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Outcome in kidney transplant recipients receiving kidneys from renal donors screened using 51CR-EDTA to determine glomerular filtration rate

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OUTCOME IN KIDNEY TRANSPLANT RECIPIENTS RECEIVING KIDNEYS FROM RENAL DONORS SCREENED USING ^{51}CR-EDTA TO DETERMINE GLOMERULAR FILTRATION RATE

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Nuclear Medicine

Johannesburg 2013

DECLARATION

I, Nico Malan, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Nuclear Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this university or any other university.

October 2013

DEDICATION

For all transplant patients, patients that show courage and let us not forget the donors.

ABSTRACT

Introduction

There is a global preponderance of renal disease and many of these conditions are associated with renal failure. A significant proportion of these patients will develop advanced kidney disease with ultimately end stage renal failure. Kidney transplantation is the most successful treatment of patients in end stage renal failure.

Currently, the majority of transplantations in the University of the Witwatersrand (WITS) Academic complex (which comprises Charlotte Maxeke Academic, Helen Joseph and Chris Hani Baragwanath Academic Hospitals) are performed with cadaveric donor kidneys. With the worldwide shortage of kidneys required for patients in renal failure, living kidney donation has increased the availability of donor kidneys.

Potential renal donors (PRD) are subjected to a battery of investigations prior to being considered for donation, amongst other, glomerular filtration rate (GFR). Potential renal donors at the WITS Academic Complex undergo GFR assessment using ^{51}Cr-ethylene-diamine-tetra-acetic acid (EDTA).

Some PRD, potentially those on a vegetarian diet, have a "low" GFR (≤80ml/min/1.73m^2) and do not meet criteria for renal donation. These potential renal donors are requested to follow a novel protocol, following a diet high in animal protein (beef and fish) for one week after which time the GFR is

determined again. Potential renal donors where the GFR increases (thus showing good renal reserve), subsequently donate a kidney.

We do not know the outcome of recipients receiving kidneys in which the GFR "normalized" following a protein load. To the best of our knowledge, the outcome of such recipients is also not described in the current available body of literature.

The aim is to determine the outcome in recipients receiving kidneys from protein loaded donors versus recipients of non-protein loaded ("normal" GFR) kidneys.

Methods

The study follows a retrospective record analysis of patient demographics and work-up results (cross-matching, GFR, infectious diseases, renal anatomy etc.) and outcomes in:

i. All potential renal donors (PRDs) from 1997 to July 2012 who had GFR determined by clearance of 51Cr-EDTA

ii. The subset of those who donated a kidney

iii. Donors with "low" (≤80ml/min/1.73m2) baseline GFR and subsequent protein-loaded GFR

iv. Donors with a "normal" (>80ml/min/1.73m2) GFR

v. The recipients receiving the grafts – outcome, complications, creatinine over time and survival

Results

In this study it was shown that ^{51}Cr-EDTA clearance correlates with calculated creatinine clearance (r^2=0.44) within the range of ^{51}Cr-EDTA clearance between 40 and 180ml/min (uncorrected for BSA). It was also shown that reproducibility of ^{51}Cr-EDTA clearance in 7 donor patients was excellent (r^2=0.86), suggesting a robust method. Two hundred and forty nine patients were screened using ^{51}Cr-EDTA clearance. Two hundred and twelve potential donors had good GFR and were not protein loaded. Of these potential renal donors, one hundred and twenty four were well matched and donated to recipients, with complete follow up in 85 cases. A total of 88 potential donors were excluded for various reasons. In this non-protein loaded group that donated kidneys, overall graft and recipient survival over 16 years was 64%.

Thirty seven potential renal donors were protein loaded since their initial GFR was ≤80ml/min/1.73m^2. Of these 37 potential donors, 15 were excluded. Twenty two potential donors donated kidneys in this group, with outcomes available in 13 recipients. Overall graft and recipient survival over 14 years was 58.3% for this group. This was not significantly different from the recipients receiving grafts from non-protein loaded donors (p=0.14; log rank test). For potential donors, upon protein loading, GFR increased significantly to 89.3±18.4 ml/min/1.73m^2 (p<0.0001), with a mean percentage increase of 26.0±24.4% (median 22.2%; range: -12.9% to +103.4%). Furthermore, when comparing only patient survival between recipients of grafts from donors that were protein loaded and donors that were not protein loaded, there is still no significant

difference between the two groups. The pre-protein loaded GFR correlated with the post-protein loaded GFR measurement. There was a negative correlation between pre-protein load (baseline) GFR and percentage increase in GFR following protein loading. In all donors, GFR declined from pre-transplant determination of 97.7±18.1ml/ min/1.73m^2 to 75.2±16.4ml/min/1.73m^2 ($p<0.005$).

Discussion and conclusion

^{51}Cr-EDTA is a reliable, reproducible technique to assess the GFR of potential renal donors. The novel protein loading technique developed in our institution is a convenient way to assess the functional renal reserve of potential renal donors that have a suboptimal estimated GFR of ≤80ml/min/1.73m^2. Although the patient numbers in this study are small, there is no significant difference between the outcome of recipients of renal grafts from non-protein loaded and protein loaded donors. The inclusion of potential donors that show a normal GFR post protein load may increase the pool of available kidney grafts and assist greatly in the treatment of many patients with end stage renal disease.

Further research in this area is encouraged, and multicenter studies may yield results that with larger number of patients that may ratify the results of this study.

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LIST OF ABBREVIATIONS

WITS	University of the Witwatersrand
PRD	Potential renal donors
EDTA	Ethylene-diamine-tetra-acetic acid
DTPA	Diethylene-triamine-penta-acetic acid
CKD	Chronic kidney disease
ESRD	End stage renal disease
LUD	Living unrelated donor
LRD	Living related donor
HLA	Human Leukocyte Antigen
PSA	Prostate Specific Antigen
HIV	Human Immunodeficiency Virus
HIVAN	Human Immunodeficiency Virus associated Nephropathy
CAPD	Continuous Ambulatory Peritoneal Dialysis
HTLV	Human T-lymphotropic Virus
HHV	Human Herpes Virus

HSV	Herpes Simplex Virus
G6PD	Glucose-6-phosphate dehydrogenase
PT	Prothrombin Time
APTT	Activated Partial Prothrombin Time
GFR	Glomerular filtration rate
C_{Cr}	Creatinine clearance
eGFR	estimated glomerular filtration rate
MDRD	Modification of Diet in Renal Disease
Kf	Filtration coefficient
P_G	Glomerular hydrostatic pressure
P_B	Hydrostatic pressure in Bowman's capsule
π_G	Colloid osmotic pressure of the capillary plasma proteins
π_B	Colloid osmotic pressure of the proteins in Bowman's capsule
IKGFR	Individual kidney glomerular filtration rate
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration Equation
FRR	Functional renal reserve

ERPF	Effective renal plasma flow
FDA	Food and Drug Administration

CHAPTER 1 INTRODUCTION

1.1 Renal failure and End Stage Renal Disease (ESRD) a South African perspective

Calculations suggest that the prevalence of chronic kidney disease (CKD) in African countries is in the range of 200 – 300 million of the general population.[1]

Causes for end stage renal failure (ESRD) include: hypertension, glomerulonephritis, Human Immunodeficiency Virus (HIV) associated kidney disease (HIVAN) and diabetic nephropathy.[2]

In sub-Saharan Africa mainly young adults aged 20 – 50 years are affected by chronic kidney disease and major causes for end stage renal disease include: hypertension and glomerular diseases. This is unlike in developed countries were middle-aged and elderly patients are affected, with the most common causes being diabetes mellitus and hypertension.[1]

Studies in South Africa show that about 25% of the adult population is affected by hypertension.[3,4] Hypertension is also the cause of chronic renal failure in approximately 21% of patients on the South African Registry that receive renal replacement therapy.[3] Although hypertension is the most common cause in Black South Africans, hypertension as cause of ESRD was only reported in 4.3% of Whites and 13.8% of Indians. [4]

Glomerular disease is more prevalent in Africa and seems to be more severe than in western countries.[2] There is also an associated poor response to treatment. Focal segmental glomerulosclerosis is commonest in South Africa.[2]

HIV infection is epidemic in sub-Saharan Africa. Although data on the prevalence of HIV-related glomerular disease in Africa is scarce, screening studies in South Africa reported proteinuria in 5.5 – 6%, with HIV associated nephropathy (HIVAN) on biopsy in 5 - 83%.[2]

Diabetes mellitus affects an estimated 9.4 million people in Africa and there is an expected increase of 140% by 2025.[2] The current estimated prevalence of diabetic nephropathy in South Africa is estimated to be 6 – 16%.[2]

1.2 Renal transplantation in South Africa

Treatment for chronic renal failure in sub-Saharan Africa includes dialysis (hemodialysis and continuous ambulatory peritoneal dialysis (CAPD)) and the most successful modality, renal transplantation. [2, 5]

Availability of continuous ambulatory peritoneal dialysis (CAPD) is limited in sub-Saharan Africa due to the high cost of dialysis fluid and the perception of a high rate of peritonitis. Futhermore, dialysis is primarily available in the private sector in Africa but in South Africa indigent patients can access chronic dialysis at government cost only if they are eligible for transplantation. Chronic dialysis is not sustainable in many countries in Sub-Saharan Africa since patients are unable to afford the costs beyond a few months. [2,5]

This highlights the need for renal transplantation, both living and cadaveric. Transplantation is currently hampered by donor shortages as well as costs, however the transplant rate in Africa averages 4 per million people and is 9.2 per million people in South Africa.[5]

There are 17 renal transplant centers in South Africa, scattered across the country (see table 1 below).[6]

Table 1 Summary of Transplant Centers in South Africa

CENTRE	PRIVATE	STATE
Bloemfontein	Netcare Universitas Hospital	Universitas Hospital
Cape Town	Netcare Christiaan Barnard Memorial Hospital Netcare UCT Private Academic Hospital	Groote Schuur Hospital Red Cross Memorial Children's Hospital Tygerberg hospital
Durban	Entabeni Life Hospital Netcare St Augustine's Hospital	Inkosi Albert Luthuli Hospital
Johannesburg	Netcare Milpark Hospital Netcare Garden City Clinic WITS Donald Gordon Medical Centre	Charlotte Maxeke Johannesburg Academic Hospital
Pretoria	Netcare Jacaranda Hospital	George Mukhari Hospital Steve Biko Pretoria Academic Hospital

The South African Transplant Society follows a protocol based on the report published by the Amsterdam Forum on the assessment of potential living kidney donors. This was endorsed at their meeting in May of 2011.[7]

CHAPTER 2 ASSESSMENT OF POTENTIAL RENAL DONORS

2.1 Living Donors

The survival of a kidney transplanted from a live donor exceeds the results achieved from that of a deceased donor.[11] Improved outcome in renal transplant grafts is largely due to improved therapy to help prevent rejection. [17]

The term 'living related' donor has implied some HLA identity with the recipient, but emotional bonds of marriage or friendship are just as valid in defining a donor as 'related' to the recipient. The outcome for the genetically and emotionally related donors are the same.[13]

The survival rate of a kidney transplant from a genetically unrelated donor is excellent, with a 10 year survival equivalent to a kidney transplant from a sibling HLA (Human Leukocyte Antigen) haploidentical to the recipient.[12]

2.2 Guidelines and consensus statements

During April 2004, kidney transplant physicians and surgeons met in Amsterdam, the Netherlands for the International Forum on the Care of the Live Kidney Donor. It was the objective of the forum to develop an international standard of care for the live kidney donor. The report of the Amsterdam Forum was derived from participants' international experience as well as from evidence based recommendations. It was highlighted that medical judgment of published data and physician experience influence the decision to

accept (or not) an individual for renal donation. The guidelines of the forum therefore is not a document of mandatory regulation.[14, 15]

The routine screening procedures for potential living kidney donors are summarized in Table 2, as published by the Forum.[14, 15]

Table 2	**Routine Screening For Potential Living Kidney Donors[14,15]**
Urinalysis	Dipstick for protein, blood and glucose Microscopy, culture and sensitivity Measurement of protein excretion rate
Assessment of renal function	Estimation / measurement of renal function
Blood Tests	**Haematological profile** Full blood count Hemoglobinopathy (where indicated) Coagulation screen (PT and APTT) G6PD deficiency (where indicated) **Biochemical profile** Creatinine, urea & electrolytes Liver functions Urate Fasting plasma glucose Bone profile Glucose tolerance test (if fasting plasma glucose > 6 - 7 mmol/l) Blood lipids Thyroid function tests (if indicated) Pregnancy test (if indicated) PSA (if indicated) **Virology and infection screen** Hepatitis B and C Toxoplasmosis Syphilis HIV and HTLV 1/2 Malaria (where indicated) Cytomegalovirus Trypanozome cruzi (where indicated) Epstein-Barr Virus Schistosomiasis (where indicated) HHV8 and HSV (where indicated) Strongyloides (where indicated) Typhoid (where indicated) Brucellosis (where indicated)
Cardiorespiratory system	Chest X-ray Stress test Echocardiography (where indicated)
Assessment of renal anatomy	Appropriate imaging investigations should allow confirmation of the presence of two kidneys of normal size and enable abnormalities of the collecting system and calcification or stone disease in the renal tract to be detected. They must also delineate the anatomy of the renal vasculature.

