure 1). He was treated with ciprofloxacin, nebulized tobramycin and DNase, and Positive Expiratory Pressure (PEP) by mask.

Despite this treatment he had a severe exacerbation of symptoms at the age of 16.5 years, when bronchoalveolar lavage fluid (BALF) showed *Streptococcus pneumoniae* and rhinovirus. No other pathogens were found. He was treated with intravenous cefuroxim and recovered only slowly and incompletely. Cystic fibrosis and primary ciliary dyskinesia were ruled out by appropriate tests. It was hypothesized that the bronchiectasis could be explained by the proven *Bordetella pertussis* infection.

Table 1. Characteristics of 5 children who had kidney or liver transplantation and subsequently developed bronchiectasis

	1. male 17 yrs	2. male 12 yrs	3. male 11 yrs	4. male 7 yrs	5. female 11 yrs
Age at Tx (yr)	12	10 (2nd Tx)	7	3	0.8
Age at respiratory symptoms (yr)	15	11	8	5	7
Primary diagnosis	renal dysplasia	asphyxia	mes prol GN	cong NS	biliary atresia
Transplanted organ	kidney	kidney	kidney	kidney	liver
Creatinin (µmol/l)	250	85	65	60	
Immunosuppression	P, CsA, MMF	P, CsA, MMF	P, CsA[a], MMF	P, CsA[a], MMF	P, CsA, MMF
Sputumcultures	Pseud. aeruginosa Str.pneumoniae	H. influenzae	H. influenzae H. parainfluenzae St. maltophilia Flavobact. sp. Pseud. aeruginosa	H. influenzae	H.parainfluenzae
FVC [b, c]	69 / 91	67 / 100	61 / 92	66 / 101	94 / 88
FEV1 [b, c]	59 / 80	56 / 103	51 / 71	72 / 101	88 / 81
FEV1/ FVC (%) [c]	71 / 73	69 / 86	70 / 64	93 / 84	80 / 79
MEF25 [b, c]	11 / 28	17 / 68	13 / 23	28 / 43	45 / 36
Location bronchiectasis	both lower lobes, right upper lobe	left lower lobe	generalized	right middle lobe, lingula	both lower lobes, lingula

Abbreviations: mes prol GN: mesangio proliferative glomerulonephritis, cong NS: congenital nephrotic syndrome, P: Prednisone, CSA: Cyclosporin, MMF: Mycophenolate Mofetil, FVC: forced vital capacity, FEV1: forced expiratory volume in 1 second, MEF 25: maximal expiratory flow at 25% of vital capacity

[a] prescribed only during the first half year after transplantation

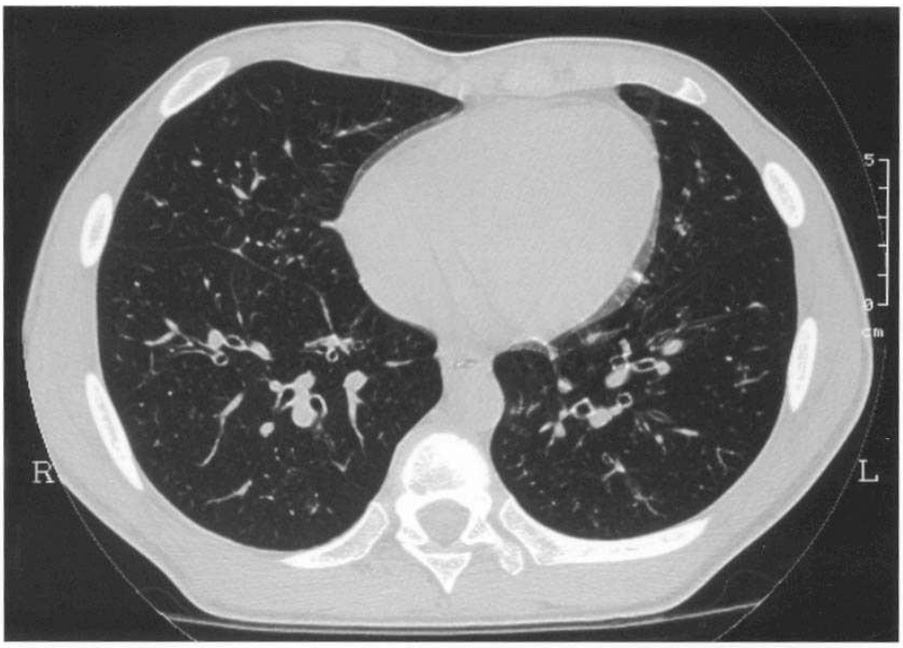
[b] values are percentages predicted for sex and height

[c] at referral to the department of pediatric respiratory medicine/ maximal value after treatment

However, within 18 months of this referral, four other patients on immunosuppressive treatment following kidney (3) or liver (1) transplantation were referred because of similar symptoms. They all presented with chronic productive cough without fever, dyspnea and with diminished exercise tolerance. The initial immunosuppressive therapy was the same for all 4 kidney transplant patients and consisted of corticosteroids, CsA and MMF. No induction therapy was administered. From 6 months post-transplant the dosage of prednisolone was 5 mg/m^2/day, and MMF 600 mg/m^2 b.i.d.; CsA dosage was titrated to a serum concentration of 100 mg/l. In 2 cases (patient 3 and patient 4) cyclosporine was discontinued at 6 months post-transplantation in the framework of a study protocol. In patient 5 CsA was discontinued and replaced by MMF because of slight impairment of renal function. Chest X-rays on the day of transplantation were normal in all patients. Kidney transplant patients 1, 2 and 4 received antibiotics continuously for urinary tract infection prophylaxis.

The diagnostic workup, similar to patient 1, did not reveal any indication for the common causes of bronchiectasis. Patients 2 and 3 are from families with low income; the families of patient 4 and 5 are prosperous.

Figure 1. Slide of HRCT-scan, showing bronchiectasis in patient 1



Patient number 2 had a renal transplant at the age of 2 because of renal failure after perinatal asphyxia. Chronic pyelonephritis lead to failure of this graft at the age of 8 years, after which he was treated with peritoneal dialysis. A second transplant with a kidney from his mother was performed at the age of 9 years. As a toddler the boy had recurrent ear, nose and throat infections, treated with adenotonsillectomy and bilateral ventilation tubes. Chest X-ray before transplantation was unremarkable. One year after his second transplant he presented with chronic productive cough and recurrent pansinusitis. Sputum culture revealed *Haemophilus influenzae* and the chest X-ray showed increased markings. On HRCT bronchiectasis in the left lower lobe was detected. The boy was treated with amoxicillin, DNase and PEP mask and only recovered slowly, partly due to low adherence to treatment.

An 11 years old boy was the third referral to our pediatric pulmonology department. He received a kidney transplant from his father at the age of 7 years and presented with respiratory symptoms 1 year later. His primary diagnosis was mesangioproliferative glomerulonephritis. At first presentation severe airway obstruction was present. Sputum cultures revealed *Haemophilus influenzae*. He was treated with antibiotics based on sensitivity tests. However, his lung function did not recover and HRCT scan showed bronchiectasis. DNase was added and he was treated with a PEP mask. When he presented with an exacerbation of his complaints a bronchoscopy was performed and BALF grew *Haemophilus influenzae*, and no opportunistic microorganisms.

At 3 years after referral sputum was colonized with *Pseudomonas aeruginosa*, and the patient was treated with ciprofloxacin and tobramycin inhalations. *Stenotrophomonas* infection was treated with cotrimoxazole. At the last follow-up visit he had a productive cough and crackles bilaterally on chest auscultation. His pulmonary function tests improved significantly, but did not recover fully.

The fourth patient was a 3 year old who received a cadaveric kidney transplant for congenital nephrotic syndrome. Two years later he presented with chronic productive cough; sputum culture grew *Haemophilus influenzae* and he was treated with amoxicilline/clavulanic acid. As symptoms persisted antibiotics were switched to clarithromycin and nebulisations with DNase and PEP therapy were started. Only after an intravenous course of cefuroxime did his pulmonary function normalized and symptoms disappeared. No other pathogens besides *Haemophilus influenzae* were cultured. Bronchoscopy and BAL were not performed.

An 11 year old girl was the 5th patient who developed bronchiectasis after a solid organ transplantation. She received a cadaveric donor liver because of biliary atresia when she was 10 months old. Initial immunosuppression consisted of prednisone, azathioprine and CsA. CsA was discontinued after 1 year, but restarted at the age of 6 years because of signs of rejection on a liver biopsy. Five years after transplantation azathioprine was replaced with MMF. CsA was withdrawn after introduction of MMF. At the age of 9 years she presented with chronic cough and diminished exercise tolerance for 1.5 years. Immunosuppression at that time consisted of MMF 500 mg bid and prednisone 12 mg on alternate days. MMF levels varied between 3.5 and 9.6 mg/L (4.0 mg/L at presentation). Immunoglobulins were within normal limits. Her pulmonary history was previously unremarkable, however, she had had recurrent ear nose and throat infections. Except for one antibiotic course the patient did not receive any antibiotic treatment. In her sputum *Haemophilus parainfluenzae* was cultured. The girl was treated with cotrimoxazole, DNase and PEP therapy. However, pulmonary function tests have not improved.

Discussion

This is the first report of bronchiectasis in children who underwent a kidney or liver transplant. Airway infection with bronchial obstruction and retention of mucus are major factors in the pathogenesis of bronchiectasis.[3] Well-known causes of bronchiectasis in children in the Western world are cystic fibrosis, mucociliary clearance defects, recurrent aspiration and immunodeficiencies, but in up to 50% of patients with bronchiectasis no cause is identified[8] In our current pediatric renal transplant population we diagnosed bronchiectasis in 4 out of 38 patients. Bronchiectasis has been reported after pediatric bone marrow, heart, heart-lung and lung transplantation.[4-7] In bone marrow transplantation bronchiectasis is associated with chronic GVHD.[4] In adult bone marrow recipients it was shown that ciliary beat frequency was severely reduced in those patients who developed bronchiolitis obliterans and chronic GVHD.[9]

Impaired maturation of antipolysaccharide responses caused by immunosuppression may be responsible for recurrent infections leading to bronchiectasis, especially in children transplanted before the age of 4 years, as was shown for pediatric heart transplant recipients.[6]

Several mechanisms may account for the development of bronchiectasis in our patients. First, all patients have secondary immunodeficiency due to immunosuppressive medication, which may have facilitated pulmonary infections. Besides, pulmonary infections may have been masked by the prophylactic antibiotic treatment these patients received to prevent urinary tract infections. We cannot exclude that the patients had opportunistic infections, but could not identify such microorganisms despite bronchoscopy with lavage in 2 patients. Patient 1 had had a serologically proven *B. pertussis* infection, a well-known but rare cause of bronchiectasis, 18 months before he presented with bronchiectasis.

As we did not feel lung biopsy would provide us with useful information or would change our treatment, lung biopsies were not performed in our patients. An alternative hypothesis is that the immunosuppressive drugs were a causative factor. All patients used one shared immunosuppressive drug, MMF, a relatively new drug in our immunosuppressive regime, for a prolonged period of time. Pulmonary side effects of MMF have been described: coughing, bronchitis and shortness of breath. Two case reports document acute respiratory failure with pulmonary fibrosis and pneumonitis most likely due to MMF, but bronchiectasis is not known as an adverse effect of MMF.[10,11] In vivo, MMF severely depresses humoral immunity making patients more susceptible to infection.[12] Before the introduction of MMF in our standard immunosuppression regime in 1997, we had not seen any patient with bronchiectasis after transplantation. We speculate that MMF may play a role in the development of bronchiectasis in our patients by facilitating or masking pulmonary infections more than other immunosuppressive drugs or by exerting a direct effect on the airway wall. Obviously, the exact mechanism remains to be elucidated and requires further research.

Therapeutic levels of CsA are not associated with serious pulmonary toxicity.

In our patients no data on pre-transplant PFT were available nor did we have any pre-transplant CT scans. Diminished lung volumes have been described in chronic renal failure, but most transplanted patients showed normal spirometry.[13,14] We cannot exclude pre-transplant abnormalities in PFT in our patients, but there were no respiratory symptoms prior to transplantation.

As prognosis and survival of children after organ transplantation increased dramatically the past decade, complications of the post-transplant therapy have become more and more important and may co-determine long-term prognosis.[1] Once structural abnormalities of the bronchi are present, even control of infection and inflammation may not be sufficient to arrest the progression to disabling irreversible airway obstruction.

In the presence of respiratory symptoms bronchiectasis should be considered and, as spirometry is not sensitive enough in detecting early structural lung damage, HRCT should be performed.[2,15] However, we recommend including follow-up of lung function in the post-transplantation protocol as well, as peripheral airway obstruction may be a sign of bronchiectasis. By aggressively treating children with bronchiectasis who are receiving immunosuppressive drugs, further damage to the lung may be prevented and quality of life and long-term prognosis improved.

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CHAPTER 9

PULMONARY COMPLAINTS AND LUNG FUNCTION AFTER PEDIATRIC KIDNEY TRANSPLANTATION

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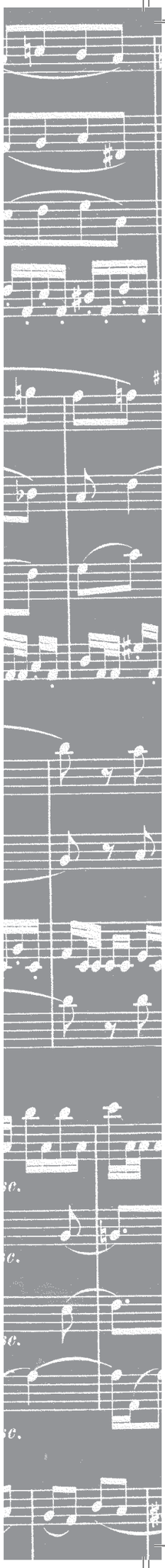

Abstract

Background

Recently four of 38 children with a kidney transplant were diagnosed with bronchiectasis. The aim of the current study was to identify patients with increased risk for pulmonary damage.

Methods

In this cross-sectional observational study, children with a functioning kidney graft in the Netherlands and Antwerp, Belgium, were screened with the use of a symptom checklist and spirometry. Maximum score for upper airway complaints was 21 (normal: <8), for lower airway complaints 28 (<10). Results of forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV1) and maximal expiratory flow at 25% of vital capacity (MEF25) were expressed as percentage predicted for height and sex.

