Innovations in Kidney Transplantation

PhD Thesis

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List of Abbreviations

ACR	Acute cellular rejection
AD	Anno Domini ("In the year of Lord…"
BC	Before Christ
BMI	Body Mass Index
BSA	Body surface area
BUN	Blood urea nitrogen
CMV	Cytomegalovirus
CsA	Cyclosporine
CTA	Computerized tomography angiography
DM	Diabetes mellitus
DSA	Digital subtraction angiography
ECD	Extended criteria donor
ESRD	End-stage renal disease
FMD	Fibromuscular dysplasia
GFR	Glomerular filtration rate
HLA	Human Leukocyte Antigen
HTN	Hypertension
LOS	Hospital length of stay
MDRD	Modification of Diet in Renal Disease Study Group
MRA	Magnetic resonance angiogram
ns	Not significant
NT	Nucleotide
OKT3	Muromonab-CD3
OPTN	Organ Procurement and Transplantation Network
PRA	Panel reactive antibody
rATG	Rabbit anti-thymocyta globulin
RNA	Ribonucleic acids
SD	Standard deviation
SRL	Sirolimus
SRTR	Scientific Registry of Transplant Recipients
UNOS	United Network of Organ Sharing
US	United States of America
VEGF	Vascular endothelial growth factor

1. Introduction

Transplantation is a revolutionary opportunity for end stage renal disease suffering patients for receiving back their normal daily life. During its history the surgical technique became straightforward. The intervention supported with modern anesthesia, fine surgical materials and technique is safe, well tolerated and low risk.

1.1. “Rebirth” - Kidney transplant history

Logo of the Transplant services at University of Texas – Houston.

Mythological tales of chimerical beings have been told all over the world, although these transplantations were by supernatural forces rather than by surgical methods. The earliest evidence of an orthotopic autograft has been preserved from the Bronze Age as a circular disk of bone was removed from the calvaria to relieve intracranial pressure and later replaced as an autograft. Written accounts from Egypt, China, and India dating back many centuries describe experimentation in grafting. One Indian text from 800 BC describes by potters of the Koomas caste that the surgeon Susrata grafted new noses created from skin flaps.[1] In 15 AD was reported of Saint Peter replacing the young Agatha's breasts, which were cut off as punishment by Roman guards. [1] The first reference to the concept of organ transplantation and replacement for therapeutic purposes was reported in c. 200 AD as Hua-To in China replaced diseased organs with healthy ones. [1] The miracle by Saints Cosmas and Damian

(brothers and patrons of physicians and surgeons can be seen on the Hungarian crown), in which the leg of a deceased Moor was grafted onto a person whose leg was diseased [1] was reported from the years c.300 AD.

Job Van Meeneren documented in 1668 the first successful bone graft from a dog's skull used to repair defect in human cranium. While in 1880 the first reported cornea transplants were done 10 years later Locke invented a preservation solution [1] Kidney transplantation started in 1902 when the Hungarian Emerich Ullmann performed the first successful experimental kidney transplant (in neck of a dog)[2]. 4 years later Jaboulay did the (unsuccessful) first human kidney transplant, using animal kidney (xenograft) and in 1908 Carrell performed the first autologous renal transplantation with survival of several years. The surgical technique of transplantation advanced in 1906 as Carrell and Guthrie performed artery replacement with segment of vein and in the same year Jaboulay transplanted en bloc pig kidney into human after perfused with Locke's solution. 1913 Schonstadt repeats experiment of transplanting a kidney from a Japanese monkey into a young girl with nephritis caused by mercury poisoning. After producing small amounts of urine, the patient died 60 hours after transplant. [1, 3] The first human kidney transplant (allograft) was done but unsuccessful in 1936 (Voronoy)[2, 3].

The reality of organ transplantation began with advances in chemical anesthesia and aseptic surgery. Alexis Carrel is known as the founding father of experimental organ transplantation because of his pioneering work with vascular techniques. Carrel and Guthrie are credited with the vascular technique of triangulation, in which 3 equidistant stay sutures are placed and a fine suture is run along the relatively flat surface between each of the stay sutures. Carrel is also credited with the "Carrel" patch technique used in replantation of major vascular structures during organ transplantation. The work of Carrel and Charles Guthrie served as the foundation of vascular surgery and organ transplantation.[4-6] Early transplantation attempts in humans, which began with transplantation of renal allografts in 1936, generally did not succeed until the discovery of immunogenetics and the implementation of immunosuppressive drugs. Experimental intra-abdominal renal grafts were being performed in animals in the 1930s and 1940s. Autografts generally survived, although homografts were rejected. On December 25, 1952, Hamburger performed a renal transplantation in a 15-year-old roofer who injured his solitary kidney. The donor of the graft was the patient's mother. The graft functioned

immediately following surgery, but it unfortunately ceased to function on the 22nd postoperative day. The patient died 10 days later due to the unavailability of immunosuppression and hemodialysis.

Joseph E. Murray and Hartwell Harrison performed the first successful kidney transplant at Peter Brent Brigham Hospital on December 23, 1954 where the donor was the living identical twin brother of the recipient, and the kidney functioned for 8 years. This success was followed by subsequent attempts by Murray and Merrill that led to 7 successful transplantations between identical twins in Boston. Most of the recipients of identical twin kidney grafts performed by Joseph Murray did well; some still have functioning kidneys more than 30 years after transplantation. However, the attempts at cadaveric renal transplantation universally resulted in graft failure due to rejection. The same hospital gave place for the first successful cadaveric kidney transplant. The kidney functioned for 21 months in 1962. In the same year András Németh in Szeged performed the first living related kidney transplant in Hungary. The patient lived for 79 days without immunosuppression[7].

In the early 1960s, cadaveric donations were thought to be impractical and impossible. Living donors were the only available source of organs for transplantation. At Massachusetts General Hospital, a liver was harvested from a police officer whose heart was beating but whose brain was deemed dead. This seminal event led to the development of the concept of brain death as death, rather than the cessation of circulation, which previously defined death. The concept of brain death greatly increased the number of organs available for donation and improved the preservation of harvested organs. Once the concept of brain death was established, a system for organ procurement was founded to ensure the quality and availability of organs as efficiently as possible.

The promising steps of kidney transplantation accelerated other parenchymal organs transplantation as in 1963, Thomas Starzl made the first successful human liver transplant at the University of Colorado or James D. Hardy at the University of Mississippi performed the first lung transplant. In l966 Richard C. Lillehei at the University of Minnesota, performed the first successful pancreas transplant. In l967, Christian Bernard in Cape Town, South Africa, performed the first successful heart

transplantation, and in 1968 the first heart transplant in the United States was performed at Stanford University Hospital[3].

The Hungarian centralized and newly organized kidney transplant program started in 1973. Since 1994 the four university transplant centers cover the whole country for kidney, liver and pancreas transplantation.

The first attempts to control the immune system used total body irradiation. In 1958, a Boston-area woman who was accidentally irradiated with 6 Gy received a functional renal graft, although the patient died from bone marrow aplasia. In 1959, Hamburger and Merrill irradiated 2 transplant recipients with a total dose of 4.5-4.8 Gy; the donors were nonidentical twins. Both of these recipients had successful outcomes. The patients survived 20 and 26 years, respectively. In June 1960, Kuss and colleagues were faced with rejection in a kidney transplant recipient who received the graft from an unselected donor. The use of 6-mercaptopurine in this patient, an immunosuppressive agent previously studied in animals (by Zukowski and Calne), reversed the rejection process and ushered in the era of medications for the prevention and treatment of rejection. In 1964, Crosnier performed another cadaveric transplantation with long-term successful function. Discovery of the fungus - *Beauveria nivea,* by Jean Borel in samples of soil from Wisconsin and the Hardanger Vidda (fjord) in Norway, leads to cyclosporine. 1983 cyclosporine, an anti-rejection drug, was approved by the Food and Drug Administration (US Government)

In the early 1960s, the pioneering work of Thomas Starzl led to further advancements. His contributions were systematic studies using azathioprine and prednisone therapy to prolong graft survival. Following the demonstration of antilymphocyte serum efficacy by Waksman, Starzl conducted the first clinical trial of antilymphocyte globulin as an adjunct to azathioprine and prednisone in human kidney transplantation.

In the current decade, not the surgery but the accompanying management needs to be answered – to decrease the waiting time with the number of the transplantable organs and the use of them; immunosuppressive therapy suitable for all recipients in lower dose and lower side effect rate; prevention and treatment of rejections effectively giving longer graft and patient survival and without losing working nephrons; cheap and easy treatment of minor side effects. Nowadays the main goal for the transplant services

is to help as many people as possible. They need to find safe sources to expand the donor pool – using related and unrelated living donors, non-heart beating donors, cross match positive transplants, using and not rejecting not perfectly functioning older kidneys, even transplanting two of them into one recipient (dual kidney transplant), or use very young kidneys separately and help two recipients not only one as more commonly done with kidneys under the age of 5, en-bloc transplanted. Use of organs with rare vascular disease affecting the renal artery may come even from living donors. Finding out it's danger to the recipients or to donors. Searching for new immunosuppressant drugs and reviewing the current protocols to diminish better rejection rate with lower side effect rates. Find solution to save already rejecting kidneys if they do not respond to the usual therapy. While we do not find the best medical and surgical therapy find cheap and easy agents, topicals to decrease the risk of any side effects.

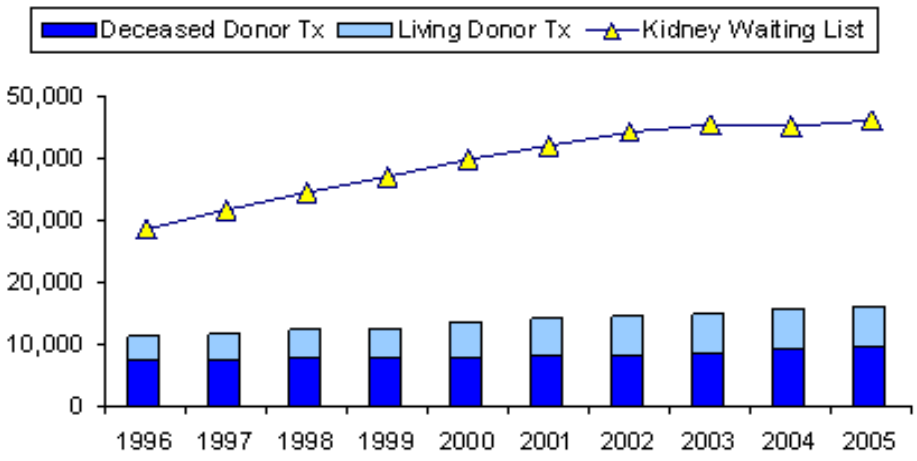
The questions needed to be answered are countless and the world's transplant society still has a lot to do even on the donor`s side and on the recipient`s side.

1.2. Expanding the donor pool

Kidney transplantation is a well-established therapy for chronic renal failure, but its application is limited primarily by the availability of transplantable organs. The number of end stage renal diseased people[8] and wait-listed patients continues to grow, and aggressive attempts to increase the number of transplants have failed to keep pace with demand. The continuing disparity between the demand for kidney transplants and the supply of organs has made efficient use of critical organs, forcing the transplant community to use organs from higher-risk donors than would previously have been considered [9].

Kidney transplantation is a surgical procedure where a diseased kidney replaced with a healthy one originated from another person. During this treatment procedure, healthy kidneys in excellent condition are the best help and they have the best results on diseased patients. As the surgical technique of transplantation reached the level of a safe and well tolerated procedure while immunosuppression gives a long well functioning life for the organ and the patient.

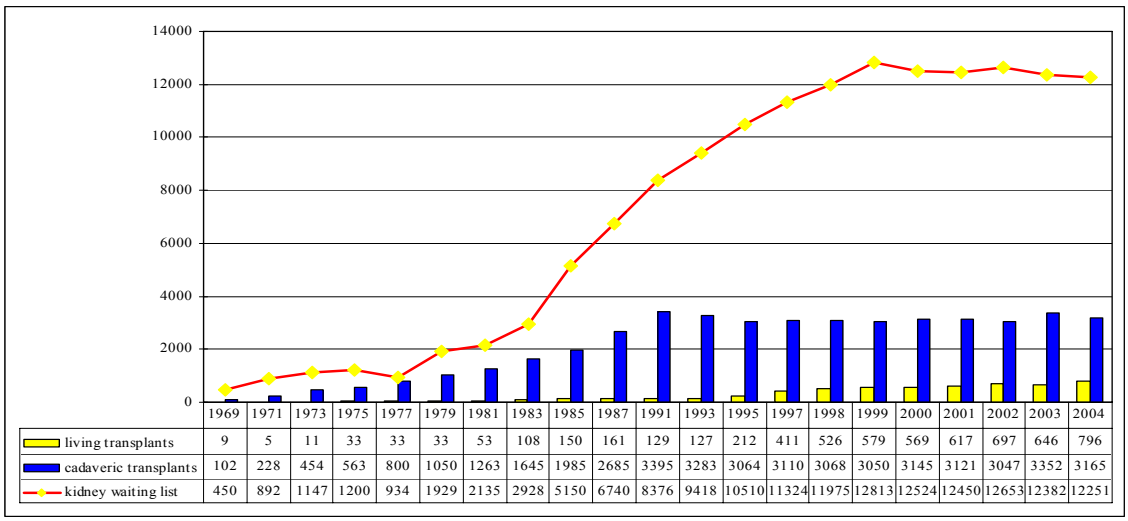
Figure 1: Waiting list and kidney transplants in USA



Source: 2006 OPTN/SRTS Annual Report, Tables 1.7, 5.1a

There is a gap between the number of patients waiting for a transplant and the number receiving a transplant. This gap has been widening, which means that the waiting times from listing to transplant continue to increase. Similar tendency is observed in the US (Figure 1) and in Europe (Figure 2).