PSA, prostate-specific antigen; HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; HHV, human herpes virus; HSV, herpes simplex virus

2.3 Acceptable donor renal function

To minimize the risk to the donor, prenephrectomy evaluations focus on selecting individuals who are healthy and will therefore be at minimum risk for an elective surgical procedure. The individual should therefore demonstrate "normal" renal function as determined by assessment of GFR, so that loss of approximately one-half of the nephron mass can be tolerated. [16]

Normal GFR has been shown to be, on average, 10% less in women than in age matched male individuals. The definition of "normal" GFR also changes with age, as renal function deteriorates over time. Cardella et al noted a decrease of approximately 1ml/min/m^2 per year after age 40.[14]

All potential living renal donors should have renal function accurately assessed, as measured by GFR. There are various methods for GFR estimation. Creatinine clearance methods (these may over or under estimate renal function in patients with normal or near normal GFR), calculated GFR values (may over estimate GFR as they are not standardized in this population. These methods may be supplemented or replaced by isotopic clearance estimation of GFR by the use of ^{99m}Tc-Diethylene-triamine-penta-acetic acid (^{99m}Tc-DTPA) or ^{51}Cr-Ethylene-diamine-tetra-acetic acid (^{51}Cr-EDTA).

Acceptable GFR in a donor is described to provide adequate GFR for both donor and recipient after donor nephrectomy. There are reports in the literature that reveal that donors with GFR ≤ 80ml/min/1.73m^2 prior to nephrectomy cannot reliably be expected to provide or maintain optimal function after nephrectomy. Despite this, up to a fifth of transplant centers in the United States will still accept donors with a creatinine clearance as low as 60ml/min/1.73m^2.[15] In view of documented cases of successful transplantation of elderly donors with GFR between 65 – 70 ml/min/1.73m^2, there is clearly a need for individualization.[14,15]

The Amsterdam Forum defined acceptable renal function as a GFR >80ml/min/1.73m^2 and corrected for age and gender. It is accepted that a GFR ≤80ml/min/1.73m^2 (and corrected for age and gender) generally precludes donation.

In South Africa, the Transplant Society follows the Amsterdam Forum document and the practice is that in order to be able to donate a kidney, GFR must be >80ml/min/1.73m^2.

There is therefore a need to look at a group of potential renal donors with GFR between 60 – 80ml/min/1.73m^2 who may following a protein load increase their GFR to >80ml/min/1.73m^2 and thus increase the number of potential renal donors.

CHAPTER 3 BRIEF RENAL PHYSIOLOGY

The kidneys are vital organs and involved in a myriad of physiological processes, amongst others: excretion of metabolic waste products and foreign chemicals, water and electrolyte balance regulation, maintenance of body fluid osmolality, arterial pressure regulation, acid-base homeostasis, calcium homeostasis, hormone secretion, metabolism and excretion as well as gluconeogenesis.

3.1 Glomerular filtration

Glomerular filtration can be defined as the volume of plasma that is cleared of a certain substance (X), over a certain period of time. The formula to calculate GFR is:

$$\text{GFR} = Ux + \frac{V}{Px}$$

Where U_x = urine concentration of substance x

V = rate of urine flow in ml/min

P_x = plasma concentration of substance x

Glomerular filtration is considered the first step in the formation of urine. This process occurs in the Bowman's capsule in cortical regions of kidneys. Afferent arterioles enter the Bowman's capsule (which leads to the proximal tubules). Here they form capillary loops

prior to exiting the capsule as efferent arterioles. The glomerular capillary loops are covered with epithelial cells (podocytes) with spaces called slit pores. Between the capillary endothelium and the podocytes, the basement membrane is found. The basement membrane is a meshwork of collagen and proteoglycan fibrillae. Fluid must be filtered through fenestrations in the capillary endothelium, through the basement membrane and slit pores between the podocytes to eventually reach the Bowman's space that surrounds the capillary loops, thereafter the filtrated fluid is directed towards the proximal tubules.

A large amount of fluid is filtered through the capillaries as glomerular capillaries have a much higher rate of filtration due to a large capillary filtration coefficient and high hydrostatic pressure. Glomerular capillaries are relatively impermeable to large molecules, e.g. proteins and cellular elements. The constituents of the glomerular filtrate are fairly similar to that of plasma, save for a few low-molecular weight substances that are protein bound, such as calcium and fatty acids.

The fraction of the renal plasma flow that is filtered is approximate 0.2. It follows thus that 20 per cent of the plasma that is flowing through the kidney is filtered through the glomerular capillaries.

In the average adult human the glomerular filtration rate is about 125ml/min/1.73m^2, thus generating 180 liters of glomerular filtrate daily. Only approximately 1.5 liters of urine is produced daily, thus 99% of the filtrated fluid is reabsorbed.[8 -10]

3.1.1 Filtering of solutes and charged large molecules

The filterability of solutes is inversely related to their size. Electrolytes and small organic molecules (such as glucose) are filtered freely, however as the molecular weight of substances approach that of albumin, the filterability rapid decreases and approaches zero.

Negatively charged molecules are filtered less easily than positively charged molecules of equal molecular size. This is due to the negative charges of the basement membrane and the podocytes.[8-10]

3.1.2 Determinants of GFR

GFR is determined by the net filtration pressure and the glomerular capillary filtration coefficient (K_f), which is mathematically expressed as:

$$GFR = K_f \times \text{Net filtration pressure}$$

3.1.2.1 Net filtration pressure

The Net filtration pressure is the sum of hydrostatic and osmotic forces that act through the glomerular membrane.

$$\text{Net filtration pressure} = P_G - P_B - \pi_G + \pi_B$$

Where:

P_G = Glomerular hydrostatic pressure

P_B = Hydrostatic pressure in Bowman's capsule

π_G = Colloid osmotic pressure of the glomerular capillary plasma proteins

π_B = Colloid osmotic pressure of the proteins in Bowman's capsule

i. Glomerular hydrostatic pressure

Glomerular hydrostatic pressure is determined by: arterial pressure, afferent arteriolar resistance and efferent arteriolar resistance. Changes in the glomerular hydrostatic pressure serve as the main mechanism for physiological regulation of GFR. This pressure denoted the pressure that forces fluid across the capillary wall into the Bowman space. It has been estimated to be 60 mmHg under normal conditions.

ii. Hydrostatic pressure in Bowman's capsule

Directs measurements suggest that estimates for Bowman's capsule pressure in humans is about 18 mmHg under normal conditions. This force attempts to push fluid back into the glomerular capillary. Under certain pathological conditions e.g. renal stones there may be an obstruction and this will lead to an increase in the hydrostatic pressure in Bowman's capsule with a resultant reduction in GFR.

iii. Colloid osmotic pressure of the glomerular capillary plasma proteins

The plasma protein concentration increases as blood passes from the afferent arteriole to the efferent arteriole, due to the fact that proteins are not filtrated.

The normal colloid osmotic pressure rises from 28 mmHg to 36 mmHg. As a result the average glomerular capillary colloid osmotic pressure is about 32 mmHg. This denotes the oncotic force that is generated by plasma proteins that attracts fluid into the capillary by osmosis, opposing glomerular filtration.

iv. Colloid osmotic pressure of the proteins in Bowman's capsule

This force is essentially non-existent under normal conditions, as plasma proteins are not filtered in normal kidneys. Under certain pathological conditions, proteins may appear in the filtrate and then contribute to this osmotic force, promoting glomerular filtration.

3.1.2.2 Filtration coefficient (K_f)

The filtration coefficient cannot be measured directly. It is estimated experimentally by dividing the GFR by the net filtration rate.

The filtration coefficient is the product of permeability and surface of the filtration area.

i. Permeability of the glomerular capillaries

Capillary permeability is increased in inflammatory conditions such as glomerulonephritis and reduced in pathologies where there is thickening of the wall of the glomerular membrane, such as hypertensive and diabetic nephropathy.

ii. Size of the capillary bed (surface for filtration)

A reduction in the size of the capillary bed, will lead to a reduction in the surface available for filtration.[8-10]

CHAPTER 4 METHODS FOR ASSESSMENT OF RENAL FUNCTION

Glomerular filtration rate (GFR) is the best index available to assess kidney function in health and in disease in an individual, since the filtration capacity correlates with the various functions of the nephron. Predictable changes in other kidneys functions (erythropoiesis, calcium and phosphate metabolism) are associated with changes in the GFR. [19]

Normal values are in the range of 120 to 130 ml/min/1.73m^2. Although literature reports that there is a decline of 0.8 – 1 ml/min/1.73m^2 per year in individuals after 40 years of age, the Baltimore Longitudinal study on aging showed that approximately 33% of the patients that were followed, did not show a decrease in GFR with age.[19]

Glomerular filtration rate cannot be measured directly in an individual, it is derived using either exogenous markers (e.g. inulin, DTPA or endogenous markers (e.g. urea, creatinine, cystatin C) in their steady state.[20] The marker that is used for measuring GFR should neither be secreted not reabsorbed by renal tubules. The characteristics for an ideal glomerular filtration agent are listed in Table 3.[21]

The concept of renal clearance of a particular substance is expressed as the volume of plasma that can be completely cleared of that substance in unit time.

Currently there is no ideal agent for the estimation of glomerular filtration rate that can be used clinically in an efficient manner, that is also cost effective. The characteristics of an ideal agent can however be used to compare various agents.

Table 3 Characteristics of an ideal Glomerular Filtration Marker[21]

The marker should be cleared at a constant rate (exogenously or endogenously)
Be freely filtered at the glomerulus
It must not be secreted or reabsorbed by the tubules
No extra-renal elimination
Convenient, safe, available and inexpensive
Readily diffusible in the extracellular space
Not protein bound
No interference from other compounds

Methods used for estimation of GFR, vary from region to region. Clearance of ^{51}Cr-EDTA is commonly used in Europe as well as in our institution. In the United States, use of creatinine clearance and serum creatinine concentration is common as ^{51}Cr-EDTA is not Food and Drug Administration (FDA) approved in the United States.[22] Table 4 lists the available methods of determining GFR.[21]

Table 4	Methods for determining GFR[21]
Exogenous markers	Inulin
	Iohexal
	^{51}Cr-EDTA
	^{125}I-iothalamate
	^{99m}Tc-DTPA
Endogenous markers	Serum creatinine
	Measured urinary clearance of creatinine
	Serum creatinine-based estimation equations: CG-Formula MDRD Formula
	Serum cystatin C

4.1 Exogenous markers

4.1.1 Inulin

Inulin is a fructose polymer that is found in Jerusalem artichoke and chicory. It has an average molecular weight of 5200 Daltons. Inulin exhibits characteristics of an ideal tracer since it is freely filtered and not secreted nor reabsorbed by the renal tubules and without any extra-renal elimination. Due to these characteristics, inulin is considered the gold standard for the estimation of GFR.

