DELAYED GRAFT FUNCTION IN RENAL TRANSPLANTATION: AETIOLOGY AND IMPACT ON GRAFT OUTCOME

Walter James Percival Douie

MBChB (UCT), DCH (SA), DA (SA), FCS (SA)

A research report submitted to the Faculty of Medicine, University of Cape Town, in partial fulfillment of the requirements for the Degree of Master of Medicine in the branch of General Surgery.

Cape Town 2001

DECLARATION

I, Walter James Percival Douie, declare that this research report is my own work. It is being submitted as partial fulfillment for the degree of Master of Medicine, in the branch of General Surgery, at the University of Cape Town, South Africa. It has not been submitted before for any degree or examination.

DR W.J.P. DOUIE

September 2001

CONGRESS PRESENTATIONS ARISING FROM THIS WORK.

Congress of the Surgical Research Society of South Africa – July 2000, Cape Town.

1. Delayed allograft function in renal transplantation – a stitch in time saves nine? W Douie, M McCulloch, A Pontin, J Halkett, M Pascoe, D Kahn.

2. What are the factors that influence renal allograft survival? W Douie, M McCulloch, A Pontin, J Halkett, M Pascoe, D Kahn.

(See Appendix A & B)

ACKNOWLEDGEMENTS

The Groote Schuur Hospital transplant team - medical, surgical, laboratory technicians, transplant coordinators and nursing staff all too numerous to mention by name who willingly and enthusiastically gave their time and assistance in data location for this analysis.

Professor D Kahn for his continued enthusiasm, encouragement and guidance in the drafting of this thesis.

Dr A Pontin, Dr M Pascoe and Dr J Halkett for willingly sharing their considerable knowledge of transplantation medicine and for setting the standards.

Professor P.A. Goldberg for technical help with the drafting of the thesis.

Dr S Isaacs for his assistance with statistical analysis.

To my wife, Martina and children, James and Thomas, for giving me the time, which should have been theirs, so that I could complete this thesis.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION AND LITERATURE REVIEW

The optimal therapy for most patients with end stage renal disease is renal transplantation[1]. This form of therapy results in improved quality of life[2], decreased medical expense[3], and perhaps a survival advantage for the recipient[4]. Renal transplantation is however a complex form of therapy which may be associated with significant complications.

The most common allograft complication post transplantation is the development of delayed graft function with a reported incidence of between 23 to 34%[5-9]. This is a costly complication requiring ongoing dialysis and prolonged hospitalization. It also has a significant emotional impact on the patient and their family[10]. Of greater concern though are the reports in the literature which associate delayed graft function with decreased graft survival[5; 9; 11-15].

With current organ shortages organ donor criteria have been expanded to match the shortage. It is thus imperative that we should understand the mechanisms of graft damage which may result in delayed graft function. A better understanding of the relationship between delayed graft function and graft survival might significantly impact on our practice in certain settings such as in the use of organs harvested from a marginal donor.

1.1 EARLY GRAFT DYSFUNCTION.

Most renal allografts function soon after implantation as evidenced by production of urine and improvement in biochemical markers. A significant proportion of grafts however experience early graft dysfunction. There are many causes of early graft dysfunction with delayed graft function being just one of them. Successful management requires that the correct diagnosis be made and appropriate therapy instituted early. This requires integration of clinical, biochemical, radiological and biopsy findings by a clinician familiar with the common causes.

The cause of early graft dysfunction may primarily be mechanical or non-mechanical in nature. The following paragraphs outline briefly the common causes of early graft dysfunction in an attempt to place the problem of delayed graft function, the subject of this dissertation, into clinical perspective.

1.1.1 MECHANICAL FACTORS

URINARY COMPLICATIONS

Obstruction. Catheter obstruction from a kink, misplacement or blood clot is a common and easily solved problem which should be excluded early post transplantation should doubt exist. Significant bleeding into the urinary system resulting in clot obstruction is unusual but is often related to the method of ureteral implantation into the bladder. The use of cystostomy with a long submucosal tunnel as proposed by Leadbetter and Politano has a higher incidence of bleeding as compared to direct external ureteroneocystostomy. In most cases bleeding from this area is minor and settles spontaneously[16; 17].

Less common causes of significant bleeding into the urinary tract may be renal biopsy or the rare complication of fulminant rejection resulting in papillary necrosis or renal rupture.

Ureteral stenosis secondary to edema at the cystoureteric anastomosis is an uncommon cause of urinary obstruction in the early postoperative period which settles spontaneously. More commonly obstruction at this level presents later and is due to ureteric ischaemia. When this is the case then surgery or interventional stents are required to correct the problem.

Lymphocele formation may cause a degree of obstruction when large. After confirming the diagnosis by biochemical analysis of aspirated fluid, therapy may be either with percutaneous drainage or marsupialization of the lymphocele into the peritoneal cavity.

Urinary leak. This problem is primarily due to distal ureteral ischaemic necrosis caused by damage to the blood supply during donor nephrectomy. The native ureter has a segmental blood supply receiving blood from numerous sources throughout its course. The donor ureter however receives its blood supply from the ureteric artery arising from the renal artery or a lower pole branch. This complication is thus more common in kidneys with small lower pole branches. This is especially important in living donor transplants when visibility during harvest is limited. This problem can be reduced by meticulous microvacular anastomotic techniques in living donor transplants if the kidney must be used or by use of an aortic patch in the case of cadaveric kidneys. The donor ureter should always be kept short to limit distal ischaemia.

Other causes of urinary leak may include technical error, infection and bladder outlet obstruction resulting in over-distension and disruption of the anastomosis.

Diagnosis of a urinary leak may be difficult as it may mimic an acute rejection episode, lymphocele formation or a perinephric haematoma. Typical symptoms of graft pain and fever in a patient with a poor urine output should alert one to this possibility. Occasionally swelling of the scrotum or labia and thigh may occur if the urine does not leak into the peritoneum, which may have been inadvertently opened during surgery. Ultrasound, biochemical analysis of the perinephric or drainage fluid, radionuclide renogram scanning and in few cases percutaneous nephrostogram may all be useful in confirming the diagnosis.

Therapy is typically surgical correction with or without temporary percutaneous stenting.

VASCULAR COMPLICATIONS

Thrombosis. Arterial and venous thrombosis early post transplantation occur uncommonly and may be confused with delayed graft function. A technical problem at the arterial anastomosis is the commonest cause of arterial thrombosis but kinking of the artery, atherosclerosis in the donor or recipient vessels and administration of OKT3 or cyclosporine have all been implicated as possible causes. Clinical suspicion of the thrombosis may be confirmed with ultrasound Doppler, radionuclide scanning or angiography. The prognosis for the graft is poor and the usual treatment is graft nephrectomy.

1.1.2 NON MECHANICAL FACTORS

Hypovolaemia. The maintenance of adequate intravascular volume is essential to optimize allograft function. Hypovolaemia in the immediate postoperative period is not uncommon due to preoperative dialysis and a reluctance on the part of the anesthetist to overload the patient under anesthesia. A brisk diuresis following graft reperfusion due to a high circulating osmotic load, a degree of proximal tubular dysfunction and intra-operative administration of a diuretic may then compound this problem.

Awareness of the problem coupled with regular fluid balance and clinical assessment is essential. Central venous pressure monitoring is used routinely in many centres while pulmonary artery pressure catheters are utilized in difficult situations. The therapy is obviously fluid resuscitation.

Rejection. Allograft rejection as a cause of early graft dysfunction is common and must be recognized early. Of the 4 basic patterns of rejection, those being hyperacute, accelerated, acute and chronic rejection, the first three are relevant to this discussion.

Hyperacute rejection causing irreversible destruction of the allograft within minutes to hours after revascularization occurs when preexisting recipient antibodies recognize donor antigens on vascular endothelium. This pre-sensitization occurs during previous pregnancies, blood transfusions and transplants. With modern crossmatching techniques this form of rejection is now rare.

Accelerated rejection causes graft destruction within a few days of transplantation. Regarded by many as a "second set" response, where previously sensitized host cells rapidly infiltrate the graft causing a combination of cellular and humoral graft destruction. This form of rejection is often irreversible but with modern crossmatching techniques it is again uncommon.

Acute rejection typically occurs within the first month of transplantation but may occur at a later stage. It is classified histologically by the varying degrees of cellular and humoral activity in the allograft. The typical features of fever, malaise, hypertension, oliguria and decreased creatinine clearance associated with graft tenderness may be confused with infection and cyclosporine toxicity. Renal biopsy is thus a useful but not infallible aid to the diagnosis.

Treatment is typically with pulsed intravenous steroids or mono/ polyclonal antibody therapy which is successful in most patients.

Cyclosporine Nephrotoxicity. Cyclosporine therapy represented a major breakthrough in immunosuppression. It is however associated with numerous problems with nephrotoxicity being a serious and well recognized cause of early graft dysfunction. The typical patient with nephrotoxicity has an elevated serum creatinine level, steadily decreasing creatinine clearance values and may develop hypertension and some degree of tubular dysfunction as evidenced by renal tubular acidosis, hyperuricaemia and decreased fractional excretion of sodium.

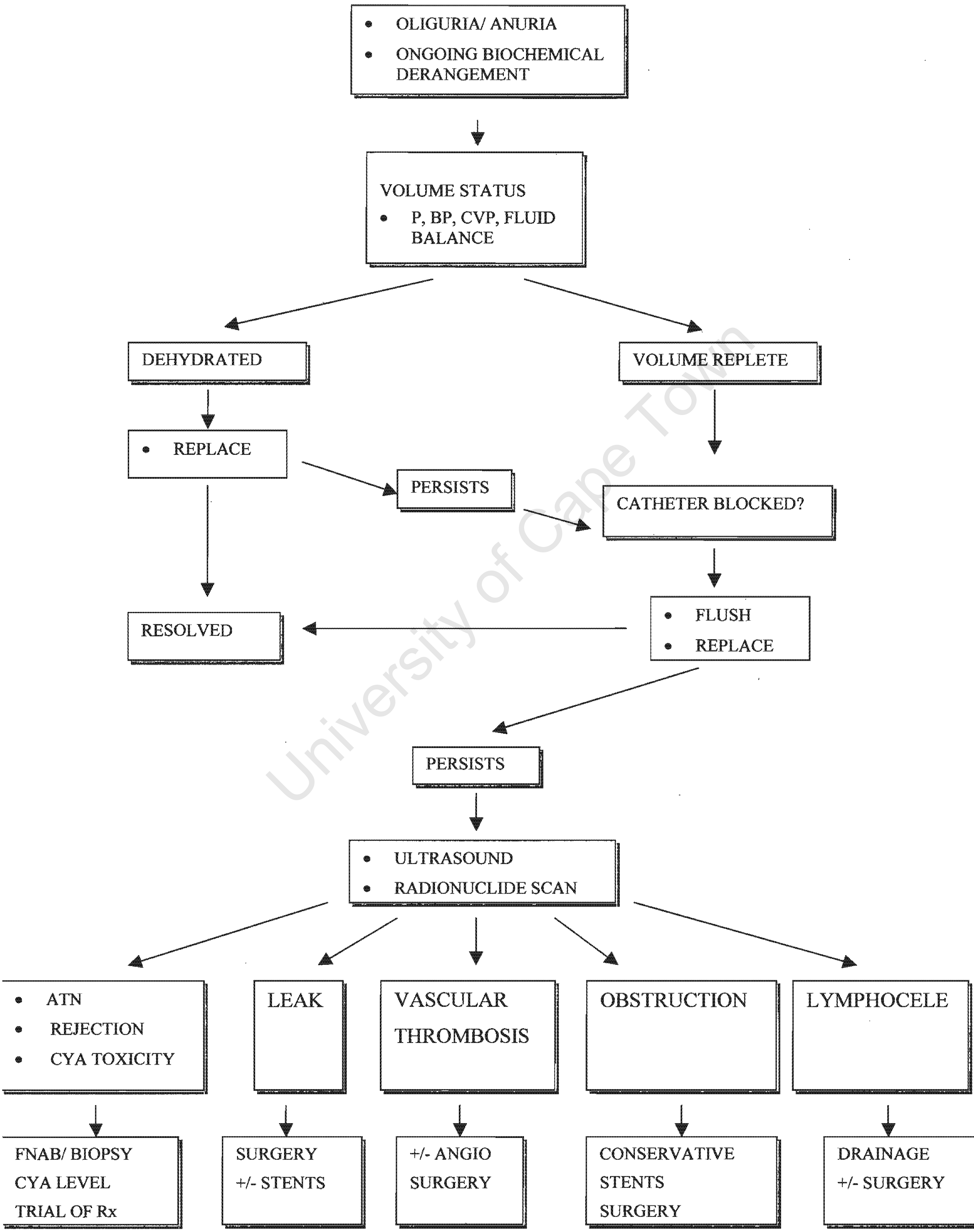
Patient response to cyclosporine may vary significantly and thus serum cyclosporine levels may or may not be elevated above the recommended guideline values.

Therapy consists of dose reduction. In those patients where response to dose reduction has been poor a renal biopsy may be helpful.