Results

Hundred and thirty-five patients completed the interview (122) and/or spirometry (103); 91 did both. Lower airways symptoms were above acceptable levels in 18 (14%) patients. Forty-nine patients (48%) had an abnormal lung function test: in 12 concerning FVC%, in 11 FEV1%, in 24 MEF25%, and in 36 FEV1/FVC. Of correlations between symptomatology or spirometry data, and clinical parameters, only that between GFR and MEF25% was statistically significant.

Conclusion

Children with a kidney transplant are at increased risk for obstructive lung disease. We recommend to monitor lung function during the follow-up after renal transplantation.

Introduction

The improved long-term survival of transplanted organs is associated with more extensive and potent immunosuppressive treatment. Adverse effects of this treatment, therefore, are becoming more apparent. The most relevant one is the increased susceptibility for (opportunistic) infections, with the lung among the target sites. Since the introduction of mycophenolate mofetil (MMF) in our standard immunosuppressive protocol, four out of 38 children with a kidney transplant followed in Rotterdam have been diagnosed with bronchiectasis.[1] They presented with persistent productive cough and showed disturbed lung function. Similarly, a later report described bronchiectasis in 5 adults with a kidney transplant.[2] Bronchiectasis is a rare disorder in children in the Western world, associated with cystic fibrosis, immunodeficiencies and ciliary dyskinesia.[3] It has been reported after bone marrow transplantation, in relation to graft-vs.-host disease,[3, 4] and after recurrent pulmonary infections in heart transplantation in very young children.[5] Bronchiectasis may be seen after lung transplantation as part of chronic allograft rejection.[6] However, pulmonary complications of kidney transplant have not been systematically studied.
Being alerted, we designed a cross-sectional study, (1) to identify pulmonary complaints and/or abnormal lung function in children with a functioning kidney transplant, and (2) to identify risk factors for disturbed lung function. In particular, we explored the hypothesis that MMF might affect respiratory symptoms and lung function.

Methods

All 166 children with a functioning kidney transplant who visited one of the 5 pediatric nephrology outpatient clinics in the Netherlands and Antwerp, Belgium during a 3 month period (between April and July) were asked to participate. The index-patients with bronchiectasis were excluded. In these institutions a shared initial immunosuppressive protocol was applied, consisting of corticosteroids, MMF and cyclosporine, after 2001 in combination with basiliximab. After a year the regimen generally was reduced to two drugs, either prednisolone and a calcineurin inhibitor or prednisolone and MMF. CMV prophylaxis consisted of a 3 months course of valganciclovir in CMV-positive donor with negative recipient combination, and valaciclovir in CMV-positive recipients. In most institutions, cotrimoxazol prophylaxis was prescribed for the first three months after transplant.

The parents were interviewed on upper and lower airway complaints of the children using a modified validated questionnaire designed to assess respiratory symptoms in patients with bronchiectasis (Table 1).[7] Items were scored on a 4-point scale, ranging from 0 (never), 1 (sometimes), 2 (often), to 3 (always). The use of antibiotics was scored as 0 (none) or 1. The maximum total score possible for upper airway complaints was 21, for lower airway complaints 28.
Item scores 0 or 1 were accepted as normal, resulting in a maximum acceptable score for complaints of the upper airways of 7, of the lower airways of 9; patients were categorized according to these cut-off values.

Table 1. Symptom checklist

Did your child during the last 3 months complain of following symptoms
Upper airway complaints:
Nose obstruction
Nasal mucus clear
Nasal mucus yellow or green
Snoring
Impaired hearing
Ear ache
Otitis
Lower airway complaints:
Cough, hacking
Cough in the morning
Cough at night
Cough at laughing/crying
Cough during exercise
Cough with mucus production
Dyspnea at rest
Dyspnea during exercise
Did your child get antibiotics for pulmonary complaints? (no=0, yes=1)
How often did episodes of coughing and dyspnea occur? (0=0, 1=1, 2=2, >2=3)

Spirometry was performed in each institution according to guidelines of the American Thoracic Society by authorized lung function technicians used to encourage the children to make the ultimate effort in expiration. After maximal inspiration, three reproducible loops with a maximum variability in FVC of 10% were obtained. The best of the three curves is selected for analysis. Results of spirometry were interpreted by pediatric pulmonologists at each local site. FVC, FEV1 and MEF25 were expressed as percentage predicted for height and sex (FVC%, FEV1%, MEF25%). The lower borders of normal values (-2 SD) were 80% of predicted for FVC, 85% for FEV1, 70% for MEF25.[8] FEV1/FVC ratio, as the most sensitive indicator of airway obstruction, was calculated; a value < 0.85 was interpreted as airways obstruction.
Collected patient data included: age, height and weight at time of the investigation, time on renal replacement therapy and after transplantation, and the immunosuppressive therapy through the years. Serum creatinine levels at the time of the study were collected, and, in one center, serum immunoglobulin levels.
Since these children did not have major complaints, we considered CT-scanning of all patients as unethical, and refrained from it.

Definitions

Acute rejection was defined as the prescription of a course of antirejection therapy, consisting of methylprednisolone or ATG. The glomerular filtration rate (GFR) was calculated according to a modified formula of Schwartz.[9, 10]

Statistical analysis

Correlations between spirometry findings and clinical parameters were assessed for statistical significance with Pearson's test. Correlations between complaints and clinical parameters were tested for statistical significance with logistic regression.

Comparison of means between 2 groups was performed by *t*-test. A *p*-value < 0.05 was considered significant.

Results

Of 166 eligible children, 135 completed the interview (122) and/or performed spirometry successfully (103); 91 did both. Clinical data of these 135 children are shown in Table 2.

Table 2. Clinical characteristics of 135 patients who performed lung function test and/or filled in the symptom checklist

	Mean	Median	Range
Age (yr)	13.9	14.6	(4.4 - 19.9)
Time from start RRT (yr)	6.8	6.2	(0.4 - 15.6)
Time from transplantation (yr)	4.3	3.9	(0.2 - 13.7)
GFR (ml/min.1.73m^2)	57	57	(12 - 113)
Creatinine (mcmol/l)	122	99	(43 - 439)
IgG (n=26, mmol/l)	8.7	8.9	(3.1 - 16.4)
Height SD score	-2.1	-2.1	(-5.7 - 1.2)
Body mass index (kg/m^2)	21	20	(15 - 39)
Pre-emptive/post-dialysis (n)	15/120		
Rank order 1/2/3 (n)	114/16/5		
Sexe m/f (n)	80/55		
Acute rejection incidence 0/1/>1 (n)	66/33/14	(missing 22)	
Donor source LD/DD (n)	29/96	(missing 10)	
MMF currently yes/no	63/45	(missing 27)	
Years on pred	4.1	3	(0 - 13)
Years on CsA	3	2	(0 - 13)
Years on MMF	1.7	1	(0 - 8)
Years on TCL	0.6	0	(0 - 5)

Pred: prednisolone, CsA: cyclosporine, MMF mycophenolate mofetil, RRT: renal replacement therapy, GFR: glomerular filtration rate, TCL: tacrolimus, LD: living donor, DD: deceased donor

Their median age was 14.6 years, they had been on renal replacement therapy for a median of 6.2 years, and received their kidney transplant a median of 3.9 years ago. As pre-existent pulmonary disease is concerned, no cases with Wegener's or Goodpasture's disease as primary kidney disease were registered, one patient had SLE.

No overt respiratory problems were present before transplantation; however, a previous history of atopy or asthma has not been registered.

The median creatinine level was 99 mcmol/L, with a mean and median GFR of 57 mL/min/1.73m^2. Forty five percent of patients were still receiving MMF at the time of the study, of whom 66% had used it for more than 12 months. Immunosuppressive therapy at time of the study consisted of double therapy in 51% of patients (37% on prednisolone and a calcineurin inhibitor, and 14% on prednisolone and MMF) and 44% on triple therapy (prednisolone, a calcineurin inhibitor and MMF). No patients were on sirolimus.

The symptom checklist scores revealed complaints of upper and lower airways above acceptable level in 15 (13%), and in 17 (15%) patients, respectively (Table 3).

Table 3. Spirometry and interview data, mean and median values, range, and number and percentage of patients with abnormal values

	Mean	Range	< -2SD n (%)
FVC (% of predicted)	104	(55 - 143)	12 (11.5%)
FEV1 (% of predicted)	107	(56 - 142)	10 (9.6%)
MEF25 (% of predicted)	89	(32 - 198)	24 (23.8%)
FEV1/FVC	0.87	(0.48 – 1.00)	36 (34.6%)
Interview score of	Median	Range	> Acceptable n (%)
Upper airways	3	(0 - 14)	15 (12.8%)
Lower airways	4	(0 - 20)	17 (15.4%)

A clear correlation between lower and upper airway complaints was found (r=0.60, p<0.001). No statistically significant correlation was found between on the one hand checklist scores of either upper or lower airway complaints, and on the other hand age, time after transplantation, time on renal replacement therapy, rank order of transplant, presence of acute rejection episodes, current use and duration of MMF therapy, GFR and IgG. Of the patients currently treated with MMF the proportion with lower airway complaints did not differ from those not treated with MMF (6/35 vs. 7/56, p=0.453).

Lung function tests revealed at least one lung function parameter value less than -2 SD in 49 out of 103 patients (48%), in 12 (12%) concerning the FVC%, in 11 (11%) the FEV1%, in 36 (35%) the FEV1/FVC ratio, and in 24 (24%) the MEF25% (Table 3). The mean FVC%, FEV1%, FEV1/FVC ratio and MEF25% were within the normal range. No correlations were found between on the one hand lung function parameters (in percentage predicted) and on the other hand age, time after transplantation, time on renal replacement therapy, rank order of transplant, presence of acute rejections, serum IgG, level of immunosuppression (double vs triple), and current use and duration of MMF treatment (Table 4). A significant but weak correlation was shown between GFR and MEF25% (r=0.216, p=0.036). The proportion of patients with below-normal MEF25% was lower in those on MMF therapy than in those not on MMF (5/34 vs. 18/49, p=0.045); no difference between these groups was noted for the FEV1/FVC ratio. Lung function parameters expressed as percentage of predicted for height and sex were not significantly correlated with body mass index (BMI).

Of the 26 patients with known IgG levels at the time of the study, 13 were currently treated with MMF. IgG levels did not significantly differ between patients with and without MMF (7.4 vs 9.3 g/L, p=0.201). Neither did lymphocyte count significantly differ between patients with and without MMF (1.9 and 2.1 x 10^9/L, respectively, p=0.549).

Table 4. Correlation of lung function with clinical characteristics

A. Pearson: correlation	fvc%		fev1%		mef25%		fev1/fvc	
	coeff	*p*	coeff	*p*	coeff	*p*	coeff	*p*
Age	0.133	*0.177*	0.070	*0.483*	0.066	*0.511*	-0.178	*0.071*
Time after transplant	0.178	*0.071*	0.085	*0.390*	-0.086	*0.392*	-0.175	*0.076*
Time after start RRT	0.110	*0.302*	0.046	*0.665*	-0.012	*0.909*	-0.127	*0.233*
Height SDS	-0.102	*0.301*	-0.085	*0.408*	0.122	*0.224*	0.072	*0.466*
BMI	0.164	*0.096*	0.179	*0.068*	-0.006	*0.955*	-0.004	*0.971*
Years on MMF	0.021	*0.845*	0.131	*0.226*	0.134	*0.215*	0.196	*0.067*
GFR	0.060	*0.562*	0.177	*0.099*	0.216	*0.036**	0.231	*0.054*
IgG	0.183	*0.382*	0.193	*0.354*	0.090	*0.667*	0.054	*0.799*
B. t-test: differences of means								
Ranking order								
first	103		108		89		87	
later	108	*0.332*	110	*0.588*	87	*0.769*	84	*0.139*
Duration MMF therapy								
max 1 year	107		109		79		86	
longer	102	*0.302*	106	*0.639*	89	*0.205*	88	*0.317*
MMF currently								
yes	100		105		88		89	
no	106	*0.131*	108	*0.404*	86	*0.753*	86	*0.266*
Nr acute rejections								
0	100		106		89		90	
1 or more	107	*0.078*	108	*0.64*	83	*0.356*	85	*0.010*[a]

[a] p=0.05

Discussion

Children with a renal transplant have functional abnormalities of upper and lower airways in a higher proportion than expected, as shown in this multi-center, cross-sectional and descriptive study. We observed pulmonary dysfunction in a significant proportion of our patients, in addition to four cases with clinically significant bronchiectasis. The findings from the symptom checklist only give a crude impression of the prevalence of airway complaints, as it has not been validated in Dutch patients with a kidney transplant. The impression is confirmed, however, by the more objective spirometry data: much more patients had disturbed pulmonary function than expected from the normal distribution in healthy children. The parameters most often aberrant were the FEV1/FVC, reflecting airway obstruction, and the MEF25, which may be indicative for obstruction of the more peripheral airways. FEV1/FVC is the most sensitive and reproducible of these. A dysbalance in production and clearance of pulmonary mucus may provide an explanation for this phenomenon.

Possible causes of overproduction are: increased infectious load from immunosuppressive therapy, or diminished clearance due to damaged ciliary clearance or lower muscle power. In this respect, myopathy has been suggested as a cause of diminished lung volumes in patients on renal replacement therapy, possibly resulting from uremia before, and corticosteroid treatment after transplantation.[11, 12] We cannot rule out the possibility that our patients might have had pre-transplant abnormalities in lung function because relevant data are missing.

Lung function disturbance in children and adults with end-stage kidney disease has been reported before. It appears to be more pronounced during dialysis than after transplantation, and more during peritoneal than during hemodialysis.[11-14] Paul et al. showed FEV1 in transplanted children to be inversely related to the duration of prior dialysis, and to be better than in conservatively treated or dialyzed patients[11] So, in the event of pulmonary function declining over the dialysis period , with return to normal in the transplantation phase, one would expect gradually improving spirometry data over time elapsing since transplantation. The absence of a correlation in the currently studied population between time after transplantation and spirometry data does not support this hypothesis, but a longitudinal survey is needed to dismiss it. One longitudinal study in adults on lung function before, and monthly for six months after transplantation, showed normalization of residual volume, but an unchanged, normal FVC and FEV1.[15]

The need for lung function measurements is highlighted by the observation that abnormal values are not always accompanied by complaints. Remarkably, we only found a statistically significant association between FVC%, not the parameters for obstruction, and the level of lower airway complaints. Though the mean FVC% differed in patients with and without complaints, it lay well within the normal range in both groups. The isolated abnormal FVC% in two patients may reflect restrictive disease, or an incomplete expiratory manoeuvre. Lung volume studies are indicated to exclude restrictive disease.

