VALUE OF BASELINE POST-TRANSPLANT MAG3 RENAL SCINTIGRAPHY IN THE EVALUATION OF GRAFT FUNCTION

BY

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DECLARATION

"I hereby declare that the dissertation entitled **VALUE OF BASELINE POST-TRANSPLANT MAG3 RENAL SCINTIGRAPHY IN THE EVALUATION OF GRAFT FUNCTION** is a product of my work, except for those that have been cited clearly in the references."

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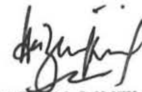

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ABBREVIATIONS

^{131}I OIH	Iodine 131-orthoiodohippuran
^{18}F-FDG	Fluorine 18-fluorodeoxyglucose
^{51}Cr-EDTA	Chromium 51-ethylenediaminetetraacetic acid
^{68}Ga-EDTA	Gallium 68-ethylenediaminetetraacetic acid
^{99m}Tc-DMSA	Technetium 99 metastable-dimercaptosuccinic acid
^{99m}Tc-DTPA	Technetium 99 metastable-diethylenetriaminepentaacetic acid
^{99m}Tc-MAG3	Technetium 99 metastable-mercaptoacetyltriglycine
ATN	Acute tubular necrosis
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
CI	Confidence interval
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease Epidemiology Collaboration
DGF	Delayed graft function
DRS	Dynamic renal scintigraphy
ERPF	Estimated renal plasma flow
ESRD	End-stage renal disease
GFR	Glomerular filtration rate

HKL	Hospital Kuala Lumpur
IQR	Interquartile range
KIM1	Kidney injury molecule 1
MAG3	Mercaptoacetyltriglycine
MRI	Magnetic resonance imaging
NGAL	Neutrophil gelatinase lipocalin
PET	Positron Emission Tomography
pmp	per million population
R20:3	Ratio of radiotracer counts in the graft at 20 minutes versus graft counts at 3 minutes
ROC	Receiver operator curve
ROI	Region of interest
ROS	Reactive oxygen species
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
SUV	Standardised uptake value
TISS	Tubular injury severity score

SYMBOLS

K^+	potassium
kg	kilogram
m^2	meter x meter
MBq	megabecquerel
mCi	millicurie
mg/dL	milligram/ decilitre
ml	millilitre
Na^+	sodium
μmol/L	micromole/ litre

ABSTRAK

Objektif. Penilaian tepat fungsi ginjal selepas pemindahan sangat penting kerana ia mempengaruhi rawatan pesakit dan graf prognosis. Walau bagaimanapun, penilaian fungsi ginjal pada tempoh masa yang awal selepas pemindahan adalah mencabar kerana ujian seperti serum kreatinin, output urin dan ultrasonografi mempunyai pelbagai kelemahan. Tujuan kajian ini adalah untuk menentukan sama ada parameter daripada ujian garis dasar MAG3 yang dilakukan dalam tempoh 72 jam selepas pemindahan dapat meramal graf yang mengalami fungsi tertunda (DGF) menggunakan titik penamat klinikal primer DGF. DGF ditakrifkan sebagai keperluan untuk menjalani dialisis dalam tempoh satu minggu selepas pemindahan. Pada masa yang sama, kajian ini turut menilai sama ada ujian garis dasar MAG3 dapat memberi nilai prognostik jangka pendek dengan menilai fungsi transplan pada 1 dan 3 bulan selepas transplantasi.

Kaedah. Ini adalah kajian retrospektif di mana semua pesakit pemindahan ginjal yang menjalani ujian MAG3 dalam tempoh 72 jam selepas transplan di antara tahun 2017-2019 di Hospital Kuala Lumpur dimasukkan dalam kajian ini. Dua parameter kualitatif; gred renogram dan *tubular injury severity score* (TISS) dan parameter kuantitatif; R20:3 dinilai. Tiga penilai berpengalaman akan menganalisa parameter skan secara rawak dan berasingan untuk menilai korelasi antara pemerhati. Data klinikal pesakit akan diteliti untuk menilai sama ada graf mengalami DGF, faktor risiko pesakit serta fungsi graf pada 1 dan 3 bulan selepas pemindahan.

Keputusan. Seramai 120 pesakit menjalani ujian MAG3 dalam tempoh 72 jam selepas pemindahan. 3 pesakit dikecualikan atas isu kehilangan data dan kegagalan transplan. 117 pesakit dimasukkan dalam kajian ini. Kadar kejadian DGF adalah 16.2% dalam kajian ini. Insiden DGF jauh lebih tinggi bagi penerima graf kadaver (53.6%) berbanding

dengan penerima graf hidup (4.5%). Ketiga-tiga parameter skan (gred renogram, skor TISS dan R20: 3) berbeza secara signifikan antara kumpulan mengalami DGF dan tidak mengalami DGF, $p < 0,05$ dan skor R20:3 mempunyai nilai (*area under curve, AUC*) yang tinggi iaitu 0.97. Korelasi antara pemerhati adalah sangat baik untuk gred renogram dan R20:3. Nilai pemotongan untuk R20: 3 adalah 1.31 dengan kepekaan 94.7% dan kekhususan 92.8% untuk ramalan DGF. Faktor risiko yang menyumbang secara signifikan kepada DGF adalah jenis transplan, ketidakserasian HLA, tempoh dialisis pra-transplantasi, tempoh masa iskemia sejuk dan tempoh masa anastomotik.