Acute tubular necrosis. Ischaemic insults to the donor kidney at any stage of the engraftment process results in acute tubular necrosis. A degree of tubular dysfunction is inevitable in all transplants but it is not typically of a severity to be clinically relevant. When clinically severe enough to require dialysis the term *delayed graft function* is used. This is the focus of the thesis and will be discussed further in the following sections.

Not withstanding the complexities of transplantation, ***Figure 1.1*** offers a simple algorithmic approach to graft dysfunction in the first days following transplantation.

Figure 1.1: **Management of early graft dysfunction.**



1.2 DELAYED GRAFT FUNCTION.

Delayed graft function is a common, complex and poorly understood consequence of transplantation. Its occurrence complicates early post transplant management in that dialysis is required and makes acute rejection and cyclosporine toxicity more difficult to diagnose. There is also concern regarding its effect on the long-term graft function. Driven by the increased demand for organs there has been a tendency to relax donor criteria to keep pace with this demand. Use of the so-called "marginal" donor for example is now common practice even though these grafts are associated with a higher incidence of delayed graft function. The renal transplantation team thus needs to be aware of developments regarding delayed graft function so as to make optimal use of donor organs and, at the same time, limit morbidity.

1.2.1 DEFINING THE PROBLEM.

The most5 frequently used definition of delayed graft function is the need for dialysis within the first week post transplanation[9; 15]. On review of current literature it is apparent that definitions used, although sharing a common flavor, that is life sustaining renal function within the first week following transplantation, differ in detail. Lehtonen et al imply that the absence of a steady decrease in creatinine even if dialysis is not required constitutes delayed function. The same authors also classified patients as having immediate graft function even if requiring dialysis within the first week if the indication for dialysis was fluid overload alone and serum creatinine was declining[18]. Koning et al define delayed graft function as the absence of life sustaining renal function requiring dialysis on 2 or more occasions within the first week post transplantation[19].

The reason for the numerous definitions is that the clinical manifestations of delayed graft function vary along a spectrum of severity, from a subtle slowing of the expected decline in serum creatinine to prolonged oliguria requiring a period of support.

Primary non-function should be distinguished from delayed graft function. With delayed graft function, sufficient renal function returns with time to permit life off dialysis. This is not the case with primary non-function.

1.2.2 PATHOLOGY AND POSTULATED PATHOGENESIS OF DGF.

The pathological correlate of delayed graft function is acute tubular necrosis. This pathological end point results through complex pathophysiological processes which may result in damage at every step along the path from procurement, through storage, to implantation and reperfusion[20; 21].

Brain dead patients often exhibit abnormal homeostasis as evidenced by haemodynamic instability and diabetes insipidus. It has been suggested that some degree of microcirculatory damage to the donor organs may occur during these periods of disequilibrium contributing to the development of delayed graft function[22; 23]. The graft may also be damaged by the underlying disease process, be subjected to periods of warm ischaemia during periods of hypotension in the donor and also by the therapeutic manoeuvres used to counter such episodes. These insults occur in the donor prior to organ harvesting and contribute to the development of delayed graft function.

Ischaemia to the donor organ is central to the development of delayed graft function[20; 21; 24-28]. Prolonged ischaemia results in prolonged anaerobic metabolism in the donor kidney. Anaerobic metabolism with degradation of ATP increases the levels of adenosine, inosine and hypoxanthine. Under normal conditions there is a high intracellular concentration of Potassium and Magnesium. With depletion of energy stores there is inactivation of the Na+/ K+ ATPase, the enzyme system that controls the Sodium pump on the cell membrane. Inactivation of this pump results in Sodium and Chloride ingression to the cell along with water, thus resulting in cellular oedema. Likewise the Calcium – Magnesium ATPase is also inactivated resulting in Calcium entering the cell and Magnesium leaving. These changes together activate cytosolic phospholipase A2, an enzyme that lyses cellular and subcellular membranes. During anaerobic metabolism lactic acid is generated which results in intracellular acidosis which in itself activates lysosomal lytic enzymes. Thus prolonged ischaemia during cold storage may cause renal cellular damage.

Probably of more significance is the post ischaemic reperfusion injury that results after graft reperfusion[28-31]. Hypoxanthine, which accumulates as a result of ATP degradation under anaerobic conditions, has been shown to be a major source of oxygen free radical species (ORS). With Calcium influx mentioned above there is activation of cytosolic proteases to produce xanthine oxidase. With reperfusion hypoxanthine is rapidly oxidized to xanthine by Xanthine Oxidase with production of superoxide free radicals and hydrogen peroxide. Any free iron in the cell reacts with hydrogen peroxide to form the highly reactive hydroxyl radical. Naturally occurring free radical scavengers are depleted during ischaemic periods and thus these ORS's produced on reperfusion overwhelms the free radical scavengers and may directly damage tissue. Alternatively they may activate vascular endothelium, which in turn may produce a variety of inflammatory mediators including various cytokines and the compliment system. This activated response is similar to that produced during an allo-immune response and may cause significant cellular damage[29; 29-33].

Immunological factors, as discussed in section *1.2.3.3* of this thesis, have been implicated in the development of delayed graft function but their relative importance and precise mechanism is still unclear. Interestingly it has been postulated that Ischaemia may be central to enhanced allo-immune responsiveness and allo-atherogenesis[20]. Both of these processes probably impact on long-term graft survival and will be discussed further later.

1.2.3 FACTORS ASSOCIATED WITH DELAYED GRAFT FUNCTION.

Numerous factors implicated in the development of delayed graft function have been identified with some having a significantly bigger impact than others. The most frequently noted factors are discussed below. For simplicity they will be discussed under the headings of (1) Donor factors, (2) Harvesting, Preservation and Transplantation factors and (3) Recipient factors.

1.2.3.1 Donor factors

Donor age. Donor age is cited as a risk factor for the development of delayed graft function. On multivariate analysis both McLaren et al and Koning et al identified donor age of greater than 50 years as a significant risk factor for the development of delayed graft function [19; 27]. In a much larger analysis of donors from the US Renal Data System, Ojo et al confirmed the above but also noted a smaller but increased risk in donors over 40 years of age. Interestingly they noted that donors younger than 10 years of age were at a slightly increased risk for the development of delayed graft function. This would suggest that an optimal age for organ donation is in the teen and young adulthood years[5].

As well as being associated with higher incidences of delayed graft function, grafts from donors at extremes of age have been associated with and decreased graft survival [28; 34]. In spite of this, the number of donors older than 55 years of age between 1990 and 1997 recorded with the United Network for Organ Sharing (UNOS) scientific registry grew from 9 to 14% [35].

It has been suggested that a number of age related factors such as hypertension, creatinine clearance and diabetes may themselves influence graft outcome. Karpinski et al assessed outcomes of donor kidneys deemed high risk preoperatively due to age, hypertension or vascular disease. These high risk grafts were subjected to preoperative renal biopsy. They found that both the predicted donor creatinine clearance values of a graft and the graft vascular pathology predicted recipient graft dysfunction[36]. These findings are reinforced by the findings of Carter et al who noted a decreased graft survival in grafts from donors with predicted lower creatinine clearances and preoperative hypertension[35].

In summary, grafts from older donors may have worsened initial function. They are however still capable of good function. Age alone should not exclude kidney donation without consideration of the comorbid factors affecting the graft structure and functioning.

Donor creatinine levels. Mean creatinine levels in the donor prior to transplantation have been identified as an important risk factor for the development of delayed graft function. In an analysis of 547 transplanted allografts, Koning et al noted on univariate analysis that there was a significant difference in the incidence of delayed graft function between donors with serum creatinine values of greater than 120 micromoles per litre. The highest donor serum creatinine value in this study was 140 micromoles per litre. Ths implies that there may be a sharp increase in the incidence of delayed graft function over a relatively narrow serum creatinine range [19].

High donor creatinine values may be chronic or just reflect a degree of acute tubular dysfunction. It has been suggested that acute tubular dysfunction, as evidenced by a raised serum creatinine, is associated with a rich tubular interstitial milieu of proximal pro-inflammatory agonists. A second ischaemic or reperfusion insult added to any acute tubular dysfunction may have an amplified effect and result in delayed graft function[5; 37].

Donor urine output. Poor donor urine output prior to procurement correlates with the development of delayed graft function post operatively. From animal experiments it has been suggested that the brain dead state leads to a decreased cardiac output secondary to decreased left ventricular contractility. The resultant decreased renal perfusion, as manifested by a decreased donor urine output may be responsible for a degree of donor renal dysfunction [22; 23; 38]. This often trivial dysfunction may become clinically relevant when combined with other insults such as a period of warm or cold ischaemia.

Non-heart-beating donors. Donation of kidneys from brain dead heart-beating (NHB) donors has plateaued with little prospect of increasing organ procurement from this source. The increasing disparity between demand and supply has prompted centres to look again at non-heart-beating donors as a potential organ source. Wijnen et al in reviewing their twelve year experience of non-heart-beating donors estimated that they were able to increase the number of available donor kidneys from their area by 20%. This increase in procurement is associated with increased financial costs, logistical problems associated with the necessary haste of the procedure and also functional problems of the graft post transplant[39].

Numerous authors have reported a significantly increased risk of delayed graft function in recipients of grafts from NHB donors. Centres experienced in this technique have reported delayed graft function rates of over 60%, a figure twice that of heart-beating donors. Interestingly this increased incidence of delayed graft function has not always been associated with an increased incidence of primary non - function or worstened graft survival figures[39-41].

It is not surprising that kidneys from non-heart-beating donors are associated with a higher incidence of delayed graft function as these grafts are associated with an unavoidable period of warm ischaemia [39]. In an analysis of their experience with non-heart-beating donors, Gonzalez- Segura et al noted a strong association between warm ischaemic times and the development of delayed graft function. They noted that patients had a significantly greater chance of loosing their graft if first warm ischaemic times were greater than 45 minutes with an associated cold ischaemic time of greater than 22 hours[42].

There is agreement that the use of less than ideal donors (extremes of age, diabetic and hypertensive donors) and asystolic donors are all capable of giving good functional results. There is however justifiable concern that recipient outcomes may be worse in both the short and longer term.

Cause of donor death. Delayed graft function is said to occur more frequently in donors who died a medical death such as a cerebrovascular accident or anoxia. Pfaff et al noted a higher incidence of DGF in patients who died of medical causes when compared to those who died a violent death. The authors noted that cerebrovascular deaths more often affected females who were older than victims of violent death. This increased incidence of delayed graft function in donors dying from cerebrovascular accidents has been noted by others[12]. This observation possibly just reflects preoperatively undetected co-morbid disease in an elderly group of donors.

Single vs. multiple retrieval donors. It has been proposed that kidneys harvested from multiple retrieval donors perform poorly in comparison to single donor organs. The reason cited for this is the level of priority, and thus care, assigned to the kidneys in multiple harvest donors is less than in single organ harvests. Scandling et al in a 4 year review of locally harvested kidneys reported a doubling in the incidence of delayed graft function when multiple organs were harvested. They noted concordance in kidney function between pairs of harvested kidneys from single or multiple harvest donors. They also noted that the incidence of delayed graft function was higher the more organs harvested. These findings would suggest that harvest related factors such as unrecognized warm ischaemia may be responsible for the poorer results in multiple retrieval harvests[8].

1.2.3.2 Harvesting, preservation and transplantation factors.

Prolonged cold ischaemic time. Prolonged cold ischaemic times are a consistently reported association with delayed graft function and appear to be an independent risk factor for its development. Numerous authors have found it to be the most predictive determinant of delayed graft function on multivariate analysis[43] [19; 27; 28; 44]Data from the United States renal Data system showed that, when analyzed as a linear variable, every 6 hours of cold ischaemia increases the incidence of DGF by 23% [5; 27].

As previously mentioned, ischaemic damage and the post ischaemic reperfusion injury are central to the development of delayed graft function. Cold storage of the graft used to minimize this damage slows cellular metabolism but does not halt it completely. Cold storage also results in a discordance of metabolic processes which usually occur in unison at 37 degrees Celsius. At 4 degrees Celsius various enzymatic processes are inhibited to differing degrees resulting in altered concentrations of metabolites. This abnormal balance may in itself be harmful. One would thus expect greater degrees of cellular damage with prolonged periods of cold storage

Warm ischaemia. The detrimental effects of warm ischaemia on graft function have been known for some time. Sacks et al proposed that 30 minutes of warm ischaemic time was more damaging than 24 hours of cold ischaemia. Kidney damage secondary to warm ischaemia is said to be predictably reversible only if it is kept to less than thirty minutes [17; 45]. With modern harvest techniques first warm ischaemic times are negligible but second warm ischaemic times, that is the time taken to complete the anastomosis has been strongly correlated with the incidence of delayed graft function. Shoskes et al identified anastomosis time as the strongest independent predictor for the development of delayed graft function in a cohort of paediatric patients[28; 46]. Every effort should be made to shorten this anastomosis time and to maintain some surface cooling of the graft during the implantation process.