Figure 2: Dynamics of the Eurotransplant kidney waiting list and kidney transplants between 1969 and 2004



	1969	1971	1973	1975	1977	1979	1981	1983	1985	1987	1991	1993	1995	1997	1998	1999	2000	2001	2002	2003	2004
living transplants	9	5	11	33	33	33	53	108	150	161	129	127	212	411	526	579	569	617	697	646	796
cadaveric transplants	102	228	454	563	800	1050	1263	1645	1985	2685	3395	3283	3064	3110	3068	3050	3145	3121	3047	3352	3165
kidney waiting list	450	892	1147	1200	934	1929	2135	2928	5150	6740	8376	9418	10510	11324	11975	12813	12524	12450	12653	12382	12251

To increase the number of transplants we should increase the donor pool, and we need to use as many organs of the current pool as possible without jeopardizing the safety

and good function of the transplanted organs. The demographics of the donor pool are also changing. The average potential cadaveric organ donor is now more likely to be older, at greater risk for co-morbid conditions such as hypertension or viral infections, and more likely to die from cerebrovascular disease. These factors have led to an expansion of the criteria that defines the suitable organ donor [10]. Quite influencing number of donors are in the age ranges of the "not optimal". According to the deceased donor characteristics in US or Europe 4-5 % of the donors under the age of 5, and 7-8 % above 60 years of age, and additional 20-25% is above 50 years of age meaning a large number for injured kidneys or extended criteria kidneys. Following these characteristics we can observe an increasing incidence of older donor since the past years than before. (Table 1)(Table 2)

Table 1: Deceased kidney donor characteristics in USA

Donor Age								**Year**
	1998	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**
Total	**5,339**	**5,386**	**5,489**	**5,528**	**5,638**	**5,753**	**6,325**	**6,700**
<1 Year	**32** (0.6%)	**45** (0.8%)	**42** (0.8%)	**48** (0.9%)	**38** (0.7%)	**29** (0.5%)	**33** (0.5%)	**36** (0.5%)
1-5 Years	**208** (3.9%)	**174** (3.2%)	**171** (3.1%)	**185** (3.3%)	**171** (3.0%)	**155** (2.7%)	**178** (2.8%)	**175** (2.6%)
6-10 Years	**156** (2.9%)	**152** (2.8%)	**148** (2.7%)	**173** (3.1%)	**137** (2.4%)	**102** (1.8%)	**133** (2.1%)	**94** (1.4%)
11-17 Years	**570** (10.7%)	**514** (9.5%)	**546** (9.9%)	**499** (9.0%)	**508** (9.0%)	**507** (8.8%)	**523** (8.3%)	**494** (7.4%)
18-34 Years	**1,469** (27.5%)	**1,398** (26.0%)	**1,437** (26.2%)	**1,486** (26.9%)	**1,608** (28.5%)	**1,586** (27.6%)	**1,728** (27.3%)	**1,861** (27.8%)
35-49 Years	**1,394** (26.1%)	**1,483** (27.5%)	**1,478** (26.9%)	**1,492** (27.0%)	**1,494** (26.5%)	**1,552** (27.0%)	**1,692** (26.8%)	**1,784** (26.6%)
50-64 Years	**1,116** (20.9%)	**1,197** (22.2%)	**1,284** (23.4%)	**1,267** (22.9%)	**1,291** (22.9%)	**1,427** (24.8%)	**1,570** (24.8%)	**1,721** (25.7%)
65+ Years	**394** (7.4%)	**423** (7.9%)	**383** (7.0%)	**378** (6.8%)	**391** (6.9%)	**395** (6.9%)	**468** (7.4%)	**535** (8.0%)

Source: OPTN/SRTR Data as of May 1, 2006.

Table 2: Demographic data on deceased organ donors in Europe

age	**2001**	**2002**	**2003**	**2004**	**2005**	**%**	**2004/2005**
0-15	98	91	103	75	91	4.2%	**21.3%**
16-55	1133	1141	1225	1152	1157	64.2%	**0.4%**
56-64	280	290	305	303	333	16.9%	**9.9%**
>=65	221	222	262	263	364	14.7%	**38.4%**
total	**1732**	**1747**	**1895**	**1793**	**1945**	**100.0%**	**8.5%**

Source: Eurotransplant web database

2.1. Extended criteria donors

At the current stage of its history organ transplantation has become a viable treatment for an increasing number of patients suffering from irreversible organ failure. In response to the stepped rising demand for transplantation, both the number of transplant centers and the number of patients on waiting lists have grown rapidly. Because organ donation has not kept pace with demand, each year a greater number of patients die while awaiting donor organs. Among the factors contributing to the organ shortage are cultural and psychological barriers to donation and missed opportunities to request donation. [11]

Solving the increasing problem of organ shortage may guide us to increase the number of living related and unrelated donor organ transplants and use marginal organs for transplant. An accompanying diminution in traumatic deaths of potential young donors has made older and other marginal, or higher-risk donors the focus of studies on expansion of the donor pool.[11]

According to the United Network of Organ Sharing (UNOS) definitions extended criteria donors (ECD) are:

1) the donor is older than 60 years of age
2) the donors age 50-59 years and one criteria meet from the following as
    a) the donors death was cerebrovascular origin
    b) donor had hypertension
    c) serum creatinine >1.5 mg/dl
3) the donors age <50 years and two above mentioned risk factors are present

In the recent past high age was a contraindication both for organ donation and transplantation. However, similar to trends in the overall general population in the United States, there has been an increasing, yet disproportionate shift toward increasing numbers of older donors and recipients in kidney transplantation. In the last decade, the proportion of deceased donors >50 years of age has increased from 21% to 31%. [12] Expanded criteria donors (ECDs) aged >60 years and donors aged 50 to 59 years with additional risk factors accounted for 177 kidney transplants in

1988 and for 1200 transplants in 2003.[13] Although the median age in the US general population has increased by only 3 years (from 33 to 36 years) since 1988, the median age of deceased donors has increased by 15 years (from 25 to 40 years) during the same time frame.[13] Since 1995, the number of patients on the kidney transplant waiting list who are >65 years old has increased steadily, although the largest proportionate increase in the waiting list has occurred in patients who are 50 to 64 years of age.[14] Also in the last decade, brain death that results from cerebrovascular causes has increased from 26% to 41%; the median waiting times for kidney transplantation have doubled, and the kidney waiting list has increased 2.6-fold.[12-14] During this same time period, the number of deceased-donor kidney transplants has increased by only 16%.[13] in addition, nearly 50% of the >60,000 candidates on the current active waiting list for kidney transplantation are >50 years of age[14]. These changes have occurred because of the convergence of demographic inevitability and medical advances. The aging donor and recipient populations have led to new challenges in kidney transplantation. Controversy exists regarding the optimal approach to the elderly donor and recipient, particularly because each have been associated independently with reduced 6-month allograft function and decreased long-term graft survival.[15-18] A number of strategies have been proposed that include matching by age, medical risks, serologic condition, histocompatibility, size, and nephron mass.[19-24]

The increasing disparity between the number of available renal allografts and the number of potential recipients has prompted novel approaches to enlarge the donor pool. The numbers of living donors has increased through the introduction of the laparoscopic technique and the use of non-related donors. The deceased donor pool has been expanded via the use of individuals at the extremes of age.

2.1.1. Using ECD donor organs – dual kidney transplantation

Among older donors the incidence of worsened kidney function or multiple co-morbidities results often rejecting these organs for transplant due to their not optimal condition. Careful changes in the current practice of donor selection may lead to more available donor organs and more patients off hemodialysis with good results. In these

conditions we can use both kidneys of a donor for one recipient where a single one would not be enough for long term function so the recipients require both kidneys of the same donor. Dual kidney transplantation ("two to one") used since the 1990s in Europe and in the USA. Short and long term follow up studies prove good final results with dual kidney transplant. Short term graft and recipients survival data and kidney functions of dual kidneys were similar compared to single adult deceased kidney transplant [25]. Long term follow up in recipients older than 55 years of age was observed in 74 single and 39 dual kidney transplants and proved non-significant difference. 5 years after the transplant the graft survival rate was 82.1% in the double kidney and 80.0% in the single kidney transplants group. At the 8 years time point these rates were 82.1% and 74.1%. (p=ns)[26].

2.1.2. Using pediatric donor kidneys

Another group of donors with extreme age are the very young ones. According to the data available from the literature approximately 5 % of the available donor kidneys are younger than the age of 5. In the 1970s, heavy skepticism surrounded the use of pediatric kidneys for transplantation. Today, although skepticism has evolved into acceptance, the optimal use of the subgroup of very young donor kidneys remains unclear. Reservations have been expressed about poor functional results and increased technical complications in these kidneys. To improve outcome, some authors have advocated en bloc transplantation, which would decrease the number of recipients by half.

Nonetheless, there is some reluctance to accept kidneys from deceased donors below the age of 5 years, because of reports of inferior outcomes compared to adult donor grafts [27-29]. For example, the concern regarding the reduced renal mass of pediatric grafts has been addressed by the use of en-bloc double-kidneys, in which both pediatric kidneys from a single donor are transplanted into one recipient. Yet en-bloc transplantation is a more technically challenging surgical procedure which has been associated with a higher incidence of both vascular and ureteral complications, although a number of centers have reported excellent long-term outcomes [30-34]. Recently there has been interest in the transplantation of single pediatric grafts into adult recipients [35-37].

2.1.3. Organs with arterial disease

As we expand the donor pool among living donors, kidneys with multiple arteries, as well as with vascular anomalies such as fibromuscular dysplasia (FMD), have been used. FMD is a non-inflammatory, non-atherosclerotic condition affecting small- to medium-sized vessels, mainly the renal artery (60 - 75%) [38]. The etiology is unknown, but there is some evidence of an autosomal-dominant inheritance pattern with variable penetrance [39]. In addition, the prevalence is higher in women (75%), which may also imply a hormonal role. According to the arterial wall involvement, [40] there are 3 types of FMD: intimal fibroplasia (<10%), medial dysplasia (90%) and adventitial dysplasia (< 1%). Medial FMD is further divided into medial fibroplasia (75 - 80%), perimedial fibroplasia (10-15%) and medial hyperplasia (1-2%).

Although the long-term follow-up for this recipient population seems to be satisfactory, we could find an early recurrence of a rare type of FMD in a living-related renal transplant recipient, bringing back the question about the security of transplantation in this setting for either the recipient or donor.

2.2. Investigations on recipient's side

Important area of donor pool extension is to decrease the number of patient getting to waiting lists for a second transplant if their previous graft functioning well. Over the past 30 years, progress in basic science, immunopharmacology, and clinical practice has engendered exciting improvements in the field of transplantation that have resulted in longer patient and graft survivals and a better quality of life for recipients. The goal of organ transplantation — an efficient yet non-toxic immunosuppressive regimen — is particularly necessary for transplants.

2.2.1. Using new combination for immunosuppression

The first generation of chemical immunosuppressants in clinical use—azathioprine, cyclophosphamide, methotrexate—as well as the more recent agent mycophenolate mofetil, all exert an indiscriminate blockade of cell division, which prevents expansion

of the number of immunocompetent elements. The next development in this therapeutic area was the introduction of lymphoid depleting modalities — antilymphocyte sera, total lymphoid irradiation, and thoracic duct drainage. The most recent stage utilizes compounds that display greater selectivity for immunocompetent than for non-specific host resistance elements. Cyclosporine (CsA), as the first drug to fulfill this goal, had an important impact on the biology and practice of immunosuppression. After decades of wide use of cyclosporine, side effects and leak in efficacy in regular dose in premature higher risk races became clear.

In the absence of selective and specific drugs the decreased incidence and severity of side effects can be achieved by the combination of synergistic drugs only. With wise selection and the use of the combination of potent immunosuppressive agents for the maintenance therapy better results can be achieved with the goal of less toxicity particularly in high-risk patients who lose their grafts prematurely. Therefore, a good combination will allow not only reducing individual immunosuppressive drug induced toxicities but will also allow achieving better graft and patient survival.

However, the currently used immunosuppressive agents significantly decreased the incidence of acute rejections, but they have several side effects. A sirolimus and reduced dose cyclosporine combination reduced the rejection rate under 10% [41], while infectious complication rate was not significantly increased [41],[42]. The side effects of this combination – delayed wound healing, higher incidence of postoperative hernia, lymphocele myelosuppression, anemia, thrombocytopenia and leukopenia are well known and easily treated [43],[41, 42],[44],[45]. Previous studies proved that among high risk African American patients the SRL-CsA combination can achieve the same <10% rejection rate [46],[47].

2.2.2. Treating acute rejection

The time to failure of a renal allograft is determined by the initial function achieved after transplantation, the number and severity of insults to the graft, and a number of tissue characteristics. The insults a graft usually encounters including ischaemia/reperfusion injury, acute rejection episodes, drug-related nephrotoxicity, hypertension and hyperlipidemia. Important tissue characteristics include susceptibility

to injury and the ability of the tissue to repair damage. Prevention and treatment of acute rejection in kidney transplant patients play a key role in their daily care due to the permanently worsening organ function and to reduce the progression of chronic allograft nephropathy. Chronic rejection results from recurrent episodes of subclinical or clinically evident acute rejection, with or without involvement of chronic rejection-specific allogeneic immune mechanisms. The tissue damage occurs over a prolonged period of time, which allows the emergence of antigen-independent tissue repair mechanisms and intrarenal adaptations in response to progressive loss of renal mass.[48] Elderly transplant recipients are considered poor immune responders but if a single acute rejection episode occurs this is more likely to significantly shorten graft and patient survival in this age group. In the case of old (>50 years of age) donor kidneys once a rejection episode occurs, the ability to mount a tissue repair process seems impaired. An explanation for the increased loss of grafts from elderly donors that have experienced acute rejection episodes is that such kidneys have fewer nephrons that function adequately and that the cumulated effect of damage results in an earlier demise of the graft compared with younger donor kidneys. We have suggested that increased graft loss of older donor kidneys results from increased incidence of acute rejection episodes in the early post-transplantation months together with a partly impaired ability to repair the tissue. [49]

Campath-1H

Campath® (alemtuzumab) is a recombinant DNA-derived humanized monoclonal antibody (Campath-1H) that is directed against the 21-28 KD cell surface glycoproteins, CD52. CD52 is expressed on the surface of normal and malignant B and T lymphocytes, NK cells, monocytes, macrophages, and tissues of the male reproductive system. The Campath-1H antibody is an IgG1 kappa with human variable framework and constant regions, and complementarity-determining regions from a murine (rat) monoclonal antibody (Campath-1G).

The mechanism of action: Campath binds to CD52, a non-modulating antigen that is present on the surface of essentially all B and T lymphocytes, a majority of monocytes, macrophages, and NK cells, and a subpopulation of granulocytes. Analysis of samples

collected from multiple volunteers has not identified CD52 expression on erythrocytes or hematopoietic stem cells. The proposed mechanism of action is antibody-dependent lysis of leukemic cells following cell surface binding. Campath-1H Fab binding was observed in lymphoid tissues and the mononuclear phagocyte system. A proportion of bone marrow cells, including some CD34+ cells, express variable levels of CD52. Significant binding was also observed in the skin and male reproductive tract (epididymis, sperm, seminal vesicle). Mature spermatozoa stain for CD52, but neither spermatogenic cells nor immature spermatozoa show evidence of staining.

The efficacy of anti-T-cell antibodies to treat moderate to severe episodes of acute cellular rejection (ACR) may be mitigated by neutralizing antibodies, by hypersensitivity reactions, or by a fundamental resistance of the immune process. Campath-1H (Millennium Pharmaceuticals, Cambridge, Mass., USA), has been prescribed for the treatment of myeloid malignancies. In addition, this reagent has been investigated for induction therapy after renal transplantation following the pioneering work of the Cambridge group. [50]

Our group was one of the first ones to use Campath-1H for the treatment of acute rejection in kidney transplantation and I was the first to publish the data of these results. Campath-1H has been primarily used for induction therapy. Calne et al [51] treated 37 patients with two doses of Campath-1H followed by low-dose maintenance cyclosporine monotherapy. There was a 10.8% incidence of acute rejection episodes and a 71% 5-year graft survival rate. A larger series using a single dose of Campath-1H (30 mg) followed by tacrolimus and mycophenolate mofetil has also been reported to be associated with a low incidence of rejection episodes (16.35%) [52]. Campath safety was showen in children. 34 children underwent renal transplantation with Campath preconditioning, steroid avoidance and reduced calcineurin inhibitor immunosuppression with good results and tolerance. [53]. In cases of pancreatic transplantation, Kaufman et al [54] reported a 5% rate of rejection episodes using Campath-1H induction. Tzakis et al [55] also reported a decreased incidence and severity of rejection episodes among liver and intestinal transplant cases using Campath-1H induction treatment.