Obstructive pulmonary disease might also be caused by asthmatic symptoms. From this study it cannot be concluded to what extent asthma might contribute to our results, since we did not register its prevalence in our patients. Assuming the same prevalence as in the Dutch population (3.5% in 1999) and considering the continuous treatment of all patients with low-dose prednisolone, asthma can only have a small additional role in the 30% patients with obstructive lung function.[16]

Body size affects spirometry data. We corrected for the smaller height of transplanted children by using the percentage of the spirometry data predicted for height and sex. One might argue that dysproportion between sitting height to body height may account for the lower pulmonary function values we found in part of the study population. However, this is unlikely since the sitting height to body height ratio hardly differs between transplanted children and healthy children: at onset of puberty this ratio is equal in both populations (0.51), at the end of puberty it is 0.49 in transplanted children and 0.52 in healthy children.[17, 18]

The rationale for the current study was the alarming finding that 4 of 38 renal transplant patients in our institution showed bronchiectasis. These cases and the 5 reported by Rook et al, all had been treated with MMF for a prolonged period of time, always in combination with prednisolone, with or without a third immunosuppressive drug.[1, 2] The above mentioned previous study of lung function in renal transplant children was done in the pre-MMF era.[11]

During the 25 years before the introduction of MMF in the standard immunosuppression regime, neither of the two centers encountered any patient with post-transplantation bronchiectasis (in 200 and 1500 transplants, respectively, $p<0.001$). Pulmonary side-effects of MMF have been described, like coughing, bronchitis and shortness of breath.

Two case reports document acute respiratory failure with pulmonary fibrosis and pneumonitis most likely due to MMF, but development of bronchiectasis has not been reported as an adverse effect of MMF.[19, 20] In vivo, MMF depresses humoral immunity making patients more susceptible for infections.[21] Therefore, one would expect lower IgG levels in MMF treated patients. We did not find this in the small number of patients in whom this was investigated. An alternative hypothesis proposes that MMF itself is a causative factor, e.g. influencing ciliary motility of pneumocytes. In both pathogenetic mechanisms retention of mucus in peripheral bronchi would be expected, accompanied by disturbance of the MEF25 in particular. The four index patients with bronchiectasis all showed a severely disturbed MEF25%. Therefore, an early decline in MEF25% should make us aware of potential risk for bronchiectasis.
However, in contrast to our expectation based upon the index patients with bronchiectasis, in the current study population no association at all was shown between the use of MMF, and pulmonary complaints or lung function.

Once structural abnormalities of the bronchi are present, even control of infection and inflammation may fail to arrest the progression to disabling irreversible airways obstruction. In contrast, the respiratory symptoms of the four Rotterdam children with bronchiectasis after renal transplant fortunately subsided after withdrawal of MMF, intensive treatment with antibiotics and DNAse, and physical therapy using a positive expiratory pressure (PEP) mask (data not published). The other 5 reported cases also clinically recovered, although structural changes seen on CT did not disappear.[2]

Conclusion

Children with a renal transplant may be at higher risk for respiratory complications, with bronchiectasis as a severe outcome. Therefore, we recommend spirometry as a useful tool for lung function monitoring during follow-up visits of children with a kidney transplant. Early signs of lung function decline should prompt referral to a pulmonary specialist for further evaluation by chest imaging studies and institution of aggressive management of pulmonary care and airway clearance to prevent progression to bronchiectasis.

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CHAPTER 10

LEFT VENTRICULAR DIASTOLIC DYSFUNCTION IN PEDIATRIC PATIENTS ON PERITONEAL DIALYSIS AND AFTER RENAL TRANSPLANTATION

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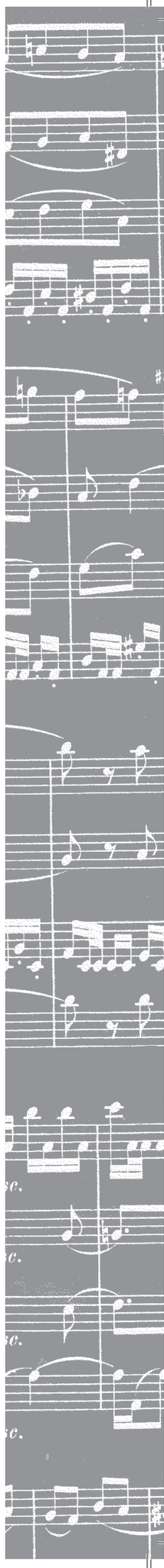
Submitted



Abstract

Introduction

Cardiovascular disease is the leading cause of death in children with end-stage renal disease. We investigated the presence of diastolic dysfunction in a group of patients on peritoneal dialysis or after renal transplantation.

Methods and results

Fourteen patients on peritoneal dialysis for a median of 1.4 years (range 0.1-5.3) and thirty nine patients with a functioning kidney transplant for a median time of 3.3 years (range 1.2-14.5) were studied and compared to controls. As assessed by echocardiography both groups showed diastolic dysfunction. The contribution of atrial contraction (A) to the mitral inflow was relatively high in both patient groups (E/A ratio in the controls 2.27 ± 0.61; transplant 1.82 ± 0.58; dialysis 1.57 ± 0.73; p<0.001). The ratio of early mitral inflow (E) to the early tissue movement of the mitral annular ring (E') was increased in the patient groups, indicating diastolic dysfunction (E/E' ratio in controls 7.97 ± 1.46; transplant 9.49 ± 1.71; dialysis 11.90 ± 2.11; p<0.001). Left ventricular mass was only increased in the transplant patients (controls 61 ± 13 g/m^2; transplant 105 ± 28 g/m^2; p<0.001). High PTH levels, more prevalent in dialysis patients, are associated with abnormal E/A ratios.

Conclusions

Abnormalities in diastolic function are present in both peritoneal dialysis and renal transplanted patients. In the dialysis group abnormalities in calcium-phosphate metabolism are a major risk factor, whereas the transplanted patients show an increased left ventricular mass.

Introduction

Cardiovascular disease is the leading cause of death in patients who started renal replacement therapy in childhood.[1-4] This includes cerebrovascular accident, cardiomyopathy, arrhythmias and cardiac arrest from unknown origin. Early signs of cardiac disease in renal patients include left ventricular hypertrophy and diastolic dysfunction of the left ventricle.[3] These abnormalities have been shown to develop in children already at the time of mild to moderate chronic renal insufficiency, and progress as renal function deteriorates.[5,6] They have been described in patients on chronic dialysis as well as after renal transplantation. Hypertension and prolonged dialysis are predictors for cardiovascular mortality.[4] Risk factors for cardiovascular disease in children include abnormalities in calcium-phosphate metabolism, nephrotic syndrome, anemia and long-term use of immunosuppressive agents, such as corticosteroids and calcineurin inhibitors.[1,7] Many of these risk factors improve after transplantation. In the present study we investigated children on chronic peritoneal dialysis or after renal transplantation. All patients underwent both conventional echocardiography and measurement of tissue Doppler variables. These may help to determine early onset diastolic dysfunction.
We hypothesized that the cardiovascular abnormalities in transplanted patients may be less prominent than in patients on dialysis and that left ventricular hypertrophy is a major risk factor in developing diastolic dysfunction.

Methods

A cross-sectional study of dialysis and transplanted patients in a tertiary pediatric nephrology department in a single university hospital in Rotterdam, the Netherlands was performed. The study population consisted of 53 patients with a median time on renal replacement therapy of 4.3 year (range 0.1-16.0), and 88 healthy controls. Thirty nine patients had a functioning kidney transplant for a median time of 3.3 years (range 1.2-14.5), 14 patients were on peritoneal dialysis for a median of 1.4 years (range 0.1-5.3).
The patients were followed by the outpatient nephrology clinic, and all underwent yearly echocardiography as part of their medical follow-up. The controls were healthy school children without any medication or illness known to influence renal or cardiac function and underwent echocardiography only. The institutional review board of the Erasmus Medical Center, Rotterdam, The Netherlands approved the study, and written informed consent was obtained from all controls and their parents. Patients' medical records were reviewed for age, cause of renal failure, duration of peritoneal dialysis or time after renal transplantation. Calcium-phosphate disturbances were prevented as much as possible by the use of dialysate with a physiologic calcium concentration (1.25 mmol/l), a protein-restricted diet, phosphate binding medication with the meals (usually sevelamer), and alphacalcidol as vitamin D analogue. In transplanted patients 24 hr ambulatory blood pressure monitoring was performed at the same occasion as the cardiac evaluation, by using an oscillometric device (SpaceLabs monitor, 90207; SpaceLabs Medical Inc., Redmond, Washington, USA).

Measurements were performed every 30 minutes during daytime and every hour during the night. Bed-time of the child was used as night-time. Hypertension was scored as a mean blood pressure above the 95th percentile for age and sex[8] during both systole and diastole and during both day and night.

Serum creatinine, calcium, phosphorus, intact parathyroid hormone (iPTH), and haemoglobin determined for each patient at time of echocardiography. GFR was estimated using the Schwartz formula, modified to conform with local laboratory practice.[9,10]

Echocardiography was performed using standard techniques on a commercially available machine (Philips Sonos 5500, Andover, MA, USA). These included M-mode measurement of left ventricular wall and septum, mitral inflow parameters, and pulsed Tissue Doppler estimates of the basal part of the left ventricle.
Left ventricular mass (LVM) was measured by two-dimensional directed M-mode echocardiography according to the American Society of Echocardiography criteria[11]
LV systolic performance was assessed by calculation of shortening fraction (SF) and heart rate-corrected velocity of circumferential shortening.
Transmitral flow was obtained with pulsed wave Doppler at the leaflet tips. The velocity of early mitral inflow (E), the velocity of the mitral inflow during active contraction (A), the E/A ratio and the decelaration time (Dt) were also calculated.[12]

Pulsed wave Doppler tissue velocities were obtained at the cardiac base in the apical 4-chamber orientation from 3 locations: the lateral mitral annulus; the interventricular septum; and the lateral tricuspid annulus. Tissue doppler measurements from each of these myocardial wall segments included peak systolic annular velocity (S), peak early diastolic annular velocity (E') and peak late diastolic annular velocity (A) waves. The ratio of early mitral inflow measured by Doppler to peak early diastolic annular velocity (E/E') was measured as well.

Statistics
Statistical analysis was performed with SPSS 11.5 (SPSS, Inc., Chicago, Il). Values are presented as mean ± SD unless stated otherwise. A two sample *t*-test was used to compare the means of continuous variables with a normal distribution, and Mann-Whitney test for continuous variables without normal distribution. Categorical values were compared using the chi-square test or Fisher exact test. The correlations between variables were assessed by Spearman correlation analysis. A p value < 0.05 was considered statistically significant.

Results

Patient characteristics (Table 1)
The peritoneal dialysis patients were significantly younger than the transplant patients. Consequently, length and weight were lower as well. The main causes of end-stage renal disease in the transplanted patients were congenital anatomic abnormalities (n=16, 43%) and glomerulonephritis (n=14, 38%).

In the dialysis patients the main causes were congenital anatomic abnormalities (n=5, 36%), glomerulonephritis (n=4, 29%), and congenital nephrotic syndrome (n=4, 29%). Phosphate and iPTH levels were significantly higher in dialysis patients as compared to transplant patients, as were urea and creatinine levels. The glomerular filtration rate in the transplanted patients had a mean value of 54 ml/min.1.73m^2 (range 16-107).

Table 1. Patient characteristics

	Controls	NTX	PD	C vs.N	C vs. P	N vs. P
Age	11 ± 1	14 ± 4	8 ± 6	ns		<0.01
Length	149 ± 10	147 ± 25	113 ± 32	ns	<0.001	<0.001
Weight	40 ± 10	49 ± 19	24 ± 15	<0.01	<0.001	<0.001
Hb (mmol/l)		7.5 ± 1.1	7.2 ± 1.0		ns	
Ht (l/l)		0.35 ± 0.05	0.33 ± 0.05		ns	
Urea (mmol/l)		9.9 ± 3.2	14.7 ± 6.0		<0.001	
Creatinine (µmol/l)		126 ± 60	692 ± 367		<0.001	
Phosphate (mmol/l)		1.39 ± 0.25	1.69 ± 0.48		<0.01	
Calcium (mmol/l)		2.47 ± 0.13	2.45 ±. 015		ns	
Ca.PO4 product		3.42 ± 0.57	4.17 ± 1.40		<0.01	
iPTH (pmol/l)		13.0 ± 9.2	36.7 ± 35.9		<0.001	

24 hour ambulatory blood pressure registration

Twenty-four hour blood pressure monitoring was performed in the transplanted patients only. Blood pressure above the 95th percentile was observed in 13 (35%) for daytime systolic blood pressure, in 9 (24%) for daytime diastolic blood pressure, in 13 (37%) for nighttime systolic blood pressure and in 11 (31%) for nighttime diastolic blood pressure. In 10 patients mean systolic and diastolic blood pressure during both day and night were below p95, in 5 all these values were above p95.

Left ventricular mass (Table 2)

In the transplanted patients both left ventricular posterior wall and interventricular septum were thicker than in the controls. This resulted in an increased left ventricular mass. In contrast, mean left ventricular mass of dialysis patients did not differ from controls. LV mass above the 95th percentile of the controls was present in 3/14 PD patients (22%), and 30/39 NTX patients (73%) (p<0.01).