Preservation solutions and technique. The commonest form of renal storage is the so-called "static storage". The graft kidney is flushed with iced preservation solution until the effluent is clear of blood. It is then immersed and stored in preservation solution at near zero degrees Celsius. Preservation solutions have been designed to minimize ischaemic damage during storage. Components are added to reduce swelling (impermeants), prevent intracellular acidosis (buffers), maintain calcium homeostasis, decrease free radical substrate formation (scavengers) and provide high energy substrates (ATP precursors such as Adenosine)[24].

Of the many preservation solutions available, the commonly used solutions are Euro-Collins and University of Wisconsin (UW) solutions or their derivatives. Which of these is the more effective is the topic of considerable debate. In a randomized multicentre study comparing 352 kidneys preserved with UW solution and 343 with EuroCollins solution, Ploeg et al noted a significant decrease in the incidence of delayed graft function in grafts preserved with UW solution (80 vs. 114, P = 0.003). Other relevant observations by the authors were improved graft functioning and graft survival in the UW preserved grafts[38; 47].

Not all authors however concur with the above findings. Hefty et al in a prospective study using paired cadaveric kidneys from heart beating donors found no benefit from the use of UW solution over EuroCollins solution[48].

UW solution is not without its problems. A small number of patients experience bradycardias and electrocardiographic irregularities which is thought to be related to the infusion of adenosine, one of its components. UW solution is also significantly more expensive than EuroCollins solution. It has been suggested that the use of UW solution may be preferable for salvage therapy of grafts from marginal donors or when cold ischaemic times of thirty-six hours or longer are predicted [17].

Pulsatile perfusion storage once enjoyed popularity particularly in the USA. In support of its use, proponents of pulsatile perfusion storage site increased preservation times, closer physiological monitoring of the graft and improved performance compared to statically stored organs[49]. It is however tedious, cumbersome and expensive. Proponents of simple cold storage cite the reduced expense of the technique, a reduced risk for endothelial damage from the pulsatile perfusion pressure and a lower risk of bacterial contamination with static cold storage[50; 51]. The superiority of one of these methods over the other is largely unproven[52; 53]. In a prospective controlled study comparing split kidneys from the same donor, one kidney being stored with static cold storage and the other with pulsatile perfusion, Merion et al found no difference in graft function and graft survival between the two groups. They noted that even when preservation times were greater than 24 hours no significant difference emerged. This study was however small with only 51 sets of kidneys for comparison, Halloran et al in another small study of 107 donors reached similar conclusions [54; 55].

Polyak et al retrospectively reviewed 650 kidneys preserved either by machine perfusion or static cold storage in their laboratory between January 1993 and March 1999. They noted improved early and long term renal function in machine perfused grafts especially when they augmented their perfusate with Prostoglandin E1[56].

With the more frequent use of extended criteria donor kidneys coupled with improvement in perfusion solutions, advances in perfusion technology and continued professionalization of transplantation it seems likely that machine perfusion will experience a resurgence in popularity so as to exploit any small benefit that may exist over static cold storage preservation[57].

Pharmacological manipulations. Numerous pharmacological manipulations to decrease the incidence of delayed graft function have been tried with varying degrees of success. Agents such as diuretics, alpha 1 blockers, Allopurinol and other oxygen free radical scavengers have all been tried but none have shown any convincing benefit.

The use of *mannitol* infusion at the time of graft revascularization is a simple measure which has yielded promising results in the prevention of ATN in numerous vascular surgical settings. In a small prospective controlled trial, Weimar et al compared the incidence of delayed graft function in two comparable groups, one group receiving 50 grams mannitol just prior to revascularization, the other half receiving an equivalent volume of normal saline. Recipients in the mannitol group experienced a significantly decreased incidence of delayed graft function compared to the control group. When comparing the patients who experienced immediate graft function the authors noted significantly higher creatinine clearance values throughout the first week post transplantation in the mannitol group of patients. Other less robust studies have noted similar benefits with the use of mannitol [19; 58].

A number of postulates have been proposed to explain this beneficial effect. Through its osmotic activity, mannitol increases plasma volume, increases cardiac output and also decreases systemic vascular resistance in man. All of these factors improve renal perfusion[59]. Mannitol is thought to have a direct effect on the kidney, reducing renal vascular resistance and increasing glomerular filtration rates. It also has oxygen free radical scavenging properties which may play a role[32].

Calcium channel blockers have shown promise in improving initial graft function as well as decreasing the nephrotoxic effects of cyclosporine. In three prospective randomized trials diltiazem improved initial graft function. The beneficial effects are noted when treatment is initiated in either donor or recipient [60]. Possible mechanisms for these beneficial effects include proximal arteriolar vasodilatation and also decreased lipid peroxidation during reperfusion.

The beneficial effects are not limited to diltiazem alone but are seen with other calcium channel blockers as well[60-62].

The addition of *Prostaglandin E1* to machine perfusate has recently been reported to improve early graft function compared to machine perfusion using standard perfusate or static storage alone. PGE1 is thought to improve perfusion characteristics by decreasing the release of calcium into the perfusate and decreasing mitochondrial ischaemia, this translating into a clinical benefit[56]. This finding, if confirmed by others, may have significant implications in the future.

The *21- aminosteroids*, a new class of agents which prevent lipid peroxidation and thus limit reperfusion injury have shown promise in experimental renal reperfusion injury in rats[63]. Their role in humans is still to be established.

Intra operative observations. A number of intra-operative observations have been noted, the occurrence of which often precedes the development of delayed graft function.

Quality or reperfusion. Koning et al in a prospective review of 547 renal transplants noted a significantly increased incidence of delayed graft function in those grafts noted not to reperfuse smoothly on revascularization. In this series DGF occurred in 21% of grafts noted to reperfuse smoothly as compared to 44% of grafts noted to have spotty, patchy reperfusion or areas of renal infarction[19].

Immediate Intra-operative diuresis. In the same study Koning et al noted that the absence of intra-operative diuresis was prognostic for the development of DGF in 50% of the grafts vs 12% when immediate urine production was present. This has been noted previously in a randomized study[19; 64].

Observing either of these phenomena intra-operatively would serve to alert the attending physician to the potential problems and may help plan further management.

1.2.3.3 Recipient factors

Preoperative recipient urine output. Preoperative urine output has been sited as an independent predictor of the development of delayed graft function. Koning et al, when comparing recipients with a preoperative urine outputs of greater or less than 500 ml/day noted a tendency towards a decreased incidence of delayed graft function, p= 0,06[19]. McLaren et al noted a decreased incidence of delayed graft function in recipients who had pre-emptive renal transplantations, P< 0.0001[27; 27].

The above observations were probably predictable. Certainly in the case of pre-emptive transplantation the decreased incidence of delayed graft function may just reflects a degree of residual renal function in the recipients native kidneys rather than an improved functioning of the implanted graft. The same logic probably applies, but to a lesser degree and hence a lesser statistical significance, to the preoperative oliguric recipient. Inthese patients fluid management is simplified considerably by the production of urine and the need for dialysis for fluid overload is lessened.

Immunologic risk factors. A number of immunologically mediated factors have been associated with an increased incidence of delayed graft function.

Number of previous transplants. Although not a universal finding, the number of previous transplants has been frequently sited as an independent predictor on multivariate analysis for the development of delayed graft function[19; 28].

Panel reactive antibody status, both highest and latest, is routinely recorded in potential graft recipients. Again, although not a universal finding it seems that high sensitization levels do impact negatively on the incidence of delayed graft function [21; 27; 28].

Ischaemic and immunological factors have been found to be synergistic with regard to the development of delayed graft function in paediatric transplantation[28]. Although there is presently no way to decrease sensitization of a recipient, early function rates may perhaps be improved in patients with high immunological risk by selecting kidneys with minimal ischaemic risk factors.

Race has been sited as a risk factor for the development of delayed graft function. In a review of over 37 000 patients on the US Renal Data System, Ojo et al noted African-American recipients to be at increased odds of developing delayed graft function. They did not however note donor race to significantly affect the incidence of delayed graft function[5]. Although immunological factors may be responsible for this observation it is also conceivable that certain race groups use different health care facilities. The increased incidence of delayed graft function may well reflect other institutional influences rather than race alone.

1.2.4 DELAYED GRAFT FUNCTION AND LONG TERM OUTCOME

1.2.4.1 The Implications

Post transplant oliguria is a commonly encountered problem. The cause may be trivial and easily rectified or may reflect a more serious underlying problem with far reaching consequences. A diagnostic algorithmic approach to post transplant oliguria was discussed at the beginning of this thesis.

Once the diagnosis of acute tubular necrosis has been established then the treatment is essentially supportive. The use of haemodialysis has to be balanced against the vasomotor changes that may occur and which have been implicated in prolonging the course of Acute tubular necrosis. Measures to avoid dialysis such as fluid restriction, control of serum Potassium with cautious electrolyte administration or pharmacological measures may limit the need for dialysis. Pharmacological control of blood pressure is also important in this regard.

Fluid management may be simplified if a diuresis can be induced with the use of a diuretic or low dose dopamine. The benefit of this therapy is controversial and probably does not alter the course of acute tubular necrosis.

The elimination of cyclosporine from the immunosuppressive protocol in patients who develop delayed graft function has been suggested. The reasons for this are two fold. Firstly cyclosporine has been shown to increase the recovery time of post transplant acute tubular necrosis. Secondly the difficulty of diagnosing and thus the higher incidence of undiagnosed acute rejection in these patients makes the option of conversion to an antilymphocytic immunosuppressant such as OKT3 attractive.

Not withstanding the above problems most patients with delayed graft function recover useful renal function over a variable period of time. The usual course is a conversion from oliguric to non-oliguric renal failure. This is then followed by a decrease in serum creatinine values.

Over the last three decades there has been much research into what effects delayed graft function has on long term graft function. The earliest study of this relationship was performed by Williams et al who found no relationship between the two[65]. Whittaker et al in 1973 first proposed that delayed graft function was associated with a poorer long-term outcome[66]. Since these early studies, newer studies with varying design have been published with conflicting results[21; 67-69].

It is very difficult to compare these studies and draw meaningful conclusions for a number of reasons. Firstly the diagnosis of delayed graft function is often based on the need for dialysis within the first week alone with no reference to declining serum creatinine values or urine output. As mentioned previously, there are certainly patients who may require dialysis for other reasons (hyperkalaemia, fluid overload etc) who have functioning grafts. Likewise patients receiving pre-emptive transplants may well have delayed functioning in the implanted graft who do not receive dialysis due to residual renal functioning in the native kidneys. Secondly, significant changes in transplantation during three decades makes comparison difficult. Major changes in immunosuppressive management, with a shift from steroids and azathioprine through cyclosporine to newer antilymphocyte preparations, and the resultant significant improvement in graft survival has unmasked significant prognostic factors previously missed due to poor survival[21].

Within the cyclosporine era most single centre univariate studies showed between 20 – 30% improvement in 1 yr survival of kidneys with early graft function[65; 70-73]. This has also been noted in paediatric transplantation[74; 75].

More recently multicentre trials in the cyclosporine era have consistently shown delayed graft function to negatively correlate with graft survival[76]. The detrimental effects have become more apparent in recent times. This is evidenced by initial published results from Minnesota which showed delayed graft function to have no detrimental effect to long-term graft survival. Over an eight year period the same group reviewed their results and have subsequently correlated delayed graft function with poorer graft survival[25; 77; 78].

More recently numerous authors have recognized that the degree of graft dysfunction impacts on graft survival. In particular a need for dialysis of greater than 1 week resulted in worse 1 and 5 year graft survival than those grafts requiring no dialysis or dialysis limited to the first week post transplantation[72; 79-81].

Graft survival in grafts exhibiting early function has also been analyzed against quality of early function as measured by rate and degree of decline of serum creatinine values. Najarian et al noted that even in grafts exhibiting early function that the quality of that function correlated with overall graft outcome[82].

In summary there is mounting evidence that in modern transplantation delayed graft function is an independent predictor of graft survival. In addition the duration of delayed function and also the quality of early function appear to impact on graft survival.

1.2.4.2 Proposed pathogenesis

Although most authors now accept that delayed graft function impacts negatively on long term graft function there has been little published research into possible mechanisms to explain this. From the available literature both immunological and non-immunological mechanisms have been proposed.

Immunological mechanisms. As previously mentioned, recipients at higher immunological risk such as those with significant HLA graft mismatch or a sensitized PRA status are more prone to developing delayed graft function[19; 83]. Both cellular and antibody mediated rejection may also masquerade as delayed graft function, such as when there are preformed anti class 1 antibodies result in accelerated rejection[84].

Acute rejection is more difficult to diagnose during an episode of acute tubular necrosis and may go unnoticed. As early rejection has been shown to be an independent predictor of long term graft function it follows that this non recognition or delayed recognition of rejection could partly explain the decreased long term graft function in certain patients with delayed function[82; 85; 86].