Inulin is however an exogenous compound and must be administered as a continuous infusion in order to achieve a steady state concentration in the blood. Its use in clinical practice is precluded by the fact that it is a laborious process, but also has high cost and limited availability.[23]

4.1.2 Contrast media

Contrast media infusion (iohexol and iothalmate) has been used to determine GFR. It is a cumbersome technique, which makes it unsuitable for daily clinical use, but contrast urography and GFR can be done at a single examination.[24]

4.1.3 ^{99m}Tc-DTPA

^{99m}Tc-DTPA is solely excreted by glomerular filtration. Ninety per cent of the injected dose is excreted by 24 hours. Only a small percentage (5% – 10%) is bound to plasma proteins at 1 hour. ^{99m}Tc-DTPA has a rapid clearance with a half-time of 70 minutes.[25]

Mulligan et al. examined several methods of measuring GFR with ^{99m}Tc-DTPA.[25,26] GFR can be measured from the activity of a single injection, and subsequent single or multiple blood samples, from the accumulation of the activity in the bladder or from counts solely obtained from the gamma camera assessing the clearance of the tracer from the blood. Mulligan et al. found that the dual plasma sample technique developed by Russell and the urinary sample technique developed by Jackson were the most accurate methods over a large range of renal function.[25, 26]

The Russell method involves obtaining blood samples at either 30 and 180 minutes, or at 60 and 180 minutes. This data is then applied to the dual compartment model of Sapirstein.[25]

In the Jackson urinary method, the GFR is calculated from the terminal slope of a plasma disappearance curve. The total urinary activity and the GFR is corrected for unexcreted residual bladder activity. This method was found to be as accurate as the two-sample plasma method, it is however inconvenient and prone to collection errors.[25]

Gamma camera techniques for measuring GFR have been developed. Most of these techniques do not require urine samples, and although most do not require plasma samples, several do use a single plasma sample. Global and differential GFR measurements can be obtained after a single bolus injection of ^{99m}Tc-DTPA, and apply the data of the renogram to the following equation to obtain the individual kidney glomerular filtration rate (IKGFR):[25]

$$\frac{dR(t)}{dt} = \alpha \cdot P(t)$$

where dR is the rate of renal uptake

P is the plasma concentration

α is the constant of proportionality and represents IKGFR

The IKGFR is constant for up to 2.5 minutes following tracer injection, once correction for background has been made. There are many variations on the equations, notably reported by Rutland and Rehling.[25, 27]

Background correction is a main source of error for this technique. Various methods have been used in an attempt to approximate background activity correctly. These include: peri-renal, subrenal, suprarenal and heart activity regions. Piepsz et al proposed a method of double background correction that combines the area ratio method and the linear fit method.[25]

4.1.4 ^{51}Cr-EDTA

Glomerular filtration rate is a commonly accepted standard measure of renal function. The generally accepted gold standard technique for GFR assessment uses inulin infusion.[23] This technique is time consuming and difficult to perform and is considered inappropriate for routine clinical use. Moreover, methods that need collection of urine are prone to errors due to urinary losses and incomplete urinary bladder emptying.[23]

Plasma clearance of radiopharmaceuticals has emerged as an accepted standard routine method of assessing glomerular filtration rate.[69, 70] Among the various agents solely cleared by glomerular filtration, ^{51}Cr-EDTA is probably the most reliable.[70] Garnet et al. described ^{51}Cr-EDTA as an alternative method to measure GFR in 1967.[61] In a study by Medeiros et al. in 2008 involving 44 kidney recipients and 22 kidney donors there was good correlation

between GFR measurement with Inulin and ^{51}Cr-EDTA (R = 0.94) in this population of patients.[62]

Total plasma clearance is determined from the injected amount of the tracer divided by the total area under the curve. This requires taking multiple samples over several hours. GFR is thus calculated using the fundamental definition:[63]

$$GFR = Q / \int_0^\infty P(t)dt$$

where $\int_0^\infty P(t)dt$ is the area under the plasma concentration curve (AUC) from time zero to infinity.

This is however a time consuming method, but it may be useful for research studies or when assumptions involved in estimating a correction for the AUC when only the terminal exponential is sampled, is to be avoided.[63]

EDTA clearance results in a bi-exponential plasma concentration curve as shown Sapirstein et al.[63] Most of the area under the curve is under the second exponential. The slope intercept technique attempts an approximation by measuring the area under the second exponential. The first exponential is thus neglected. This has the advantage that the number of samples required is considerably reduced.[69] The slope intercept method will always overestimate GFR, as the area under the curve will be underestimated by neglecting the first exponential.[64, 69]

'Slope – intercept' method[28, 29]:

$$Cl1 = \frac{D \text{ x } \ln\left(\frac{P1}{P2}\right)}{T2 - T1} \exp \frac{(T1lnP2) - (T2lnP1)}{T2 - T1}$$

Where: D = administered activity in counts per minute

P1 = activity at T1 in counts/min/ml

P2 = activity at T2 in counts/min/ml

*Cl*1 = preliminary estimate of GFR and should be corrected for body surface

Empirical methods have been derived for correcting the error, namely the linear or quadratic equations.[69]

Chantler[64] has introduced the linear correction factor for adults:

$Cl_1 = 0.093 \text{ x } Cl_2$

where

Cl_1 = the clearance corrected for the first exponential

Cl_2 = the non-corrected clearance

The Chantler method is adequate for normal and high clearance levels but underestimates in low clearance values.[28, 29]

Brochner-Mortensen introduced the quadratic correction for adults:[65, 66]

$Cl_1 = 0.99 \times Cl_2 - 0.0012 \times Cl^2_2$

where

Cl_1 = the clearance corrected for the first exponential

Cl_2 = the non-corrected clearance

The effect of neglecting the first exponential clearance is negligible when the clearance is very low and it increases non-linearly for higher clearance. The choice of a quadratic correction factor allows a higher correction for higher clearance but the parabolic shape of a quadratic function could lead to inconsistency at very high values of clearance.[67]

Chantler's global linear correction factor uses a mean correction factor for a wide range of clearance values. It is obvious that the two correction factors will give different results. The differences between the two methods are small for normal or reduced clearance values, but become quite large for clearances greater than 140ml/min. Both Chantler and Brochner-Mortensen clearances were found to give lower results than by means of multiple sample techniques as shown by De Sadeleer et al.[67]

It is commonly viewed that four samples are necessary to ensure quality control in calculation of the slope, since an outlying sample can be ignored in calculations.[69] It has however been shown that differences in precision achieved when using four and two samples is very small.[71] Criticism of the two sample technique relates to the lack of quality

control on the measurement of the slope in that a straight line can always be drawn between two points, so there is no measure of goodness-of-fit.[69] An alternative approach to quality control is to estimate the volume of distribution, which can be calculated from the slope intercept technique. This value can then be checked for consistency with body surface area or body weight.

The methodology of using ^{51}Cr-EDTA clearance underwent even further simplification by reducing the number of samples to one by Fisher et al.[68] and Constable et al.[72] The method relies on the experimental determination of an equation of regression between the multiple sample clearance and a "volume of distribution" calculated from the activity of a single blood sample.[72] Fischer et al. concluded that the single sample technique provides an estimate of the GFR whose accuracy is comparable with that of endogenous creatinine clearance but without 24 hour urine collection and which is rapid.[68] Picciotto et al. performed a comparative assessment of simplified techniques using ^{51}Cr-EDTA plasma clearance in 1991 and reported that for low clearances (between 10 and 30ml/min) the single sample techniques failed to give reliable results. However in patients with clearance greater than 30ml/min, the single sample techniques provide a good alternative to the multi-sample techniques.[70]

GFR is calculated using the formula of Constable and is corrected to a body surface area (BSA) of 1.73m^2:

$$GFR = \sqrt{V - 6.3} - 67$$

(where V is the apparent volume (L) of distribution at sample time 240 minutes)

4.2 Endogenous markers

4.2.1 Serum urea nitrogen

Urea was first isolated from human urine in 1773 by Rouelle and first used as a clinical diagnostic test of renal function in 1903. Urea is a poor marker of renal activity owing to its limitations. Its rate of production is not constant and production is determined by protein intake.[31, 32]

Futhermore there are a myriad of factors that may lead to an increase in urea, amongst others, liver cirrhosis, congestive heart failure, corticosteroids, tetracyclines and others.[31,32]

Urea is also reabsorbed in renal tubules and furthermore highly diffusible, with resultant increased reabsorption in hypovolaemic conditions and pre-renal conditions. This accounts for the disproportionate increase in pre-renal states.[31,32]

Urea must be interpreted along side serum creatinine to be meaningful, and tend to underestimate GFR.

4.2.2 Serum creatinine

Creatinine is a metabolite of creatinine phosphate, which is found in skeletal muscles. Although it is produced at a constant rate in men (~ 15 – 25mg/kg body weight) and women (~ 10 – 20mg/kg body weight), it is proportional to muscle mass and dietary meat intake.[33]

Creatinine is freely filtered, but undergoes variable tubular secretion. Various factors influences creatinine, listed in Table 5.[34]

Table 5 Factors altering serum creatinine[34]

Overproduction	Rhabdomyolisis Vigorous sustained exercise Anabolic steroids Dietary supplements (creatine)
Blocked tubular secretion	Trimethoprim Cimetidine Asprin
Assay interference	Cephalosporins Flucytosine Ketosis Methyldopa Levodopa Ascorbic acid
Decreased production	Decreased muscle mass Cirrhosis

It is well described that with advanced renal failure (serum creatinine >6mg/dl), there is intestinal bacterial overgrowth and increased bacterial creatinase activity, with resultant extra-renal creatinine clearance and lower than expected serum creatinine concentration.

Conversely in early renal disease, there is only a minimal increase of 0.1 to 0.2 mg/dl as a result of increased proximal secretion of creatinine. However, with progress in renal dysfunction, the proximal tubular secretion process becomes saturated, and only then is a rise in serum creatinine detected.

The practical implication is that normal serum creatinine values do not necessarily imply normal GFR or stable disease and that there may already be a significant loss of renal function, before increased GFR values are recorded.[34]

4.2.3 Creatinine clearance

Creatinine clearance (Ccr) is a commonly used tool to estimate GFR in clinical practice and requires the use of 24 hours urine collection. This is quite cumbersome and frequently inaccurate volumes are collected.

The patient should be instructed to void the bladder in order to start collection with an empty bladder. The time should be noted and all urine produced over the next 24 hours should be collected. The blood sample of serum-creatinine is taken during the urine collection period.[32] It has been shown that the use of 24 hours urine collection for estimation of GFR is more reliable than serum-creatinine based equations.[34]

Tubular secretion increases in progressive renal failure as the GFR falls and can increase by over 50%, which may lead to a gross over estimation of GFR. By giving a single dose of cimetidine (which inhibits tubular secretion), a more accurate estimate of GFR may be obtained.[35]

4.2.4 Formulae equations

Several formulae were developed in an effort to provide an accurate estimation of GFR based on plasma creatinine. The two most widely used formulae are the Cockcroft and Gault (CG) formula and the Modification of Diet in Renal Disease (MDRD) formula.

The Cockcroft and Gault (CG) formula can only be used in patients with stable renal function. This formula takes into account the patient's age, weight, gender and plasma creatinine.[38] The following equation describes the formula[38]:

$$Ccr = \frac{[140 - age] \text{ x } [weight]}{PCr \text{ x } [gender\ factor]}$$

where: Ccr is creatinine clearance (ml/min)

PCr is plasma creatinine in µg/dl

age in years

weight in kilogram

gender factor: 72 for males; 85 for females

The Cockcroft and Gault formula takes into consideration increased production of creatinine with increasing weight as well as decreased production with age and gender differences in muscle mass. The formula will thus overestimate GFR in obese or edematous individuals, and should therefore not be used in these patients. Good correlation was noted comparing the Cockcroft and Gault formula and measured GFR.[38]

The Modification of Diet in Renal Disease (MDRD) formula may prove more accurate in patients with known renal disease. It is a complex formula that is based on serum creatinine, age, gender, serum albumin, blood urea nitrogen and race to estimate GFR.[39] A

simplified formula has since been developed, and only requires serum creatinine, age, gender and race[39]:

$$GFR = 186.3 \,.serum\ creatinine^{-1.154}.age^{-0.203}.(0.742\ if\ female).(1.21\ if\ black)$$

where:

GFR in ml/min/1.73m^2

The MDRD formula can be used reliably in patients with significant renal dysfunction as it was validated in kidney transplant recipients. The MDRD equation is not validated in patients with normal or near normal renal function.[39]

4.2.5 Plasma Cystatin C

Due to the problems with creatinine production and secretion, other endogenous substances were evaluated to find a more accurate estimation of GFR.