However, the indications for Campath-1H treatment of acute rejection episodes have not yet been defined. Schneeberger et al [56] reported a case of severe steroid- and rATG-

resistant rejection in a bilateral forearm transplant recipient. After two doses of 20 mg Campath-1H, most of the skin lesion's disappeared. Reams et al [57] also successfully treated an rATG-resistant acute rejection episode in a lung transplant recipient using four doses of Campath-1H (53 mg total). Finally, Basu et al [58] reported 36 kidney transplant recipients who experienced steroid-resistant rejection episodes and were treated with Campath-1H two doses of 20 mg). The patient survival was 94.4% and the graft survival 67%. In the series presented herein, four of the five patients were clearly experiencing more severe rejection episodes than those reported by Basu et al [58], since they had previously failed not only to steroid therapy but also to administration of antilymphocyte antibodies. Despite the use of much higher doses of Campath-1H than previously reported, only two, entirely reversible, adverse events were observed at a minimum follow-up of 2 months.

Recently, an association has been reported between steroid-resistant renal allograft rejection and the presence of a $CD20^+$ B-cell infiltrate [59], which may be susceptible to Campath-1H therapy. Since Campath-1H is a humanized antibody, an infusion reaction is relatively rare, although rigors, fever, nausea, vomiting, and hypotension have been reported in other series but not in the experience presented herein. After the first few days of the infusion anemia and thrombocytopenia can occur and the therapeutic effect of lymphocytopenia with recovery of monocytes generally in 2 to 3 months, of B-cells in 6 months, and of T cells in 12 months, although the CD4 fraction only reached 20%[60].

2.3. A complication of transplantation – wound impairment

Advances in surgical techniques and immunosuppression have led to an appreciable reduction in postoperative complications following transplantation. Complications after kidney transplantation most commonly vascular, urinary tract, peri-organ complications as lymphocele or haemorrhage and wound complications. Vascular complications varied 10-30% of cases [61]. Arterial thrombosis occurs less than 1% of cases while stenosis most common in days 2-22 post-transplantation in 2-10 % [62], venous complications are less common 0.3-4.2 % [63, 64]. Urinary complication found in 2-10% [65], mostly urinary leak. Compared the leak incidence in the most common

ureteroneocystostomies Leadbetter-Politano versus Lich-Gregoir turned to be 9.4% versus 3.7%[66]. Nonanastomotic postoperative bleeding is a not-uncommon complication caused by uremic coagulopathies unappreciated injuries to small host or torn hilar donor vessels, as well as anticoagulant therapy to maintain the potency of vascular access grafts or to treat coagulation disorders. [67] Haemorrhage is not common, while lymphocele formation can reach 0.6-18% [68], however using sirolimus the incidence can reach even 38-45.5%[69, 70].

Complications may occur due to technical misadventures or to an unanticipated evolution of minor problems. While the overall incidence of technical complications is approximately 5%, most problems are neither life-threatening nor hazardous to the outcome of the graft.

However, wound complications as probably the most common type of post-transplantation surgical complication can still limit these improved outcomes and result in prolonged hospitalization, hospital readmission, and reoperation, consequently increasing overall transplant cost. Corticosteroids disadvantageous effect on wound healing is well known. Compared to the previously used immuno suppressant agents, in regimes with sirolimus added the impaired surgical site healing occurs in 20% to 50%, even without receiving concomitant corticosteroids[71]. Generally, wound complications are categorized as superficial and deep wound dehiscences, perigraft fluid collections and seroma, superficial and deep wound infections, cellulitis, lymphocele and wound drainage. Lymphocele requires intervention significantly more frequent in patients on sirolimus. In a study 38% of the patients had lymphocele and 18% of them needed surgical reinterventio to solve it [72]. The results of several studies showed that the most important risk factors for wound complications are immunosuppression therapy and obesity rather than surgical and/or technical factors, including type of incision, reoperation, and the surgeon's expertise, as well as comorbidities such as advanced age, diabetes mellitus, malnutrition, and uremia. Immunsuppression modalities and agents, especially sirolimus (SRL), and steroids (ST) should be adjusted according to the patient's co-existing risk factors. Therapeutic modalities must focus on the most efficient and cost-effective medications and/or interventions to facilitate and improve wound healing [73]. 31.8% of kidney transplanted patients developed wound complications as lymphocele, bladder leak, wound dehiscence, cellulitis or an abscess.

The main factors turned to be immunosuppression therapy and obesity. Seventy-one percent of obese sirolimus treated patients experienced complications compared with 24.3% (P = 0.025) of non-obese sirolimus patients. In a study [71] surgical treatment was required in 29% of these patients.

2.3.1. Topical RNA derivates enhanced wound healing

Wound healing is a well regulated process with 4 main phases: coagulation, inflammation, migration/proliferation and remodeling with continuous absence of immune cells [74]. Normal wound healing can be impaired by chronic infection, protein malnutrition, poor blood supply, vitamin deficiencies, previous radiation exposure, diabetes mellitus, various drug therapies (corticosteroids, immunosuppressant) and deficiencies in the components of the host wound response. The process of wound healing involves a complex system of local and remote (systemic) energy and substrate requirements and uses. Lymphocyte participation in wound healing has been demonstrated. [75] Alteration in the host's T-cell dependent immune response has also been shown to influence wound healing. Beside the nutrients needed for these processes nucleotides (NT) including ribonucleic acids (RNA) have been shown to stimulate the immune system and NT-free diet suppressed immune response [76]. The usefulness of dietary nucleotides in certain medical contexts is documented. Dietary nucleotides are required for maintenance and recovery of host immune response [77, 78] Nucleotide supplementation has also been shown to provide an increase in both immunohemopoiesis [79] and resistance to infectious microorganisms. [80] Nucleotide supplementation has also been described as reversing immunosuppression induced by protein starvation [81]. Normal cellular immune response has therefore been postulated to require a source of preformed nucleotides. Dietary sources of nucleotides are important to support optimal growth and function of metabolically active cells such as lymphocytes, macrophages and intestinal cells. Besides the results of enhanced wound healing with dietary supplementation of nucleotides the first results of local therapy with nucleotide enhanced tear in rabbit corneal wound.[82]

According to the above mentioned results the aim of our study was to observe whether the use of extended criteria donors, donors of extreme ages or arterial disease

can safely expand the donor pool. We were interested in the protection of the grafts with immunosuppressant drug combinations and newer acute rejection treatments to decrease the number of patients getting back to the waiting list. One of the most common complications is still the wound complications. Due to our groups results on dietary RNAs on wound healing we were intend to prove sirolimus effect on wound healing on experimental basis using a mouse model and using the same model to observe whether the application of topical RNA gel to dermal wounds stimulates local immune response to accelerate the wound healing process.

3. Main objectives:

A. To find unusual sources for expanding the donor pool
    a. Using older (extended criteria donor), usually not used kidneys as dual transplants ("two to one")
    b. Small, very young pediatric kidneys as single transplants ("one to two")
    c. Checking the future risk for donor or recipient if kidneys used with fibromuscular dysplasia
B. To preserve the already transplanted organs
    a. Observe sirolimus-cyclosporine combination efficacy among premature high rejectional risk recipients compared to the side-effects
    b. Trying Campath-1H with a new indication for saving organs during acute rejection not responding the usual therapy
C. In an experimental animal model prove sirolimus to delay wound healing and try to prevent it with a topical containing nucleotide derivates

4. Methods

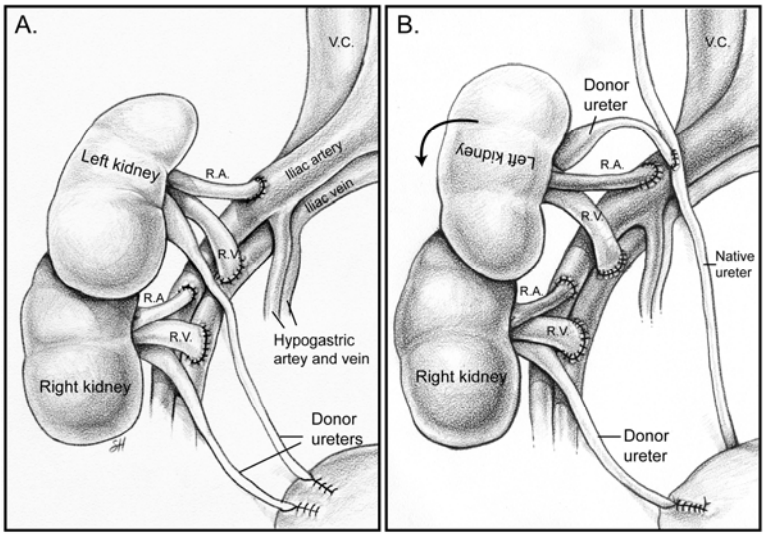
4.1. Investigations on the donor side

During my studies, I used the cases and data occurred during my completed organ transplant fellowship (2003-2005) at the University of Texas. For the retrospective analysis I have used the data of the Division of Immunology and Organ Transplantation at the University of Texas – Houston in the period of 1998 January and 2004 July.

4.1.1. Dual kidney transplantation

In the period of 2003 July and 2004 June, I was involved in the first 5 double kidney transplantations performed at this transplant center. All local transplant services regretted these kidneys for single transplant. According to our protocol we implanted both kidneys into one recipient if the glomerular filtration rate (GFR) was 45-66 ml/min and/or the kidney biopsy proved glomerulosclerosis rate less than 20%. If GFR was <45ml/min and/or the glomerulosclerosis rate >20%, we did not use the kidneys for transplant. Kidneys were implanted to the same side into the iliac fossa; arterial connection was made to the common or external iliac artery, venous anastomosis to the femoral vein. Ureters were implanted to the urinary bladder (Lich-Gregoir technique) [83-85] or to the recipients native ureter with an internal stent guarding the anastomosis. (Figure 3)

Figure 3: Anatomy of dual kidney transplantation



RA, renal artery; RV, renal vein; VC, caval vein

In the iliac fossa; arterial connection was made to the common or external iliac artery, venous anastomosis to the femoral vein. Ureters were implanted to the urinary bladder or native ureter

The abdominal wall was closed with interrupted suture in more layers. No mash graft was needed. We compared the patients and grafts survival rates and kidney function

after the transplant and in a two years follow-up period. To estimate the glomerular filtration rate the Modification of Diet in Renal Disease Study Group (MDRD) estimation was used. [86]

4.1.2. Pediatric kidney transplantation

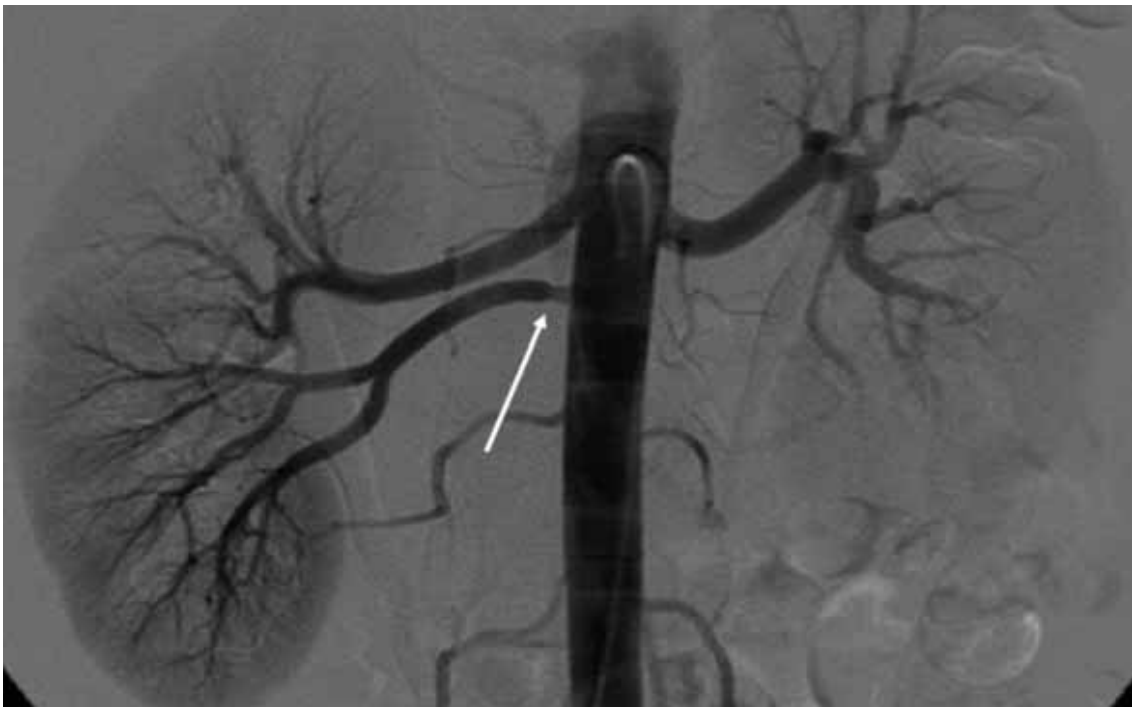
We performed the single center review of all 38 recipients of single pediatric donor grafts less than 5 years of age transplanted between January 1998 and July 2004. All pediatric donors were above 24 months of age. Recipients of pediatric en-bloc grafts were excluded from the evaluation. Recipient selection for pediatric donor grafts was at the discretion of the individual transplant surgeon. Typically a recipient with a low BMI was selected in order to minimize the risk of technical problems associated with obesity and to optimize the donor to recipient size match. The outcomes of pediatric grafts were compared with those in 121 non-obese (BMI < 25 kg/m^2) recipients of kidneys from "ideal donors", defined as deceased individuals between the ages of 18 and 45 years, who were transplanted during the same time. Both groups were limited to recipients of first transplants.

4.1.3. Fibromuscular dysplasia

During my stay I met a rare case of fibromuscular dysplasia. Following the case I became interested in the possible danger of uncommon conditions affecting donors or recipients and observed this case and the previous additional five ones at this transplant center.

The disease appeared in the case of a 32-year-old Asian woman who presented with end-stage renal disease (ESRD) secondary to a congenital bladder defect. She received a kidney transplant from her sister, who was a 35-year-old healthy woman with normal physical examination. An angiogram during the donor evaluation revealed a widely patent single left renal artery and 2 right renal arteries. The lower pole artery of the right kidney showed a mild (30 -40%) focal stenosis (less than l cm) suggestive of FMD (Figure 8). An open right nephrectomy distal to the stenotic segment of the lower pole artery was performed without complications.