Numerous studies have suggested that delayed graft function even in the absence of acute rejection is deleterious to graft survival[85-88]. A possible explanation for this is that ischaemic changes, known to be central to the development of delayed graft function, may affect the immune system. It is noteworthy that many inflammatory cytokines released during recovery from ischaemic renal damage are identical to those released during the allo-immune response. It is likely that these pathways of renal regeneration and rejection, with their common components, interact in complex ways that may augment the immune response. This may result in decreased renal recovery and perhaps initiate cellular proliferative responses culminating in chronic allograft failure[21; 88].

Nonimmunological mechanisms. It is possible that post transplant acute tubular necrosis which results in a lower functional nephron mass could contribute to poorer long-term graft survival for a few reasons. Firstly, the fewer nephrons to start off with allows little room for normal attrition associated with time before this become clinically relevant. Secondly, with fewer nephrons there is a resultant increased single nephron glomerular filtration rate with resultant glomerular sclerosis, proteinuria and hypertension. This is also seen in the early stages of diabetic nephropathy and in patients with decreased renal mass following cancer surgery[21; 89; 90].

With the ever-increasing demand for cadaveric organs and the need for transplant teams to stretch the limits for organ donation it is noteworthy that the graft survival is increasing. In an analysis of over 93 000 renal transplants performed through the United Network of Organ sharing between 1988 and 1996 Hariharan et al noted a substantial increase in both short and long term graft survival. After censoring the data for patients who died with functional grafts they reported an increase in cadaveric graft half life from 11 to 19,5 years[91].

The Groote Schuur Hospital renal transplant unit is responsible for the majority of the renal transplants performed in the government sector throughout the Cape Province, performing about 80 transplants per year. Its practice may differ from other centers around the world in a number of ways:

- Service area. The geographical area serviced by our unit is vast mandating a considerable movement of donor organs and recipients about the province. Medical staff without specialist training in transplantation medicine are required to assume greater responsibility in the management of these patients pre and post transplantation.
- Organ sharing. Unlike the USA and Europe, where organ sharing networks are well established, ours is very limited. There is no inter-provincial organ sharing and minimal sharing between government and private institutions. This depleted organ pool may significantly compromise our short and long term transplant results.

- Stringent financial constraints. The stretched hospital facilities impact on the process of transplantation in a number of ways. For example, the cost of newer immunosuppressive agents and organ preservation solutions prohibit their usage in our unit. Operating theatre and other services essential to the practice of transplantation are all affected by these financial constraints and may impact on our practice.

The general findings reported in the literature may not be totally applicable to our own situation and this prompts an analysis of our own results.

2. AIM OF THE STUDY

To analyze a cohort of renal transplant recipients from the Groote Schuur Hospital transplant unit who developed delayed graft function. Specifically I will:

- Ascertain the incidence of delayed graft function in our setting.
- Attempt to identify factors that have significant influence on the development of delayed graft function.
- Assess which factors impact on longer-term graft function.
- Discuss these findings in the light of the current literature.

3. PATIENTS AND METHODS

3.1 BACKGROUND AND DATA COLLECTION

Between September 1995 and May 1998 a total 245 renal allograft transplants were performed by the Groote Schuur Hospital Transplant team. During this period 50 recipients developed delayed graft function and these patients were prospectively identified and form the index population in this study. For comparative purposes, a control group of 100 patients was generated by identifying those patients who received grafts immediately before and after the indexed patients. Information was extracted from patient hospital records for all the study patients. This information consisted of details of the donor, the harvest, the recipient, the tissue match as well as the process of transplantation. In addition, data pertaining to the first post transplant month (dialysis, rejection episodes, recipient serum creatinine levels etc) as well as the long term functioning of the graft was obtained. The long-term follow-up of the transplants was assessed up until December 1999. Data was collected on a prescribed proforma (Appendix C) and subsequently entered into a database specifically designed for this study. Both living and cadaveric donor transplants were included in the study.

3.2 SURGICAL TECHNIQUE

Renal harvesting was performed throughout the Cape Province by a surgeon from our transplantation team. Conventional surgical techniques were used for the procurement of the organs from the donors. The preservation solution of choice was Euro-Collins solution except in harvests involving simultaneous procurement of the liver. In these harvests University of Wisconsin solution was used. The implantation of the cadaver kidneys included the anastomosis of the renal vein to the external iliac vein, the renal artery to the external iliac artery and the ureter to the bladder. For living related donor kidneys, the renal artery was anastomosed end to end to the internal iliac artery.

3.3 DEFINITIONS

3.3.1 DONOR AND RECIPIENT CLASSIFICATION

Any organ harvested from donors in a hospital other than our own, irrespective of the distance from our unit, was classified as a distant donor harvest. The majority of these distant harvests were performed in centres outside Cape Town itself. Harvests performed in Groote Schuur Hospital and Red Cross War Memorial Hospital were classified and local donor harvests.

Recipients who were dialyzed in centres other than by the Groote Schuur or Red Cross War Memorial Hospitals and were not followed up in these units post transplantation were classified as distant recipients. The majority of these patients resided in rural centres.

3.3.2 HLA MISMATCH SCORING

HLA mismatching was assessed as a score out of 6 using the HLA loci A, B and DR. A point was assigned for each donor- recipient mismatch of the two alleles at these loci.

3.3.3 PRA STATUS

Panel reactive antibody status of the recipients was routinely assessed every 6 to 8 weeks in the dialysis clinic. Patients were classified as sensitized if the PRA status was reactive to over 30% of the antigens in the panel. The most recent sensitization status prior to the transplant as well as the highest sensitization status at any time prior to transplantation was recorded.

3.3.4 COLD ISCHAEMIC TIME

Cold ischaemic time was defined as the time, in hours, between placing the graft on crushed ice to the time of removal of the graft from the ice for implantation.

3.3.5 DELAYED GRAFT FUNCTION

Delayed graft function was defined as the requirement for dialysis in the first week post transplantation. The duration of delayed function was calculated to the time when the patient became dialysis independent again. Patients who never regained function were classified as primary non-function.

3.3.6 ACUTE REJECTION

An acute rejection episode was defined using the usual clinical and biochemical parameters and was considered acute if it occurred within the first month post transplantation. Fine needle and core renal biopsy were not routinely performed to diagnose rejection but were performed in difficult cases were the diagnosis was in doubt. Therapy for acute rejection was pulsed methyl-prednisilone 500 mg /d IVI for 4 days. Steroid resistant rejection was managed with Anti-thymocyte globulin.

3.3.7 GRAFT FAILURE.

Graft failure was defined as the need to return to long-term dialysis. Graft survival was calculated from time of transplantation to the date of return to dialysis. A few patients died from uraemia as they were deemed unsuitable for return to the dialysis program for various reasons. They were also classified as graft failures. This however was not the norm and most patients who required re-dialysis were accommodated on a dialysis program with or without return of their names to the transplantation waiting list. Those patients who died with functional grafts from other causes were not classified as graft failure.

3.4 IMMUNOSUPPRESSION.

The Immunosuppression protocol consisted of triple therapy with Cyclosporine, Azathioprine and Prednisilone. The Cyclosporine dose was adjusted to maintain trough blood levels between 150 – 300ng/ml. Azathioprine was given at 1,5 mg/kg/d adjusted were necessary according to the white cell count. Prednisolone was started at a dose of 30 mg/d. The dosages of these agents were slowly decreased over the ensuing months.

3.5 FOLLOW-UP

The majority of patients were followed up in the long term by transplantation physicians from our unit. A number of patients however were attending follow-up at other institutions as they resided in centres some distance from Cape Town. These patients attending physicians were however in regular contact with our unit and accurate follow-up data for these patients was attainable.

Data was complete in all respects in 118 patients. The data from the remaining patients was *not* excluded from analysis as significant data on these patients was available, data being incomplete in one or two aspects only in each case.

3.6 STATISTICAL METHODS.

Univariate data analysis of factors pertaining to delayed graft function was analyzed comparing discrete variables with Chi square test and continuous variables with the Student t-test. Multivariate analysis of factors affecting graft survival was analyzed using a Cox regression analysis. Data expressed in tables reflect absolute patient numbers or median values for non parametric data with ranges expressed in parentheses unless stated otherwise. In all analyses a significance level of $p < 0.05$ was assumed. Actuarial graft survival was calculated according to the Kaplan- Meier method. The statistical package used was Statistica.

3.7 ETHICAL APPROVAL.

Ethical Approval for this study was obtained from the University of Cape Town Faculty of Health Sciences Research Ethics Committee on 29 September 2000 – REC REF: 210/2000.

4. RESULTS

4.1 INCIDENCE OF DELAYED GRAFT FUNCTION

Between September 1995 and May 1998, a total of 245 renal transplants were performed in the Renal Transplant Unit at Groote Schuur Hospital. During this study period 50 patients developed delayed graft function giving an incidence of 20.4%.

These 50 patients were compared with 100 control patients who had immediate graft function after the kidney transplant.

4.2 DONOR CHARACTERISTICS

The demographic and other relevant data pertaining to the 150 donors are shown in ***Table 4.1***. The median age of the donors was 24 years (mean 24.7 years) with a male to female ratio of 109 to 40. 47 donors were White, 65 were of mixed race, and 32 were Black. There were 132 cadaver donors and 18 living-related donors. The cause of death in the cadaver donors was mainly related to trauma and included 43 assaults and 59 motor vehicle accidents. Of the 150 procurements, 59 were classified as local and 91 involved distant donors. Euro-Collins solution was used as the preservation solution in 131 donors and Wisconsin solution in 19 donors.

Table 4.1: **Donor Data**

Age (years)	24.0 (3- 56)
Sex (M: F)	109: 40
Race (W: C: B)	47: 65: 32
Local: Distant	59: 91
Cadaver: Living	132: 18
Cause of death	
Assault	43
MVA	59
Other	48
Preservation Solution (EC: W)	131: 19

4.3 RECIPIENT CHARACTERISTICS

The demographic data of the recipients are shown in ***Table 4.2***. The median age of the recipients was 39 years (mean 37.7 years) with a male to female ratio of 89 to 60. There were 30 White recipients, 85 of mixed racial origin and 31 were Black. The majority of renal transplant recipients were locally based.

Table4.2: **Recipient demographics**

Age (years)	39.0 (5 – 63)
Sex (M: F)	89:60
Race (W:C:B)	30:85:31
Local: Distant	102:39

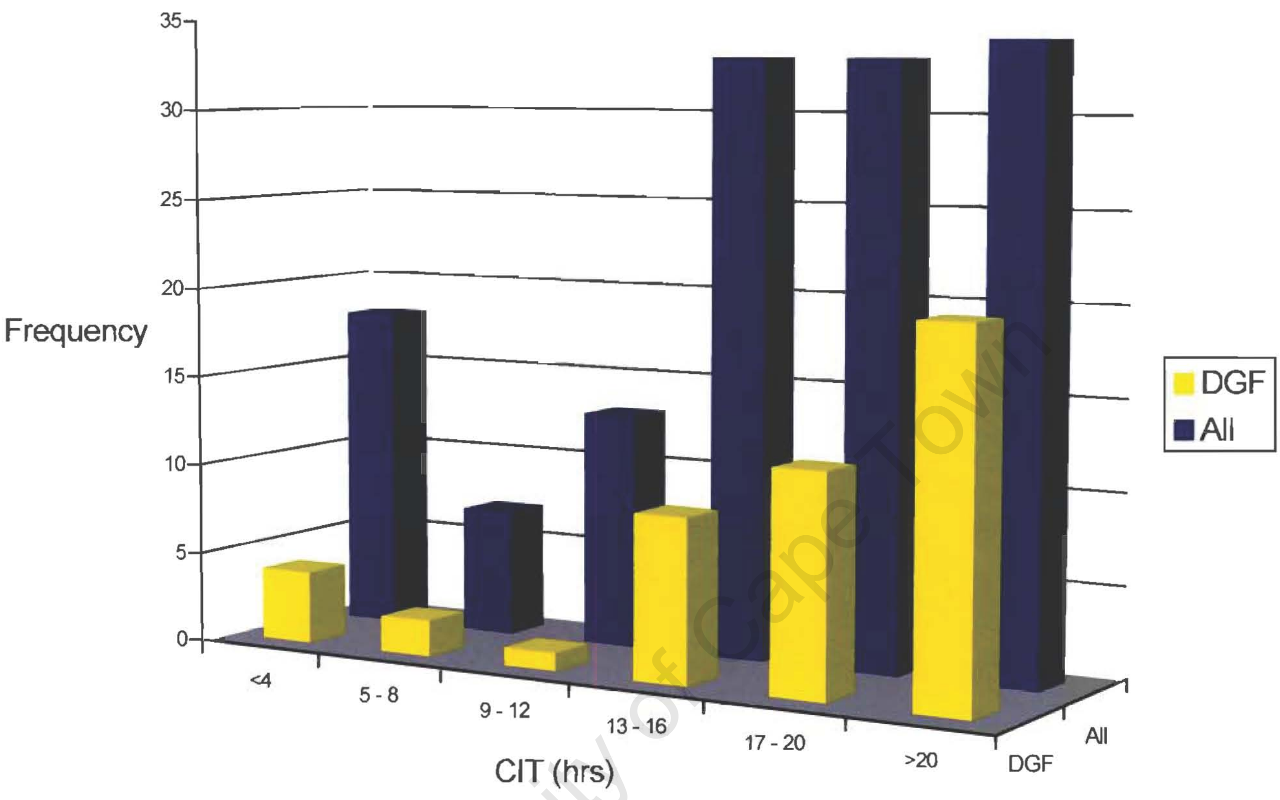
4.4 FACTORS ASSOCIATED WITH ONSET OF GRAFT FUNCTION

The results of the univariate analysis of the various factors in relation to the onset of graft function are shown in ***Table 4.3***. There was no significant difference in donor and recipient ages between the patients who had delayed graft function compared to those who had immediate graft function. Similarly, the proportion of local donors and local recipients compared to distant donors and distant recipients were similar between the patients who had delayed graft function and those who had immediate graft function. Furthermore, the gender distribution in the donors and the recipients were similar between the patients with delayed and immediate graft function. There was also no difference in the type of preservation solution used, the HLA mismatch and the PRA status between the patients with delayed or immediate graft function.