Table 2. M-mode values of left ventricular function

	Controls	NTX	PD	C vs.N	C vs. P	N vs. P
IVSd	6.6 ± 1.2	10.4 ± 2.3	7.3 ± 2.1	<0.001	ns	<0.001
LVPWd	5.8 ± 1.3	9.0 ± 2.0	6.7 ± 2.1	<0.001	ns	<0.01
LVmass	61 ± 13	105 ± 28	72 ± 25	<0.001	ns	<0.001
SF	0.37 ± 0.05	0.38 ± 0.12	0.36 ± 0.11	ns	ns	ns
LVED	44 ± 4	45 ± 5	33 ± 9	ns	<0.001	<0.001
LVES	27 ± 3	27 ± 4	20 ± 6	ns	<0.001	<0.001
VCFC	1.17 ± 0.19	1.21 ± 0.22	1.25 ± 0.29	ns	ns	ns

IVSd = thickness of the interventricular septum in diastole (mm), LVPWd = thickness of the left ventricular posterior wall in diastole (mm), LVmass = left ventricular mass (g/m^2), SF = shortening fraction (%), LVED = left ventricular enddiastolic dimension (mm), LVES = left ventricular endsystolic dimension (mm), VCFC = Velocity of circumferential fiber shortening, corrected for heart rate

Systolic function as assessed by shortening fraction was similar between patients and controls. There was a significant correlation in the transplanted patients between 24-hour systolic blood pressure and left ventricular mass (R=0.38; p<0.05).

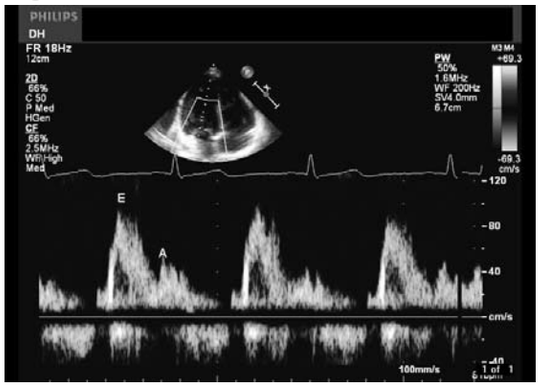
Doppler parameters (Table 3, Figures 1 and 2)
Diastolic function as assessed by the mitral E/A ratio was decreased in both transplant and dialysis patients. This abnormality was more prominent in the dialysis patients. Right ventricular function in all patients was similar to the control population.

Table 3. Mitral inflow parameters

	Controls	NTX	PD	C vs.N	C vs. P	N vs. P
MV-E	1.02 ± 0.16	0.98 ± 0.17	0.97 ± 0.24	ns	ns	ns
MV-A	0.47 ± 0.12	0.57 ± 0.14	0.69 ± 0.28	<0.001	<0.05	ns
MV E/A	2.27 ± 0.61	1.82 ± 0.58	1.57 ± 0.73	<0.001	<0.01	ns
DecTime	0.16 ± 0.03	0.17 ± 0.04	0.18 ± 0.04	<0.05	ns	ns
TV-E	0.73 ± 0.16	0.64 ± 0.13	0.64 ± 0.18	<0.01	ns	ns
TV-A	0.40 ± 0.12	0.39 ± 0.14	0.44 ± 0.21	ns	ns	ns
TV E/A	1.94 ± 0.57	1.75 ± 0.54	1.76 ± 0.91	ns	ns	ns
DecTime	0.17 ± 0.03	0.17 ± 0.05	0.15 ± 0.05	ns	ns	ns

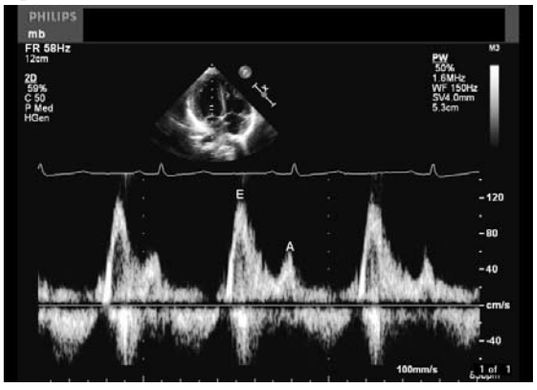
MV-E = early mitral inflow (m/sec), MV-A = active mitral inflow (m/sec), MV E/A = ratio of early mitral inflow to active mitral inflow, Dec Time = deceleration time (msec), TV-E = early tricuspid inflow (m/sec), TV-A = active tricuspid inflow (m/sec), E/A = ratio of early tricuspid inflow to active tricuspid inflow

Figure 1.



Mitral inflow in a healthy control, showing an E-peak of 0.91 m/sec, an A-peak of 0.36 m/sec and an E/A ratio of 2.5

Figure 2.



Mitral inflow in a patient with a renal transplant, showing an E-peak of 1.21 m/sec, an A-peak of 0.59 m/sec and an E/A ratio of 2.0.

Tissue Doppler velocities (Table 4, Figures 3 and 4)
All tissue Doppler values were smaller in the patient groups than in the controls, both at the basal part of the left ventricular posterior wall as well as at the basal part of the interventricular septum. Especially a lower E wave and an increased E/E' ratio were seen in the patients. Also for the tissue Doppler values differences from controls were more prominent in the dialysis patients. iPTH was inversely related to E/A ratio (R=-0.34; p<0.05). This correlation was not found between iPTH and E/E' ratio.

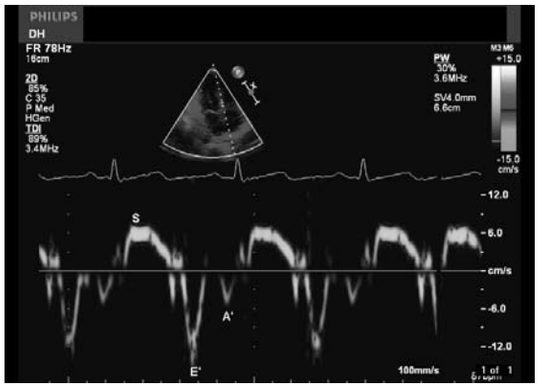
The E/E' ratio was significantly higher in patients with blood pressure above the 95th percentile (p<0.01). Significance was reached for both systolic and diastolic blood pressure during daytime as well as during nighttime. In transplanted patients left ventricular mass was significantly correlated with E/E' ratio (R=0.36, p<0.05).Since there was an age difference between dialysis and transplanted patients we tested the age effect in the normal controls. Both inflow parameters and tissue Doppler values were independent of age. Only left ventricular enddiastolic and endsystolic dimensions increased with age and body weight.

Table 4. Tissue Doppler parameters

	Controls	NTX	PD	C vs.N	C vs. P	N vs. P
TDI-sys LV	0.11 ± 0.08	0.08 ± 0.02	0.07 ± 0.02	<0.01	<0.001	ns
TDI-E LV	0.17 ± 0.04	0.15 ± 0.03	0.13 ± 0.04	<0.001	<0.01	ns
TDI-A LV	0.05 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	ns	ns	ns
TDI E/E'	6.22 ± 2.49	6.70 ± 1.76	8.20 ± 2.98	ns	<0.05	ns
TDI-Sys IVS	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	<0.001	<0.05	ns
TDI-E IVS	0.13 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	<0.001	<0.001	<0.001
TDI-A IVS	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.02	ns	ns	ns
TDI E/E'	7.97 ± 1.46	9.49 ± 1.71	11.20 ± 2.11	<0.001	<0.001	<0.01

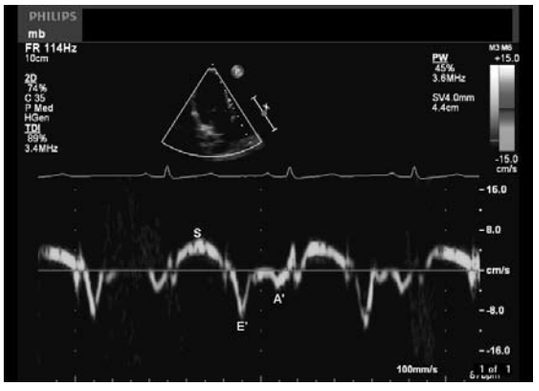
TDI-Sys LV = systolic movement of the basal part of the posterior wall of the left ventricle, TDI-E LV = early diastolic movement of the basal part of the posterior wall of the left ventricle, TDI-A LV = late diastolic movement of the basal part of the posterior wall of the left ventricle, TDI-Sys IVS = systolic movement of the basal part of the interventricular septum, TDI-E IVS = early diastolic movement of the basal part of the interventricular septum, TDI-A IVS = late diastolic movement of the basal part of the interventricular septum

Figure 3.



Tissue Doppler of the basal part of the interventricular septum in a healthy control. The E'-wave is 13 cm/sec. The E/E' ratio is 7.0.

Figure 4.



Tissue Doppler of the basal part of the interventricular septum in a renal transplant patient. The E'-wave is 9 cm/sec. The E/E' ratio is 13.5

Discussion

In our pediatric patients we noted significant cardiac abnormalities, which may underly the well known increased cardiovascular risks associated with end-stage renal disease in childhood. The main findings are the preservation of systolic function in all patients, an increase in left ventricular mass in the transplanted patients, and not in the peritoneal dialysis patients, and diastolic dysfunction of the left ventricle in all patients, but more prominent in the dialysis patients.

A correlation was found between systolic blood pressure and left ventricular mass in transplanted patients, and between increased iPTH and diastolic dysfunction. An increased E/E' ratio was found in patients with hypertension, and was correlated to left ventricular mass in transplanted patients.

Diastolic dysfunction of the left ventricle, as observed in both transplanted as well as dialyzed patients, has been reported previously.[5,12,13,14] The underlying mechanisms, however, remain unclear. Diastolic dysfunction is one of the first signs of cardiovascular disease in renal patients, eventually leading to death in many patients.[1,4] Correlations between diastolic dysfunction and increased left ventricular mass, like the one we found in transplanted patients, have frequently been reported. In the present study we found an increased left ventricular mass in the transplanted patients, but not in the dialyzed patients, who showed left ventricular hypertrophy in only 22%. Others found an increased left ventricular mass already in pre-dialysis patients.[6,7] The left ventricular mass has been reported to increase gradually before and during the time on chronic dialysis treatment.[7] During this period left ventricular mass is especially related to the presence of systolic hypertension.[7] However, most studies in children reflect the changes during hemodialysis. In four reports the contribution of PD patients to the dialysis population was only 25% (16/61).[3,5,13,15] Further comparison between hemodialysis and peritoneal dialysis patients has to be made. Despite the normal LV mass our dialysis patients showed inferior diastolic function of the left ventricle compared to the transplanted patients. This suggests that left ventricular hypertrophy is not the only cause of diastolic dysfunction. An increased PTH level was present in our dialysis patients, and this was associated with diastolic dysfunction. Dysregulation of calcium phosphate metabolism in end-stage renal disease may lead to calcification of arterial walls, resulting in arterial stiffness. Mitsnefes et al, who studied cardiovascular disease in children in all phases of chronic kidney disease, showed a relationship between arterial stiffness and poor diastolic function in dialysis patients.[15] Since calcium-phosphate metabolism normalises with renal function after kidney transplantation, new vascular calcifications are not expected to develop. Nevertheless existing calcified vascular lesions are not expected to diminish considerably.
After renal transplantation a gradual decline of the increased left ventricular mass has been reported, although the prevalence of left ventricular hypertrophy remained unchanged.[16] Others did not find any change in left ventricular hypertrophy or diastolic dysfunction after renal transplantation.[2,17]
Further studies are necessary to unravel these mechanisms, and to give insight in the cardiovascular changes that occur when a dialysis patient receives a kidney transplantation.

Limitations of the study
Since the present investigation is a cross-sectional study, differences between dialysis and transplant patients may be due to differences in patient characteristics. Only long-term follow-up studies can answer the question if subsequent transplantation in dialysis patients will improve left ventricular hypertrophy and diastolic dysfunction found in the dialysis patients in the present study.

Age differences between the transplant and dialysis patients may influence echocardiographic parameters. However, when the youngest and oldest controls were compared with each other, corresponding to the age ranges of the dialysis and transplanted patients, the only differences found were small increases in left ventricular enddiastolic dimension and in left ventricular endsystolic dimension according to age and weight, whereas all other inflow and tissue doppler values were similar. This makes this factor an unlikely explanation for the found differences between dialysis and transplanted patients.

Conclusions

Abnormalities in diastolic function are present in both peritoneal dialysis and renal transplant patients. Risk factors associated with these abnormalities are different between the groups, abnormalities in calcium phosphate metabolism being most prominent in dialysis patients. In the transplant group an increased left ventricular mass is prominent, probably related to arterial hypertension and toxicity of immunosuppressive agents. Measures to improve cardiovascular function in patients with end stage renal disease should account for these differences between dialysis and transplant patients.

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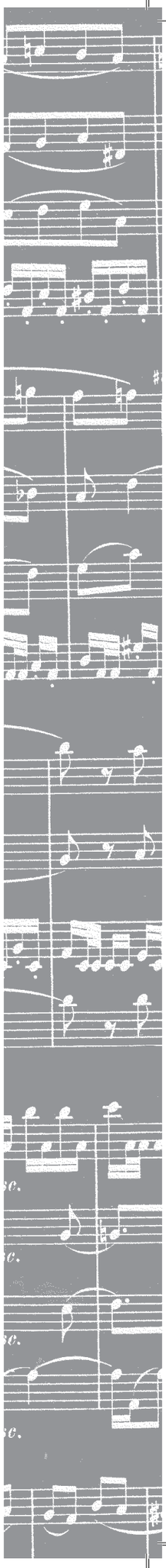
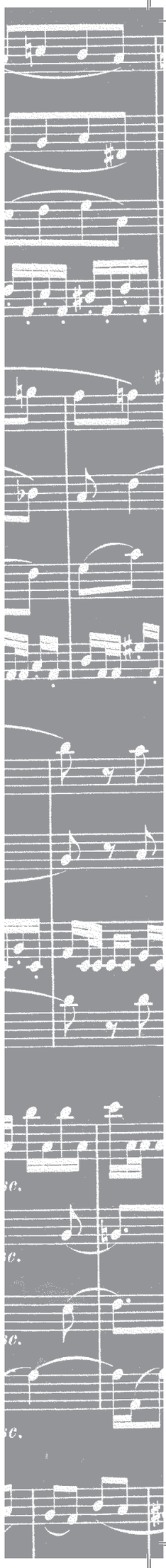
PART V

General discussion and summary



CHAPTER 11

GENERAL DISCUSSION: WAYS TO IMPROVE PEDIATRIC KIDNEY TRANSPLANTATION



General discussion

Kidney transplantation is the preferred mode of treatment of children with end stage renal disease. Unlike dialysis modalities, it replaces most functions of the native kidneys. Moreover, the burden of treatment following transplantation is lighter than during dialysis. Still, renal replacement therapy is a lifelong affair, at best made up of decades of transplantation alternated with months of dialysis. It is notably the extrarenal comorbid conditions – such as cardiovascular disease, bone disease, disturbed growth, and developmental problems – that determine life expectancy of a child on renal replacement therapy. These conditions may improve after transplantation, given a good clearance. Nevertheless, transplantation brings along comorbidity of its own, not least caused by toxic side effects of immunosuppressive therapy. These mostly affect the cardiovascular system and the skeleton. In addition, height growth may not catch up as hoped for, resulting in a below normal final height.