Figure 8: Donor angiogram: right lower pole artery stenosis



The vascular anastomoses of both arteries were done in an end-to-side fashion with the upper pole vessel to the common iliac artery and the lower pole vessel to the external iliac artery of the recipient. At Day 5, the recipient was discharged after an uneventful recovery on cyclosporine, sirolimus and prednisone with a serum creatinine of 0.7 mg/dl. 6 months after transplantation, she presented with a progressive increase in serum creatinine to 1.9 mg/dl. A magnetic resonance angiogram (MRA) using gadolinium revealed a moderate stenosis (Figure 8a) of the upper pole renal artery (the donor had the disease in the lower pole renal artery). This finding was confirmed during the arteriogram (Figure 8b) and treated by percutaneous angioplasty with an excellent cosmetic result (Figure 8c). However, 40 minutes later, the patient experienced an episode of hypotension with a fall in the hematocrit. Ultrasound revealed a moderate amount of fluid in the abdomen and the patient was brought to the operating room for exploration. At surgery, the site of hemorrhage appeared to be a bleeding point distal to the anastomosis and adjacent to the stenotic segment that had undergone angioplasty. A new anastomosis was performed connecting the internal iliac artery to the upper pole renal artery after resection of the stenotic segment.

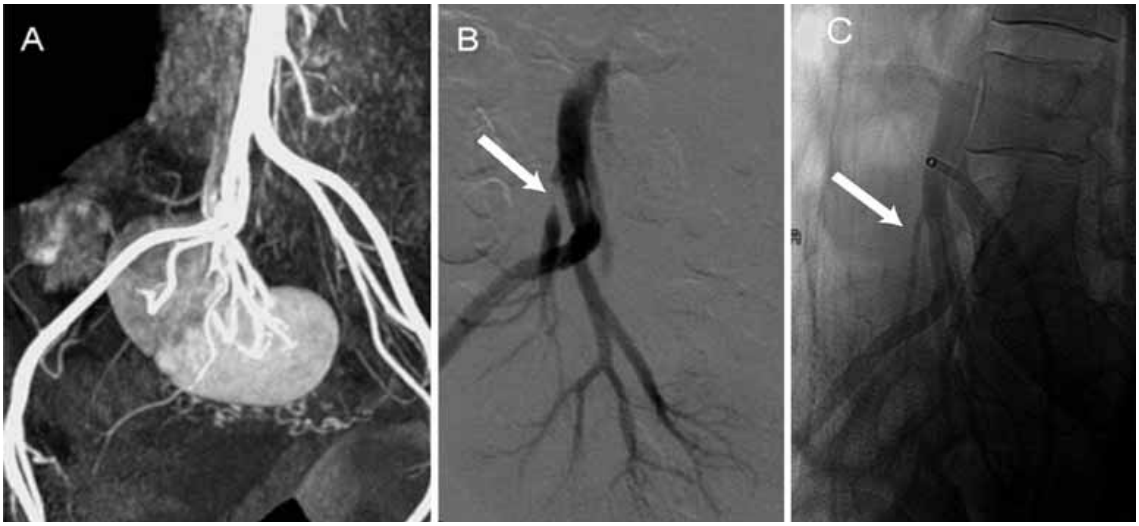
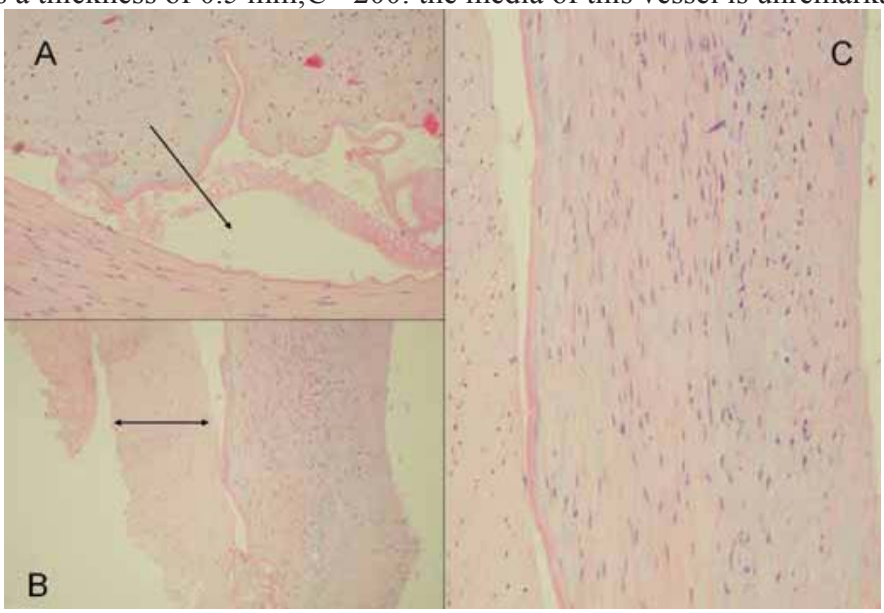


Figure 8 a, b,c: MRA and arteriogram, with renal artery stenosis of the upper pole vessel; C: arteriogram after dilatation

After this procedure, the creatinine improved and the patient was discharged without any other complication. At 12 months post transplantation, her serum creatinine was 1.2 mg/dl. The pathology results on the resected stenotic segment revealed intimal fibroplasia of the renal artery with fibromyxoid intimal thickening and fragmentation of the internal elastic lamina (Figure 9).

Figure 9: Fibromyxoid intimal thickening with areas of fragmentation of the internal elastic membrane; A ×200: there is separation between the thickened intima and the internal elastic lamina; B × 100: the normal intima is usually 1 or 2 cells thick. This intima has a thickness of 0.5 mm;C× 200: the media of this vessel is unremarkable.



4.2. Investigations on recipient side

4.2.1. Genetically high risk patients - Sirolimus-cyclosporine-steroid combination

All of our patients received the same immunosuppressive regimen included induction treatment with either basiliximab (Novartis, East Hanover, NJ) for non-African-American recipients with a PRA <25% or Thymoglobulin (Genzyme, Cambridge, MA) for all other recipients. Maintenance therapy consisted of sirolimus, reduced doses of cyclosporine and steroids [87]. Basiliximab was delivered intravenously at a dose of 20 mg in the operating room prior to graft revascularization as well as a second dose on post-operative day 4. Thymoglobulin (1.5 mg/kg/day) was begun in the operating room prior to revascularization of the graft and continued until the serum creatinine fell below 2.5 mg/dl or for a maximum of 10 days. Sirolimus (Rapamune, Wyeth, Philadelphia, PA) was initiated on the first postoperative day using an oral loading dose, followed by 5-10 mg/day doses to maintain a trough level between 10 and 15 ng/ml for weeks 1 through 12 and thereafter at 10±2 ng/ml. Treatment with oral cyclosporine (Neoral, Novartis) was initiated when the serum creatinine fell below 2.5 mg/dl. The doses were adjusted to achieve an average target concentration (C_{av}), which was calculated as the quotient of the area under the concentration curve and the dosing interval, of 100-200 ng/ml for weeks 1 through 12, generally correlating with a trough level of 75-125 ng/ml. Thereafter the target value was reduced to 100-150 ng/ml (trough level between 50-75 ng/ml). At 6 months the exposure was further lowered to 75-100 ng/ml (trough level <50 ng/ml). Methylprednisolone 500 mg was given on the day of transplantation; oral prednisone therapy was tapered to 2.5-5 mg/day by the third month post-transplantation.

To asses the 6-year impact of a sirolimus-based regimen with modest exposures to cyclosporine among three ethnic groups with different rejection risk, we performed a retrospective analysis of 470 renal transplant recipients who were treated contemporaneously: Group 1, high risk African Americans (n=122), Group 2, moderate risk Hispanics (n=132) and Group 3, mild risk Caucasians (n=216). The average follow up period was 78.7, 84.6, and 81.6 months, respectively. Multivariate models were used to compare the outcomes in Group 1 with those of the other groups.

Our goal with this study was to explore the toxicity of the drug combination (hemostatus, serum glucose, lipid profile) beside the recipient and graft survivals in the

different rejection risk ethnic groups. The threshold for the laboratory parameters were: hemoglobin <10g/dl, white blood cell count $<3.5x10^3/cm^3$, platelet count $<100x10^3/cm^3$, serum cholesterine >200 mg/dl, triglyceride >200mg/dl and fasting blood sugar level >120mg/dl.

4.2.2. Campath-1H in acute rejection

Between January and August 2004, five patients presented with ACR with or without combined humoral rejection as detected by positive staining for C4d. These subjects either had a medical history of previous acute rejection episodes treated with rabbit anti-thymocyte globulin (rATG) or muromonab-CD3 (OKT3) or displayed allergic reactions to these antibodies on the initial exposure. After a transplant biopsy-proven ACR (classified by the Banff score), the patients received peripheral intravenous infusions of Campath-1H, starting with an initial dose of 3 mg, increasing to a total dose of 55 to 93 mg divided over 4 to 5 days. Before each dose, the patients were premedicated with methylprednisone intravenously (125 to 500 mg). Oral valgancyclovir was prescribed for cytomegalovirus (CMV) prophylaxis for 3 months. The baseline immunosuppressive regimen of cyclosporine (C_2 = 200 ± 50 ng/mL), sirolimus (C_0 = 10 ng/mL), and occasionally prednisone (5 to 15 mg/d) was not changed during the period of Campath-1H treatment. We assessed the recovery of renal function by the serum creatinine and blood urea nitrogen (BUN) levels, as well as by estimated creatinine clearance using the Cockcroft-Gault equation [88], and the glomerular filtration rate (GFR), using the equation described in the Modification of Diet in Renal Disease Study (MDRD) [86]. Patient responses were assessed at inception as well as after 2 weeks and 2 months of Campath-1H therapy. The mean follow-up was 107.4 days**.**

The following five cases represent the first cases treated by Campath-1H not only in our department but in the literature. We have used Campath-1H in kidney transplant recipients to rescue rejection episodes in five patients who were refractory not only to steroids but also to thymoglobulin or OKT3. These kidney transplant patients were the first ones who received Campath-1H at our center and according to the literature review

even the first ones who received this agent with this indication. Their cases are unique to prove the advantage to report thir case individually.

Patient 1, a 30-year-old Hispanic woman with end-stage renal disease (ESRD) secondary to lupus erythematosus received her second kidney as a cadaveric transplant in 1999 after losing her first living related kidney due to her non compliance. Due to the retransplant setting, thymoglobulin was used for induction therapy. At approximately 1 year, OKT3 treatment was required to treat a grade 2, biopsy-proven ACR. She experienced a third acute rejection due to noncompliance, returning for evaluation after a 3-week "drug-free" holiday. She presented with decreased urine output and a serum creatinine value of 7.7 mg/dL. On her second hospital day, administration of Campath-1H (total dose = 90 mg) was initiated. Although her initial response was modest, 2 weeks later, her serum creatinine decreased to 2.5 mg/dL without hemodialysis. At 2 months, the value was 2.9 mg/dL. Her kidney function remains stable, albeit impaired, and she has experienced no adverse events from the therapy.

Patient 2, a 36-year-old Asian man whose kidneys failed due to hypertension, received a living unrelated nondesignated donor graft in October 2003. After initially excellent graft function, his serum creatinine of 1.0 mg/dL rapidly deteriorated to 3.5 mg/dL. Following the diagnosis of a biopsy-proven ACR, thymoglobulin was initiated, but during the first dose, the patient experienced severe chills, hypotension, and angina pectoris, requiring the termination of the treatment. He was administered a Campath-1H therapy (total dose = 83 mg). When the C4d staining was reported to be positive 24 hours later, additional therapy directed to humoral rejection was implemented, namely, plasmapheresis, intravenous immunoglobulin (IVIG), and rituximab. Two weeks after the completion of this therapy, his serum creatinine was 1.6 mg/dL; at 2 months, it was 1.4 mg/dL; and after another month, 1.2 mg/dL. He did not experience any adverse events associated with the treatment.

Patient 3, a 31-year-old Hispanic woman, originally presented with ESRD of unknown origin. After rejecting her first cadaveric transplant kidney she received a second, living related kidney transplant in November 2002. When her nadir serum creatinine of 1.6 mg/dL increased to 2.2 mg/dL during an outpatient visit, a kidney biopsy showed ACR. She was treated with Campath-1H because thymoglobulin had been used for induction treatment during her second transplant and OKT3 had been

used for rejection treatment with her first kidney. Two weeks after the treatment with 53 mg of Campath-1H, her serum creatinine fell to 1.9 mg/dL. Two months after the therapy, the patient presented with a bout of easily controlled herpes zoster infection.

Patient 4, a 39-year-old male Caucasian, had an original disease of hypertension. After a living related transplant, he was admitted for a serum creatinine elevated from 1.3 to 4.6 mg/dL. When a biopsy revealed acute cellular and humoral rejection, he was treated with OKT3, plasmapheresis, rituximab, and IVIG, resulting in improvement. When his serum creatinine increased again 2 months later, another biopsy showed ACR without positive C4d staining. Campath-1H (55 mg) was administered, leading to a decrease in the serum creatinine from 4.6 mg/dL to 1.4 mg/dL within 2 weeks. At 2 months, the value was 1.7 mg/dL. Subsequently, the patient suffered a complicated evolution of an idiopathic pneumonitis, leading to modestly impaired renal function; namely, a serum creatinine value of 2.0 mg/dL.

Patient 5, a 37-year-old Hispanic woman with adult dominant polycystic kidney disease, received a cadaveric transplant in 2000, which displayed a nadir serum creatinine of 2.1 mg/dL. She was transferred from an outside hospital on July 6, 2004, with oliguria and a serum creatinine of 10 mg/dL. A biopsy showed ACR. After failure of a 7-day course of OKT3 treatment, she was discharged on hemodialysis due to the concern that the relatively mild ACR on renal biopsy had precipitated acute tubular necrosis. Upon failure of resolution of this putative process 32 days later, she was readmitted for Campath-1H therapy. However, she failed to respond during or after the treatment and remained on hemodialysis.

4.3. Experimental studies for wound healing

The experimental studies were designed and performed according to the regulation and rules of the Animal Welfare Committee at University of Texas – Houston Medical School.

For both experimental studies we used forty, eight week old, female Balb/c mice (weight: 19-20 grams) each, kept in individual cages with free access to water and devided into four equal groups. All wounds were created in the same way; after shaving

the hair at the back of the animals with an electric shaver the dorsal skin was cleansed aseptically and wounds made under intra-peritoneal anesthesia (ketamine/xylazine at 100-200mg body weight), with an 8 mm in diameter full thickness skin punch.

In the study for determining the effect of sirolimus on wound healing the mice had free access to regular food and oral sirolimus was administered (gavaged) daily in four groups as saline (control), 4mg/kg, 8mg/kg and 12mg/kg for 2 weeks prior to the wound creation. No wound coverage was used.