There was a statistically significant difference in cold ischaemic time between the patients with delayed graft function (median 20 hours, mean 18.6 hours) compared with those patients with immediate graft function, (median 15.5 hours, mean 14.7 hours). No patients experienced a cold ischaemic time of greater than 30 hours.

Figure 4.1 compares graphically the cold ischaemic times of patients who developed delayed graft function and the cold ischaemic times of the group as a whole. Of note is the sharp increase in the incidence of delayed graft function with relatively short increases in the cold ischaemic times over 12 hours.

Figure 4.1: **Plot of graft function against cold ischaemic time**



Grafts harvested from distant donors were subjected to significantly longer cold ischaemic times (mean 19.2, median 18 hours, range 8-30) compared to grafts harvested within our Institution (mean 10.9, median 10 hours, range 2-24) with a significance level $P<0.001$. Distant recipients however did not as a group experience longer cold ischaemic times (mean 16.6, median 18 hours, range 2-29) compared to those living in Cape Town (mean 15.4, median 15 hours, range 2-30) with a significance level $P=0.22$.

It is noteworthy that kidneys harvested with livers were not subjected to longer cold ischaemic times (mean 18, median 17 hours, range 3-26) compared to those harvested alone (mean 16.6, median 16 hours, range 2-30) with a significance level P=0.14.

The patients who were recipients of first transplants had significantly lower incidence of delayed graft function compared to those patients who received more than one transplant previously. There was no correlation between cold ischaemic time and transplant number, P=0.399.

Table4.3: **Univariate analysis of factors in relation to onset of graft function**

	Delayed Function	Immediate Function	P value
Donor age (years)	26 (6-52)	24 (3-56)	0.17
Recipient age (years)	38.5 (13-60)	39 (5-63)	0.65
Donor location (L/D)	17:33	42:58	0.34
Recipient location (L/D)	38:10	64:29	0.19
Living/ Cadaveric donor	5: 45	13:87	0.59
Donor Sex (M:F)	38:11	71:29	0.51
Recipient Sex (M:F)	32:17	57:43	0.56
Graft Number (1 vs. 2 or3)	32:18	77:18	**0.02**
Preservation soln (W: EC)	8:42	11:89	0.39
CIT (hours)	20 (2-28)	15.5 (2-30)	**0.002**
HLA mismatch	4 (0-6)	4 (0-6)	0.35
PRA – highest	4:44	16:74	0.13
PRA – latest	1:47	2:86	0.71

Where *data* expressed as median and range, *Location* L = local, D= Distant, *Preservation soln* W= Wisconsin solution, EC= Euro-Collins, *CIT*= Cold ischaemic time.

4.5 FACTORS ASSOCIATED WITH GRAFT SURVIVAL

The results of the multivariate analysis of the factors which affected graft survival are shown in ***Table 4.4***. There was no difference in donor age and recipient location between the grafts that were functional and those that were not. Furthermore, the incidence of delayed graft function was similar between the functioning and the non-functioning grafts. The duration of DGF in the non-functioning grafts (mean 16.4, median 17 days) was not significantly different from that experienced by the functional grafts (mean 12.5, median 12 days). The cold ischaemic time was similar between non-functional grafts (mean 18.8, median 17 hours) and the functional grafts (mean 15.2, median 16 hours).

75% of the patients in this study were receiving their first renal transplants with only 21% receiving second transplants and 4% receiving their third transplants – ***Figure 4.2***. Recipients of second and third grafts had significantly shorter medium to long-term graft survival compared to recipients of first transplants. The HLA mismatch in the patients with non-functional grafts (mean 3.8, median 4 mismatches) was significantly greater than in the patients with functional grafts (mean 3.47, median 4 mismatches) suggesting that a higher degree of HLA mismatch between the donor and recipient was associated with significantly poorer graft survival. The HLA mismatch status of patients receiving first transplants compared to those receiving second and third transplants was similar, P=0.27, repeat transplant recipients not being subjected to tighter HLA matching. This is represented graphically in ***Figure 4.3***.

Figure 4.2: **Plot of number of patients receiving first and subsequent renal transplants.**

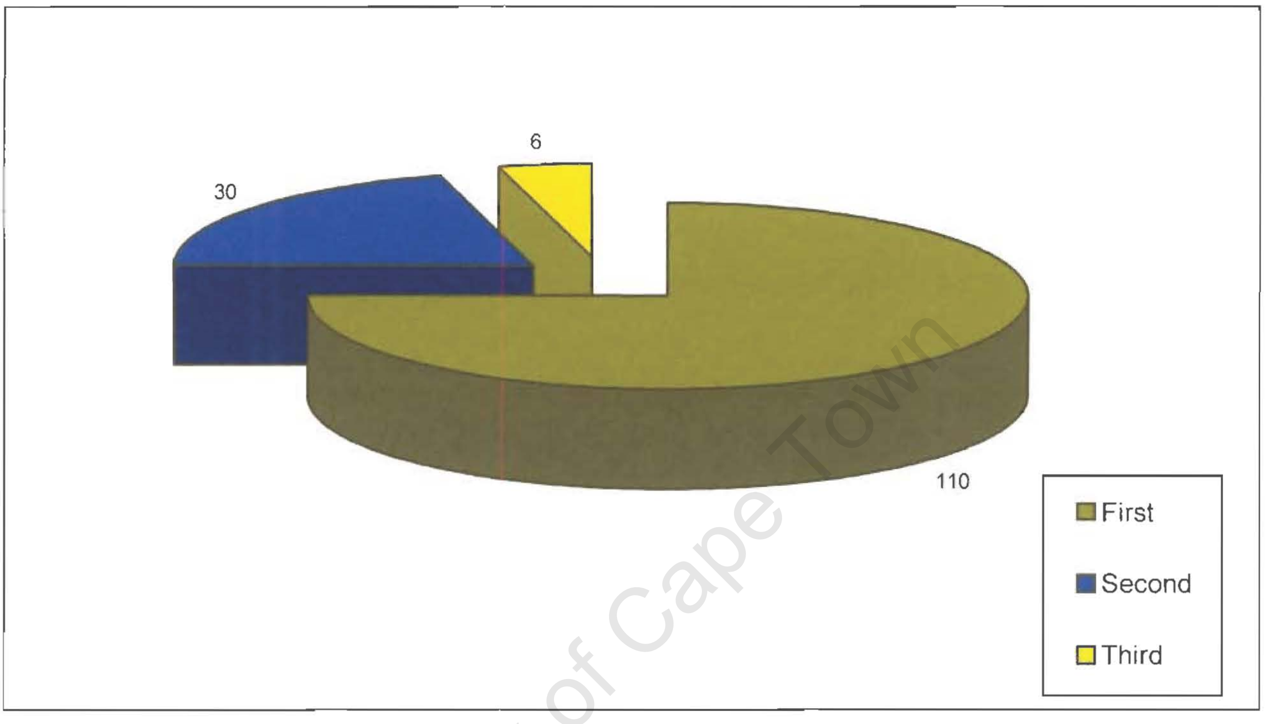
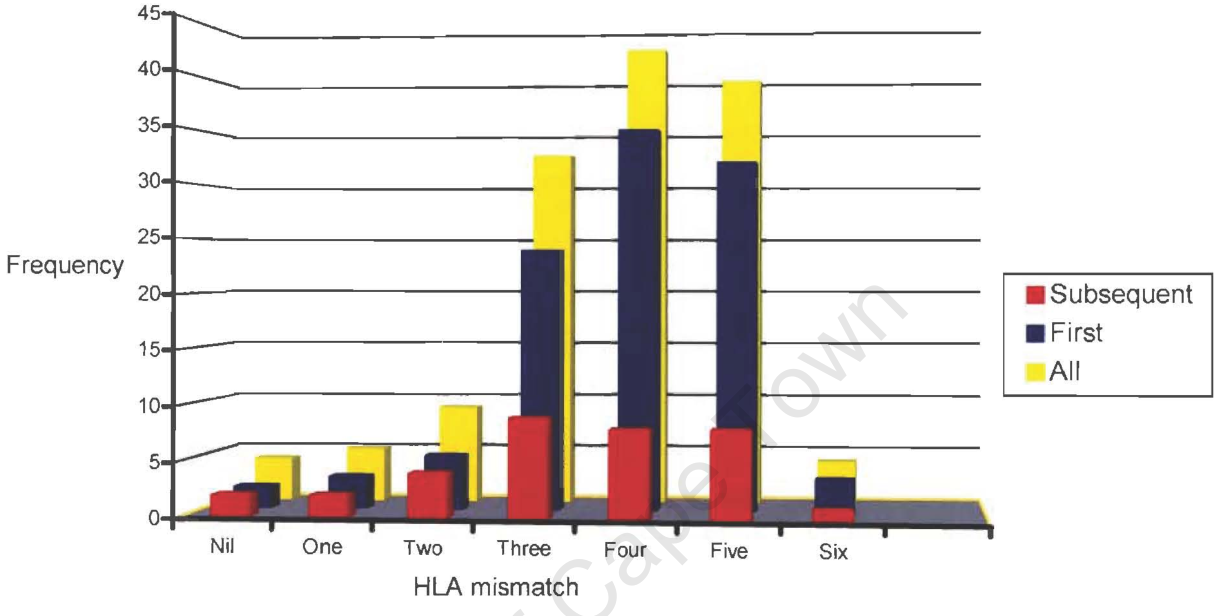


Figure 4.3: **Plot of Donor- Recipient HLA mismatch status for recipients of first and subsequent grafts.**



In this study, 86% of the patients had a PRA of less than 30%. This is represented graphically in ***Figure 4.4***. A historical PRA reactivity of greater than 30% was associated with a significantly poorer long-term graft survival compared to patients with a less than 30% PRA reactivity.

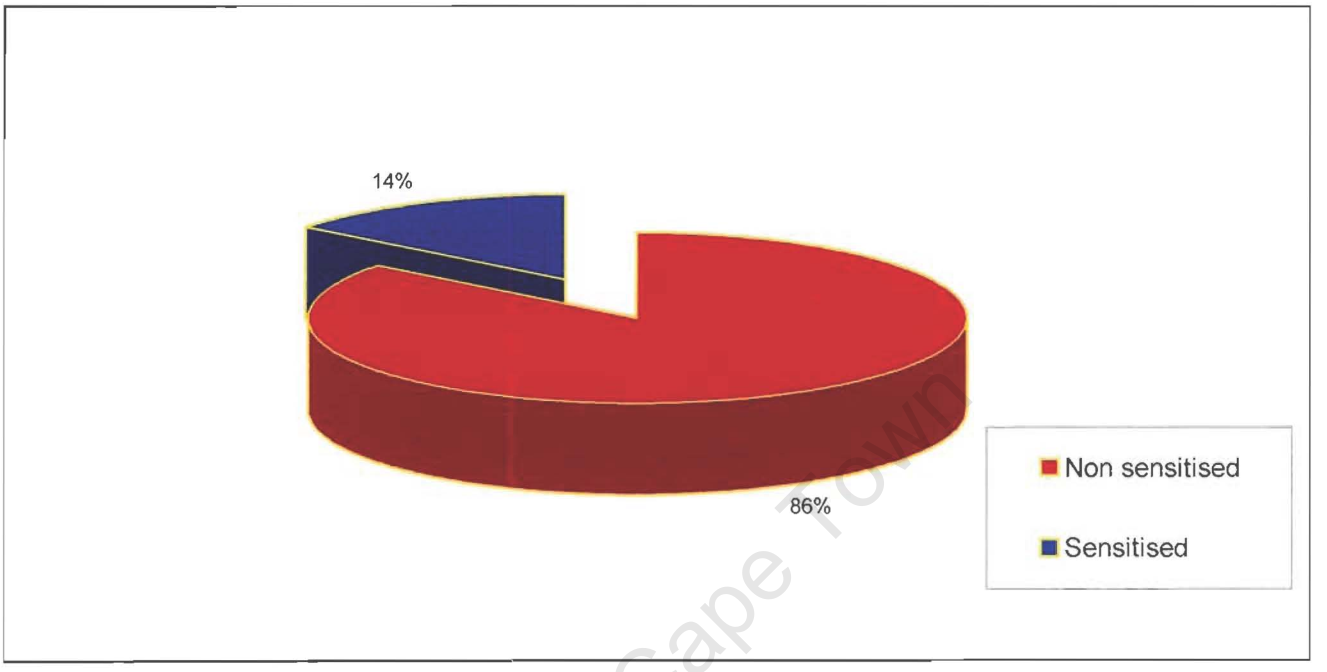
The incidence of acute rejection did not influence graft survival.