Cystatin C is a low molecular weight protein and member of the cystatin protease inhibitors family.[36] Cystatin C is produced by all nucleated cells and its production fairly constant since it is not altered by inflammatory conditions, changes in diet or affected by volume status. It is eliminated by glomerular filtration and is not secreted by the tubules.[37]

It has further been shown that cystatin C correlates more closely with GFR than plasma creatinine. Measurement of cystatin C is however still not widely available.[37]

CHAPTER 5 FUNCTIONAL RENAL RESERVE (FRR)

In the early 1980's there was interest in the possible protective effect of protein-restricted diets on the progression of renal insufficiency. Ter Wee reports that observations in several models of experimental renal failure had shown that glomerular hyperfiltration occurred in remnant glomeruli to compensate for the loss of renal mass, except in rats that were fed a protein-restricted diet.[53, 54] Concomitant research then started to establish methods to show the presence of glomerular hyperfiltration in humans.

It was shown in 1983 by Bosch et al.[40,41] that glomerular filtration depended on diet and could be increased in the order of 20% following a protein load. "Renal functional reserve" was defined as the difference between the maximum GFR after a protein load and the baseline GFR. This increase in GFR can be elicited by oral protein load or by intravenous infusion of amino acids or dopamine.

GFR is thus a dynamic parameter that is diet dependent and can be altered. For most individuals, the kidneys are capable of working at higher filtration rates than the baseline measured GFR.

Renal functional reserve is a measure of nephron function and may be a useful measure of renal function in following progression of renal disease (Bosch 1995).[40,41]

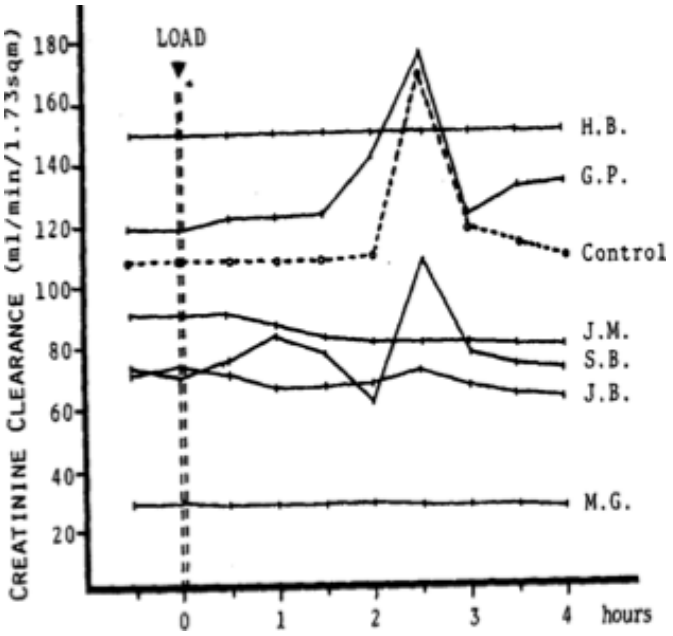


Figure 1. Effect of acute protein load on glomerular filtration rate in patients with variable renal function.[40,41]

Figure 1 shows the effect of an acute protein load on glomerular filtration rate in 6 patients. The increase in GFR two hours after the protein load is clearly visible. Of note is that patients with good and poor GFR, may not increase their GFR following the protein load, whilst other patients with reduced GFR in the range of 60 - 80ml/min/1.73m^2 may increase their GFR to the normal range (>80ml/min/1.73m^2).

Ter Wee et al subjected healthy individuals and patients with varying degrees of impaired renal function to separate infusions of an amino acid solution (Vamin N) and dopamine. The glomerular filtration rate and effective renal plasma flow were measured before and after the infusions. Healthy individuals showed an increase in GFR during amino acid infusion (while the filtered fraction remain unchanged), but not patients with impaired renal function. Amino acids were shown to increase GFR by recruiting 'dormant cortical nephrons' and increasing net ultrafiltration pressure of other filtrating glomeruli, both due to afferent vasodilatation.[54] Dopamine infusion led to an increase in the glomerular filtration rate and a fall in the filtered fraction in healthy individuals. Similar to the findings following the amino acid infusion, patients with varying degrees of renal dysfunction did not show a significant functional renal reserve. Dopamine increases GFR trough a decrease in total renal vascular resistance.[55] Ter Wee et al further concluded that already early in renal disease there exists a diminished functional renal reserve and that if the GFR is less than 50ml/min/1.73m^2, the functional renal reserve is exhausted.[56] Furthermore, renal donors that donated, showed an increase in GFR for years after kidney donation most likely on the basis of compensatory hypertrophy of the remaining kidney.[57, 58]

Rook et al showed in a study of 125 consecutive donors, that assessment of pre-donation GFR gives a reliable prediction of post-donation GFR and is improved by taking age and stimulated (amino acids, dopamine or both) GFR into account.[58] GFR post donation was predicted by baseline GFR (r^2 = 0.54), amino acid stimulated GFR (r^2 = 0.56), dopamine stimulated GFR (r^2 = 0.35) and stimulated GFR by amino acids and dopamine (r^2 = -0.22) ($p<0.001$ for all). [58]

Rook et al also showed that function renal reserve was absent in donors post donation and obesity had even more impact in younger donors. Donor nephrectomy thus unmasked an age- and overweight-induced loss of functional renal reserve. [58 – 60]

Chan showed that pre-nephrectomy renal response to protein ingestion did not predict creatinine clearance of the kidney after donor nephrectomy.[72]

Loo et al performed a study in which the protein meal was used to assess functional renal reserve in normal subjects as well as various groups of renal patients. They showed that renal reserve may be used to assess suitability of living related transplant donor for nephrectomy.[73]

Functional reserve is usually measured by subjecting the patient to a high animal protein meal, usually 100g of beef or fish. Following this, the GFR may increase transiently about 2 hours after the protein load and is usually in the order of a 20% increase from the baseline GFR. [51] Various techniques have been used to determine functional renal reserve, most commonly by using inulin infusion till steady state blood concentration is reached. The protein meal is administered and blood sampling is continued and the transient increase in GFR measured. This procedure is laborious and takes considerable time.

A novel protocol was developed at our institution (*Candy, Esser et al 1994)* whereby baseline GFR would be measured after an overnight fast. The patient would be requested to consume a diet that is rich in fish and/or beef protein for one week. The GFR as estimated by ^{51}Cr-EDTA clearance would then be repeated and the change in GFR calculated after which the functional renal reserve would be measured.[47 - 50]

This novel protocol is used to increase the GFR in otherwise suitable potential renal donors who has a baseline GFR of ≤80ml/min/1.73m^2 and protein loaded where the GFR would increase to >80ml/min/1.73m^2. These potential renal donors have subsequently donated kidneys. The outcome of both these donors and recipients is not known. To the best of our knowledge the outcome of such recipients and donors have also not been described in literature.

The aim of the study is to determine the outcome in recipients of renal transplants from protein-loaded donors versus recipients of non-protein loaded ("normal" (>80ml/min/1.73m2) GFR) donors.

The following objectives were set:

i. Create a database of all potential renal donors (PRDs) screened using 51Cr-EDTA clearance as a measure of glomerular filtration rate (GFR) to determine which potential donors were used for donation, to the recipients as well as the documentation of outcome of the recipients (survival, morbidity and mortality).

ii. Determine the outcome of those recipients receiving kidneys from potential renal donors (PRDs) with "low" (≤80ml/min/1.73m2) baseline GFR, who were subsequently protein loaded, compared to those receiving kidneys from PRDs with normal (>80ml/min/1.73m2) GFR.

iii. To determine follow-up GFR in the donors post donation where this was undertaken.

CHAPTER 6 METHODS

6.1 Study design, study population and sampling

The study follows a retrospective record analysis of patient demographics and work-up results (cross-matching, GFR, infectious diseases, renal anatomy etc.) and outcomes in:

i. All potential renal donors (PRDs) from 1997 to July 2012 who had GFR determined by clearance of ^{51}Cr-EDTA
ii. The subset of those who donated a kidney
iii. Donors with "low" (≤80ml/min/1.73m^2) baseline GFR and subsequent protein-loaded GFR
iv. Donors with a "normal" (>80ml/min/1.73m^2) GFR
v. The recipients receiving the grafts – outcome, complications, creatinine over time and survival

The data of all potential renal donors that underwent GFR assessment with ^{51}Cr-EDTA (whether as baseline or following protein load) from January 1997 to July 2012 are kept in a database at Scintillation Services, which form part of the Department of Nuclear Medicine, University of the Witwatersrand. This database was accessed and names of all potential renal donors were obtained (n = 249). A list of the names of all potential renal donors was made.

The files (hardcopies) of all potential renal donors are being kept in the Nephrology department at Charlotte Maxeke Johannesburg Academic Hospital. The files of all the potential renal donors that donated a kidney were located (n = 146). Potential renal donors that did not donate a kidney (n = 103) were excluded. Table 7 shows the reasons why these potential donors did not donate.

These potential renal donors that did donate were placed into two groups: those that donated a kidney without having been subjected to protein loading (n=124) (baseline GFR > 80ml/min/1.73m^2) and those that donated after having had protein loading (n = 22) (baseline GFR ≤ 80ml/min/1.73m^2). Demographic data of these potential donors were collected from their files as well as the names of the recipients of the respective kidneys that were donated. Demographic data that were collected from the potential renal donors included: age, gender, ethnicity, GFR assessed by ^{51}Cr-EDTA and GFR post protein load as assessed by ^{51}Cr-EDTA (if this was done). From the information found in the hardcopy files of the potential renal donors that donated – a list was made of which potential renal donor donated to which renal transplant recipients.

The list of renal transplant recipients was taken to the Renal Transplant Unit and the hardcopy files of the recipients were located. All the files could be located, however, there were some patients that were lost to follow up and have presumably relocated as they could not be contacted (n = 39 from the group that received kidneys from non-protein loaded donors and n = 9 from the group that received kidneys from the protein loaded group). The recipients that were lost to follow-up were excluded from the analysis. Demographic data from the files were collected (age, gender, weight, height, ethnicity). In

addition the yearly creatinine level was also recorded (as recorded at the yearly follow-up visits) as well as the status of the patient at the time of collection of data (July 2012). This included whether the recipient was deceased (as well as cause), rejection of the transplant kidney, other complications or whether the recipient was doing well.

6.1.1 Inclusion and exclusion criteria

Potential renal donors (PRDs) that did not undergo ^{51}Cr-EDTA clearance assessment were excluded.

Recipients of renal grafts that were lost to follow-up or could not be found were excluded as well as their respective renal donors.

6.1.2 Ethical considerations

Ethics approval for this study was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand: number M110969 on 30 September 2011.

A retrospective study design was followed involving data collection from archived records, thus there was no need to obtain informed consent.

The expected benefit of embarking on this research is if no difference in outcome between the two groups is shown, then those potential renal donors in whom a protein load increases the GFR to "normal" (>80ml/min/1.73m^2) may increase the pool of potential renal donors. Conversely, if there is a difference in outcome, then these donors should be excluded.

6.2.3 Confidentiality

Patient names and hospital numbers was recorded only for the purpose of matching the donors to recipients. Once this was achieved, the personal information was deleted and a sequential study number used – i.e. one record for both donor and recipient. Dates of transplant and follow-up times were converted to days and the actual dates deleted from the database. Names Hospital numbers and other patient identifiers are not relevant and thus are not in the database used for statistical analysis.