For the observation of RNA derivates enhanced topical effect on wound healing of sirolimus treated animals, the mice had free access to a nucleotide-free diet (Product code: 48238, Purina Mills, LLC, St Louis, MO, USA) for two weeks. They received the same diet during the whole experiment and 8mg/kg oral sirolimus was administered (gavaged) daily for three weeks prior wounding and during the experiment. The wound surfaces were treated with different concentration RNA enhanced KY jelly as topical. The RNA enhanced topical (Zhen-Ao Group, China) contained a mixture of nucleosides and nucleotide mono- and diphosphates and extracted from yeast RNA with average MW of 350. We had a control group in which wound was treated with daily application of pure KY jelly (Group A). RNA gels were formulated with Zhen-Ao RNA/NTs mixture (Zhen-Ao, China) and KY jelly. Three groups were treated with daily application of nucleotide supplemented KY jelly as 0.025%(0.4mg RNA/100gm gel)(Group B), 0.25%(4mg RNA/100gm gel)(Group C) and 2.5%(40mg RNA/100gm gel)(Group D) nucleotide concentration.

All animals were weighted every 4 days for checking weight loss due to the special diet we used. For the experimental studies we used the same technique as digital pictures of the wounds were taken every other day (Nikon Coolpix 4500), using the same 1cm x 1cm template around the wound, starting on the day of wounding. Picture analyzing software (Optimas 6, Optimas Inc) was used to measure the wound surface area as percentage of the standard template area we used. During the analysis of the photos we compared the healing wound area as the percentage of the initial one of the same animal. We followed the healing process for 14 days. (Figure 11a-c)

Figure 11: template (a, b) and area measuring software (c)

a b c

4.4. Statistical analysis

Group comparisons were performed using the Chi-square or Student's t-test as appropriate, while multivariate analysis (ANOVA) was used for the continuously changing parameters as laboratory test results. The Kaplan-Meier equation was used to estimate survival rates and the log-rank test to compare outcomes. Results were considered significant when $p<0.05$.

5. Results

5.1. Results of the investigations on the donor side

5.1.1. Dual kidney transplantation

During the research period 5 recipients received double kidneys from deceased adult donors rejected all local transplant services for transplant as single organs. All of them were males, mean age of 44.4 years (±10.67). According to the genetical rejection risk two recipients were in the high risk African American race, two of them in the mild risk Caucasian and one Oriental. This was the first transplant for all cases. The cause of the recipients renal failure was hypertension in 4 patients (80%) and IgA nephropathy in 1 patient (20%). The mean Body Mass Index (BMI) was 28.0±6.4 kg/m^2. (Table 3)

Table 3 Recipients Demography

	Recipient 1	recipient 2	recipient 3	recipient 4	recipient 5	Mean	SD
Age (years)	62	41	33	43	43	44.4	10.67
Sex	male	male	male	male	male		
BMI (kg/m^2)	30.1	28	37.3	24.5	20.4	28.06	6.34
Race	AA	Cau	AA	Cau	As		
Primary disease	HTN	HTN	HTN	HTN	IgA		
Renal scan	9	9	4	9	7	7.6	2.19
Acute rejection		Yes	Yes				
LOS (days)	7	5	8	6	7	6.6	1.14

(AA=African-American, Cau=Caucasian, As=Oriental, HTN=hypertension, IgA=Ig A nephropathy, BMI=Body Mass Index, LOS= Length of Stay)

The donors mean age was 54.2±26.2 years. Two of them was >70 years of age, two >60 years and one was 8 years old. Their BMI was 29.0±4.9 kg/m^2 and the estimated GFR 68.2±16.3 ml/min. Before the organ harvesting all donors received medical cardiac support (Table 4)

Table 4: Donor demography

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
Age (years)	70	70	63	61	8
Sex	female	female	male	female	male
BMI (kg/m^2)	33.4	33.4	30.6	24.8	22.9
Race	Cau	As	Hisp	AA	AA
Serum creatinine mg/dl (µmol/l)	0.7 (61.6)	1.2 (105.6)	0.7 (61.6)	1.2 (105.6)	1.7 (149.6)
GFR (ml/perc/1.73m^2)	61.6	105.6	61.6	123.2	65.6
Cardiac support	+	+	+	+	+

(BMI=Body Mass Index, GFR=Glomerular Filtration Rate, AA=African-American, As= Oriental, Cau=Caucasian, Hisp= Hispanic))

The immediate renal scan score was 8/10 (maximal: 10/10). The mean hospital length of stay (LOS) was 6.25 days. All five recipients were alive and avoided hemodialysis 1 year after their transplant.

We used the same immunosuppression regime in all cases as sirolimus (Rapamune®, Wyeth), cyclosporine (Neoral®, Novartis) and steroid. In the case of the African-American high risk patients we used 5 days anti-thymocyta globuline induction (Thymoglobulin®, Genzyme); the lower risk patients received basiliximab (Simulect®, Novartis) induction. We followed the patients for 24 months.

There was no severe surgical complication, no ureteral or kidney necrosis and no disturbances in the graft blood supply. In the case of an obese patient (BMI=37.3kg/m^2) wound debridement was needed due to local wound infection and in an other case 30 days after the transplant, graft pyelonephritis needed antibiotic administration.

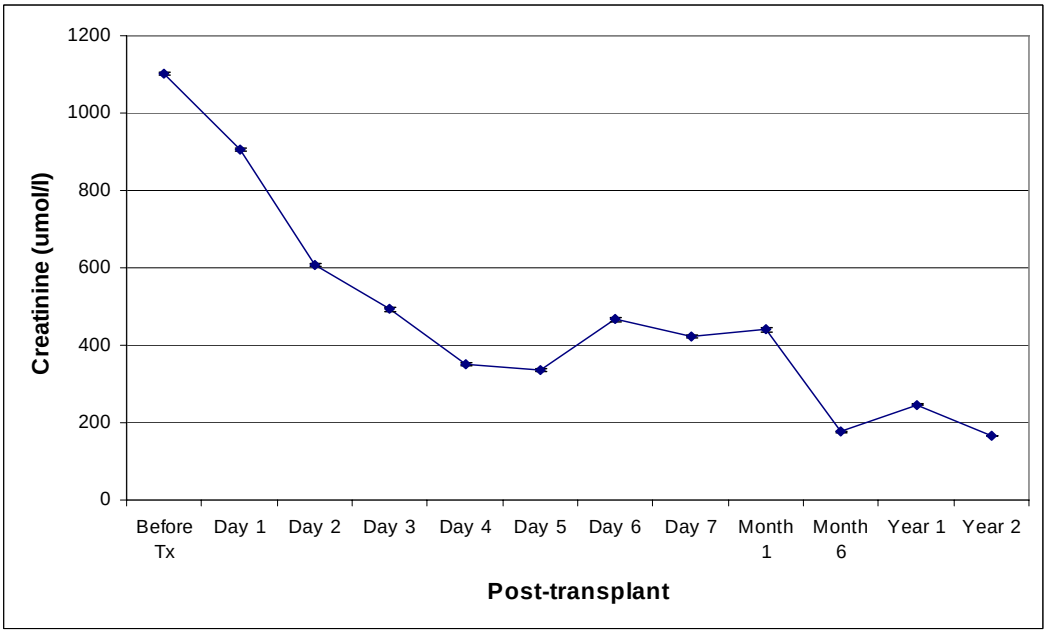
Two patients suffered acute rejection. Recipient 3 had cellular and humoral rejection 2 weeks after the transplant and Recipient 2 had cellular rejection 2 months after the transplant.

All patients suffered hyperlipidemia as a side effect of the immunosuppressive regime.

Immediate kidney function was observed in all cases after the transplantation. Serum creatinine levels rapidly decreased from the pre-transplant mean 12.5mg/dl (1100μmol/l) level to the 3.8 mg/dl (334.4 μmol/l) level on the 5th post-operative day. 1 year after the transplant the creatinine concentration in the serum was 2.8mg/dl (246.4 μmol/l), 2 years after the transplant 1.9 mg/dl (167.4 μmol/l) (Figure 4)

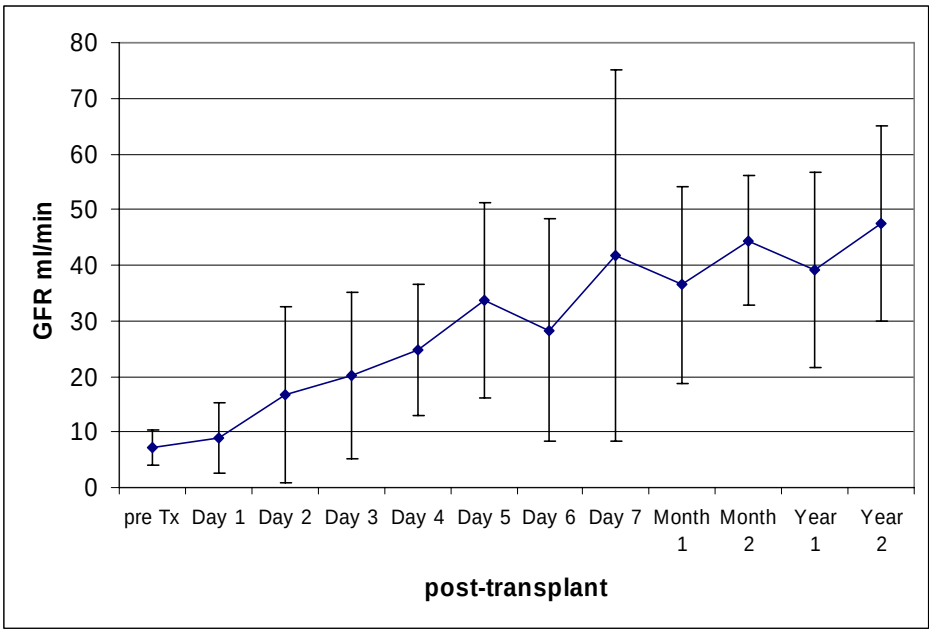
During the rejection episodes kidney functions significantly worsened. The patients kidney function suffering humoral rejection was worsened at all time points however the patient could avoid hemodialysis.

Figure 4: Serum creatinine in the post-transplant period



The mean GFR increased from 7 ml/min to 41.8 ml/min within a week and 1 year after the transplant it was 39.07 ml/min and 2 years after transplant 47.5ml/min. (Figure 5)

Figure 5: Glomerular filtration rate (GFR) in the post-transplant period



5.1.2. Pediatric kidney transplantation

Using pediatric kidneys the mean ages of the pediatric donors were 2.8±1.0 versus 31.3±9.2 years for the adult donors (p<0.01). The mean ages of the recipients of pediatric donors were 42.0±12.4 versus 45.7±14.8 years for recipients of adult grafts (p=ns). The mean recipient BMI values of pediatric donors were 21.8±2.9 versus 22.4±2.0 kg/m^2 for recipients of adult donors (p=ns). Sixty-six percent (n=25) of pediatric donor recipients were women versus 44% (n=53) of adult donor recipients (p=0.03). The mean pre-transplant recipient PRA was similar between groups (Table 5).

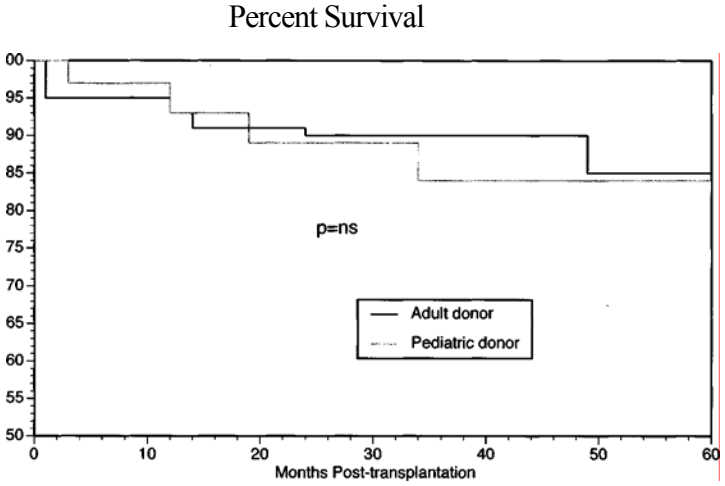
Table 5: Donor and recipient demographics

	Pediatric donor (n=38)	Adult donor (n=121)	p value[1]
Donor			
Mean age (years)	2.8±1.0	31.1±9.2	<0.01
Recipient			
Mean age (years)	42.0±12.4	45.7±14.8	ns
Mean BMI (kg/m^2)	21.8±2.9	22.4±2.0	ns
Female (%)	25 (66)	53 (44)	0.03
Mean pre-transplant PRA (%)	5.7±16.3	14.7±28.0	ns

[1] Student's T-test and Chi-square test as appropriate.

Thirteen pediatric donor recipients (34%) suffered an acute rejection episode compared to 16 (13%) adult graft recipients (p<0.01). Death-censored actuarial graft survival rates at one and 5 years for recipients of pediatric donor grafts were 93% and 84% compared with 93% and 85% for recipients of adult donor grafts (p=ns, Figure 6).

Figure 6: Death censored actuarial graft survival (Kaplan-Meier) of adult and pediatric donor groups.



There were no graft losses due to technical complications among the pediatric donor cohort. The most common causes of graft loss for both pediatric and adult donor groups were death with a functioning graft (13 vs. 5%, p=ns) and chronic allograft nephropathy (8 vs. 4%, p-ns). (Table 6)

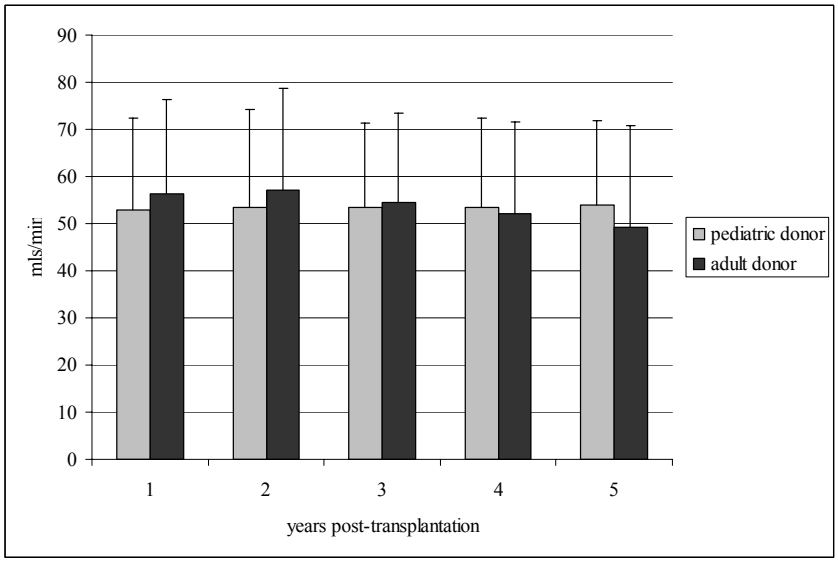
Table 6: Causes of graft loss

	Pediatric donor N (%)	**Adult donor** N (%)	**p value**[1]
Death with functioning graft	5 (13)	6 (5)	ns
Vascular complication	0	2 (2)	ns
Initial non-function	0	4 (3)	ns
Chronic allograft nephropathy	3 (8)	5 (4)	ns
Other	1 (3)	0	ns

[1] Chi-square test

At one and 5 years post-transplantation, the mean estimated creatinine clearances (Cockcroft-Gault)[88] of pediatric donor graft recipients were 52.9±19.6 and 54.0±17.8 ml/min, respectively, compared with 56.4±19.8 and 49.1±21.7 ml/min for recipients of adult donor grafts at the same times (p=ns, Figure 7).