The studies compiled in this thesis aimed to improve medical outcome of children undergoing kidney transplantation in the Netherlands. Most studies were performed within the framework of the Netherlands - Antwerp collaboration. The results support the assumption that outcome is determined by 1. the child's condition at the time of transplant (**Chapter 5**), 2. success of transplantation in terms of improvement of patient and graft survival (**Chapters 3 and 4**), and 3. comorbidity (**Chapter 6**). Here, we will explore how adaptation of protocols, new clinical trials, and organizational streamlining may hold promising potential for better outcome of kidney transplantation in children. Issues to be considered are: pre-transplant factors, source of the allograft, outcome of transplantation, patient survival, comorbidity and logistics.

Pre-transplant factors

Many children at the verge of kidney transplantation already have a long and complicated medical history. It begins with the primary kidney disease. Some of these diseases are likely to recur in the grafted kidney, other may lead to distinctive post-transplant complications, such as in diseases with lower urinary tract dysfunction, congenital nephrotic syndrome, Alport disease. Chronic severe proteinuria, as seen, for example, in focal segmental glomerulosclerosis, raises the risk of vascular disease, due to dyslipidemia and hypertension. Patients with chronic nephrotic syndrome typically will have been treated with cyclosporine for years. Patients with long-standing renal disease, especially those on dialysis and with hypertension, are at risk for cardiac and vascular lesions. One of the predictors of cardiovascular disease is hyperparathyroidism. Hyperparathyroidism is part of renal osteodystrophy, another form of comorbidity of renal disease, which may occur with or without skeletal deformities. Growth is frequently disturbed, in many cases despite temporary treatment with growth hormone.

A number of factors place a heavy psychoemotional burden on these children: dietary restrictions, in combination with loss of appetite, and the obligation to take medication, loss of freedom due to the dialysis treatment, deteriorating physical condition. And then, parents' anxiety for the well-being of their child may be an emotional load for the child as well.Severe renal failure and chronic dialysis – while the child is awaiting transplantation – seriously hampers both school performance and the development of self-esteem, independency and normal psychosocial skills. All these factors together may negatively affect quality of life and social performances for life, and make the child dependent on ongoing psychosocial support.

Limiting pre-transplant comorbidity to a minimum would seem all-important for the outcome of transplantation. Though not always easy, treatment should aim at normalizing blood pressure and biochemical parameters of bone disease. Good nutrition, as well as correction of metabolic disturbances such as acidosis and hyperparathyroidism, contributes to better growth. Tube feeding may be necessary to avoid malnutrition, especially in infants and toddlers. Proper management of bladder function, e.g. in children with urethral valves, is essential as well.
In 2007 a study has started aimed at improving quality of care of patients on renal replacement therapy by means of central registration and peer reviewing. This is a multicenter cohort study in all pediatric dialysis centers in the Netherlands and Belgium, by the name RICH-Q: Renal Insufficiency Therapy in Childhood – Quality assessment and improvement (www.rich-q.nl).

Ultimately, the best way to improve comorbidity of end-stage renal disease is to limit the duration of it by early transplantation. For children in particular it is critical to keep the waiting time for transplantation as short as possible. They are at a critical stage of development, both physically and psychosocially, which makes them vulnerable to lifelong damage – more than adults. From a health care perspective such damage is relatively costly since children's life expectancy obviously is longer than that of adults. The waiting time – from the moment that renal transplantation is indicated until the moment the donor kidney becomes available – has gradually increased during recent years. A dual cause explains this effect: a drop in number of potential donors combined with more and more adult patients requiring transplantation.

Source of the allograft
The strategy to reduce the waiting time and the dialysis period is threefold: curbing the influx of new patients, raising the numbers of donors and striving at pre-emptive transplantation in combination with earlier start of searching for a donor.

Concerning the influx of new patients: almost half of cases of end-stage renal disease in children are due to congenital disease, some based on consanguinity, and half to acquired diseases. Regrettably, better understanding of these disorders has not yet reduced the frequency of end-stage renal disease in this age group. Large scale international prospective studies are needed to answer the question whether general interventions, such as inhibition of the RAAS system, could slow down the progression of renal failure in children.

Prevention of diabetes mellitus might be more effective, however, as this disease causes end-stage renal disease in more and more adults, thereby inflating the waiting list. In contrast, improvement in graft survival may gradually reduce numbers of patients returning on the waiting list.

Several methods could be proposed to enlarge the donor pool. First of all, by increasing the proportion of living donors from the current 30% to the 60% level achieved in the USA.[1,this thesis] This might prove feasible by further encouraging family members, including grandparents, by considering ABO incompatible donor-recipient combinations, and by including pediatric recipients in the national program for cross-over exchange of potential living donors.[2] Since the start of this program in 2004, 100 more patients could be transplanted with a living donor graft.[3] The total number of living donated kidney transplantations last year increased as well, from 278 in 2006 to 355 in 2007.[3]
Second, living adult donors could be allowed for young children, despite the surgical implications. Experience with this kind of transplantation is accumulating. These youngest recipients will require extra intensive care as the circulating volume must be significantly increased in the presence of young children's relatively low blood pressure. In these children, plasma creatinin level will be a less sensitive indicator of rejection, due to the large functional reserve of such a kidney. This would mandate surveillance biopsies to detect subclinical rejection of the allograft.[4] As such transplantations will still be rare, it would be desirable to have them performed by a single team in the Netherlands, composed of dedicated surgeons, anesthetists, intensivists and pediatric nephrologists.

More deceased donors could be acquired in the Netherlands, as demonstrated by Ploeg et al.[5] Donors may be missed for several reasons. The delicacy of putting a donation request to the next of kin just after the death of their relative is one reason. The presence of well trained procurement coordinators will facilitate the donation request. Currently, in 70% of these cases, however, relatives do not consent in organ donation.[3] This is related to the fact that less than one third of Dutch inhabitants has registered in the donor registry, and fewer still have registered positively for organ donation. Repetitive reminders might lead to more potential donors. Regrettably, a proposal to change the law from an informed consent system to a presumed consent system was rejected. Finally, the need to have a team available to harvest the organs heavily draws on human resources. The current organization with regional procurement teams on duty aims at improving this situation. Nevertheless, thanks to the efforts of the Dutch Transplantation Foundation, recent publicity generated by the *'De grote donorshow*' on television, and NIGZ[e] publicity campaigns, more people have registered as potential donors. In parallel, numbers of transplantations with kidneys from deceased donors rose from 360 in 2006 to 435 in 2007. Numbers of non heart beating donors did not change.
Recently Eurotransplant adopted a 'Young for young' policy, implying that kidneys of young donors will preferably be used for young recipients.

[e] Nationaal Instituut voor Gezondheidsbevordering en Ziektepreventie

However, so far none of the 16 available kidneys from donors younger than 10 years has been allocated to a child (personal communication Dr J de Boer, Eurotransplant). This policy might become more effective through easing of the HLA-match requirement of at least 2 DR-matches and extending the age-criteria for donor and recipient up to 16 years. Such measures will be of very little consequence for the waiting time of adults, given their at least 30-fold bigger population.

Kidneys from very young donors, from 1 to 5 years of age, can be used more effectively. Many centers discard these kidneys. Nevertheless, provided these kidneys are of relatively large size, they can be transplanted as single grafts into pediatric patients. Relatively small kidneys can still be used for children, when transplanted *en bloc*.[6-8]

Finally, pre-emptive transplantation and starting the search for a suitable donor prior to the actual start of dialysis are measures that will shorten the dialysis episode and thus alleviate its impact on children's physical and psychosocial outcomes. Such pre-emptive transplantation in Dutch children has been associated with superior graft survival (**Chapter 5**).

Outcome of transplantation

Graft survival

The past 20 years have seen impressive improvement in graft survival and fall in rejection rate **(Chapters 2 to 5)**. Findings parallel those of large registries, e.g. NAPRTCS. The latest figure of 1 year graft survival, 92%, corresponds to the 91-92% in recent reports of the OPTN/SRTR and NAPRTCS registries.[1,9] This improvement may largely be explained by the expansion of immunosuppressive treatment and the use of more modern agents. In addition, collaboration between the centers, with meetings aimed at finding consensus on protocols, and the faster developing experience with larger numbers of patients, will have contributed to these results. Unfortunately, the extent of this contribution is hardly measurable. The incidence of acute rejections is slightly higher than the 16% in the latest NAPRTCS report.[1] This may be due to the relatively low rates of biopsy proven acute rejection in our studies. NAPRTCS surveys for that matter do not report proportions of biopsy proven acute rejections.
The 81% graft survival after 5 years in the cohort 1998-2004 (data not shown) – tallies with the 79% reported by the NAPRTCS, if calculated to the same LD/DD distribution as in our population.[1]

Thrombosis of the graft

We found that 8% of grafts failed within the first year. Most of these graft failures were caused by thrombotic events, which partially might have been avoided. Incidence of thrombotic events (4.3%) have remained unchanged over the past two decades, despite the introduction of a shared protocol on antithrombotic prophylaxis in high-risk transplantations.

NAPRTCS reports a lower rate of 1.85%, in striking contrast with the 10% observed in the Great Ormond Street Hospital for Children in London.[1,10,11] In our population we could not confirm the previously reported negative association with pre-transplant peritoneal dialysis, nor the protective effect of IL-2 receptor antagonists on the incidence of thrombosis.[10,12-14] We expect that several antithrombotic measures might be effective in lowering the rate of graft failure due to thrombosis. For one, more aggressive perioperative fluid management seems promising, provided that central venous pressure is monitored. Another option is limiting cold and warm ischemia times. No longer considered urgent, kidney transplantation usually is performed at the end of the surgical program. Together with more efficient allocation logistics of Eurotransplant the average cold ischemia time in the deceased donor transplants of our last cohort was 20 hours. As graft loss due to thrombosis is associated with longer cold ischemia time, surgery should be scheduled such that ischemia times are shortened, especially when young donors and/or recipients are involved.The second warm ischemia time is dependent on quality of the graft and blood vessels and on the surgical technique.
Widening the criteria for anti-thrombosis prophylaxis might help to prevent thrombosis. Pre-transplant evaluation of thrombophilia could identify extra patients at risk for thrombosis.[15] Another alternative would be prophylaxis for all patients. This could result, however, in hemorrhagic complications, also in patients who would not need anticoagulation therapy. Summarizing, as possible measures to reduce graft loss due to thrombosis we propose extended pre-transplant evaluation of thrombotic risk factors, improvement of perioperative fluid management, and reduction of ischemia times.
A report from our group is in preparation on graft thrombosis in our patient population, and the effects of two different approaches to thrombosis prophylaxis.

Delayed graft function
Partly overlapping with the above mentioned problem is that of ischemic injury of the donor organs, leading to delayed graft function, which occurred in 10-13% of transplants (**Chapter 5**). Wider donor criteria may have contributed to this phenomenon: the use of non heart beating kidneys is associated with delayed graft function, and even early graft loss.[16] The increase in number of non heart beating donation over the last decade unfortunately has been associated with a decrease in number of heart beating donors. Non heart beating donor kidneys with a prolonged first warm ischemia time of more than 30 minutes are associated with early graft loss due to ischemic injury. Moreover, for a certain time before cardiac arrest the donor's circulation will not function optimally. It would seem very important therefore, and the trend apparently was set in 2007, to stimulate heart beating donation. Medical teams should not decide for non heart beating donation if heart beating donation is a feasible option. As in the case of thrombosis, shortening of cold ischemia time and more aggressive fluid management may improve delayed graft function as well.
One would expect that avoiding vasoconstrictive calcineurin inhibitors shortly after the ischemia/reperfusion injury should diminish the incidence of delayed graft function This was not the case, however, in our study described in chapter 5.

Immunosuppressive therapy

Our study described in **Chapter 5,** showed fewer acute rejections after induction with basiliximab with delayed onset of cyclosporine therapy. Nevertheless, this delay in the start of cyclosporine did not affect the occurrence of delayed graft function, nor did it improve graft function or graft survival twelve months after transplantation. It is too early to tell, however, whether improvement is possible on the longer term. The question arises whether initial immunosuppressive therapy for all patients should consist of this quadruple, sequential therapy, or whether it should be reserved for selected patients, e.g. those receiving deceased donor transplants, or even those undergoing retransplants. The addition of basiliximab brings along extra costs, which are not counterbalanced by the postponed onset of cyclosporine. Maintenance immunosuppressive therapy should ideally be tailored to the patient's immunological profile. We showed that the combination MMF and corticosteroids beyond 1 year post-transplant leads to more stable glomerular filtration rate than does treatment with cyclosporine and corticosteroids (**Chapter 6**). Still, as we observed late acute rejection episodes in 2 of 18 patients on MMF, there is much to say for restricting this regimen to patients at low risk of acute rejection, e.g. recipients of living donor grafts.

Suspected noncompliance to immunosuppressive therapy calls for discussion of the patient's concerns about specific side effects of the medication. If, for instance, cosmetic side effects are feared, maintenance therapy could consist of tacrolimus and MMF, without corticosteroids or cyclosporine. As an added benefit, this combination will also improve blood pressure and bone composition. Noncompliant patients will benefit from the once daily formula of tacrolimus that may be available in the near future. Minimizing immune therapy requires caution in patients who show strong immune reactivity to other HLA-antigens, measured as panel reactive antibodies, or in those with a high number of HLA-mismatches with the donor. In patients with chronic allograft nephropathy who are on cyclosporine therapy, a decrease in dosage of cyclosporine or a switch to sirolimus is recommended.[17] Substitution of calcineurin inhibitors by sirolimus could also be considered in the case of toxicity.