Table 4.4: **Multivariate analysis of various factors and their affect on graft survival.**

	Non Functional graft	Functional graft	Beta	p value
Donor age (yrs)	27 (6-44)	24 (3-56)	.01	0.31
Recipient location (D/L)	6/14	20/75	-.27	0.31
Delayed Function (IF/DGF)	16/10	62/35	.50	0.23
Duration of DGF (days)	17 (11-20)	12 (3-20)	-.04	0.15
CIT (hrs)	17 (4-29)	16 (2-30)	.02	0.27
Previous Transplant (1/>1)	22/5	71/28	-.56	**0.01**
HLA mismatch	4 (0-6)	4 (0-6)	-.28	**0.005**
PRA highest (ns/s)	20/5	78/11	-.78	**0.04**
PRA latest (ns/s)	24/1	86/2	.21	0.79
Acute rejection	6/20	39/55	-.02	0.94

Data expressed as median or absolute number. *Recipient location* D = distant center, L = Groote Schuur patient. *Delayed function*, IF = Immediate graft function, DGF = Delayed graft function. *CIT* = cold ischaemic time. *Previous transplant* 1 = first transplant, >1 = subsequent transplants. *PRA* = Panel reactive antibody status, ns = non-sensitized ie less than 30% reactivity, s = sensitized ie > 30% reactivity.

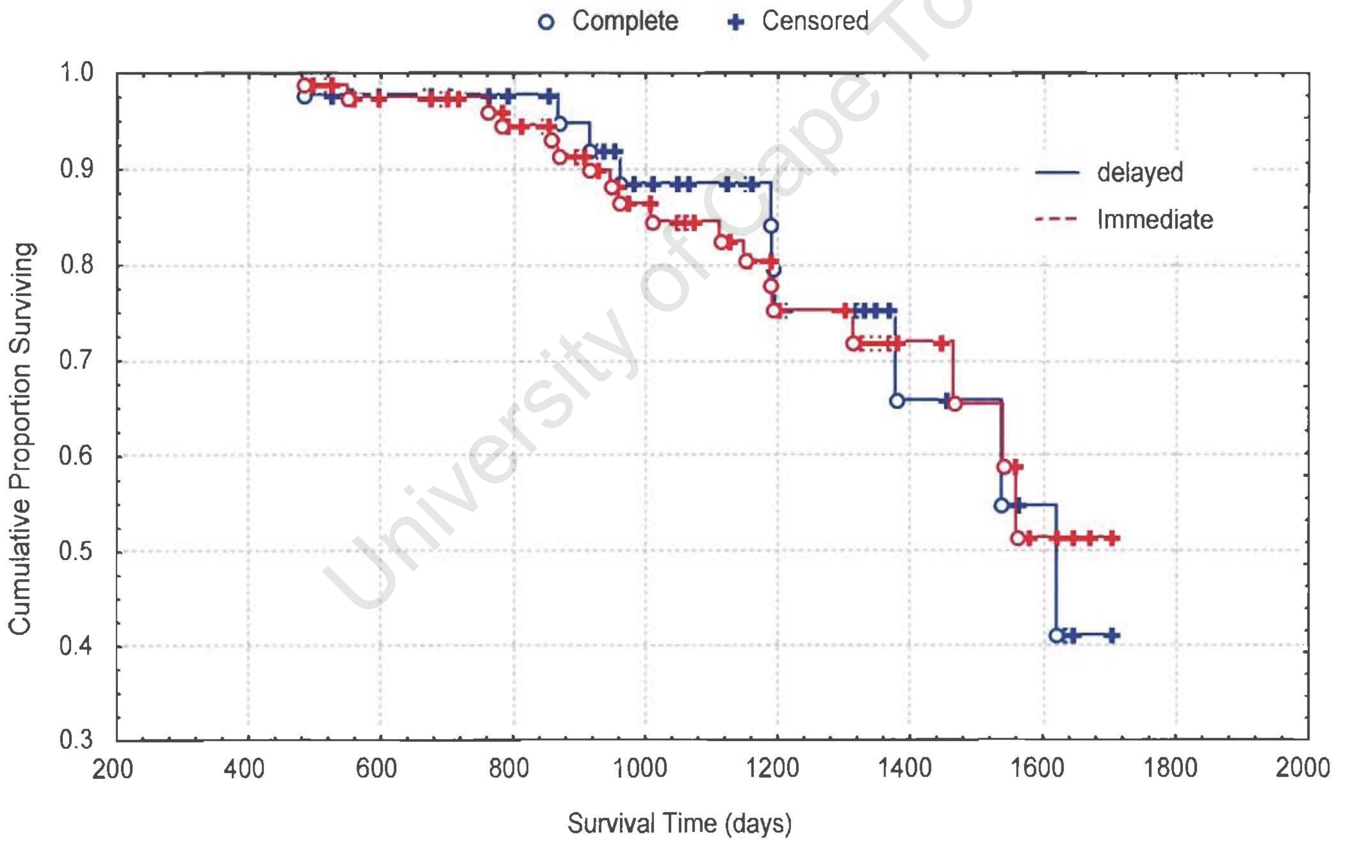
Figure 4.4: **Plot of Panel reactive antigen sensitization status of patients studied with > 30% reactivity to panel representing sensitization.**



4.6 GRAFT SURVIVAL.

The cumulative graft survival of patients with delayed graft function and those with immediate graft function are shown in ***Figure 4.5***. There was no difference in graft survival between patients with delayed graft function compared to those with immediate graft function.

***Figure 4.5*: Kaplan Meier survival curves of grafts experiencing delayed graft function and those experiencing immediate function.**



5. DISCUSSION AND CONCLUSION

Despite the significant advances made over the last few decades in donor care, organ procurement, tissue typing and immunosuppression, delayed graft function still occurs and complicates post transplant management. The incidence of 20.4% as reported in this study compares favorably with an incidence of between 7 to 34% reported in the literature [5-9; 11; 12; 18].

In this study there was a significant male predominance amongst the donor population with 73% being male. Other reported series in the literature also report a male donor predominance but none to the extent reported in our series [5; 9; 13; 38]. Likewise, a mean donor age of under 25 yrs and recipient age of under 38 years reported in this series is notably lower than that reported by many in the literature [5; 13; 38]. These observations may be explained by the unusually high number of trauma victims amongst our donor population with violent assault alone accounting for 29% of donor diagnoses. A positive spin off of this observation is that trauma donors and young donors have a lower reported incidence of delayed graft function which may have positively influenced our results [9; 12; 14; 19; 27].

Indigent black donors made up 21% of donors in our series. Population Census figures conducted over the same period of the study indicate that this racial group contributed to over 76% of the population of South Africa as a whole, 61% of the population in the geographical area served by the transplant unit but only 21% of the population of the Western Cape [92]. One may thus have expected a higher proportion of black donors in this series on demographic grounds alone. It is however known that consent rates for organ donation are lower amongst black families. The reason for this is certainly multifactorial with cultural, socioeconomic status (education levels, exposure to media etc) and transplantation awareness being just a few important factors.

Living related and living unrelated donors contributed only 12% to our organ donor pool. Such donors may represent an inadequately utilized source of potential donors. To date we have not utilized organs from non-heart-beating donors. Non-heart-beating donor kidneys are associated with an increased incidence of delayed graft function with some centres reporting incidence rates of up to 60%, a figure twice that reported for cadaveric transplantation [39; 40; 42]. Currently our recipient waiting period for a cadaveric renal transplant is approximately 1 year, a figure that compares favorable with most centres around the world. It is debatable whether this waiting period could justify the extra expense, manpower and complications which would result from actively pursuing non-heart-beating donors as an organ source.

Patients receiving dialysis at GSH received preference for grafts, with 72% of recipients in this group being GSH patients. For this reason recipient race closely mirrored the racial constitution of the Western Cape rather than the demographics of the area served by the transplant unit - 58% of recipients were of mixed racial origin, a population group constituting 27.8% of the area served by the transplantation unit but 54% of the population of the Western Cape. The reason for this disparity is understandable when one appreciates the limited resources for dialysis and post transplantation care available in peripheral state institutions in South Africa. This is also coupled with the difficulties in contacting and coordinating rapid transfer of potential recipients over long distances.

As in most series Cold ischaemic time was the most significant of factors analyzed associated with delayed graft function [5; 9; 14; 19; 27; 43; 44; 81; 93; 94]. Compared to other series however the CIT's were relatively short with no graft being subjected to a CIT of greater than 30 hours. Renal grafts harvested with livers were not subjected to longer CIT's than those kidneys harvested alone. This is significant if it is appreciated that the same small team of surgeons was responsible for both the harvest and transplantation of both organs. In practice, liver donors represent the best quality donor. As grafts from these donors are not subjected to longer CIT's than kidney donors alone, it is interesting that the incidence of delayed graft function between these two groups was not significantly different, P : 0.39.

Organ sharing between transplant units in South Africa based on best HLA match is very limited with occasional organ sharing between the two government transplant units in Cape Town. Transplant co-ordination is perhaps easier when kept "in house" as it were. This may account for both the relatively short cold ischaemic times in our series compared to other series as well as minimal delay when multiple organs required transplantation.

Figure 1 illustrates graphically the sharp increase in the incidence of delayed graft function with short incremental increases in cold ischaemic times of greater than 12 hours. This suggests a need for haste even at relatively short CIT's. One factor identified as being associated with prolonged periods of cold ischaemia was the distant donor, those not harvested in Groote Schuur Hospital experiencing significantly longer periods of cold ischaemia. However, as evidenced by the significant overlap in CIT's between local and distant donors, factors other than distance alone are involved in lengthening periods of cold ischaemia.

The current practice in our unit is to avoid performing renal transplantation after midnight. Transplantation of grafts harvested after hours is planned for early the following morning. In reality this does not always happen. With considerable competition for limited theatre facilities and the perception that grafts can be safely stored for extended periods, priority is often given to other urgent cases resulting in prolonged cold ischaemic times.

Heightened awareness of the problem amongst those involved in the implantation process as well as a prospective assessment of the causes of transplantation delay needs to be performed. Shortening CIT has potential to significantly decrease the incidence of DGF at minimal extra cost.

First transplants experienced significantly less delayed graft function than subsequent transplants suggesting that immunological factors may play an important role in its development. HLA mismatch and PRA status however were not significantly different between those who did and did not develop delayed graft function. Numerous authors have noted similar findings[19; 27; 28]. ***Figure 1*** demonstrates that 14% of grafts that developed delayed graft function were subjected to cold ischaemic times of less than 12 hours when preservation induced injury should be minimal. This observation may add support to an immunological role in the development of delayed graft function.

It has been suggested that ischaemic and immunological factors may work synergistically to effect the incidence of delayed graft function[28]. While there is currently no way of decreasing levels of sensitization, patients at increased immunological risk such as those receiving second and subsequent transplants should perhaps receive kidneys with minimal ischaemic risk. In our series we noted no difference in cold ischaemic times between those receiving first and subsequent grafts suggesting an area we need to address in the future.

In the multivariate analysis of factors influencing graft survival as well as by Kaplan Meier survival analysis a significant finding was the lack of association between delayed graft function and ultimate graft survival. This is contrary to current thinking. In this study the longest graft surveillance was 53 months with most grafts being followed for shorter periods. With the currently improved overall graft survival rates reported in recent years it is possible that longer periods of follow-up are required to identify the factors which may influence survival. This has been the experience from other units whose initial assessment of their results failed to identify an association between delayed graft function and survival. However, subsequent publications from these units with longer follow-up have now shown the detrimental effects of delayed graft function on graft survival[25; 77; 78]. It is also possible that the more subtle associations between factors such as delayed graft function and survival may not become statistically evident with the small numbers in this study.

The Cape Town transplant unit is the referral transplant centre for four rural dialysis centres situated between 400 and 1200 km from Cape Town. After discharge following transplantation local patients are followed up by nephrologists in Groote Schuur Hospital. Rural patients are discharged back to the care of the referring practitioner, these being either general physicians or general practitioners both with limited nephrological experience. Graft survival figures were not significantly different between patients followed up in our unit and those followed up in the rural area from where they were referred. In a significantly larger study with longer follow-up, Pontin et al reported the same finding. He concluded that transplantation of organs in rural dialysis patients represented an appropriate use of valuable organs and that a central transplant unit servicing several rural dialysis units optimized the use of highly trained personnel[95]. This study would support his views.