Figure 7: Mean (±SD) calculated creatinine clearances (Cockcroft-Gault) of adult and pediatric donor groups.



A subset of recipients was screened for proteinuria by examining 24-hour urine collections. Seven of 19 adult donor recipients (27%) and 2 of 7 (28%, p=ns) pediatric donor recipients displayed evidence of moderate proteinuria (<500 mg/24h) at 2 years post-transplantation.

5.1.3 Fibromuscular dysplasia

On the basis of our unique case we reviewed our previuous 6 cases. This patient presents without hypertension but with elevation in the serum creatinine only 6 months after the transplantation. Unfortunately, after a successful percutaneous angioplasty, the patient presented a bleeding complication requiring a surgical intervention. In our series, after a median follow-up of 36 months, we have not found any complications in the donors with FMD and only one recurrence of the recipients.

5.2. Results of the investigations on the recipient side

5.2.1. Sirolimus-cyclosporine-steroid combination

During our observation on drug combinations effect on different races was no significant difference in age, gender, body weight, BMI, antibody titer, number of transplantations, and number of diabetic patients. The rate of cadaveric transplants among African American recipients were higher than in the other ethnic groups where the living related transplants were more frequent. The primary cause of kidney failure was more frequently hypertension among African Americans and Hispanics than among Caucasians. (Table 7)

Table 7: Demographic data

	African American n=122	**Hispanic N=132**	**Caucasian n=216**
Age (years±SD)	43.78±13.09	42.2±13.3	46.06±13.03
Body weight (kg, mean±SD)	81.11±18.86	72.6±16.7	77.9±17.96
BMI (mean±SD)	28.32±18.86	25.9±6.4	26.27±5.46
Pretransplant PRA (mean±SD)	6.98±16.03	7.9±17.1	5.37±13.25
HLA mach (mean±SD)	4.52±1.45	4.6±1.4	4.08±1.66*
Male (%)	73 (59.8)	74 (56.1)	134 (62.0)
Deceased donor (n (%))	92 (75.4)	85 (64.4)	134 (62.0)*
First transplant (n (%))	103 (84.4)	115 (87.1)	175 (81)
Pretransplant HTN (n (%))	117 (97.5)	122 (92.4)	190 (88.4)*
Pretransplant DM (n (%))	30 (25.9)	34 (25.8)	57 (26.5)
Delayed graft function (n (%))	21 (17.2)	17 (12.8)	24 (11.1)
Steroid withdrawal (n (%))	47 (38.5)	57 (43.1)	96 (44.4)

BMI = body mass index, HTN=hypertension, DM= diabetes mellitus, HLA= Human Leukocyte Antigen, PRA=Panel Reactive Antibody, *= $p<0.05$ Group 3 vs Group 1

In the entire cohort the target serum concentration for SRL was 10±2 ng/ml. There was no significant difference in the groups. While the drug dose was similar in all groups during the first 30 days in the 3rd, 6th, 12th, 24th, 48th months, the high risk African American patients required significantly higher dose of SRL compared to the mild risk Caucasians but this difference disappeared at the 60th month. To reach the target serum drug concentration, the African American recipients needed higher dose than the Hispanic ones (Table 8).

Table 8: Sirolimus mean dose and serum concentration (C_o) (mean±SD)

	African American n= 122		Hispanic n=216		Caucasian n=132	
Months Posttransplant	Dose (mg/day)	C_o	Dose (mg/day)	C_o	Dose (mg/day)	C_o
1	7.4±5.2	10.9±10.5	5.4±4.9*	10.8±7.7	7.0±5.0	12.3±8.4
3	7.1±5.1	11.8±7.4	4.5±3.9*	13.2±9.2	5.4±3.8**	12.3±7.6
6	6.1±4.9	13.0±7.4	4.1±3.4*	14.9±9.1	4.9±3.1**	11.7±7.1
12	5.2±4.4	11.7±6.1	3.6±2.9*	13.7±7.4	4.3±2.8**	11.3±6.1
24	5.0±4.2	12.2±7.0	3.0±2.5*	11.6±6.8	4.1±2.6**	10.9±5.4
48	4.4±2.7	10.2±5.4	3.2±2.5*	11.4±7.1	4.0±2.0**	10.7±5.2
60	4.7±2.2	8.6±4.9	2.9±1.5*	11.4±4.5	3.9±1.8	10.7±5.4
72	4.0±2.4	11.4±5.5	3.9±1.9	9.3±3.9	3.4±1.8	10.5±3.0

* = $p<0.05$ Group 2 vs Group 1
** = $p<0.05$ Group 3 vs Group 1

The CsA serum level (C_2 – 2 hours after taking the drug) and the daily dose was similar in all groups during the whole follow-up period. We decreased the CsA daily dose to about 50% of the regular dose. The target serum concentrations were 900ng/ml in the first 3 months, 600 ng/ml in 6-12th months, 550 ng/ml for the next year, 450ng/ml in the 24-48th months and 350ng/ml for the later period. We needed to administer similar dose of CsA in each group for reaching the same and equal plasma levels.

The patient survival rates were similar at one and six years after transplantation (95.5% vs 94.4% vs 94.9% and 87.7% vs 85.3% vs 81.9%). There was also no difference in the graft survival rates. The acute rejection rates were similar at 1, 3 and 6 year. The cumulative incidence of acute rejection episodes over the entire follow-up period was similar among the groups: Group 1 22%, Group 2 24.2% and Group 3 23.0%. Transplant biopsy proved chronic rejection rate proved to be similar in all groups 1, 3 and 6 years after the transplantation.

Although there were no significant differences in overall or individual infection rates, Group 1 and 2 recipients displayed higher but not significant incidence of postoperative wound infection (14.0%, 16% and 10.6% p= ns). In Group 2 and 3 pneumonia was significantly more frequent than Group 1 (25.7%, 32.0%, 22.0% p=0.05). The Hispanic patients suffered significantly less CMV infection then the others (3.8% vs 8.2% and 6.9% p=0.01) (Table 9). All recipients showed similar rates of lymphocele formation.

Table 9: Infectious complications (%)

	African American n=122	Hispanic n=132	Caucasian n=216
Wound infection	14.0	10.6	16.0
Urinary tract infection	49.0	53.0	50.0
Pneumonia	22.0	25.7*	32.0**
CMV	8.2	3.8*	6.9
Buccal ulcer	2.4	12.8*	17.5**

CMV = cytomegalovirus, * = $p<0.05$ Group 1 vs Group 2, ** = $p<0.05$ Group 1 vs Group 3

Thrombocytopenia, anemia and leukopenia were similar in all groups. However, Group 1 displayed a reduced incidence and decreased severity of hypertriglyceridemia than Group 2 or Group 3 (89.3% vs. 97.2% vs. 93.2%), a similar incidence of hypercholesterinemia (94.3% vs. 97.2% vs. 98.5%) was observed. Hypertriglyceridemia and hypercholesterinemia are the most frequent at the sixth months. 5 years after the transplantation 40% of African American recipients, 57.3% of Hispanic patients and 59% of Caucasian patients (p=ns) suffer of hypertrigliceridemia none the less of adequate medical treatment. The occurrence of post-transplant diabetes mellitus (16%, 11% and 9%) was greater in Group 1 than Group 3 but similar to Group 2. (Table 4)

Among stable patients with good kidney function we tried to withdraw steroids. During the 6 years of follow-up the withdrawal was successful and permanent in 38.5% of African American recipients, 43.1% of Hispanics and 44.4% Caucasians (p=ns). Following the steroid withdrawal the occurrence of acute rejections were 0%, 5.0% and 0.5% ($p<0.05$) and was completed at 21.2, 20.8 and 21.0 months after the transplantation.

5.2.2. Campath-1H in acute rejection

The five patients included three women and two men raging from 30 to 39 years of age. Two of them had acutely rejected previous transplants (Table 10) and displayed pretransplant panel reactive antibody (PRA) values of 35% and 60%. The three

recipients of first transplants showed 0% PRA at the time of the operation. A living donor provided the graft in three cases and deceased donation was in two cases. All patients had experienced repeated previous acute rejection episodes. Two subjects experienced a third rejection episode, and two patients had previously been treated with OKT3. One man developed a severe allergic reaction toward rATG despite no medical history of previous exposure.

Table 10: Demographic Features of the Patients Treated With Campath-1H

	Patient				
Characteristics	**1**	**2**	**3**	**4**	**5**
Age (years)	30	36	31	39	37
Ethnicity	Hispanic	Asian	Hispanic	Caucasian	Hispanic
Gender	Female	Male	Female	Male	Female
Donor source	CAD	LURD	LRD	LURD	CAD
Original disease	SLE	HTN	Unknown	HTN	PCKD
No of transplants	2	1	2	1	1
Pretransplant PRA (%)	35	0	0	60	0
Immunosuppression	CsA-Pred SRL	CsA-Pred-FTY720/MMF *	CsA-Pred-MMF	CsA-Pred-FTY720/MMF*	CsA-Pred-SRL
Previous OKT3/rATG	Yes/Yes	No/Yes	Yes/Yes	Yes/No	Yes/No

CAD, cadaveric donor; LURD, living unrelated donor; LRD, living related donor; SLE, systemic lupus erythematosus; HTN, hypertension; PCKD, polycystic kidney disease; PRA, panel reactive antibody; MMF, mycophenolate mofetil; SRL, sirolimus; CsA, cyclosporine; Pred, prednisone. * The patient received either FTY720 or MMF as part of a double-blind clinical trial.

Four of the five patients showed good responses to Campath-1H therapy as shown by the summarized serum creatinine levels and biopsy results for all patients. (Table 11)

At the time of Campath-1H administration for cellular rejection according to the patients rejection type (cellular and humoral rejection together) supportive treatment was needed (Table 12).

Table 11: Serum Creatinine and Biopsy Results in Patients Treated With Campath-1H

	Patient				
	1	**2**	**3**	**4**	**5**
Age of transplant (mo)	50	5	19	4	45
Creatinine (mg/dL)					
Nadir	1.8	1.3	1.6	1.3	2.1
At biopsy	7.9	3.2	2.2	4.6	6.0
At the end of therapy	4.9	3.5	2.1	3.6	8.4
2 weeks after therapy	2.5	1.6	2.0	1.4	8.6
2 months after therapy	2.9	1.4	1.9	1.7	NA
Latest measurement	2.9	1.2	1.9	2.0	3.9*
ACR (Banff)	Type 2 (ci2, ct2, cv2, cg2, mm0, ah2)	Type 1 (i2, t2, g2, v0)	Type 1b (g1, i3, t2, v0)	Type 1b (i3, t2, v0)	Type 1a (g1, i3, t2, v0)
C4d	+	+	−	+	−
CAN grade	II	—	II	—	I

* Patient on hemodialysis.

Table 12: Antirejection Treatment at Time of Campath-1H Administration

Treatment	Patient				
	1	**2**	**3**	**4**	**5**
Campath-1H total dose (mg)	90	83	53	55	95
Number of doses	5	5	4	4	4
Plasmapheresis	−	+	−	−	−
IVIG total dose (mg)	—	660	—	—	—
Rituximab total dose (mg)	—	1882	—	—	—

The overall patient survival was 100%, and renal function recovery rate was 80%. As a group, the serum creatinine levels fell to 20% of the peak value by the end of the treatment. Two weeks later, the creatinine level was 35% to 50% of the pre-treatment value (Figure 10A). The BUN was halved at 2 weeks, although the decrease was delayed compared to the recovery of serum creatinine. In the four cases showing good

responses to Campath-1H therapy, the estimated creatinine clearance increased to 125% by the end of the therapy, and 2 weeks later, by 200% to 300% (Figure 10B). By the MDRD estimation, the GFR increased to three- to fourfold above the pretreatment value (Figure 10C). The treatment course was associated with a transitory decrease in absolute lymphocyte and monocyte count with recovery after 6 months (data not shown). Only two of the patients who received Campath-1H infusion experienced adverse events, which were mild and easily controlled.

Figure 10. Renal function's evolution during and after Campath-1H treatment. **(A)** serum creatinine; **(B)** estimated creatinine clearance (by Cockcroft-Gault equation[88]); **(C)** estimated glomerular filtration rate (MDRD formula[86])

Figure 10A

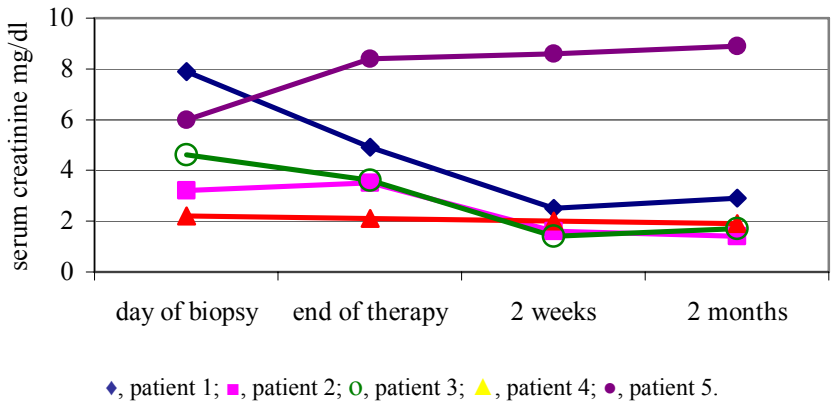


Figure 10B

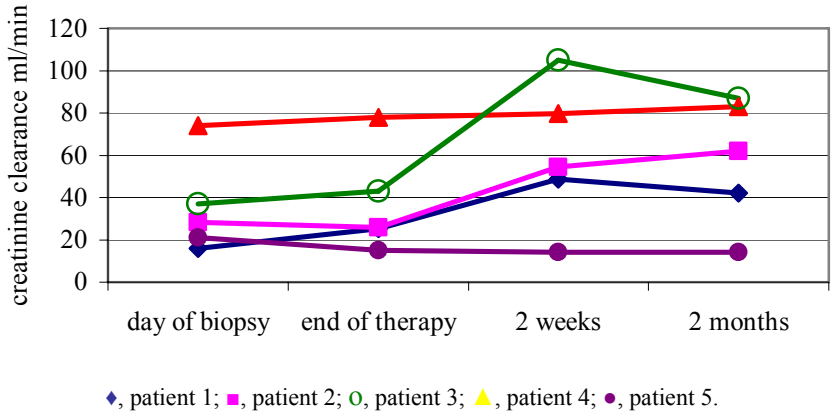
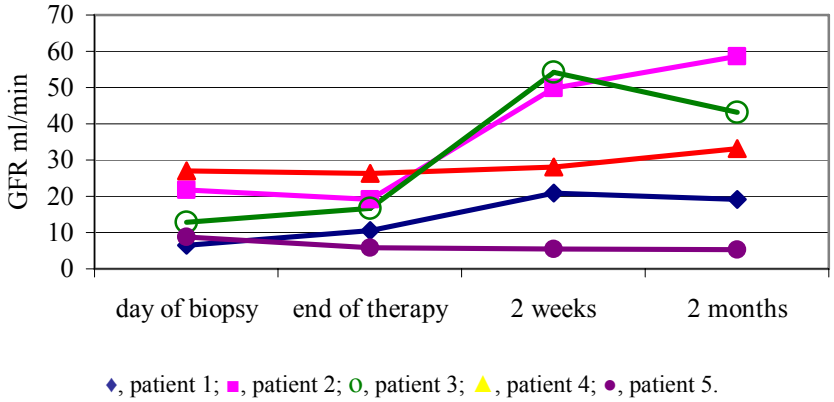


Figure 10C



♦, patient 1; ■, patient 2; o, patient 3; ▲, patient 4; ●, patient 5.