Compliance with the prescribed medication

It has been suggested that adolescent non-compliance lies at the basis of the outcome of transplantation in this age group, i.e. the least favorable outcome as compared with younger children. Non-compliance covers the spectrum from skipping a pill every now and then to systematically refusing to take them. The medical and social teams would do well to support the adolescent in taking responsibility for his own life, while gradually separating from his parents. Negotiating the appropriate extent of parental involvement – neither overprotective, nor lacking support – is a challenge for all parties. Acceptable, individualized, immunosuppressive regimens could be designed when adolescents are given room to freely talk about aversion to certain drugs. Reducing the frequency of drug administration may help. Nurses and social workers could be in a better position to dig up the reasons for reluctance or negligence. Young persons who have had their disease since early childhood, may in their teenage years be regarded as 'new users' of medical care.

After all, it was the parents who took responsibility until then. Gradually and repetitively they should be made acquainted with all aspects of the disease, medication, and risks of certain forms of behavior.[18]

It could be helpful to get the adolescents together in peer groups coached by a social worker or psychologist. This would enable them to exchange experiences. Regular meetings in hospital are often hard to realize in practice however. A worthwhile alternative is inviting them to camps together. Young adults who recently made the transition from pediatric to adult care could act as buddies to the younger patients. In our experience, camps meet the expectation of bringing patients in contact with each other, and several of them have intensive contact since meeting in camp, and visit each other during admissions.

Transition
The transition from pediatric to adult care should be well prepared to meet a number of objectives. The health care should be uninterrupted, patient-centered, age and developmentally appropriate. It should be flexible and comprehensive. Before and after transition, the adolescent should be skilled in communication, decision making, assertiveness, self-care and self-advocacy. Furthermore, parents of the young person should be supported during transition to enhance their child's advocacy skills.[18] The whole team, on the pediatric and the adult side, would do well to dedicate themselves to these tasks and let the young person start safely and self-responsibly at the adult department.

Patient survival

Cardiovascular disease
While the risk to die early is not large, life expectancy of a child on renal replacement therapy is still much shorter than that of a healthy child. Nevertheless it has improved over the years.[19] It is the patient's cardiovascular state that mainly determines lifespan. This is why prevention or restriction of cardiovascular disease, both pre- and post-transplant, should have highest priority. The load of risk factors for cardiovascular morbidity appears to be heavier in the dialysis phase than in the transplant phase. As mentioned before, one of the best ways to curb cardiovascular morbidity, therefore, is to transplant as early as possible, provided it is safe at the time. Other steps include optimalization of calcium phosphate metabolism during the pre-transplant period. This strategy aims to limit precipitation in the walls of blood vessels, which otherwise could lead to stiffening of the vessels. Furthermore, blood pressure should be normalized, both pre- and post-transplant, aiming at the 50th rather than the 95th percentile for age and sex.[20] ACE inhibition may be the treatment of choice after transplantation. For, apart from better blood pressure control, its use was found to improve graft function both in adults and children, especially those with chronic allograft nephropathy.[21,22] A large, multicenter, prospective randomized controlled trial to determine the effect of ACE-inhibition on blood pressure, graft function and mortality will start in 2008 in Canada.[23]

Other risk factors for cardiovascular disease are hypercholesterolemia and hyperhomocysteinemia. Statins are known to reduce the risk of atherosclerosis in hypercholesterolemia. To date, however. the use of statins in children is still debated, and for the time being restricted to older children.[24] Folic acid, cyanocobalamin and pyridoxin are known to lower homocystein levels. Randomized controlled studies are underway to determine whether vitamin therapy is effective in secondary prevention of myocardial infarction and stroke.[25]
The use of drugs that are toxic to blood vessels and kidneys – such as calcineurin inhibitors and corticosteroids – should be restricted as much as possible, both pre- and post-transplant. Strategies could aim at limiting doses and/or duration of treatment. The study described in **Chapter 6** documents an approach to limit the use of cyclosporine, i.e. by replacing it with maintenance treatment with MMF. Withdrawal of cyclosporine had no effect on blood pressure, however, and only a temporary effect on hypercholesterolemia. Sirolimus in combination with MMF is another alternative to calcineurin inhibitor, as it is neither nephrotoxic, nor cardiotoxic. Yet it has been associated with hyperlipidemia and could possibly lead to fertility disturbances in boys.[17,26]

Comorbidity

Infections and malignancies

Risks of infections and malignancies apparently have increased over the years, parallel to the expansion of immunosuppressive therapy.[27] Vaccination before transplantation would seem a good strategy to prevent the alarming primary infections with EBV and CMV, but is not yet clinically applicable. Post-transplant lymfoproliferative disease is the type of malignancy with the highest incidence in children after solid organ transplantation. In most cases a primary infection with EBV is the underlying cause. Treatment of viral diseases, including the EBV-related PTLD, consists first of minimizing immunosuppression as soon as possible, and secondly, of starting additional therapy, i.e. gancyclovir for CMV, and rituximab for EBV. Monitoring of viral load in the blood is critical to adequate management of these infections.

Lung disease

Our studies (**Chapter 7 and 8**) showed that pediatric kidney transplant patients are at risk for irreversible lung disease, in association with pulmonary infection. It would seem important, therefore, not to underestimate respiratory complaints, especially persistent coughing, and to monitor lung function yearly. Patients with persistent symptoms should be referred to a pediatric pulmonologist who may consider invasive diagnostics so as to establish an appropriate antibiotic drugs therapy. Physical therapy, with or without mucolytic aerosol therapy, had a positive effect on airway clearance: extensive therapy made respiratory symptoms disappear in all patients with bronchiectasis. Increased general physical activity could facilitate clearance of the airways.

Growth

Most patients on renal replacement therapy show suboptimal growth with stunted pubertal growth spurt, even many of those with good renal function. Growth may be stimulated by various medical interventions. Several investigators have reported that early age at transplantation and good graft function predict a better final height.[28,29] This is one of the considerations to limit dialysis treatment before transplantation as much as possible. Treatment with growth hormone has been proven beneficial in growth retarded patients with renal disease, before and after transplantation.[30-32] Such treatment achieves maximal effect in the first year of treatment, but each subsequent year brings further improvement in the mean standard deviation score.[33] Growth hormone treatment stops at the day of transplantation, because the high doses of corticosteroids administered during the first months after transplantation, most certainly will antagonize the effect of growth hormone. If the child shows insufficient catch-up growth, treatment could be resumed 12 or 18 months after transplantation, when corticosteroid dosage is low. Regrettably, the Netherlands health care system provides for reimbursement of growth hormone treatment only when the child's glomerular filtration rate is below 50 ml/min.1.73m^2. This policy, therefore, excludes prescription to patients with a well functioning graft. At the moment, final height has a disappointing average of -2 standard deviation score, which is in the lower range of normal body height. This would seem to implicate that 50% of children in need of renal replacement therapy remain as short as or even shorter than the 2.5% shortest healthy children.

It is unknown whether extended use of growth hormone both before and after transplantation actually improves final height. Most patients in studies received growth hormone either before or after transplantation.[30,33] Assuming an additional effect of extended use, it seems to be justified to offer renal disease patients growth hormone treatment from the start of chronic kidney disease until the end of growth, with the exception of the first year after transplantation.
Growth hormone treatment has additional benefits in children with chronic kidney disease. First, it may improve bone structure, by enhancing bone mineral density.[34,35] Secondly, it has been reported to alleviate endothelial dysfuction as described in renal failure patients as well as in patients with endothelial dysfunction due to growth hormone deficiency.[36] Thus, growth hormone treatment may help to cut back cardiovascular morbidity.

Physical condition

The transplanted child should be able to take up physical activity, more than before transplantation. Physical condition will typically decrease in the period of end-stage renal failure. For most patients, sports is not an option in the dialysis phase. Still, physical condition usually does not recover completely after transplantation, as demonstrated in adult patients.[37] In a cross-sectional study in children several years after kidney or liver transplantation, all children's physical fitness was significantly below that of age- and sex-matched controls.[38] Even corrected for delayed growth and pubertal development, fitness level was alarmingly low.

In adult transplanted patients, increased and sustained physical training could improve cardiorespiratory fitness to normal values, as demonstrated by participants of the World Transplant Games.[39,40] Some children will become overweight after transplantation, which obviously has an inhibitory effect on physical activity. Parents are pleased to see that their child finally starts eating with appetite, and corticosteroids trigger the weight gain. Together with habituation to little physical activity before transplantation, overweight will result in a downward spiral in which patients remain in their sedentary way of life. Furthermore, some types of medication, e.g. antihypertensives, may interfere with the capacity of physical activity.
Low cardiorespiratory fitness is associated with cardiovascular morbidity and mortality in the general population, as shown in the Framingham study.[41] The standard of care for transplanted patients who already are at increased cardiovascular risk should therefore include a meticulously designed and coached rehabilitation program. An additional advantage is that physical activity may improve bone condition in patients with osteopenia.[42,43]

Bone disease
A large proportion of young adult patients (18%) had crippling complaints of bone disease, in part caused by renal osteodystrophy prior to transplantation.[43] Long duration of pre-transplant dialysis treatment contributes to this morbidity. Avascular bone disease and osteopenia, with its onset post-transplant, may be prevented by minimizing corticosteroids treatment. Osteopenia may be counteracted by alfacalcidol, intranasal calcitonin, in addition to the above mentioned increased physical activity.[44] There are no effective treatment options for avascular bone disease in childhood. Total hip replacements have been performed in adults, with a cumulative implant survival of 99% at 10, and 64% at 20 years.[45]

Cognitive development
Children on renal replacement therapy may show impaired cognitive development in, the extent of which appears to be related to the duration of dialysis.[46,47] It has been reported to catch up after transplantation.[48,49] Again, shortening the duration of pre-transplant dialysis is essential to limit cognitive impairment. It is recommended to assess cognitive function and development in an early stage and during follow-up. Testing should be at a general level in combination with additional testing for the more specific domains of attention, memory, executive function and visual-spatial skills in school-children.[50] Educational support through individual educational plans, tutoring or special classes will likely optimize educational outcome for children with renal replacement therapy.

Organisation of pediatric renal tranplantation in the Netherlands

No more than 30 renal transplantations are performed in our country each year. This number has been stable for over 30 years now. Close collaboration between the four centers has obvious advantages. During the past 10 years we agreed on shared treatment protocols, discussed individual cases and considered further studies and improvements.

In the years to come we should like to invite surgeons and urologists to participate in our meetings. This would be the way to develop and discuss perioperative surgical and urological protocols, including techniques, use of *en bloc* donor kidneys, perioperative fluid management, reduction of cold and warm ischemia times, among other things. Similarly, the social workers, teachers, psychologists and dieticians in the four centers could be encouraged to share their experiences on a more regular basis.

In addition, we need to devise a strategy by which the expertise of the surgical teams can best be consolidated and guaranteed for the years to come. The low number of transplantations calls for close collaboration, either with the surgical teams of the other pediatric transplant centers or with the local transplant teams for adults.
Establishing a single national pediatric renal transplantation team may even be a worthwhile option. Obvious advantages of this strategy are greater efficiency and experience in both operative and post-operative care. On the other hand, it would come with disadvantages as well. Patients and parents often have a history from early childhood in the local dialysis unit. They will have become familiar with pediatric nephrology staff, nurses on the ward, social workers, dieticians, laboratory and radiology personnel. Transfer to a different, centralized hospital with unknown staff at the critical moment of transplantation introduces risk of miscommunications and may have repercussions on the child's further recovery. In addition, centralization would also artificially separate the centralized transplant team from the local follow up team.

Study endpoints

In future studies, effects of changes in treatment need to be assessed from suitable parameters. Clinical studies may be evaluated by clinical endpoints or laboratory markers, e.g. measuring immunologic or endothelial functions. Some remarks:
One year graft survival has reached a 92% rate. Studies demonstrating higher rates will need vast numbers of patients to obtain adequate statistical power. As such numbers cannot be provided for in the Netherlands, these studies only could be performed in collaboration with neighbouring countries. Instead, surrogate endpoints may be considered in children, for example estimated glomerular filtration rate 1 year post-transplant.[51]
Various endpoints may serve to study cardiovascular disease. At the vascular level, the effect of stiffening of the walls of the main arteries on blood flow patterns may be determined by pulse wave pattern analysis and measurement of transit times at the carotid and femoral arteries.[52] These parameters have been evaluated for children as well as for adults.[53] It is one of the endpoints of the Rich-Q study in Dutch and Belgian children on renal replacement therapy. The function of the endothelium may be studied in several ways, as performed by the group of Dr. M.R. Lilien, UMC Utrecht. In vivo flow mediated dilatation after an ischemic insult reflects endothelial function.[54] In vitro, the number of endothelial precursor cells type I present in the peripheral blood, and the amount of endothelial precursor cells type II that can be cultured from peripheral blood monocytes are associated with the risk of cardiovascular disease.

Structural and functional disease of the myocardium may be visualized by imaging techniques, e.g. echocardiography including tissue Doppler examination.
The optimal way to study exposure to calcineurin inhibitors is measuring the area under the concentration-time curve, which is a cumbersome method. While trough level does not adequately reflect the exposure, two hour postdose level has been shown to be a more reliable predictor of exposure.[55,56] Fine-tuning of the treatment with calcineurin inhibitors in children is probably best done by therapeutic drug monitoring, e.g. by estimation of the area under the concentration time curve using population pharmacokinetics (as performed by Dr. R. Mathot, Erasmus MC Rotterdam).