Graft number, HLA mismatch and PRA sensitization at any stage prior to transplantation all significantly influenced graft survival. These factors all support immune mediated chronic rejection as a cause for chronic graft failure. Most of these factors are beyond our control and attempts at modifying them to improve long-term results would be difficult. ***Figure 3*** graphically demonstrates the HLA mismatch status of patients receiving first and subsequent grafts to be very similar. One possible way of modifying these factors would be subjecting patients who are receiving subsequent grafts to tighter HLA matches with their grafts. However with limited organ sharing in South Africa this might prove difficult to implement.

In conclusion this study demonstrates that any prolongation of cold ischaemia even for short periods is associated with an increasing incidence of delayed graft function. It also supports the view that immune mediated injury is a significant cause of delayed graft function. Efforts to decrease the duration of cold ischaemia to less than 12 hours, particularly in patients who may be at increased immunological risk, seem likely to decrease this incidence of delayed graft function. The importance of Immunological factors as determinants of graft survival is also apparent. Patients at increased immunological risk by virtue of a previous transplant may benefit from a closer HLA match than is normally accepted in first time recipients.

Reference List

1. Hunsicker LG. A survival Advantage for Renal transplantation. *N Engl J Med* 2001;**341**:1762-1763.

2. Simmons RG, Abress L. Quality-of-life issues for end -stage renal disease patients. *Am J Kidney Dis* 1990;**15**:201-208.

3. Eggers P. Comparison of treatment costs between dialysis and transplantation. *Semin Nephrol* 2001;**12**:284-289.

4. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ellenger RE, Agodoa LYC, Held PJ, Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999;**341**:1725-1730.

5. Ojo AO, Wolfe RA, Held PJ, Port FK, Schmouder R. Delayed graft function: risk factors and implications for renal allograft survival. *Transplantation* 1997;**63**:968-974.

6. Perez Fontan MP, Rodriguez-Carmona A, Gaarcia-Falcon T, Moncalian J, Oliver J, Valdes F. Renal Transplantation in patients undergoing chronic peritoneal dialysis. *Perit Dial Int* 1996;**16**:48-51.

7. Sanfilippo F, Vaughn WK, Spees EK, Lucas BA. The effects of delayed graft function on renal transplantation. *Transplant Proc* 1985;**17**:13

8. Scandling JD, Izzo JLJ, Ricatta JJ, Paprocki S. Influence of cadaveric organ source on delayed renal allograft function. *Transplantation* 1989;**47**:245-249.

9. Troppmann C, Gillingham KJ, Benedetti E, Almond PS, Gruessner RW, Najarian JS, Mattas AJ. Delayed graft function, acute rejection, and outcome after cadaver renal transplantation. *Transplantation* 1995;**59**:962-968.

10. Almond PS, Troppmann C, Escobar F, Frey DJ, Matas AJ. Economic impact of delayed graft function. *Transplant Proc* 1991;**23**:1304

11. Nicholson ML, Wheatley TS, Horsburgh T, Edwards CM, Veitch PS, Bell PR. The relative influence of delayed graft function and acute rejection on renal transplantation survival. *Transpl Int* 1996;**9**:415-419.

12. Pfaff WW, Howard RJ, Patton PR, Adams VR, Rosen CB, Reed AI. Delayed graft function after renal transplantation. *Transplantation* 1998;**65**:219-223.

13. Halloran P, Aprile M, Farewell V, Ludwin D, Smith EK, Tsai SY, Bear RA, Cole EH, Fenton SS, Cattran DC. Early function as the principal correlate of graft survival. A multivariate analysis of 200 cadaveric renal transplants treated with a protocol incorporating antilymphocyte globulin and cyclosporine. *Transplantation* 1998;**46**:223-228.

14. Pirsch J, Ploeg R, Gange S, D'Alesandro AM, Knechtle SJ, Sollinger HW, Kalayoglu M, Belzer FO. Determinants of graft survival after renal transplantation. *Transplantation* 1996;**61**:1581-1586.

15. Humar A, Johnson EM, Payne WD, Wrenshall L, Sutherland DE, Najarian JS, Gillingham KJ, Matas AJ. Effects of initial slow graft function on renal allograft rejection and survival. *Clin Transplant* 1997;**11**:623-327.

16. McDonald JC, Landreneau MD, Hargroder DE, Venable DD, Rohr MS. External ureteroneocystostomy and ureteroureterostomy in renal transplantation. *Ann.Surg* 1987;**205**:428

17. Morris PJ. *Kidney Transplantation. Principles and Practice*. London: W.B.Saunders Company, 1994; 86-108.

18. Lehtonen SR, Isoniemi HM, Salmela KT, Taskinen EI, Willebrand EO, Ahonen JP. Long term graft outcome is not necessarily affected by delayed onset of graft function and early acute rejection. *Transplantation* 1997;**64**:103-107.

19. Koning OHJ, Ploeg RJ, Hajo van Bockel J, et al. Risk factors for delayed graft function in cadaveric kidney transplantation. *Transplantation* 1997;**63**:1620-1628.

20. Land W. Delayed graft function and renal allograft outcome. *Transplantation* 1999;**68** :452-453.

21. Shoskes DA, Halloran P. Delayed graft function in renal transplantation: Etiology, management and long-term significance. *J Urol* 1996;**155**:1831-1840.

22. Mertes PM, el Abassi K, Joboin Y, Burtin P, Pinelli G, Carteaux JP, Burlet C, Boulonge M, Villemot JP. Changes in hemodynamic and metabolic parameters following induced brain death in the pig. *Transplantation* 1994;**58**:414-418.

23. Rabanal JM, Teja JL, Quesada A, Cotorruelo J. Does diabetes insipidus in brain dead organ donors protect against acute tubular necrosis in renal grafts? *Transplant Proc* 1993;**25**:3143

24. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation* 1988;**45**:673-676.

25. Brophy D, Najarian JS, Kjellstrand CM. Acute tubular necrosis after renal transplantation. *Transplantation* 1980;**29**:245-248.

26. Lee CM, Carter JT, Alfrey EJ, Ascher NL, Roberts JP, Freise CE. Prolonged Cold Ischaemia time Obviates the Benifits of 0 HLA Mismatches in Renal Transplantation. *Arch Surg* 2000;**135**:1016-1020.

27. McLaren AJ, Jassem W, Gray DWR, Fuggle SV, Welsh KI, Morris PJ. Delayed graft function: risk factors and the relative effects of early graft function and acute rejection on long-term survival in cadaveric renal transplantation. *Clin Transplantation* 2001;**13**:266-272.

28. Shoskes DA, Churchill BM, McLorie GA, Khoury A. The impact of ischaemic and immunologic factors on early graft function in paediatric renal transplantation. *Transplantation* 1990;**50**:877-881.

29. Jassem W, Roake J. The molecular and cellular basis of reperfusion injury following organ transplantation. *Transpl Rev* 1998;**12**:14

30. Lehr HA, Guhlmann A, Nolte D, Keppler D, Messmer K. Leukotrienes as mediators in ischaemia-reperfusion injury in a microcirculation model in the hamster. *Clin Invest* 1991;**87**:2036-2041.

31. McCord JM. Oxygen-derived free radicals in post-ischaemic tissue injury. *N Engl J Med* 1985;**312**:159-163.

32. Delmonico FL, Marshall VC, Jablonski P, Scott D, Howden B, Morris PJ. *Oxford Textbook of Surgery*. New York, Oxford, Tokyo: Oxford University Press, 1994;642-679

33. Chuluyan HE, Schall TS, Yoshimura T, Issekutz AC. IL-1 activation of endothelium supports VLA-4 (CD49d/CD29)- mediated monocyte transendothelial migration to C5a, MIP-1a, RANTES and PAF but inhibits migration to MCP-1: a regulatory role for endothelium derived MCP-1. *J Leucoc Biol* 1995;**58**:71-79.

34. Basar H, Soran A, Shapiro R, Jordan ML, Grilsh HA, McCauley J, Rondhawa P, Irish W, Hakala TR, Fung JJ. Renal transplantation in recipients over the age of 60. *Transplantation* 1999;**67**:1191-1193.

35. Carter JT, Lee CM, Weinstein RJ, Lu AD, Dafoe DC, Alfrey EJ. Evaluation of the older cadaveric kidney donor: the impact of donor hypertension and creatinine clearance on graft performance and survival. *Transplantation* 2000;**70**:765-771.

36. Karpinski J, Lajoie G, Cattran D,Fenton S, Zaltzman J, Cardella C, Cole E. Outcome of kidneys from high risk donors is determined by both structure and function. *Transplantation* 1999;**67**:1162-1167.

37. Goes N, Urmson J, Ramassar V, Halloran PF. Ischaemic acute tubular necrosis induces an extensive local cytokine response: evidence for induction of interferon-gamma, transforming growth factor-beta 1, granulocyte-macrophage colony-stimulating factor, interleukin-2, and interleukin-10. *Transplantation* 1995;**59**:565-572.

38. Ploeg RJ, Van Bockel JH, Langedijk PHT, Groenewegen M, van der Woude FJ, Persijn GG, Thorogood J, Hermens J. Effect of preservation solution on results of cadaveric kidney transplantation – The European multicentre study group. *Lancet* 1992;**340**:129-137.

39. Wijnen RMH, Booster MH, Strubenitsky BM, de Boer J, Heineman E, Kootstra G. Outcome of transplantation of non-heart-beating donor kidneys. *Lancet* 1995;**345**:1067-1070.

40. Strong RW. Renal grafts from non-heart-beating donors. *Lancet* 1995;**245**:1064-1065.

41. Kootstra G, Kievit JK, Heineman E. The non heart-beating donor. *British Medical Bulletin* 1997;**53**:844-853.

42. Gonzalez-Segura C, Castelao AM, Torras J, Moreso F, Riera L, Lopez-Costea MA, Pascual M, Grinyo JM, Alsina J. A good alternative to reduce the kidney shortage. *Transplantation* 1998;**65**:1465-1470.

43. Halloran PF, Aprile M. Factors influencing early renal function in cadaver kidney transplants.A case-control study. *Transplantation* 1988;**45**:122-127.

44. Merkus JW, Hoitsma AJ, Koene RA. Detrimental effect of acute renal failure on the survival of renal allografts: influence of total ischaemia time and anastamosis time. *Nephrol.Dialysis Transplant.* 1991;**6**:881-886.

45. Florack G, Sutherland DER, Ascherl R, Heil J, Erhardt W, Najarian JS. Definition of normothermic ischaemia limits for kidney and pancreas grafts. *J.Surg.Res.* 1986;**40**:550-63.

46. Halloran PF, Shoskes DA. *Early transplant nonfunction - influence on ultimate graft survival and function.* New York: Marcel Dekkar, Inc., 1991;387

47. Ploeg RJ, Goossens D, McAnulty JF, Southard JH, Belzer FO. Successful 72 hour cold storage of dog kidneys with UW solution. *Transplantation* 1988;**46**:191-196.

48. Hefty T, Fraser S, Nelson K, Barry J, Bennett W. Efficacy of UW vs Euro-Collins solution in paired cadaveric kidneys: a prospective study. *Transplant Proc* 1991;**23**:2370-2371.

49. Belzer F, Southard JH. The future of kidney preservation. *Transplantation* 1980;**30**:161

50. Marshall VC. Renal preservation prior to transplantation. *Transplantation* 1980;**30**:165

51. Saks SA, Petritsch PH, Kaufman JJ. Canine kidney preservation using a new perfusate. *Lancet* 1973;**1**:10241

52. Spees EK, Vaughn WK, Mendez-Picon G, Humphries A. Preservation methods do not affect cadaver renal allograft outcome: the SEOPF prospective study 1977-82. *Transplant Proc* 1984;**16**:177

53. Vaughn WK, Mendez-Picòn G, Humphries A, Spees EK. Method of preservation is not a determinant of graft outcome in kidneys transplanted by Southern Organ Procurement Foundation Institutions. *Transplantation* 1981;**32**:490

54. Halloran P, Aprile M. A randomised prospective trial of cold storage versus pulsatile machine-perfusion for cadaver kidney preservation. *Transplantation* 1987;**43**:827-832.

55. Merion RM, Oh HK, Port FK, Toledo-Pereyra LH, Turcotte JG. A prospective controlled trial of cold-storage versus machine-perfusion preservation in cadaveric renal transplantation. *Transplantation* 1990;**50**:230-233.

56. Polyak MMR, Arrington BO, Stubenbord WT, Boykin J, Brown T, Jean-Jacques MA, Estevez J, Kapur S, Kinkhabala M. The Influence of pulsatile preservation on renal transplantation in the 1990's. *Transplantation* 2000;**69**:249-258.

57. Tesi RJ, Elkhammas EA, Henry ML, Ferguson RM. Pulsatile kidney perfusion for preservation and evaluation: use of high risk kidney donors to expand the kidney pool. *Transplant Proc* 1993;**25**:3099-3100.

58. Weimer W, Geerlings W, Bijnen AB, Obertop H, Van Urk H, Lameijer LD, Wolff ED, Jeckel J. A controlled study on the effects of mannitol on immediate renal function after cadaver donor kidney transplantation. *Transplantation* 1983;**35**:99-101.