Perhaps because we restricted therapy to high immune responders, we did not observe any severe adverse reactions.

5.3 Experimental wound studies

5.3.1. Effect of sirolimus on wound healing

The animals mean weight was 20.6g (±1.1g) and the mean weight loss on the continuous nucleotide free diet was 0.35gr (±0.4g). There was no animal mortality or wound infection during the study period.

4mg/kg sirolimus dose showed no effect on wound healing. 8mg/kg and 12mg/kg sirolimus dose had the same effect however; the animals tolerated the 8 mg/kg better. 8mg/kg daily oral dose of sirolimus significantly ($p<0.04$) impaired wound re-epithelization after day 3 during the whole healing period, however, after day 12 all wounds were less than 10% in size. (Table 13, Figure 12)

Figure 12: Wound healing on different oral sirolimus doses in mice

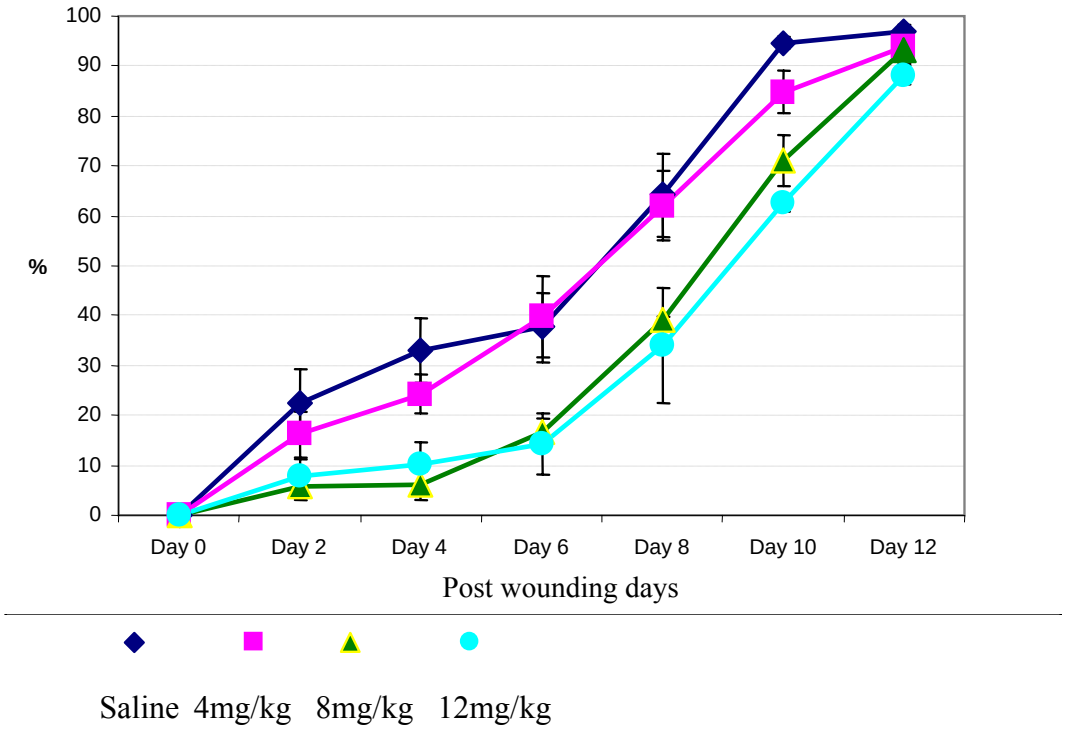


Table 13: p values saline vs 8mg/kg sirolimus

	Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**	**Day 12**
Saline vs 8mg/kg sirolimus	0.0593	0.013	0.0346	0.0369	0.0258	0.0507

When we administered the effective dosage of sirolimus orally to the animals they tolerated the sirolimus well. No wound infection or other complication observed. There was no significant weight loss during the study. Wound healing was faster with 0.25% nucleotide topical. Compared to the control group Day 4-Day 10 the wound shrinkening was significantly faster. There was no significant difference between the 0.25% concentration containing topical and the 2.5% concentration containing one. At Day 14 the treated animals wound was still smaller, but at this point there was no significant difference. (Figure 13, Table 14)

Figure 13: Wound healing with nucleotide topical

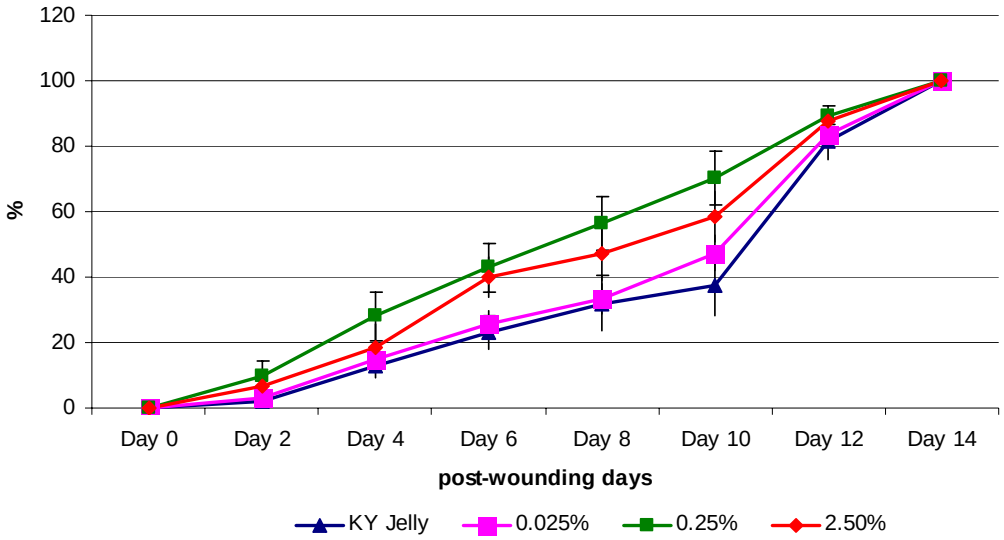


Table 14: Significance level, KY jelly versus group B,C and D

	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Group A (KY Jelly)							
Group B (0.025%)	0.2718	0.328	0.3460	0.4373	0.1939	0.3472	0.3470
Group C (0.25%)	0.0863	0.0513	0.018	0.0219	0.0064	0.0981	0.2309
Group D (2.50%)	0.0863	0.2312	0.0248	0.0965	0.0404	0.1251	0.3528

5. Discussion

6.1. Dual kidney transplantation

Surgical technique for dual kidney transplantation is similar to single kidney transplantation. During the surgical procedure both kidneys can be implanted to the same iliac region or to both sides. Using only one side gives better chance for a re-transplant later if it will be needed, however the surgery technically more challenging. To operate on both iliac regions means longer operative time and elevates the risk of infection.

Dual kidney transplantation seems to be a good solution for using cadaveric kidneys with marginal function not enough for single kidney transplant. Short and long term graft and patient survival and graft functions are good especially for older recipients.
Using the UNOS database (1997-2000), Bunnapradist compared 403 double kidney transplants and 11033 single kidney transplants. In the double kidney transplant group the recipients and the donors mean age was significantly higher, hypertension, diabetes and the donors' cerebrovascular lesion caused death was more frequently. One year graft survival was 81% in the double kidney group and 88% in the single kidney group and the 3 years graft survival was 62% to 77%.[89] Similarly to Tan's findings these differences disappeared among patients above 55 years of age. [26, 89].
1994-2002, Alfrey et al. performed 287 dual kidney transplantations and compared the results with the single transplants done during the same period. They could not find any difference in the 1 and 5 year survivals, but comparing kidneys from donors in the age 50+ the results were significantly better with dual kidney transplant (64% vs 51%).[90]
In Europe, German and Italian transplant groups published more frequently about dual kidney transplantation. In Germany among 26 dual kidney transplanted patients the 2 years graft and patients survival rate proved to be 92% [91]. Italian groups performed 79 dual kidney transplantations in 3 centers. Graft survival was 90% after three years. [92]
With careful selection we can use these marginal organs. Shortening waiting time even for recipients older than 50 years of age decreases complication and even death on hemodialysis, helps to improve their quality of life and decrease health costs.

6.2. Pediatric kidney transplantation

Pediatric deceased donors are an underutilized source of renal grafts. Because of their small size, many transplant surgeons are hesitant to utilize this resource due to the increased risks of thrombosis and urologic complications. Furthermore, it has been proposed that a small graft into an adult recipient will display an hyperfiltration syndrome and early graft loss [93, 94], concerns that are supported by a report derived from the information in The United States Renal Data system. Large individuals, with a body surface area (BSA) $>2.2m^2$, who received kidneys from small donors

(BSA<1.6m^2) experienced an increased risk of graft failure compared to medium-sized recipients of medium-size donor kidneys (BSA 1.6-2.2 m^2)[28]. A parallel analysis of the United Network for Organ Sharing database revealed that the long-term outcomes of grafts from donors less than 18 years of age were significantly worse than those of adult donors. Moreover, when pediatric donors were further stratified by age, recipients of donors aged 0-5 years displayed significantly lower graft survivals compared to recipients of donors who were 6-11 or 12-17 years. Additionally the incidence of graft thrombosis was greater among infants compared to older pediatric donor transplants. Among pediatric donor transplants, en-bloc transplants provided better results than single transplants particularly if the recipient BMI was less than 25 kg/m^2[33].

The implantation of en-bloc pediatric grafts provides greater renal mass, but remains a more technically challenging procedure than single adult donor transplants. Nevertheless, in contrast to these registry reports, a number of single centers have documented excellent outcomes utilizing pediatric kidneys [30-34]. In order to expand the donor pool, other workers have reported good outcomes with single pediatric donor grafts transplanted into adult recipients[35-37]. The obvious advantages of this approach are that single pediatric grafts provide kidneys for two rather than only one recipient as with an en-bloc graft.

In my series were no graft losses due to technical reasons among pediatric transplants. The death censored graft survival at 5 years was equivalent to that of adult donors. Pediatric kidney function was equivalent to that of "ideal" adult kidney transplants not showing deterioration over time. Additionally, there was no difference in the incidence of proteinuria among a cohort of recipients studied at 2 years post-transplantation. These findings agree with other reports suggesting that the function of pediatric kidneys improves over time as they adapt to the demands of the adult recipient[95, 96].

The limitations of the present study include its retrospective nature with a limited number of single pediatric kidney recipients. The usual recipient of a pediatric graft in this series was a young, thin female because the procedure is less technically challenging in a small patient and because this choice minimizes the donor: recipient size discrepancy. To highlight the excellent long-term outcomes using pediatric grafts, the adult donor group for comparison was restricted to non-obese (BMI < 25 kg/m^2) recipients of grafts from donors between the ages of 18-45 years.

6.3. Fibromuscular dysplasia

In our institution, we have been accepting asymptomatic healthy subjects with unilateral FMD as kidney transplant donors, and this case is the only complication in a series of 6 recipients.

In an extensive multicenter review of 1862 renal angiograms obtained from potential kidney donors, [97] an incidence of FMD of 3,8% with bilateral lesions in 71%, right-side lesions in 24%, and left-side lesions in 4% were found. In our institution, digital subtraction angiography (DSA) is considered the "gold standard" to diagnostic FMD. Recent studies attempting to use even less invasive evaluations, namely computerized tomographic angiography (CTA) or MRA, have shown less sensitivity compared with DSA for the diagnosis of FMD, especially in mild or moderate distal lesions[98-100]. Angiographic patterns of FMD vary according to the type of disease from a concentric stenosis in the medial hyperplasia and intimal fibroplasia types to the "string of beads" appearance in medial fibroplasia, where the beading is larger than the diameter of the artery, particularly when it occurs in the middle-to-distal portion of the vessel [101].

The natural history of FMD is controversial. Many years ago medial fibroplasia was thought to be a stable lesion, whereas other dysplasias (intimal fibroplasia, medial hyperplasia and perimedial fibroplasia) were known to cause progressive disease[102, 103]. In a reported series of 66 patients who underwent 2 or more renal angiograms for suspected renal artery disease, after a mean follow-up of 45 months angiographic progression of medial fibroplasia was observed in 33% of the patients but a 20% increase of the creatinine in only 3%. Most of these results have been in hypertensive patients, but the long-term outcome for healthy patients with incidental findings of FMD has not been studied.

In theory, nephrectomy could avoid hypertension in donors with FMD. Andreoni et al [98] followed 7 cases of FMD out of 159 potential living donors. These 7 patients were rejected as donors. After a short follow-up (2.46 -1.76 years), 2 of these patients (28.6%) required antihypertensive medications, with l requiring angioplasty of a progressive FMD stenotic lesion.

The long-term outcome of patients undergoing donor nephrectomy is controversial. Cragg et al. [97] reported 71 patients with FMD; 8 out of 30 patients who did not undergo

nephrectomy developed hypertension over 7.5 years, and 5 out of 19 patients who underwent nephrectomy developed hypertension over 4.4 years. However, some of these patients did have bilateral disease.

In our case, on the other hand, imaging studies did not reveal FMD in the contra-lateral kidney. Therefore, although we cannot be certain of freedom from disease in the donor, it seemed likely that she would continue to experience a benign course, which has been evident to date at 2 years post nephrectomy. In addition, Indudhara [104] evaluated 37 potential donors with FMD, 19 of these patients underwent nephrectomy on the side with the lesion or on the side with more advanced disease when the FMD was bilateral. After a mean follow-up of 4.5 years, no patient had hypertension, proteinuria or any significant change in serum creatinine. Of the remaining 18 patients who did not undergo nephrectomy, only 11 were contacted at a mean follow-up of 4 years without any complications. With respect to the recipient of a kidney with FMD, few articles have reported the course of the illness. Linder [105] reported 3 patients receiving kidneys with FMD (2 cadaveric and 1 living donor) with only one recurrence of the stenosis associated with hypertension that was successfully treated by percutaneous angioplasty. Nahas et al. [106] reported 4 recipients of kidneys with FMD without any post-operative complication and creatinine around 1.4 mg/dl at a follow-up of 21 - 115 months. Kolettis et al. [107], in a retrospective review of 36 recipients of living donors with medial fibroplasia, reported a graft survival rate of 89% with a median serum creatinine of l.6 mg/dl (0.5 - 82 mg/dl) at a median follow-up time of 37.l months. Pfeiffer et al. [108] reported 2 living donor transplants with extensive FMD requiring venous interposition with good results after 25 months of follow-up.