Summary

Summarizing, a range of issues play a role in further improvement of pediatric kidney transplantation in the Netherlands. These are listed below, each with the objectives to be derived from them:

1. Pre-transplant factors
   - Prevention and treatment of hypertension, bone metabolism, growth, development. The Rich-Q study is expected to contribute by setting evidence based standards of care (design Dr. J. Groothoff, AMC Amsterdam).
   - Shortening of pre-transplant dialysis treatment.
2. Source of the allograft
   - Evaluation of potential higher yield of deceased pediatric donors in the Netherlands.
   - Considering transplantation of adult living donor kidneys in younger children, preferentially to be performed in one institution in the Netherlands so as to develop experience within a dedicated team of surgeons, anesthetists, intensive care physicians, and pediatric nephrologists.
   - Widening the criteria of the Eurotransplant ‘Young for young’ program, by raising the age limits of both recipient and donor from 6 and 10 years, respectively, to 16 years.
   - Participation in the national cross-over program in case of incompatible donor-recipient pairs.
3. Outcome of transplantation
   - Minimizing immunosuppressive therapy one year after transplantation, in order to curb toxic effects (e.g. cardiovascular, bone, cosmetic, malignancy), notably of corticosteroids and calcineurin inhibitors.
   - Adaption of surgical schedules aimed at limiting cold ischemia time, as well as initiating more aggressive fluid management could prevent graft loss due to thrombosis. Coagulation function test should be added to pretransplant evaluation so as to identify high risk patients.
   - Surveillance biopsies at 3 and 12 months could be considered in young children with relatively large allografts to detect and treat subclinical acute rejection.
   - Transition care needs to be elaborated on.

4. Comorbidity
   - Retrospective evaluation of the effects of growth hormone therapy on final height, in relation to dosage and duration of treatment. Dependent on the results the treatment indication should be extended to transplanted patients with good graft function.
   - Effect of treatment with selected antihypertensives and/or statins on cardiovascular comorbidity.
   - Restricting corticosteroid administration could prevent bone disease .
   - Structural rehabilitation programs may improve the child's physical condition after transplantation. Worth studying are the effects on cardiac structure and function, and on bone mineral density.
5. Reaching these objectives requires our shared database to be maintained by a dedicated person fully responsible for data-collection and data-entry.

Conclusion

The collaboration between the departments of pediatric kidney transplantation in the four centers in the Netherlands and in Antwerp, Belgium, has been in place for 10 years now. It no doubt has been instrumental in improving the outcome of pediatric kidney transplantation in the Netherlands. The studies documented in this thesis support this observation. Still, efforts should be directed at optimizing these children's life expectation and quality of life, as described in the general discussion. On the horizon, children with a functioning kidney transplant will enjoy optimal education, sports, having fun with friends, in anticipation of a normal adult life.

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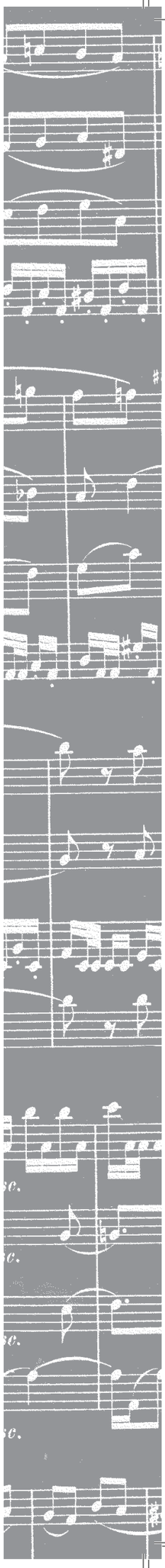
CHAPTER 12

SUMMARY



Kidney transplantation is the optimal modality of renal replacement therapy in children. It restores renal functions, more and better than dialysis, and has the potential to reduce the comorbidity of end-stage renal disease. It provides better conditions for normal physical and psychosocial development. Nevertheless, a graft usually does not function for life, and brings along comorbidity of its own. This thesis comprises studies dealing with this objective. Most are collaborative efforts from five institutions for pediatric kidney transplantations, four in the Netherlands and one in Antwerp, Belgium.

Chapter 1 gives an overview of the current state of affairs of pediatric kidney transplantation. Currently in the Netherlands, children with renal failure from the age of 3 years or a minimum body weight of 12 kg qualify for kidney transplantation. Yearly 25 to 30 children are transplanted in the Netherlands, distributed over four medical centers. After 10 years approximately 55% of kidneys are still functioning. Mortality rate is low at short term, but considerably higher in longer term follow up. Kidneys may be retrieved from living or from deceased donors. At this moment 30% of kidney grafts is from a living donor, and this figure is rising. Waiting times for children on the Eurotransplant waiting list for a deceased donor kidney are shorter than for adults, but have been increasing in recent years.

Here we review differences between pediatric and adult transplantations, as well as immunological aspects, including immunosuppressive agents and their side effects. We also report on risk factors for graft failure, such as rejection of the graft, thrombosis, ischemia/reperfusion injury, chronic allograft nephropathy. Primary kidney disease may recur in the grafted kidney, leading to loss of the graft. Non-compliance may contribute to deterioration of graft function. Unfortunately, immunosuppressive medication brings along comorbidity of its own, such as cardiovascular damage, bone disease, infections and malignancies.

Part I aims to review the outcome of former kidney transplantations in children in the Netherlands. All centers worked independently at the time, and the low numbers of patients interfered with proper evaluation of protocols.
Chapter 2 describes the outcome of the 269 transplantations performed between 1985 and 1995. Immunosuppressive therapy consisted of different combinations of corticosteroids, cyclosporine and azathioprine. CMV prophylaxis and thrombosis prophylaxis was not prescribed in all centers yet. Early graft loss was caused by thrombosis in 4.5%, and by acute rejection in 6.9% of transplantations. Only 26% of all transplants were free from acute rejection. Graft loss at long term was associated with number of acute rejections early post-transplant, but even more with late occurrence of the first rejection episode. The graft survival at 1 year was 73%, at 5 years 60%.

Chapter 3 reviews the status of young adults who received a kidney transplant as a child between 1972 and 1992, the first cohort of pediatric transplantation in our country.

The graft survival rate of all 397 transplants was 59%, 45%, 35% and 30% at 5, 10, 15 and 20 years, respectively. In comparison with azathioprine, cyclosporine was associated with better graft survival in retransplantation, but not in first transplantation. Patients treated with cyclosporine showed more comorbidity than the other patients, including more hypertension, hypertrichosis, severe warts, general fatigue, and a history of epileptic insults.

Part II contains reports of trials to improve immunosuppressive therapy in Dutch pediatric kidney transplantation.
Chapter 4 describes the effects of the introduction of a shared initial immunosuppressive regimen in all centers, consisting of a combination of corticosteroids, cyclosporine and MMF, and comparing these to the findings in the historical cohort documented in Chapter 2. A spectacular improvement in one year graft survival of first kidney transplant recipients was noted: the 73% of chapter 3 had jumped to 92%. Proportions of patients who remained rejection free had increased from 28% to 63%. Rates of steroid resistance of acute rejections had dropped from 30% to 16% (n.s.).

Chapter 5: Having treated 100 patients with this shared immunosuppressive regimen, we adapted it by delaying the start of cyclosporine and by adding basiliximab. We hypothesized that, by avoiding the vasoconstrictive action of cyclosporine in the immediate post-transplant-phase, this would reduce the incidence of delayed onset of graft function. This change in initial immunosuppressive therapy was thought to improve 1 year graft survival.
The outcome was compared to that of the cohort described in Chapter 4. Delayed graft function was measured in two ways: 1. the indication to dialyse in the immediate post-transplant period, and 2. the time period in which creatinine level reached half its pre-transplant value. We did not find an effect of the shift in initial immunosuppression on the incidence of delayed graft function, on estimated glomerular filtration rate at 1 year post-transplant, or on 1- and 2-year graft survival. The incidence of acute rejections had dropped significantly, though.

Chapter 6 contains a report of a randomized clinical trial, starting at 1 year after transplantation, to examine safety and efficacy of MMF and prednisolone as maintenance immunosuppression. The triple immunosuppressive regimen of the first year after transplantation was reduced to a dual prophylactic therapy, by slow withdrawal of the third drug over a period of 3 months. Patients treated with prednisolone and MMF showed better preservation of glomerular filtration rate over the 2 years of follow-up than did those treated with prednisolone and cyclosporine. Cholesterol levels improved over the 1st year in the MMF group, but did not significantly differ from the cyclosporine group after 2 years. In the MMF group 2 out of 18 patients had a late first acute rejection episode in the second year of the study (third year after transplantation), leading to chronic allograft nephropathy and graft loss in one patient, and proteinuria and decreased glomerular filtration rate in the other.

Part III, Chapter 7 reports the effect of restricting the time on dialysis on the outcome of pediatric kidney transplantation. Since pre-emptive transplantation was not performed very often in the Netherlands, we extended the field of investigation to the Eurotransplant region. First kidney transplants performed between 1990 and 2000 (n=1113) included 156 transplants without prior dialysis (14%), 70 with a graft from a deceased donors, and 86 from a living donor. Pre-emptive transplantation yielded the better graft survival, notably in the first 5 years, but also in the later 5 years of the study period. The effect was strongest in transplantations with kidneys from deceased donors. The favorable effect of pre-emptive transplants was absent when compared to transplantations preceded by less than 6 months dialysis. Patients with slowly progressive renal disease on the basis of congenital structural abnormalities who were enlisted before dialysis, had a 55% chance of being transplanted without dialysis.

Part IV contains reports on pulmonary and cardiovascular comorbidity of patients with a functioning kidney transplant in the Netherlands.
Chapter 8 describes findings in five patients with chronic respiratory complaints due to bronchiectasis, four after kidney transplantation and one after liver transplantation. These findings led to a cross-sectional study of respiratory complaints and pulmonary function of all children who had undergone kidney transplantation in the Netherlands, described in **Chapter 9.** More children than normal had respiratory complaints, possibly caused by a higher infectious load due to the immunosuppressive therapy, and more children had disturbed pulmonary function. To prevent bronchiectasis in the future, we recommend yearly check-ups with the pediatric pulmonologist and more aggressive treatment of respiratory infections.

Chapter 10 is a report of the prevalence of diastolic dysfunction in a single center population of children on renal replacement therapy, either peritoneal dialysis or transplantation, as compared with healthy children. The diastolic function was measured both by regular echocardiography and by tissue Doppler. Both methods yielded abnormal values in patients, more deviating in the dialysis patients than in the transplanted patients. Diastolic dysfunction is an early finding of cardiac disease and is a reason for alarm when observed in young patients.

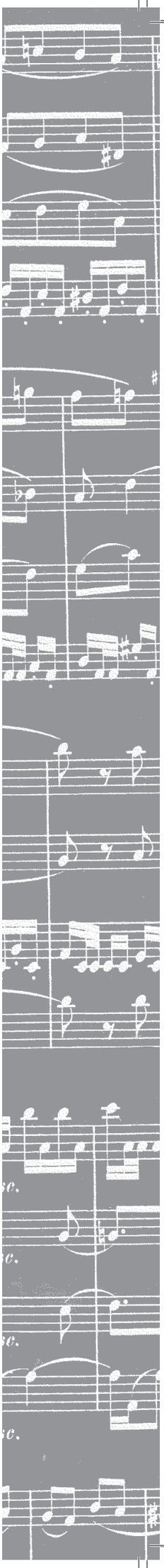
Chapter 11, the general discussion, is focused on ways to further improve the outcome of these patients. These involve pre-transplant conditions, direct transplant results as well as comorbidity issues. A major objective is shortening of the time on dialysis, since end-stage renal failure and chronic dialysis are associated with decreased patient survival, worse graft survival, increased cardiovascular risk for the long term, growth failure, and bone disease. Moreover, longer dialysis seriously interferes with development, school performance and psychosocial skills. To limit time on dialysis, a larger pool of available donors is needed.
Living donor transplantation can be further stimulated, and may be extended to younger recipients. Cross-over transplantation is an option for incompatible donor and recipient combinations. The Eurotransplant ‘young for young’ allocation program might be extended to recipients and donors up to the age of 16 years, since in the

current program (recipients younger than 6 and donors younger than 10) no transplants have been realized in almost a year. Graft losses due to thrombosis and the incidence of delayed graft function could be reduced by shortening cold ischemia times, for example by more effective scheduling of surgery. Graft survival in adolescents could be improved by more intensive coaching by social workers and psychologists to combat non-compliance. This is of special importance to prepare for transition from the pediatric to the adult medical system.

A major effort should be made in the battle against cardiovascular disease. This is the number one cause of early death in our population. Parameters to be used as study endpoints are being discussed. Minimization of immunosuppressive therapy is indicated to treat or prevent long term toxicity, including cardiovascular disease, infections, malignancies and bone disease. This strategy is preferentially controlled by immunologic tests to control the risk of acute rejection. Finally, changes in two fields could improve general well-being of the transplanted children. First, offering growth hormone treatment to growth retarded children also with good graft function, in order to achieve more appropriate adult body height. Second, offering a structured physical rehabilitation program following transplantation in order to improve children's cardiorespiratory function and fitness.

HOOFDSTUK 13

SAMENVATTING



Als de nierfunctie van een kind door een ziekte verloren is gegaan, kan deze het best vervangen worden door een niertransplantatie. Een transplantaatnier kan veel meer functies van de eigen nieren vervangen dan dialyse, en doet dat ook beter. Eindstadium nierfalen, met of zonder dialyse, brengt ziekteverschijnselen met zich mee, die door een niertransplantatie teruggedrongen kunnen worden. Bovendien neemt door een niertransplantatie de kans op normale lichamelijke, psychische en sociale ontwikkeling toe. Dit proefschrift bevat studies die als doel hebben de omstandigheden voor kinderen met een niertransplantatie te verbeteren. De meeste zijn gezamenlijk uitgevoerd door de vier Nederlandse centra voor niertransplantaties bij kinderen en dat van Antwerpen.

Hoofdstuk 1 geeft een overzicht van de huidige stand van zaken in niertransplantaties bij kinderen. Op dit moment kunnen kinderen vanaf de leeftijd van 3 jaar of een lichaamsgewicht van 12 kg in aanmerking komen voor een niertransplantatie. Per jaar ondergaan in Nederland 25 tot 30 kinderen een niertransplantatie, verdeeld over 4 ziekenhuizen. Na 10 jaar werkt 55% van de getransplanteerde nieren nog. Het sterftecijfer is op korte termijn laag, maar op de langere termijn aanzienlijk. Transplantaatnieren kunnen afkomstig zijn van een levende of een overleden donor. Op het moment van schrijven is 30% van de nieren afkomstig van een levende donor, en dit percentage loopt nog op. De wachttijd voor kinderen op de wachtlijst van Eurotransplant voor een nier van een overleden donor is weliswaar korter dan voor volwassenen, maar neemt de laatste jaren toe.