The results and conclusions in this research are in no way linked to any patient, thus complete patient confidentiality was ensured.

6.2.4 Method of ^{51}Cr-EDTA clearance used

Potential renal donors at Charlotte Maxeke Johannesburg Academic Hospital undergo measurement of GFR using ^{51}Cr-EDTA clearance for determination of GFR. A single dose of 3.7MBq of ^{51}Cr-EDTA , in a volume of 1 ml is injected intravenously through a catheter. The exact injected dose is determined by weighing the syringe before and after injection on a high precision analytical balance. The catheter is flushed through with 10 ml of saline. 10 ml blood samples are drawn from the opposite arm at exactly 240 minutes post injection. The blood sample is centrifuged at 1738 g for 10 minutes. 3 ml of the supernatant is drawn off and counted in a gamma counter for 10 minutes. A radioisotope control, taken as an aliquot from 3.7MBq 51Cr-EDTA diluted to 500ml in saline is also counted. We use a Cobra Auto-gamma manufactured by Packard.

GFR is calculated using the formula of Constable and is corrected to a body surface area (BSA) of 1.73m^2:

$$GFR = \sqrt{V - 6.3} - 67$$

(where V is the apparent volume (L) of distribution at sample time 240 minutes)

When the GFR determination of potential renal donors is ≤ 80ml/min/1.73m^2 the individual is asked to protein load 7 days prior to the test. They are requested to take 200 g of red meat or fish as well as 1 egg daily. All other foods are optional and no restriction is placed on any other foods or beverages.

6.2.5 Data management and analysis

All data generated was recorded in an Excel® spreadsheet and analysed using SAS V9.1® software.

The data was reported in tables and figures as frequency (n), mean±SD. Comparison assessment was done using t-test or Mann-Whitney test (as appropriate for normally / not-normally distributed data or Chi-squared test. Survival (graft and recipient) of recipients who received kidneys from those with a "low" baseline (and "normal" GFR following protein load) versus "normal" GFR was compared with a Kaplan Meier Survival curve.

CHAPTER 7 RESULTS

7.1 Donor and recipient demographics

The demographic characteristics of the 249 potential renal donors with routinely performed GFR using ^{51}Cr-EDTA clearance and the recipients to whom they donated are shown in Table 6. A schematic flow diagram for patients included in the study is shown in Fig. 4.

7.1.1 Non-protein loaded potential donors

Two hundred and twelve potential renal donors, mostly female, had good GFR and were therefore not requested to protein load. One hundred and twenty four of these potential donors were well matched and donated to recipients (Table 6), with complete follow-up available on the files in 85 cases.

The reasons for the remaining 88 potential donors being excluded from donating are listed in Table 7. These reasons included physiological abnormalities, such as thyroid dysfunction, anatomical variants, which would be problematic during the transplant procedure, infected donors (HIV, Hepatitis) and poor cross-matching between the donor and recipient. Social issues included non-compliance, the potential donor changing their mind, etc. In many cases there were several reasons for non-donation and only the major reason listed in Table 7.

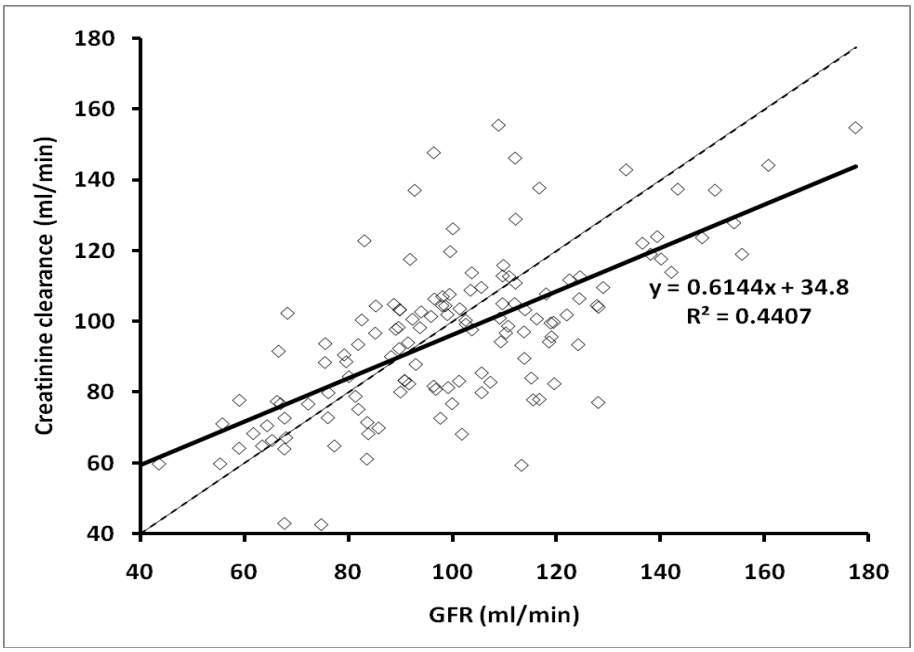


Figure 2. Association between 51EDTA clearance and creatinine clearance calculated using the Cockcroft and Gault formula from serum creatinine concentrations. Line of unit is shown as a stippled line.

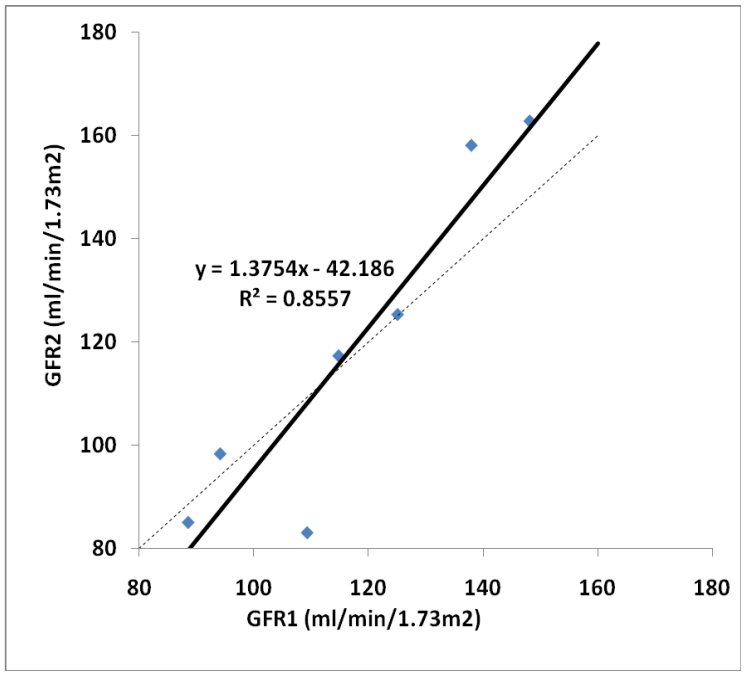


Figure 3. Reproducibility of 51Cr-EDTA clearance on the same patients(n=7) with a range of GFR.

Table 6. Demographic characteristics of all potential donors with glomerular filtration rate determined using ^{51}Cr-EDTA. *Top panel:* Potential donors were divided into those who were not protein loaded and those who were protein loaded. *Lower panel:* Characteristics of recipients who received kidneys from the donors in the panel directly above. Numbers in brackets are total number of cases and data within the Table may not add to this total as a result of missing data. Differences between non-protein loaded donors vs protein loaded-donors: p: *<0.05; Abbreviations: *p=probability; n=number; f=female; m=male; A=Asian; B=black; C= coloured; W=Caucasian; BMI = body mass index; BSA=body surface area

Parameter	All donors	Potential donors not protein loaded		Potential donors protein loaded		
	Screened (n=249)	Donated (n=124)	Non-donor (n=88)	All donors (n=37)	Donated (n=22)	Non-donor (n=15)
Age (years)	36.4±8.8	35.0±8.8	37.7±8.6*	38.6±8.9	40.3±7.3	35.4±11.3
Gender (n:f/m)	139/88	75/45	51/37	14/6	10/4	5/2
Ethnic grouping (n:A/B/C/W)	19/92/24/84	13/47/12/51	5/45/8/30	5/12/4/15	2/6/3/10	3/6/1/5
BMI (kg/m2)	26.2±4.0	26.3±3.7	26.6±4.5	24.0±3.8	24.9±3.6	22.6±4.0
BSA (m2)	1.8±0.2	1.8±0.2	1.8±0.2	1.7±0.2	1.7±0.2	1.6±0.1
GFR (ml/min/1.73m2)	93.4±18.6	99.3±16.2	94.3±16.5*	70.0±11.7	71.8±11.5	67.6±11.8
Creatinine (mmol/L)	89.0±16.2	86.9±16.0	91.4±16.2	94.8±14.8	95.0±16.0	94.0±12.7
Creatinine clearance	95.2±21.8	99.1±21.0	92.3±22.1	76.2±15.7	77.9±17.6	71.5±8.2
Urea (mmol/L)	4.6±2.8	4.7±3.5	4.6±1.4	5.0±1.2	4.9±1.3	5.2±0.7

	Recipients with known outcome (n =129)	**Recipients (n =116)**	**Recipients (n=0)**		**Recipients with known outcome (n=13)**	**Recipients (n=0)**
Age	24.8±13.4	24.6±13.5	N/A		27.8±12.2	N/A
Gender (n:f/m)	59/74	53/67	N/A		5/8	N/A
Ethnic grouping (n:A/B/C/W)	12/46/16/47	12/47/13/48	N/A		0/4/3/6	N/A
Pre-op serum creatinine	883.0±321.1	906.6±310.5	N/A		717.9±364.8	N/A
Day 1 Post-op creatinine	241.8±222.5	224.4±227.5	N/A		223.0±192.7	N/A

Recipient characteristics that received kidneys from donors in the panel directly above on the previous page (page 55).

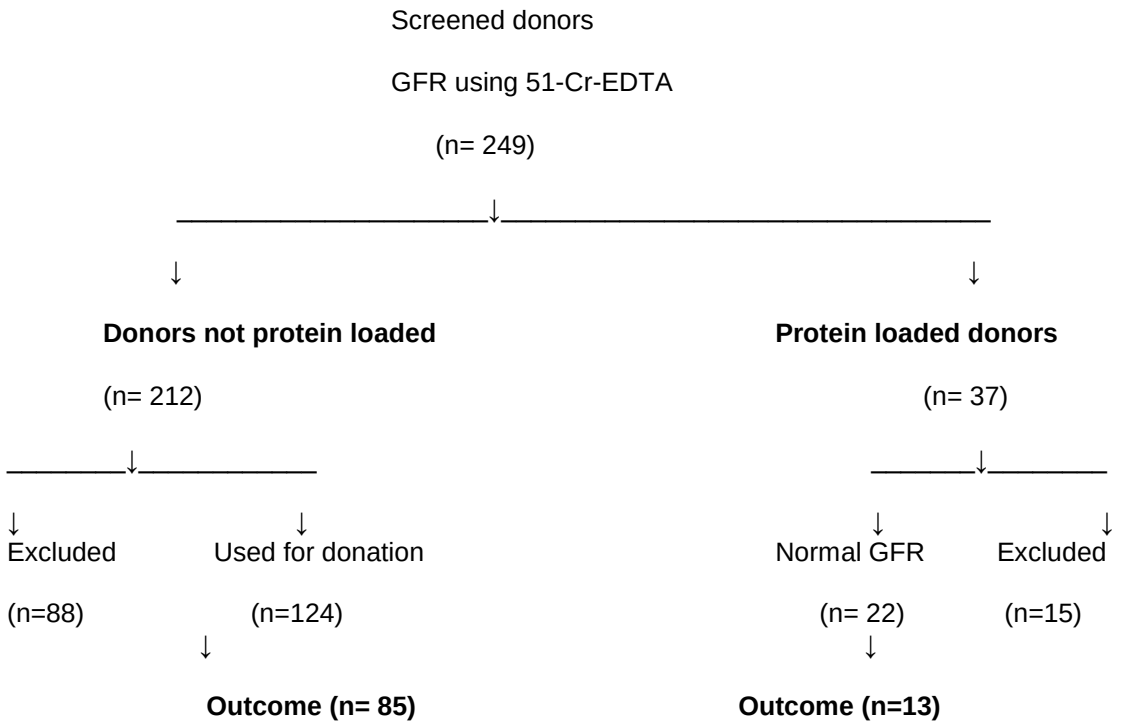


Figure 4. Schematic diagram of the study showing potential donor and recipient numbers