Among patients with symptomatic stenotic FMD, the results of percutaneous trans-luminal angioplasty of the renal arteries show overall success rates ranging from 89 - 97%, with cure of hypertension in 33 - 63% [109]. Complications of the procedure are rare, namely artery perforation (2.l%) and arterial thrombosis (l.0%) [110]. FMD is a progressive disease among symptomatic patients. However, no studies have shown deleterious results of unilateral nephrectomy in asymptomatic donors of kidney transplantations. The data concerning renal donation by young patients with FMD are controversial, and the theoretical benefit of this "prophylactic procedure" to avert hypertension requires further

evaluation. Finally, although the overall risk to the potential donor remains unknown, the risk to the recipient is low compared with the potential benefits of transplantation.

6.4. Sirolimus-cyclosporine-steroid combination in high risk patients

Using different drug combinations such as a concentration-controlled sirolimus-cyclosporine-prednisone regimen (with steroid withdrawal at 3 months) reduced the incidence of acute rejection episodes and increased 6-year graft survivals among high-risk African Americans to rates similar to other ethnic groups without an augmented toxicity profile.

Graft survival is shorter among African-American patients then other races due to immunological, pharmacological and socio-economical reasons.[111] However cyclosporine improved survival rates, its narrow therapeutic index (good immunosuppressive effect with low toxicity rate) makes their care more challenging. African-American patients' cyclosporine absorption is lower, the drug metabolism is faster and due to unclear processes they are more resistant to drug effects. [42, 112] In spite of the higher dose of cyclosporine the survival rates are lower in living related and deceased donor transplantation than in Caucasian recipients.[42, 111, 113]

Neylan et al. treated African-American recipients with azathyoprin and mycophenolate mofetil therapy. Acute rejection was more frequent and more severe than non-African patients. [114] Our results with sirolimus/cyclosporine combination showed the one year survival rate higher among African-Americans than among Caucasians (95,5% vs 94.9%), and higher then in other experiments with cyclosporine/azathyoprin/prednisone combination(92.4%)[115], but similar to tacrolimus/prednisone and tacrolimus/ mycophenolate mofetil/prednisone combinations (95%, 97%)[116, 117]

Generally African-Americans can benefit of higher sirolimus dose due to the agent's effect – through the inhibition on fibroblast and myocyta proliferation - to interrupt antibody mediated immune processes while remains not nephrotoxic.[42] Our data suggests that the incidence of acute rejections and graft losses can be decreased with sirolimus and serum level controlled cyclosporine combination.

Approximately 25 % of our patients are African-Americans giving us invaluable opportunity to observe these patients. In African-American recipients sirolimus addition

to a cyclosporine/prednisone regime could experience significantly less biopsy proved acute rejections during the first two years after transplantation.[118] In another paper the authors proved lower rejection incidence in all ethnic groups with tacrolimus/sirolimus combination than with cyclosporine/sirolimus combination (4% vs 14% p=0.03).[119] Unfortunately no long follow up studies are available.

According to our results the acute rejections are the most frequent in the 3rd year (23%), while chronic rejections become stagnate after the 5th year on the rate around 32%. The rejection rates were similar among African-Americans while graft and patient survival time was longer in the cyclosporine/sirolimus group. This is important due to the former results of significantly shorter survival rates on cyclosporine regime.[43, 113, 120]

The benefits of sirolimus are important. The number of African-Americans on the transplant waiting list is increasing, ESRD incidence is 4 fold higher among African-Americans then among Caucasians.[121, 122] It is difficult to find HLA compatible organs in the mainly Caucasians originated donor pool.[123]

Large databases prove that half life of the transplanted kidneys is definitely shorter among African Americans than Caucasians (4, years vs 7.5 years).[124, 125] Studies suggest that steroid withdrawal can be safe in low risk groups.[126-130] In a prospective study African-American patients on tacrolimus and sirolimus regime had 7 % acute rejection rate after steroid withdrawal and 10% needed resume steroid therapy.[131] In our study steroid withdrawal was successful in 38.5% of our patients without acute rejection episode.

According to the side effects of sirolimus we would like to highlight that, while sirolimus regime is beneficial with less toxic side effects among African Americans, the sirolimus caused dyslipidaemia can cause severe problems.[42-45, 117] Type II diabetes is 50% more frequent among African-American females and by 100% in males than in Caucasians.[121] Still serious problem remains that African-Americans on tacrolimus/mycofenolate mofetil/prednisone experience post-transplant diabetes in 22-45%. In our series we found 16%.[43, 116, 132-134] Cyclosporine/sirolimus synergistic effect causes myelosuppression, but this easily can be controlled with the drugs' serum level monitoring. Sirolimus caused myelosuppression occurs at the serum level >15ng/ml.[42] In our study leucopenia, thrombocytopenia and anemia incidence was

not significantly different between the races, and there was no need to change the therapy.

According to the data available in the literature and compared to our results the rejection rate among African-Americans is higher the graft survival is shorter then in other ethnic groups. Sirolimus based immunosuppression for African-Americans after kidney transplantation can be advantageous. However they require higher dose, the side effects are fewer then in other ethnics, and they can earn higher chance for safe steroid withdrawal.

6.5. Campath-1H

Our group was one of the first to administer Campath-1H in kidney transplant patients for acute rejection and the first to publish this data. With this indication no other studies available. In our patients Campath-1H was well tolerated and easily administered via the peripheral venous route. The previously described first-dose side effects were avoided by premedication with methylprednisone. When used to treat ACR refractory to conventional antilymphocyte antibodies, an extremely high immunological risk situation, Campath-1H is both effective and relatively free of adverse events. Since the publishing of our data Woodside and Lick presented a single case of a heart transplant recipient who presented twice in profound cardiogenic shock at months 4 and 8 post-transplant. The patient had unsuccessful conventional rejection therapy, but responded dramatically to alemtuzumab salvage therapy. Both times, her recovery onset was strikingly parallel to that described after using alemtuzumab as salvage therapy in renal transplantation.[135]

6.6. Experimental wound studies

Sirolimus has been implicated in impaired wound healing in several clinical studies. In a current review of wound complications included 194 renal transplant recipients received sirolimus based immunosuppression the authors found overall 36% (n = 70) incidence of wound complications within the first year post-transplantation

including infection in 12% (n = 23), lymphocele formation in 18% (n = 34), and incisional hernia in 18% (n = 34) of patients. Seventeen patients suffered more than one wound complication. With multivariate analysis they showed that a cumulative dose of sirolimus of at least 35 mg by post-transplant day 4 (odds ratio 2.694, p = 0.023) as independent risk factor was significant for the development of wound complications [136]. Beside the kidney transplant patients incisional wound healing, impairment was found in heart transplant patients[137] and Altomare et al. published their observation about sirolimus delayed gastric ulcer healing.[138]

Experimentally investigated effect of sirolimus on wound healing, show that sirolimus impairs wound healing, and this is reflected by diminished expression of VEGF and nitric oxide in the wound. Splenic lymphocyte proliferative activity was significantly decreased by sirolimus ($p < 0.05$). Sirolimus levels in wound fluid were found to be approximately two- to fivefold higher than blood levels ($p < 0.01$). Sirolimus (2.0 and 5.0 mg kg(-1) day(-1)) reduced wound breaking strength ($p < 0.01$) and wound collagen deposition ($p < 0.05$). This was paralleled by decreased expression of VEGF and nitric oxide in wounds. [139]

Another study using Sprague-Dawley rats with standard midline incision received different doses of SLR (2 and 5 mg/kg) with or without loading dose (10 mg/kg x3 days), and with or without steroids (20 mg/kg x3 days followed by 5 mg/kg for 2 weeks). Rats were humanely killed on postoperative days 5, 10, or 15. Wound breaking force was measured and tensile strength was calculated Wounds in control animals had gradual increase in tensile strength during the 15-day observation. In contrast, high and loading doses of SLR caused reduction in wound strength until day 10, but the wounds' tensile strength became equivalent to control by day 15. Low doses of SLR in non-steroid-treated animals had a short-term (5-day) impact on wound healing; high dose and loading doses delayed healing for 10 to 15 days [140]. According to our data we could observe wound healing impairment in the early phase of wound healing in the mice model. 8mg/kg sirolimus oral dose significantly delays wound healing. All animals tolerated nucleotide-free diet well. Topical application of 0.25% RNA gels enhanced the inflammatory and proliferative stages in wound healing between days 3-8 usually seen in the first three days after injury. This increase in innate and adaptive responses accelerated wound closure and healing. By expediting wound closure, the risk

of future infectious complications would be dramatically lowered, with significant reduction in morbidity and mortality.

Based even on our experimental results and the data published by various authors we think the advantageous immunological effects of sirolimus over-weight the disadvantages on wound healing.

7. Conclusions

A. Expanding the donor pool.
    a. Dual kidney transplantation's short and long term graft and patient survival and graft functions are good especially for older recipients. Using one side of the recipient is more challenging surgical technique but gives better re-transplant chances.
    b. The use of single pediatric (under the age of 5) deceased donor graft transplanted into selected adult recipient provided long-term graft survivals that were comparable to that of putatively optimal donors.
    c. With careful selection marginal organs can be used safely. For example kidneys with fibromuscular dysplasia can be used, although the overall risk to the living related donors remained unknown and has to be discovered. The risk to the recipient is low compared with the potential benefits of transplantation.

B. To preserve the transplanted organs
    a. Even among genetically high risk recipients the concentration controlled sirolimus-cyclosporine-steroid regimen (with steroid withdrawal at 3 months) reduces the incidence of acute rejection episodes without an augmented toxicity profile.
    b. I was the first to publish Campath-1H - as rescue therapy - in acute rejection among kidney transplanted patients and we found Campath-1H to be efficient in extremely high immunological risk.
C. Experimental studies for wound healing enhancement among sirolimus treated mice
    a. In an experimental model we could prove significant wound impairment with orally administered sirolimus
    b. Using our animal model we observed significant improvement in wound healing among sirolimus fed animals with low concentration nucleotide enhanced topical, especially in the early fase (2-6 days).

8. Summary

The author reviews his own clinical experiences and the international literature how to expand the number of used kidneys for transplantation, to achieve longer functioning graft and patient survival, and experimentally use topical for avoiding drug side effects. In the case of an extended criteria donor dual kidney transplantation seems to be a good solution. According to our 5 patients, 2 years graft and patient survival and graft functions are good, especially for older recipients without major surgical complication. In the case of donors under the age of 5 we used the kidneys separately as single transplants instead of the most widely used en bloc transplants. We compared 38 pediatric kidney transplants to 121 “ideal adult donor” (18-45 years of age) transplants. Without surgical complications the pediatric kidney function was equivalent to that of "ideal" adult kidney transplants not showing deterioration over time. There were no graft losses due to technical reasons among pediatric transplants. Smaller pool of potential kidneys for transplant are influenced with progressive arterial diseases – such as fibromuscular dysplasia - and questioned to be suitable for transplants. Observing our 7 patients with FMD even count with the only case we reported we think that the overall risk to the potential donor remains unknown, the risk to the recipient is low compared with the potential benefits of transplantation.

The author observed new immunosuppressant combinations to prevent the transplanted organs in prematurely high risk ethnic groups. A concentration-controlled sirolimus-cyclosporine-prednisone regimen in 470 patients reduced the incidence of acute rejection episodes and increased 6-year graft survivals without an augmented toxicity profile. The author was one of the first clinicians who used Campath-1H, if the rejection was refracter to the usual therapy. Campath-1H is accepted in leukemia therapy and for induction due to its broad immunosuppressive act. We used it as rescue therapy in 5 patients became well tolerated, effective and relatively free of adverse events.

In an experimental study the author could prove significant wound healing impairment effect of sirolimus in mice using daily 8mg/kg sirolimus orally. He could document enhanced wound healing with the use of nucleotide topical especially in the first 2-6 days in the same experimental settings.

9. Összefoglalás

A disszertáció a jelölt saját klinikai munkája során végzett tapasztalatok alapján a beültethető vesék számának növelésének lehetőségeivel, a már beültetésre került szervek élet tartamának meghosszabbításával és az immunszuppresszív szer okozta mellékhatások kivédésének kísérletes vizsgálataival foglalkozik.

A kiterjesztett kritériumú donor vesék egy recipiensbe való páros beültetését 5 betegnél végezte és vizsgálta igazolva azok két éven túli kielégítő vesefunkcióját. 5 évnél fiatalabb donor vesék esetében az elterjedtebb en-bloc beültetés helyett a vesék szeparált beültetését vizsgálta. 38 gyerekvese transzplantáció rövid és hosszútávú eredményeit hasonlította 121 „ideális felnőtt donor"-tól (18-45 év közötti) származó transzplantált vesék eredményeivel. A vese artériát érintő megbetegedés - fibromuscularis dysplasia - esetén klinikai vizsgálatban keresi a választ, hogy élődonoros vese átültetésnél a betegek veszélyeztetése változik-e. 7 beteget utánvizsgálva arra a következtetésre jutott, hogy mind a donor, mind a recipiens rizikója lényegesen alacsonyabb, mint a transzplantáció adta előnyök.

Az immunszuppresszív gyógyszerek újabb kombinációinak vizsgálatát végezte, különös tekintettel a kilökődésre magas genetikai rizikóval rendelkező népcsoportok esetében. 470 transzplantált beteg esetében a szérum koncentráció kontrollált sirolimus-cyclosporin-steroid kombináció esetében hatéves utánkövetési időt vizsgálva a kilökődések csökkenését találta a gyógyszer mellékhatások előfordulásának érdemi növekedése nélkül. Már kialakult rejekció kivédésre használt gyógyszerek hatástalansága esetére mentőövként 5 beteg esetében Campath-1H-t használt, és hatását vizsgálta klinikai vizsgálatában. A leukémia kezelésére elfogadott, transzplantáció során a beültetés időpontjában indukcióra kipróbált szert elsők között próbálta vese transzplantáltak akut rejekciójának gyógyítására használni. Az általa követet betegeknél a gyógyszer biztonságosnak és jól tolerálhatónak bizonyult, kielégítő hatás mellett.

A szövődmények csökkentését célzóan kísérletes körülmények között igazolta a sirolimusnak a seb gyógyulására kifejtett kedvezőtlen hatása, majd ugyanezen körülmények között kimutatta, hogy 0,25% koncentrációjú, kevert nukleotid tartalmú kenőcs helyi alkalmazásával a seb gyógyulása szignifikánsan felgyorsul a sebzést követő 2-6 napon.

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