59. Jarnberg PO. Acute effects of furosemide and mannitol on central haemodynamics in the early postoperative period. *Acta Anaesthesiol Scand* 1978;**22**:184-193.

60. Neumeyer HH, Kunzendorf U, Schreiber M. Protective effects of calcium antagonists in human renal transplantation. *Kidney Int., Suppl* 1992;**36**:S87

61. Ferguson CJ, Hillis AN, Williams JD, Griffin PJ, Salaman JR. Calcium-channel blockers and other factors influencing delayed function in renal allografts. *Nephrol.Dialysis Transplant.* 1990;**5**:816-820.

62. Green CJ, Gower JD, Healing G, Cotterill LA, Fuller BJ, Simpkins S. The importance of iron, calcium and free radicals in reperfusion injury: an overview of studies in ischaemic rabbit kidneys. *Free Radical Res.Commun.* 1989;**7**:255-264.

63. Stanley JJ, Goldblum JR, Frank TS, Zelenock GB, D'Alecy LG. Attenuation of renal perfusion injury in rats by the 21-aminosteroid U74006F. *J.Vasc.Surg* 1993;**17**:685-689.

64. Sakagami K, Takasu S, Kawamura T. A comparison of UW and EC solutions for simple cold storage in non-heart- beating cadaveric kidney transplantation. *Transplantation* 1990;**49**:824-826.

65. Williams GM, White HJ, Hume DM. Factors influencing long term functional success rate of human renal allografts. *Transplantation* 1967;**suppl 5**:837-843.

66. Whittaker JR, Veith FJ, Soberman R, Lalezari P, Tellis I, Freed SZ, Glieman ML. The fate of the renal transplant with delayed function. *Surg, Gynec.& Obst.* 1973;**136**:919-922.

67. Marcen R, Orifino L, Pascual J, de la Cal MA, Teruel JL, Villafruela JJ, Rivera ME, Mampaso F, Burgos FJ, Ortuno J. Delayed graft function does not reduce the survival of renal transplant allografts. *Transplantation* 1998;**66**:461-466.

68. Troppmann C, Gruessner A.C, Gillingham K.J, Sutherland D.E.R, Matas A.J, Gruessner R. Impact of Delayed Function on Long-Term graft Survival After Solid Organ Transplantation. *Transplant Proc* 1999;**31**:1290-1292.

69. White SA, Jain S, Absalom H, Murphy G, Williams ST, Knight A, Zickerman AM, Nicholson ML. Influence of Delayed Graft Function in Renal Transplants From cadaveric or Non-Heart-Beating Donors. *Transplant Proc* 2000;**32**:189

70. Belitsky P, MacDonald AS, Gajewski J, Boudreau J, Bitter-Suermann H, Cohen A. Significance of delayed function in cyclosporine-treated cadaver kidney transplants. *Transplant Proc* 1987;**19**:2096-2099.

71. Paramasivan ON, Griffin PJ, Ross WB, Sulaman JR. The effect of acute tubular necrosis on cyclosporine-treated kidney transplants. *Transplant Proc* 1987;**19**:2085

72. Rocher LL, Landis C, Dafoe DC, Keyserling C, Swatz RD, Campbell DAJr. The long term deliterious effects of delayed graft function in cyclosporin treated renal allograft recipients. *Transplant Proc* 1987;**19**:2093-2095.

73. Sumrani NB, Miles AM, Daskalakis P,Hong JH, Markell MS, Friedman EA, Sommer BG. Intermediate-term outcome of renal transplants in the cyclosporine era. *ASAIO J* 1993;**39**:47-50.

74. Churchill BM, McLorie GA, Williot P, Merguerian PA. Influence of early function on long-term paediatric cadaveric renal allograft survival. *J.Urol.* 1990;**143**:326-329.

75. McMullin ND, Koffman CG, Rigdon SP, Haycock G, Chantler C, Bewick M. The negative influence of delayed renal allograft function on longer- term graft survival in the paediatric population. *J.Pediatr.Surg.* 1994;**29**:66-70.

76. The Canadian Multicentre Transplant Study group. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. Analysis at three years. *N Engl J Med* 1986;**314**:1219

77. Heil J, Sutherland ER, Fryd DS, Ascher NL, Simmons RL, Najarian JS. Acute tubular necrosis of cadaver renal allografts does not corelate with organ sharing or preservation time but portends a poorer prognosis for long-term graft survival. *Transplant Proc* 1984;**16**:270

78. Kjellstrand CM, Casali RE, Simmons RE, Shideman JR, Buselmeier TJ, Najarian JS. Etiology and prognosis in acute post-transplant renal failure. *Amer.J.Med.* 1976;**61**:190-199.

79. Cacciarelli T, Sumrani N, Delaney V, Hong JH, DiBenedetto A, Sommer BG. The influence of delayed renal allograft function on long-term outcome in the cyclosporine era. *Clin.Nephrol.* 1993;**39**:335-339.

80. Rocher LL, Landis C, Dafoe DC, Swatz RD, Keyserling C, Campbell DAJr. The importance of prolonged post transplant dialysis requirement in cyclosporine-treated renal allograft recipients. *Clin.Transplant.* 1987;**1**:29

81. Yokoyama I, Uchida K, Kobayashi T, Tominanga Y, Orihara A, Takagi H. The effect of prolonged delayed graft function on long term graft outcome in cadaveric kidney transplantation. *Clin.Transplant.* 1994;**8**:101-106.

82. Najarian JS, Gillingham KJ, Sutherland DER, Reinsmoen NL, Payne WD, Matas AJ. The impact of the quality of initial graft function on cadaver kidney transplants. *Transplantation* 1994;**57**:812-816.

83. Shoskes DA, Cecka M. Deleterious effects of delayed graft function in cadaveric renal transplant recipients independent of acute rejection. *Transplantation* 1998;**66** :1697-1701.

84. Halloran PF, Schlaut J, Solez K, Srinivasa NS. The significance of the anti-class 1 response. Clinical and pathological features of renal transplants with anti-class 1-like antibody. *Transplantation* 1992;**53**:550-555.

85. Ferguson R. Acute rejection episodes - best predictor of long-term primary cadaveric renal transplant survival. *Clin.Transplant.* 1994;**8**:328-331.

86. Howard RJ, Pfaff W, Brunson ME, Ramos EL, Peterson JC, Crokes BP, Scornik JC, Parris CJ, Fennell RS. Delayed graft function is associated with an increased incidence of occult rejection and results in poorer graft survival. *Transplant Proc* 1993;**25**:1304

87. Cosio FG, Pelletier RP, Falkenhain ME, Henry ML, Elkhammas EA, Davies EA, Bumgardner GL, Ferguson RM. Impact of acute rejection and early allograft function on renal allograft survival. *Transplantation* 1997;**63**:1611-1615.

88. Nicholson ML, Horsburgh T, Edwards C, Morgan JDT, Veitch PS, Bell PRF. Relative influence of delayed graft function and acute rejection in renal transplant survival. *Abstracts of the British Transplantation Society* 1994;41

89. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potential advesre response to renal ablation. *Amer.J.Physiol.* 1981;**241**:F85-93.

90. Novick AC, Gephardt G, Guz B, Steinmuller D, Tubbs RR. Long-term follow-up after partial removal of a solitary solitary kidney. *N Engl J Med* 1991;**325**:1058-1062.

91. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved Graft survival after Renal Transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000;**342**:605-612.

92. Statistics South Africa. Census 1996. http//education.yahoo.com/reference/factbook/sf/popula.html

93. Troppmann C, Gillingham KJ, Benedetti E, Gruessner RW, Dunn DL, Payne WD, Najarian JS, Matas AJ. Delayed graft function in the absence of acute rejection has no long term impact. *Transplantation* 1995;**61**:1331-1337.

94. Sanfilippo F, Vaughn WK, Spees EK, Lucas BA. The detrimental effects of delayed graft function in cadaver donor renal transplantation. *Transplantation* 1984;**38**:643-648.

95. Pontin AR, Pascoe MD, Botha JF, Tandon V, Kahn D. Does rural follow-up of renal allografts give impaired graft survival? *Transpl Int* 2000;**13[Suppl 1]**:S92-S94

Appendix A: Abstract presented to the Surgical Research Society of Southern Africa in July 2000, Cape Town.

DELAYED GRAFT FUNCTION IN RENAL TRANSPLANTATION – A STITCH IN TIME SAVES NINE?

Douie WJP, McCulloch M, Pontin A, Halkett J, Pascoe M, Kahn D.

Department of Renal Transplantation, Groote Schuur Hospital, Cape Town, RSA

Delayed graft function following renal allograft transplantation has an incidence of about 23%. It is associated with prolonged hospitalization, increased expense, emotional repercussions and possible detriment to long-term graft function. The aim of this study was to identify risk factors that may predispose to delayed graft function.

PATIENTS AND METHODS

Between September 1995 and May 1998, 50 patients from a total of 245 renal transplant recipients developed delayed graft function. Data collected retrospectively from the patient notes was compared to that of 100 control patients with immediate graft function. Data was analyzed using Epi Info 6 (CDC), comparing discrete variables with Chi squared test and continuous variables with Student t test.

RESULTS

Factors found to significantly influence the development of delayed graft function included Cold ischaemic time (p:0. 002) and a second or subsequent transplants (p:0. 02). Donor or recipient age or location, the use of cadaver donors, the preservation solution used, the occurrence of acute rejection episodes and the HLA mismatch and PRA status did not influence the occurrence of delayed graft function. The only identifiable factor resulting in prolonged CIT's was the location of the donor (local vs. distant donor, p: <0.002). Patients receiving subsequent grafts did not preferentially receive grafts subjected to shorter cold ischaemic periods.

CONCLUSION

The cause of delayed graft function is multifactorial. Even short increases in the duration of cold ischaemic impacts negatively on immediate graft function. Patients receiving subsequent transplants should receive grafts with minimal ischaemic risk. The reasons for implantation delays need to be prospectively assessed.

Appendix B: Abstract presented to the Surgical Research Society of Southern Africa in July 2000, Cape Town.

WHAT ARE THE FACTORS THAT INFLUENCE RENAL ALLOGRAFT SURVIVAL?

Douie WJP, McCulloch M, Pontin A, Halkett J, Pascoe M, Kahn D.

Department of Renal Transplantation, Groote Schuur Hospital, Cape Town, RSA

The loss of a functional renal allograft has emotional, financial and quality of life implications on the patient and their family. The aim of this study was to look at factors responsible for renal allograft loss and in particular, explore the relationship between early dysfunction and long-term graft function.

PATIENTS AND METHODS

Of the 245 renal transplant recipients in our unit between September 1995 and May 1998, 150 recipients were analysed, 50 with delayed graft function and 100 with immediate function. Data relating to the acute and long-term outcome of the grafts was collected retrospectively. Multivariate analysis using Cox regression analysis of factors affecting graft survival was performed. Factors entered into the analysis included donor age, previous transplants, presence and duration of delayed graft function, HLA mismatch, Panel reactive antibody status, cold ischaemic time and presence of acute rejection. Cumulative survival was calculated according to the Kaplan Meier method.

RESULTS

Kaplan Meier cumulative proportional 5-year graft survival was 76%. 3 factors were identified as negatively influencing survival. Recipients of subsequent transplants (p:0.01), the greater the HLA mismatch (p:0.005) and a previously sensitized PRA status (p:0.04) impacted negatively on graft survival. Most patients had a non-sensitized PRA status (86%) and were receiving their first transplant (75%). Patients receiving subsequent transplants were not subjected to tighter HLA matching. Of note was that the presence and duration of delayed graft function, acute rejection episodes or the follow-up of recipients in peripheral centers did not adversely affect graft survival.

CONCLUSION

The factors identified as influencing graft survival are very difficult to modify. Recipients of subsequent grafts should however be subjected to tighter HLA matches than first time recipients. Delayed graft function did not impact negatively on graft survival in this study. Distant recipients can be adequately managed in the periphery after transplantation and represents an appropriate allocation of donor organs.

Appendix C: Data base record sheet.

DONOR DETAILS
Name
Folder Number
Age
Sex
Race
Cadaver/ Living
Cause of death
Organs harvested
Harvest Location

RECIPIENT DETAILS
Name
Folder Number
Age
Sex
Race
Recipient location
Transplant Number

TRANSPLANT DETAILS
HLA mismatch
Highest PRA
Latest PRA
Cold Ischaemic time
Transplant date

POST OPERATIVE COURSE
Serum Creatinine
D1
D2
D3
D4
D5
D6
D7
D8
D9
D10
DGF? Y/N
Duration (d)

POST OPERATIVE COURSE
Cyclosporine levels in first 10 days

Number > 300umol/l

Number < 150umol/l

POST OPERATIVE COURSE
Rejection episodes and outcome

Acute rejection ? Y/N

Chronic rejection Rx ? Y/N

Number of Rx

Serum Cr 1 yr
2 yr
3 yr
4 yr

POST OPERATIVE OUTCOME
Patient alive? Y/N
Date of death
Graft functional? Y/N
Lost graft survival (m)