Table 7. Reasons for excluding potential donors from donation

Reason	Not Protein loaded (n=88)	Protein loaded (n=15)
Physiological	16	
Anatomy	13	2
Viral infection	11	
Cross match	11	1
Social	9	1
Recipient died	8	
Low renal function	6	9*
Hypertension	6	1
Other donor used	4	
Other	1	
Not specified	3	1

*potential renal donors that did not increase GFR to > 80ml/min/1.73m^2 after protein load

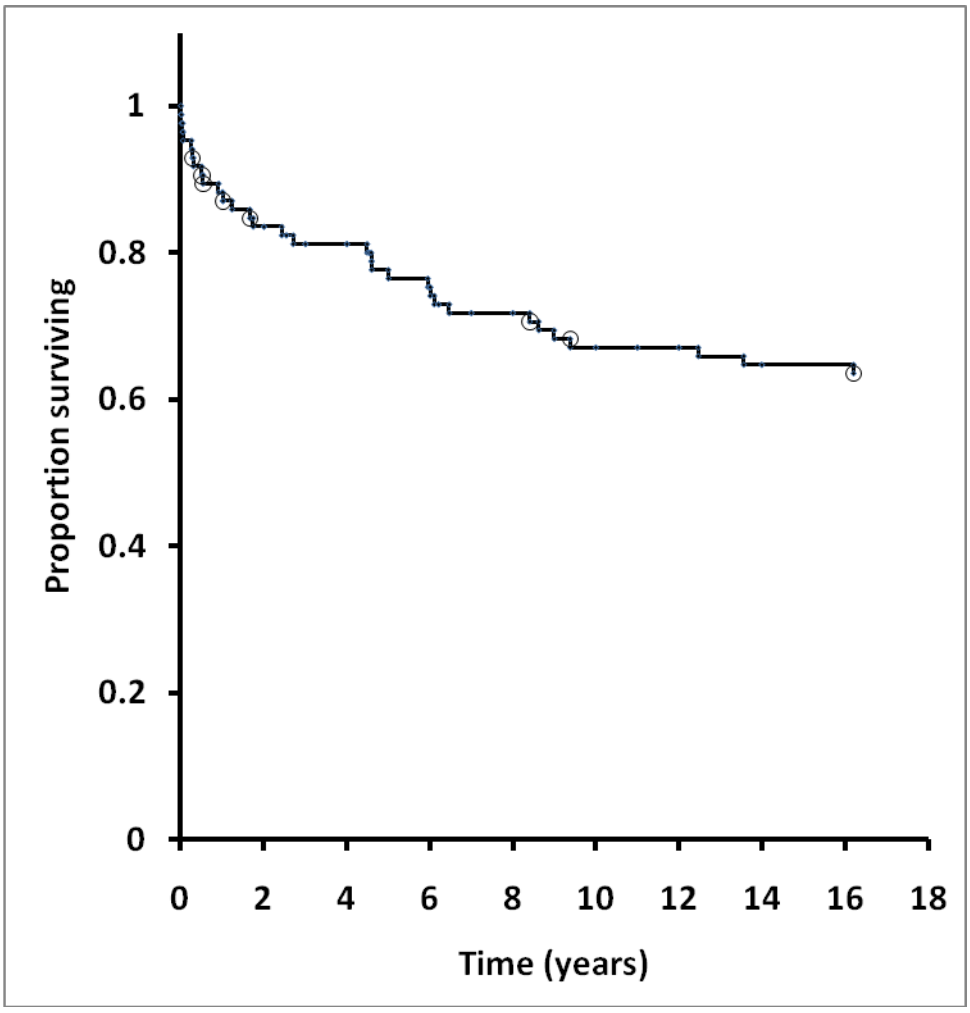


Figure 5. Kaplan-Meier survival function for recipients receiving grafts from non-protein loaded donors. Overall graft and recipient survival was 63.5% at 16 years.

A significant number of patients (n=39) were lost to follow up and could not be contacted. In this group, overall graft and recipient survival over 16 years was 64%. Twenty seven patients (32%) rejected the transplant kidneys which included 4 associated deaths. Another 4 patients died (pneumonia (n=1), pulmonary embolism (n=1), myocardial infarction (n=1) and one patient due to unknown cause).

7.1.2 Protein loaded donors

The 37 potential renal donors who were protein loaded were older than the group not protein loaded ($p<0.04$) (see Table 6). The glomerular filtration rate in these protein loaded donors was 70.0±11.7ml/min/1.73m^2, significantly less than those who were not protein loaded (97.2±16.5 ml/min/1.73m^2; $p<0.0001$). Three of these protein loaded potential donors had GFR of >80 ml/min/1.73m^2. The pre-protein loading creatinine clearance was also significantly less ($p=0.0018$) than the donors not loaded (Table 6.). For potential donors, upon protein loading, GFR increased significantly to 89.3±18.4 ml/min/1.73m^2 ($p<0.0001$), with a mean percentage increase of 26.0±24.4% (median 22.2% range: -12.9% to +103.4%; Fig. 7). In 6 donors, GFR determined after transplantation was not significantly different from the pre-transplant measurement (pre- 74.7±9.2 to 71.4±11.4 ml/min/1.73m^2; $p=0.54$; Fig. 6.). The pre-protein loaded GFR correlated with the post-protein loaded GFR measurement (Fig. 7 *A*) and as the pre-protein load GFR increased so the percentage increase in GFR following protein loading declined (Fig. 7 B). Of the 15 potential donors who did not donate, the GFR did not increase above 80 ml/min/1.73m^2 in 9 and they were excluded from donating. The other 6 potential donors were excluded for various reasons (Table 7).

Twenty two protein loaded donors subsequently donated kidneys and outcome was available for 13 recipients. Overall graft and recipient survival over 14 years was 58.3% (Fig. 8 *A*). This was not signifantly different from the recipients receiving grafts from non-protein loaded donors ($p=0.14$; log rank test). If

survival is measured in recipients with functioning grafts at 6 months, to avoid immediate post-transplant complications, the survival curves are almost identical (Fig. 8 *B*).

Futhermore, when only patient survival is compared between the recipients that received grafts from protein loaded and non-protein loaded donors, the survival curves are still almost identical (Fig. 9). Patient survival for recipients that received grafts from protein loaded donors were 83% at 14 years and for those recipients that received grafts from non-protein loaded donors 85% at 14 years.

7.2 Changes post transplant

GFR declined in all donors from a pre-transplant determination of 97.7±18.1ml/min/1.73m^2 to 75.2±16.4ml/min/1.73m^2 ($p<0.005$) (see Fig. 6). Two donors were subsequently protein loaded after donation with an increase in GFR from 57.3 to 75.5ml/min/1.73m^2 (pre-transplant 103.7 ml/min/1.73m^2) and from 69.5 to 87.2ml/min/1.73m^2 (pre-transplant 88.0ml/min/1.73m^2).

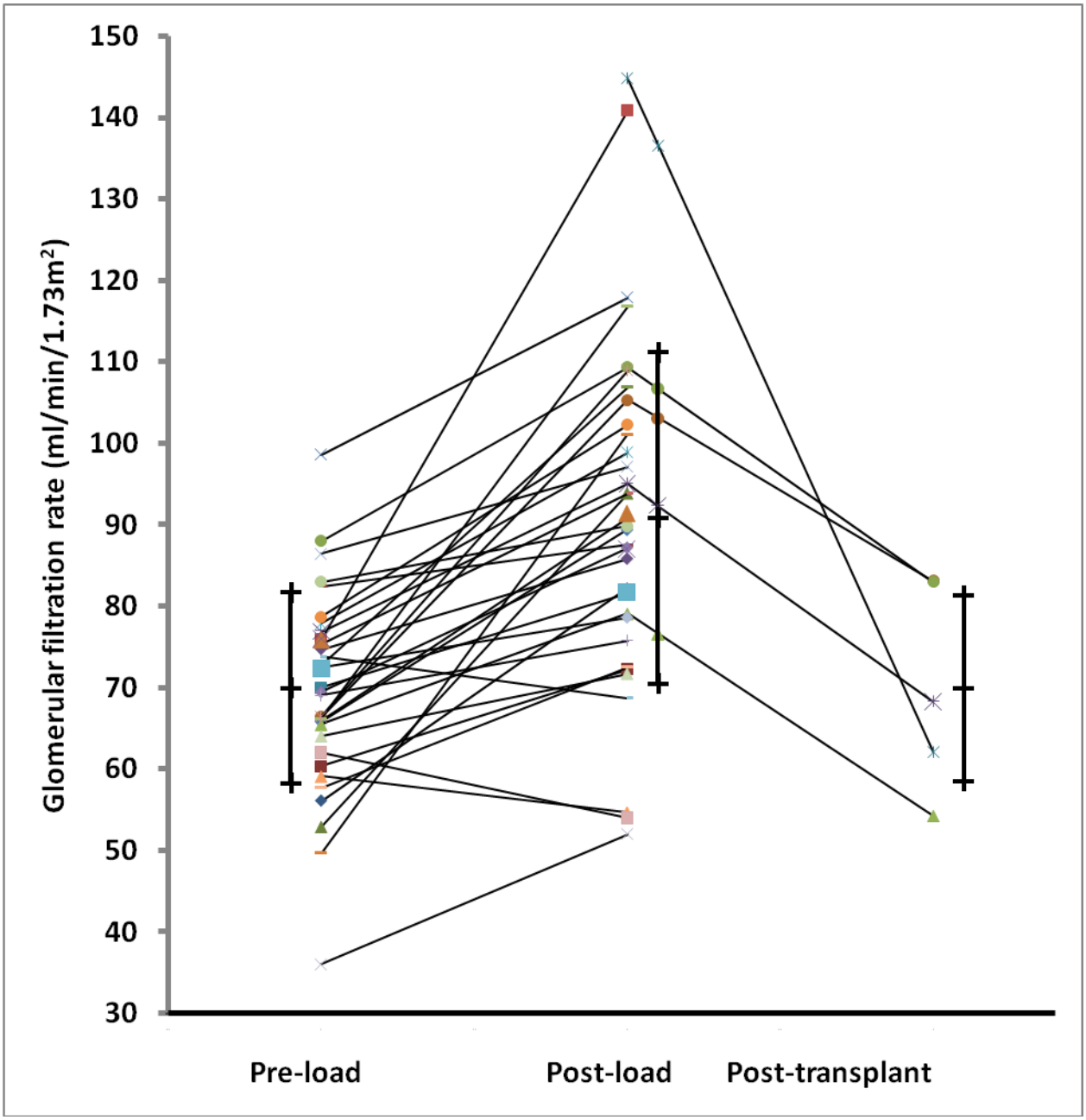
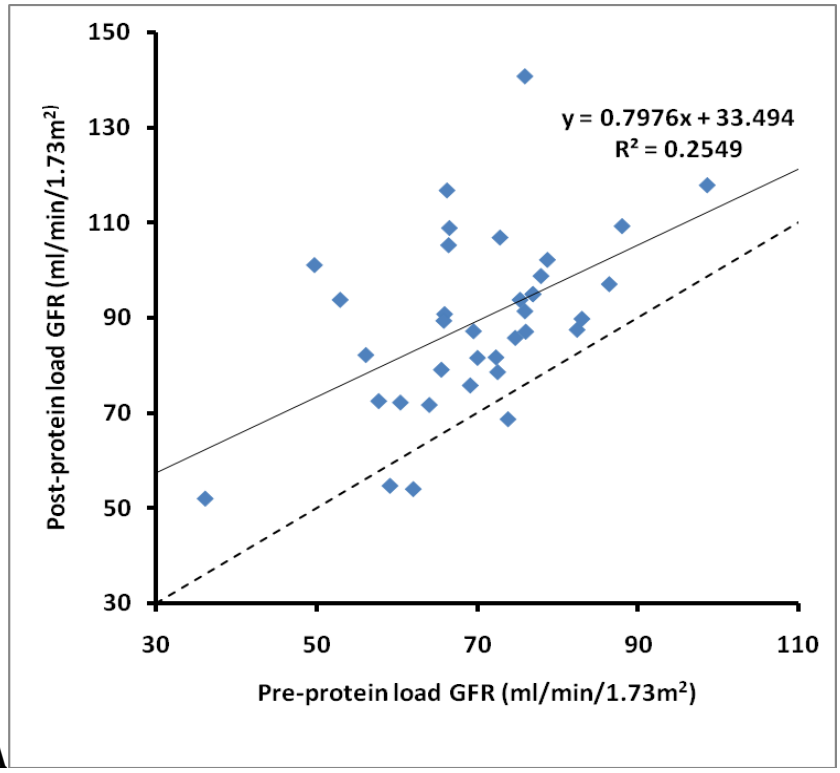
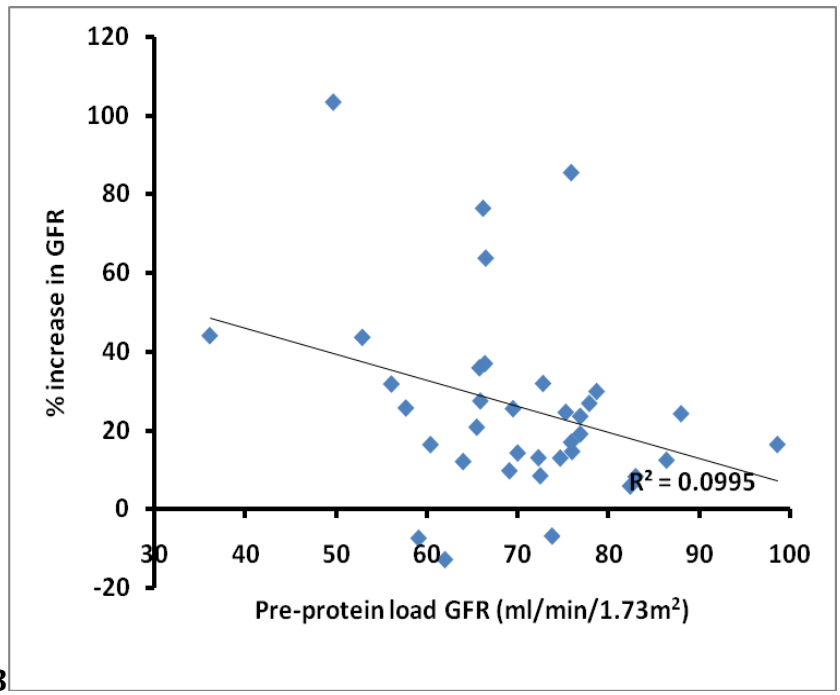