We beschrijven de verschillen tussen niertransplantaties bij kinderen en volwassenen, de immunologische processen, de medicatie die de afweer onderdrukt en de bijwerkingen ervan. Risicofactoren voor het verlies van transplantaatfunctie komen aan de orde, zoals acute afstoting, trombose, schade door zuurstofgebrek in de nier, chronisch transplantaatlijden. Sommige van de oorspronkelijke ziektes kunnen terugkeren in de nieuwe nier, en tot transplantaatverlies leiden. Een ander risico voor disfunctioneren van de nier vormt therapieontrouw die in de puberteit vaak voorkomt. Helaas brengt ook de niertransplantatie zelf en de immunosuppressieve medicatie ziekteverschijnselen met zich mee, zoals schade aan hart en vaten, aan de botten, het optreden van infecties, en maligniteiten.

Deel I bevat twee studies die terugkijken op de geschiedenis van niertransplantaties bij kinderen in Nederland, voor het begin van de samenwerking tussen de centra.
Hoofdstuk 2 beschrijft de resultaten van 269 niertransplantaties die tussen 1985 en 1995 uitgevoerd zijn. De immunosuppressieve behandeling bestond toen uit verschillende combinaties van corticosteroïden, cyclosporine en azathioprine. In die tijd werd er nog niet door alle centra profylaxe gegeven ter preventie van CMV ziekte en trombose. In de eerste weken ging 4.5% van de nieren verloren aan trombose, en 6.9% aan acute afstoting. Slechts in 26% van alle transplantaties werd geen acute afstoting gezien.

De transplantaatoverleving op de lange termijn was geassocieerd met het aantal acute afstotingen in de vroege fase na transplantatie, maar meer nog met het late optreden van de eerste afstotingsepisode. De transplantaatoverleving na 1 jaar was 73%, en na 5 jaar 60%.

Hoofdstuk 3 geeft een overzicht van jonge volwassenen die op kinderleeftijd een niertransplantatie hadden ondergaan. Deze jong volwassenen vormden het eerste cohort van getransplanteerde kinderen in Nederland. De transplantaat overleving van de hele groep van 397 transplantaties bedroeg 59%, 45%, 35% en 30% na respectievelijk 5, 10, 15 en 20 jaar. In vergelijking met azathioprine gaf initiële behandeling met cyclosporine een betere transplantaatoverleving alleen bij patiënten met een retransplantatie, maar niet in degenen met een eerste transplantatie. Patiënten die met cyclosporine behandeld werden toonden meer comorbiditeit dan anderen, onder andere hypertensie, sterkere lichaamsbeharing, wratten, vermoeidheid, en epileptische insulten.

In **Deel II** worden studies beschreven met als gemeenschappelijk doel het verbeteren van de immunosuppressieve medicatie in niertransplantaties bij kinderen in Nederland.
Hoofdstuk 4 beschrijft de effecten van een gemeenschappelijk immunosuppressief protocol in alle centra. Dit bestond uit corticosteroïden, cyclosporine en MMF. De resultaten werden vergeleken met die van de historische controlegroep beschreven in hoofdstuk 2. De transplantaatoverleving na 1 jaar was spectaculair verbeterd met het nieuwe regime, van 73% naar 92%. Het deel van de patiënten zonder acute afstoting was toegenomen van 28% naar 63%. Een groter deel van de afstotingsreacties kon afdoende behandeld worden met corticosteroïden, nl. 84%, in vergelijking tot 70% in de controlegroep.

Hoofdstuk 5: Nadat 100 patiënten met het bovenbeschreven protocol behandeld waren, werd het aangepast door de start van behandeling met cyclosporine uit te stellen en basiliximab toe te voegen. Onze hypothese was dat, door het vermijden van het vasoconstrictieve effect van cyclosporine in de herstelfase van de getransplanteerde nier, het vertraagd op gang komen van de nier minder vaak zou voorkomen. We verwachtten dat dit de 1 jaars transplantaatoverleving zou verbeteren. De resultaten werden vergeleken met het cohort beschreven in hoofdstuk 4. Het vertraagd op gang komen van de nier werd op 2 manieren gemeten: 1. door tijdelijke behandeling met dialyse in de eerste week na transplantatie, en 2. het aantal dagen dat nodig was om de helft van de kreatininewaarde voor transplantatie te bereiken. Helaas zagen we geen effect van het veranderde initiële immunosuppressieve protocol op het op gang komen van de nier, op de nierfunctie een jaar na transplantatie, of op de transplantaatoverleving na 1 en 2 jaar. Wel traden er minder acute rejecties op in de groep met basiliximab.

In **hoofdstuk 6** wordt een prospectieve, gerandomiseerde studie beschreven waarbij de effectiviteit en veiligheid van MMF werd vergeleken met cyclosporine in de onderhoudsfase, te beginnen 1 jaar na niertransplantatie.

Beide immunosuppressiva werden gegeven in combinatie met corticosteroïden. Het immunosuppressieve schema, dat in het eerste jaar uit 3 middelen bestond, werd over een periode van 3 maanden teruggebracht tot 2. Patiënten die behandeld werden met MMF en prednisolon behielden een betere nierfunctie gedurende 2 jaar dan de met CsA behandelde patiënten. De cholesterolconcentratie in het bloed verbeterde in de MMF groep over het 1e jaar, maar was niet significant verschillend van die in de CsA groep na het 2e studiejaar. Twee van de patiënten die met MMF behandeld werden, kregen echter een late acute afstoting, nl. in het 2e studiejaar. Deze leidde in de ene patiënt tot transplantaatverlies een half jaar later, en in de andere tot blijvend verlies van nierfunctie en eiwitverlies in de urine.

Deel III, Hoofdstuk 7 beschrijft het effect van het beperken van dialysetijd vóór transplantatie op de resultaten van transplantatie. Omdat transplantatie zonder voorafgaande dialyse, zgn. pre-emptieve transplantatie, niet vaak werd uitgevoerd in Nederland, werd het onderzoeksgebied uitgebreid tot de Eurotransplant-regio. Van alle 1113 eerste niertransplantaties bij kinderen, die tussen 1990 en 2000 plaats hadden gevonden, waren er 156 (14%) zonder voorafgaande dialyse, 70 met een nier van een overleden donor, en 86 van een levende donor. Pre-emptieve transplantaties hadden een duidelijk betere transplantaatoverleving, vooral in de 1e 5 jaar van de studieperiode, en vooral in de groep met nieren van een overleden donor. Er was geen verschil in transplantaatoverleving tussen kinderen getransplanteerd zonder, of na een korte periode van dialyse, maximaal 6 maanden. Van de patiënten met een langzaam progressieve nierziekte op basis van aangeboren anatomische afwijkingen, die op de wachtlijst geplaatst werden voor start van de dialyse, kon 55% ook daadwerkelijk getransplanteerd worden voordat dialyse nodig was.

Deel IV bevat rapporten van complicaties op het gebied van hart en longen in kinderen met een niertransplantatie in Nederland.
Hoofdstuk 8 beschrijft de bevinding van chronische longklachten ten gevolge van bronchiectasieën in 5 kinderen, 4 na een nier- en 1 na een levertransplantatie. Deze bevinding leidde tot het transversele onderzoek, beschreven in **hoofdstuk 9**, naar klachten van de luchtwegen en longfunctie bij kinderen met een functionerende transplantaatnier. Opvallend veel kinderen hadden klachten van de luchtwegen. Dit zou te verklaren zijn uit het grotere aantal luchtweginfecties dat kon ontstaan door de immunosuppressieve behandeling. Ook hadden meer kinderen dan normaal een afwijkend longfunctieonderzoek. Om bronchiectasieën in de toekomst te voorkomen adviseren we een jaarlijkse controle bij de longarts, en agressievere behandeling van, m.n. lagere, luchtweginfecties.

In **hoofdstuk 10** wordt het vóórkomen van een verminderde diastolische functie van de linker hartkamer beschreven in kinderen die nierfunctievervangende behandeling, peritoneaal dialyse of niertransplantatie, ondergaan in één behandelcentrum, in vergelijking tot gezonde kinderen. De diastolische functie werd gemeten met standaard echocardiografie, aangevuld met tissue-Doppler onderzoek, en vergeleken met gezonde kinderen.

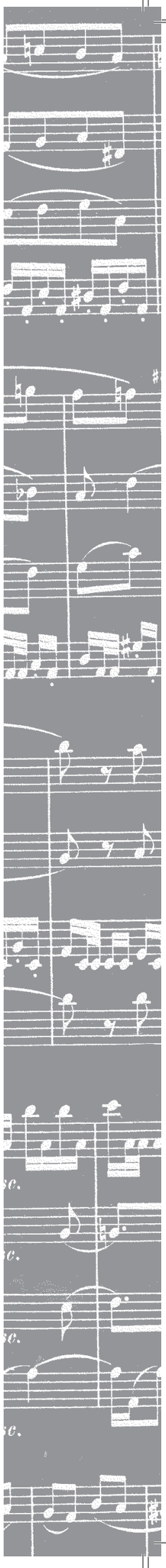
Met beide methoden werden afwijkende gemiddelde waarden gevonden in zowel de groep peritoneaal dialyse patiënten, als de getransplanteerde patiënten. Diastolische disfunctie is een vroege uiting van verminderde hartfunctie, en een alarmteken bij jonge kinderen.

Hoofdstuk 11, de algemene discussie, richt zich op manieren waarop het leven van kinderen met een niertransplantatie verder verbeterd kan worden. Het gaat om aspecten vóór transplantatie, directe transplantatieresultaten, en de aandoening van andere organen. Een belangrijk doel is het beperken van de duur van eindstadium nierfalen door beperking van de dialyseduur voor transplantatie, aangezien die periode geassocieerd is met hogere sterfte, slechtere transplantaatoverleving, groter risico op hart- en vaatziekte, slechtere groei, en botziekte. Bovendien heeft dialyse een duidelijke weerslag op de psychomotorische ontwikkeling van het kind, en de schoolprestaties. Om dialyse te kunnen beperken, zijn meer donoren of een andere verdeling van donoren nodig.
Transplantatie met nieren van levende donoren kan verder gestimuleerd worden, waarbij de capaciteit voor deze transplantaties in de ziekenhuizen aangepast dient te worden. Misschien moeten ook de jongste kinderen een nier kunnen ontvangen van één van hun ouders. Het cross-over programma is een goede mogelijkheid om transplantatie met een levende donor ook in het geval van een niet passende donor-ontvanger-combinatie mogelijk te maken. Het ‘young-for-young’ programma van Eurotransplant kan uitgebreid worden tot donoren en ontvangers van 16 jaar, aangezien het programma in de huidige versie nog niet tot transplantaties heeft geleid. Zo kunnen nieren van overleden kinderen getransplanteerd worden in kinderen, wat nu meestal niet gebeurt. Transplantaatverlies door trombose en het optreden van vertraagd op gang komen van de nier zou beperkt kunnen worden door het verkorten van koude ischemie tijd, bijvoorbeeld door het verlenen van voorrang op het operatieprogramma aan een niertransplantatie. Transplantaatoverleving in adolescenten zou bevorderd kunnen worden door een poging de therapietrouw te verbeteren door intensievere begeleiding door lotgenoten, psychologen en maatschappelijk werkenden. De overgangsfase van kinderziekenhuis naar ‘volwassen’ ziekenhuis draagt extra risico voor afname van de therapietrouw.
Een van de belangrijkste aspecten van comorbiditeit waar we de strijd tegen aan moeten binden is hart- en vaatziekte bij onze patiënten. Het is de belangrijkste doodsoorzaak. Daarvoor is terugbrengen van bepaalde immunosuppressieve medicijnen gewenst, maar ook om andere toxische effecten te beperken, o.a. het optreden van infecties, van maligniteiten en botziekte. Er zijn aanwijzingen dat dit terugbrengen gevolgd kan worden met immunologische bepalingen, die het risico op afstoting weer kunnen geven. Tenslotte zou het algemeen welbevinden van kinderen met een niertransplantatie door twee aspecten kunnen verbeteren. In de eerste plaats door een poging de volwassen lengte in een normalere range te brengen door behandeling met groeihormoon ook bij goede nierfunctie na transplantatie mogelijk te maken. In de tweede plaats zou het zinvol zijn om een revalidatieprogramma na transplantatie onderdeel van de behandeling te laten zijn, ter verbetering van de cardiorespiratoire functie en lichamelijke conditie.

DANKWOORD

CURRICULUM VITAE

PUBLICATIONS



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Eric Wolff, beste Eric, in 1990 was ik blij te kunnen komen werken bij de leukste subafdeling in het Sophia, de kindernefrologie, die op dat moment uit Jeroen en jou bestond. Naast het feit dat ik ontzettend veel kindernefrologie van je geleerd heb, droeg je ook enorm bij aan mijn dagelijkse werkplezier gedurende vele jaren. Ik ben je daar heel dankbaar voor!

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Curriculum Vitae

1972
eindexamen gymnasium beta, Rhedens Lyceum te Rozendaal (Gld)

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studie medische biologie, RU Utrecht, doctoraalexamen cum laude. Doctoraalvakken: neurofysiologie (Nederlands Herseninstituut, Amsterdam), farmacologie (Rudolf Magnus Instituut, Utrecht), immunologie (Lab Immunologie van het Wilhelmina Kinderziekenhuis, Utrecht). Een deel van het vak farmacologie werd bewerkt aan de Addiction Research Foundation in Toronto, Canada.

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opleiding tot kinderarts, Wilhelmina Kinderziekenhuis, Utrecht (Prof Dr JW Stoop)

1988-1990
werkzaam als kinderarts bij de afdeling Intensive Care Chirurgie (Prof Dr J Molenaar), Erasmus MC Sophia, Rotterdam

1990-heden
werkzaam bij de subafdeling kindernefrologie van het Erasmus MC Sophia (Dr ED Wolff, opgevolgd door Dr J Nauta), de eerste jaren als fellow, vervolgens als kindernefroloog. Vanaf 1995 vormden niertransplantaties een aandachtsgebied. Een nationaal samenwerkingsverband werd opgezet en gecoördineerd. Daarnaast werd geparticipeerd in de landelijke transplantatieorganisaties Nederlandse Orgaan Transplantatie Registratie, Transplantatie Werkgroep Nederland en het Landelijk Orgaan voor NierTransplantaties.

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