Figure 6. Glomerular filtration rate changes in potential donors pre- and post protein loading and after transplant. Data for individual potential donors and as mean and standard deviations. Change from pre-load to post loading $p<0.0001$; pre-loading vs post transplant ($n=5$; $p=0.54$).

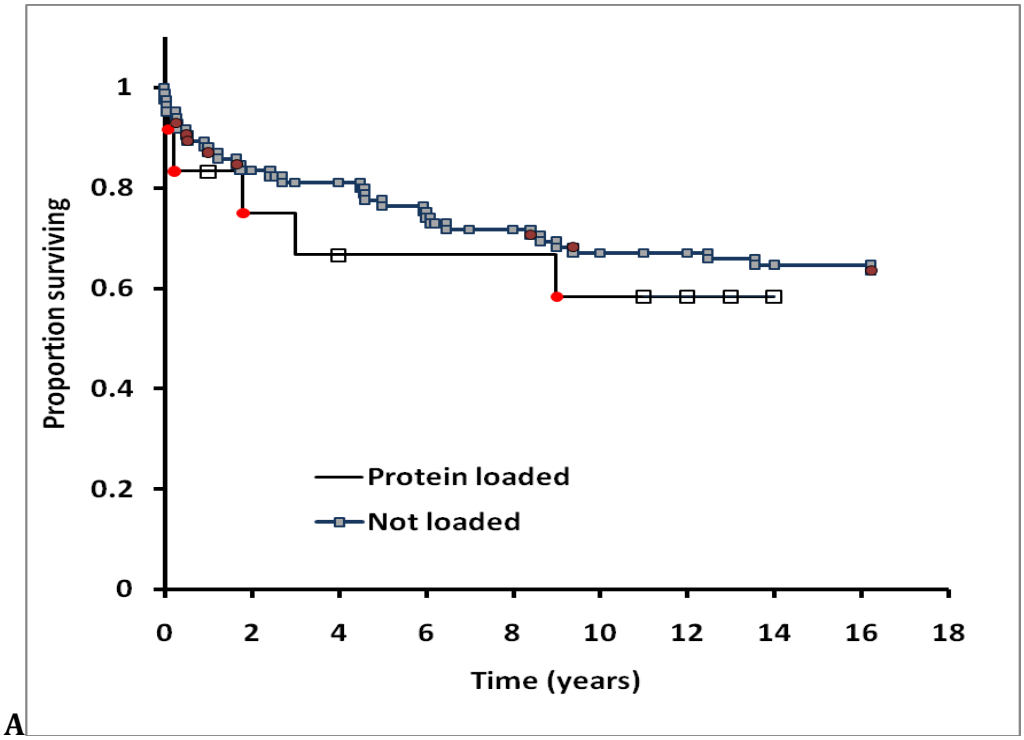


A



B

Figure 7. ***A***: Relationship between pre- and post-protein load. The 1:1 line of agreement is shown as the stipped line. ***B***: Relationship between pre- load GFR and the percentage change in GFR following a protein load. The percentage increase is less at high initial GFRs.



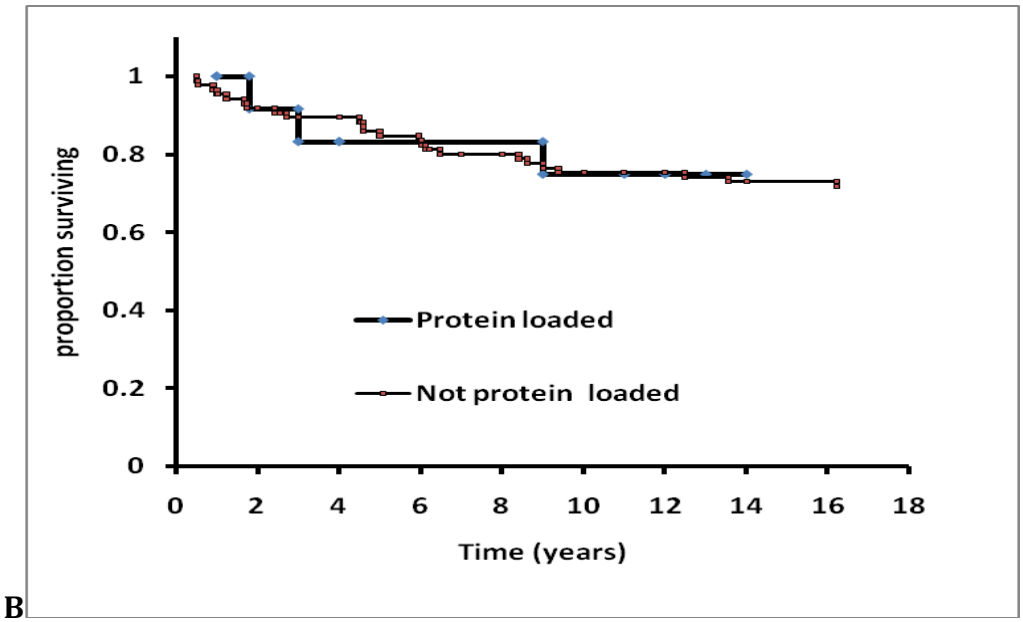


Figure 8. Kaplan Meier survival curves showing overall graft and recipient survival for recipients receiving grafts from protein loaded and non-protein loaded donors. ***A*** shows all data, without differences between groups; (p=0.14) and ***B*** shows the same data adjusted to exclude acute effects of transplantation (less than 6 months). Closed circles indicate recipients who died.

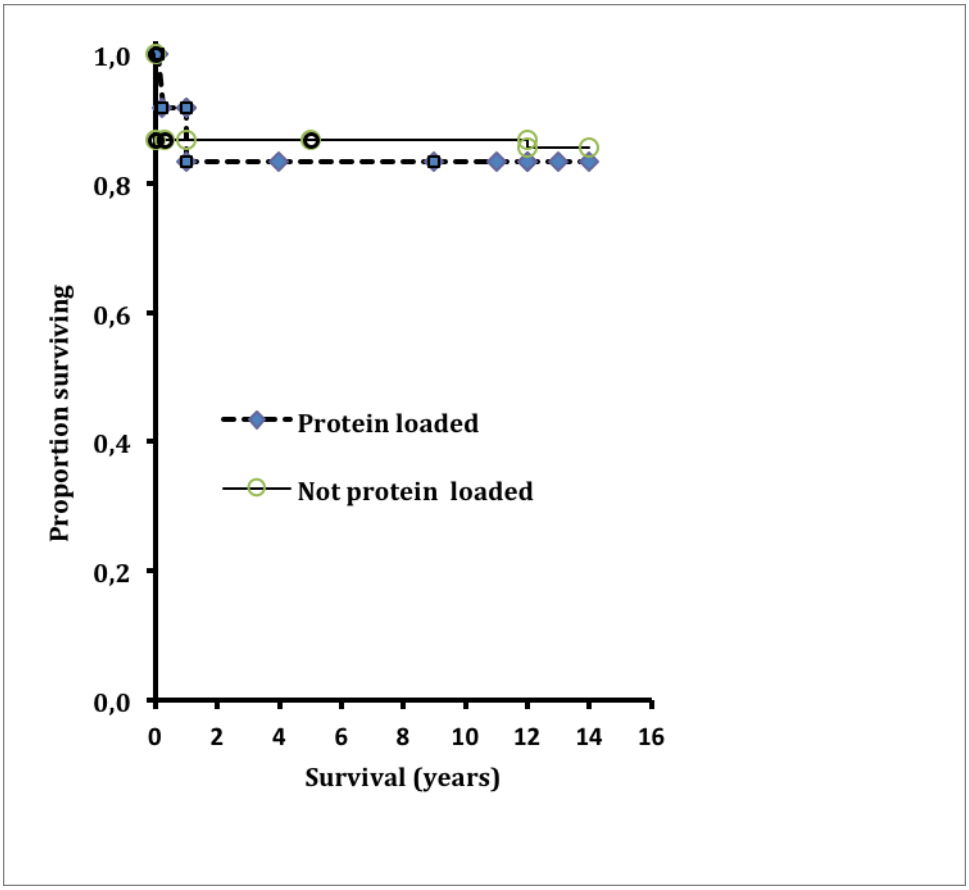


Figure 9. Kaplan Meier survival curves showing patient survival of recipients that received grafts from protein loaded and non-protein loaded donors.

CHAPTER 8 DISCUSSION AND CONCLUSION

8.1 Discussion

There is a global preponderance of renal disease and many of these conditions are associated with subsequent renal failure. These diseases include hypertension, glomerulonephritis, HIV associated nephropathy (HIVAN) and diabetic nephropathy. [1, 2]

There is a worldwide increased demand for renal grafts for transplantation as it is the most effective treatment for end stage renal disease. Potential live renal donors need to have adequate renal function in order to qualify for renal donation, and it has been shown that a glomerular filtration rate of >80ml/min/1.73m^2 is necessary to be able to provide adequate renal function for the donor post donation.[14, 15]

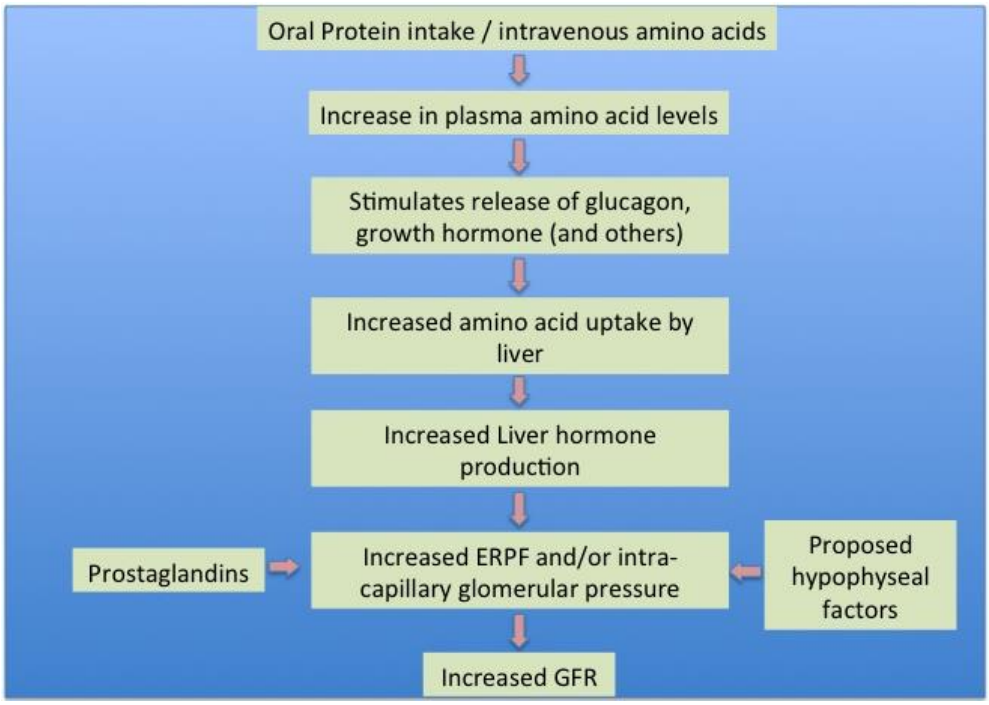
There is however a great shortage worldwide of live renal donors. Potential live renal donors with a glomerular filtration rate of ≤80ml/min/1.73m^2 but otherwise suitable for donation of a kidney, may increase the pool of available donor kidneys. It is well known that protein loading may increase the GFR to the acceptable levels, but the outcome of these protein loaded kidneys are not known.

Although the exact mechanism of this increased glomerular filtration in response to protein loading is not completely understood, the following mechanism had been proposed[40, 41]: intravenous infusion of amino acids and acute protein load will lead to an increase in plasma amino acid levels.

This may lead to hypersecretion of various substances, amongst others: glucagon and growth hormone. Following this, there is a resultant increased uptake of amino acids by the liver, with subsequent increased production of liver hormones (growth hormone, liver derived insulin-like growth factor (IGF1)).

These liver hormones, as well as prostaglandins and proposed hypophyseal factors and suspected direct renal effect of the amino acids lead to an increased effective renal plasma flow and/or intracapillary glomerular pressure and ultimately increased GFR[42 -46] as summarized in scheme 1, below.

Scheme 1: Proposed mechanism of functional renal reserve



ERPF, Effective Renal Plasma Flow; GFR Glomerular Filtration Rate

Functional renal reserve in practice can be measured by subjecting a patient to a meal that is rich in animal protein, usually 100g beef or fish. There have been various techniques used to determine functional renal reserve and these

commonly involve a steady state infusion. These methods may prove laborious and cumbersome.

The validity of estimating GFR is critical. Inulin infusion and clearance is the accepted gold standard for assessment of GFR.[23] This technique has been simplified and currently single sample measurement of clearance of ^{51}Cr-EDTA is used in our institution, as described in chapter 6. It is widely believed that the area under the plasma clearance curve following a single injection of ^{51}Cr-EDTA is a gold standard method for determining glomerular filtration rate. There are however reports that ^{51}Cr-EDTA may have a significant extra renal clearance.[75] Moore et al showed in a study of seventy healthy post-menopausal women (mean age 60 years, range 45 – 79 years) that measurements of GFR using ^{51}Cr-EDTA overestimate the true renal clearance of tracer by approximately 10%.[75] This overestimation of the true renal clearance by the area under the clearance curve is believed to be due to the failure of the plasma clearance curve to reach the true terminal exponential by 2 hours after injection as is usually assumed.[75]

The rate constant (α_2) of the terminal exponential of ^{99m}Tc-DTPA plasma clearance curve is close to the ratio of glomerular filtration rate to extracellular fluid volume and it is therefore a convenient, already normalized measure of renal function.[76] Gunasekera et al showed the validity of ^{99m}Tc-DTPA for measurement of GFR as the rate constant is a convenient measure of GFR and can be based on the terminal exponential of inulin of ^{99m}Tc-DTPA curves. This was done in 15 patients undergoing routine renography injected with 50 milliliters of ^{99m}Tc-DTPA (250MBq) and 10% inulin, mixed in the same syringe.[76]

Delanaye et al demonstrated that in 12 healthy subjects, reproducibility of GFR measured by iohexal (4.5%) was slightly better than for ^{51}Cr-EDTA (7.4%).[77] The reproducibility of GFR estimation using ^{51}Cr-EDTA by this group showed relatively the same reproducibility as was shown by Bird et al (9.0%).[77]

In a study by Medeiros et al involving 44 kidney recipients and 22 kidney donors good correlation was shown between GFR measurement with inulin and ^{51}Cr-EDTA in this population (R = 0.94).[62]

It is recommended by the British Nuclear Medicine Society that the plasma clearance of EDTA from venous samples be taken as the standard measure of GFR.[78] Using ^{99m}Tc-DTPA does have some technical advantages over ^{51}Cr-EDTA but normal ranges for DTPA are no so well established. Small systematic differences were observed between measurements from EDTA and DTPA (DTPA gives higher results) but these are sufficiently small (<5%) to recommend DTPA as a suitable alternative.[78] It is advised however that all GFR studies in a centre should use the same radiopharmaceutical. The British Nuclear Medicine Society further considers that among the various methods for measuring ^{51}Cr-EDTA plasma clearance, the slope-intercept method provides the best compromise between accuracy and reliability and simplicity and that the method of using the area under the curve is too time consuming for general clinical use.[78] The single sample method is recommended by the Radionuclides in Nephrourology Committee on renal clearance. One-sample techniques are however less precise than the slope-intercept method, with systematic errors at the low and high ends of GFR.[78] A further disadvantage of the single sample method is the lack of quality control for the eventuality of extravasated tracer.[78]

Our institution developed a novel technique[47, 48] by measuring baseline GFR after an overnight fast. The patient is requested to consume a diet that is rich in fish and or beef protein for a week. The post protein loaded GFR is then calculated by ^{51}Cr-EDTA clearance. The novel method of protein loading patients by providing a protein rich meal for a week and then repeating the ^{51}Cr-EDTA GFR assessment was validated and correlated to reported literature by Bosch, that following a protein rich meal there is an resultant increase in GFR to the magnitude of about 20%.[40, 41] The different responses to protein load may depend on the underlying renal function, but genetic difference and non-standardized protein meals may also contribute to variable responses in increases in GFR. Furthermore the majority of South Africans come from a low socio-economic background with limited financial resources, and seeing that protein meals are expensive, may not be in a position to afford the protein rich meal for a week. There may also be religious objections in certain groups to consuming an animal protein rich diet.

Further research is necessary to assess the need for and develop a standardized protocol with administration of amino acids infusion. This may prove too time consuming and laborious but will be an attempt to remove possible variations in increase in GFR due to variable protein diet.

Nephrologists routinely estimate creatinine clearance by using the formula of Cockcroft and Gault. In this study it was shown that ^{51}Cr-EDTA clearance correlates with calculated creatinine clearance (Fig 2, r^2=0.44) within the range of ^{51}Cr-EDTA clearance between 40 and 180ml/min (uncorrected for BSA).

It was also shown that reproducibility of ^{51}Cr-EDTA clearance in 7 patients was excellent (Fig 3; r^2=0.86), suggesting a robust method.

Two hundred and forty nine patients were screened by using ^{51}Cr-EDTA clearance (see Fig 4). Two hundred and twelve potential donors had good GFR and were not protein loaded. Of these one hundred and twenty four potential donors were well matched and donated to recipients, with complete follow up in 85 cases. A total of 88 potential donors were excluded for various reasons (Table 7). In this non-protein loaded group that donated kidneys, overall graft and recipient survival over 16 years was 64%.

Thirty seven potential renal donors were protein loaded since their initial GFR was ≤80ml/min/1.73m^2. Of these 37 potential donors, 15 were excluded (Table 7). Twenty two potential donors donated kidneys in this group, with outcome available in 13 recipients. Overall graft and recipient survival over 14 years was 58.3% for this group. This was not significantly different from the recipients receiving grafts from non-protein loaded donors (p=0.14; log rank test). Furthermore, when comparing only patient survival between recipients of grafts from protein loaded and non-protein loaded donors, there is still no significant difference between the two groups.

This study has shown that there is no significant difference in outcome between recipients of grafts with baseline normal GFR (>80ml/min/1.73m^2) and those that achieved a GFR of >80ml/min/1.73m^2 post protein load, especially if the first 6 months are disregarded, because this may bias the analysis due to post transplant complications.

The practical implication is that protein loaded donors that increase their GFR to the range >80ml/min/1.73m^2 should be allowed to donate a kidney, as the outcome of this group of donors is not different than that of donors with a

normal baseline GFR. By doing so, this may assist in definitive treatment of many patients that would no other option but to persist with dialysis treatment, if they are able to access this service.

The study also showed that the functional renal reserve is diminished in patients with a normal or near normal GFR. This correlated with the work of Ter Wee et al.[53, 54] It was interesting however to note that there was a patient with a baseline GFR of 50ml/min/1.73m^2 that was able to increase GFR to well beyond 80ml/min/1.73m^2. This is in contrast to Ter Wee's conclusion that at a baseline GFR of 50ml/min/1.73m^2 there is no significant functional renal reserve present.[53,54] The reason for this is no clear, and it is merely an observation.

Although the numbers of this study is small, it should also be borne in mind that the number of patients undergoing transplants from living donors is also not big in comparison.

The large number of patients that were lost to follow up and the ones that were not contactable reflects challenges typical of the South African healthcare system. Many patients do not have access to reliable communication and others are forced to move to a different part of the country to keep employment. There is also, frequently, incomplete clinical notes that may also lead to the exclusion of patients. This study also shows that a considerable number of patients are lost during follow-up. This may indicate that mechanisms for close monitoring and communication should be sought to reduce the number of patients lost to follow-up for these life saving procedures.

8.2 Limitations

Patient numbers in this study are small and this is an obvious limitation. Although very precise records are kept for recipients of renal transplants in our institution, there is however a significant proportion of recipients of renal transplants that were lost to follow up (n=9 in the group that received kidneys from donors that were protein loaded and n=39 in the group in which the donors were not protein loaded) and could not be contacted. These patients have presumable relocated to other transplant follow-up centers in the country or even internationally. A proportion of these patients that could not be contacted may also have passed away. Follow-up GFR assessment was only undertaken in 2 donors post donation and this precludes any further meaningful conclusions in this group.

8.3 Conclusion

^{51}Cr-EDTA is a reliable, reproducible technique to assess the GFR of potential renal donors. The novel protein loading technique developed in our institution is a convenient way to assess the functional renal reserve of potential renal donors that have a suboptimal estimated GFR of ≤80ml/min/1.73m^2 and may assist in providing transplantation to many patients who would otherwise have no option but to remain on dialysis long term.

Although the patient numbers in this study are small, there is no significant difference between the outcome of recipients of non-protein loaded and protein loaded renal grafts. The inclusion of potential donors that show a normal GFR post protein load may increase the pool of available kidney grafts and assist greatly in the treatment of many patients with end stage renal disease.

Mechanisms for close monitoring and follow-up of patients should be sought to reduce the number of patients that are lost to follow-up for this expensive and life saving procedures.

Further research in this area is encouraged, and multicenter studies may yield results that with larger number of patients that ratify the results of this study.

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