Kesimpulan. Ujian garis dasar MAG3 dapat menilai fungsi awal buah pinggang transplan secara tepat dan juga prognosis transplan pada bulan pertama dan ketiga selepas transplan. Gred renogram dan R20:3 harus disertakan dalam laporan skan MAG3 transplan kerana menunjukkan korelasi antara pemerhati yang baik. Memandangkan insiden fungsi transplan yang lemah lebih tinggi di kalangan penerima transplan kadaver, skan MAG3 secara rutin selepas transplantasi dapat menyumbang kepada rawatan golongan pesakit ini.

ABSTRACT

Objective. Accurate assessment of graft function in the post-transplant period is crucial as it influences patient management and graft prognostication. However, traditionally used modalities such as serum creatinine, urine output and ultrasonography are hampered by various drawbacks. This study sought to determine whether baseline DRS performed within 72 hours post-transplant could accurately depict graft function. This study chose the occurrence of delayed graft function (DGF), which is defined as the requirement for dialysis within the first week post-transplant, as the primary clinical end-point of graft function. Graft function at 1- and 3-months post-transplant were used as secondary end-points.

Subjects and Methods. This retrospective study enrolled all renal transplant recipients who underwent baseline DRS using MAG3 within 72 hours post-transplant between 2017 and 2019 in Hospital Kuala Lumpur. Two qualitative parameters, renogram grade and tubular injury severity score (TISS) and a quantitative parameter, R20:3, were evaluated. Three other experienced observers independently analysed a random selection of scans to assess interobserver agreement. Clinical data was scrutinised to determine whether patients developed DGF, their corresponding risk factors and graft function at 1- and 3- months.

Results. A total of 120 patients underwent DRS within 72 hours. Three patients were excluded due to loss of scan data and primary graft dysfunction. The remaining 117 patients were enrolled. The overall incidence of DGF was 16.2%, with a significantly higher incidence amongst cadaveric graft recipients (53.6%) compared to living graft recipients (4.5%). Renogram grade ≥ 2, TISS ≥ 4 and R20:3 > 1.31 significantly predicted DGF, $p<0.05$ with high AUC for R20:3 of 0.97. The cut-off value for R20:3

was 1.31 with a sensitivity of 94.7% and specificity of 92.8% for the prediction of DGF. Grafts with parameters above the cut-offs showed significantly worse GFR at 1- and 3-months post-transplant. Both renogram grade and R20:3 showed excellent interobserver correlation. After adjustment for various donor and recipient factors, longer cold ischaemic times significantly contributed to DGF.

Conclusion. Baseline DRS was able to depict early graft function and prognosticate grafts at 1- and 3-months post-transplant. Renogram grade and R20:3, which exhibit excellent interobserver correlation, should be included in the reporting of post-transplant DRS. Due to the greater incidence of impaired graft function in cadaveric graft recipients, routine baseline DRS could provide added value in this population.

INTRODUCTION

Renal transplant is the management of choice in patients with end-stage renal disease. Various reports demonstrate a reduction in mortality (Reese *et al.*, 2015) and improvement in quality of life (Kostro *et al.*, 2016) among transplant recipients compared to patients on dialysis. A local study of renal transplant recipients established that renal transplant is more cost-effective than haemodialysis with significant improvement in quality of life (Bavanandan *et al.*, 2015).

The rates of renal transplantation in our country have remained largely stagnant in the past decade, ranging from 3-5 per million population (pmp) annually from 2007-2016 (Malaysian Society of Nephrology, 2018). The low rates are primarily due to a paucity of donor kidneys. However, the usage of marginal donors (donors with suboptimal renal function or medical co-morbidities) and improvement in immune-suppressive medications enabling successful transplants amongst immunologically incompatible donor-recipients; would be expected to propel the number of local transplants in the foreseeable future.

Locally, the majority of renal transplants are performed at two major transplant centres; Hospital Kuala Lumpur (HKL), where the study takes place; and Hospital Selayang. Smaller numbers of transplantation are performed in university and private hospitals throughout the country.

In the early post-transplant setting, evaluation of graft function is essential to determine further management and outcome of the graft. The assessment of allograft function typically uses a combination of clinical, laboratory and imaging modalities such as urine output, serum creatinine and ultrasonography. However, these modalities have various limitations in reflecting graft function, particularly in the early post-transplant

period (Kim *et al.*, 2019, Khater and Khauli, 2013, and Brown *et al.*, 2000). Herein lies the potential role of dynamic renal scintigraphy (DRS). In DRS, intravenously injected radiotracers are eliminated by the graft, imparting data on perfusion, excretion and wash out.

Due to various confounders and difficulties in interpreting serum creatinine and urine output in the early post-transplant period, this study uses the clinical end-point of delayed graft function (DGF) as a marker of poor graft function. In the literature, there are various definitions of DGF, including creatinine-based and dialysis-based definitions (Mallon *et al.*, 2013). However, the requirement for dialysis within seven days post-transplant, which is the definition used in this study, is the most practical and commonly used definition of DGF (Mallon *et al.*, 2013). Grafts with DGF are at an increased risk of acute rejection and long-term graft loss. Surprisingly, despite advances in the postoperative management of renal transplants and immune-suppressive medications, the rates of DGF worldwide have remained unchanged (Nashan, Abbud-Filho and Citterio, 2016). However, there are no published data on the incidence rates of DGF in this country.

Baseline post-transplant DRS have been performed in many centres internationally, usually within 72 hours post-transplant using either Tc^{99m} diethylenetriaminepentaacetic acid (DTPA) or Tc^{99m} mercaptoacetyltriglycine (MAG3). However, its exact utility and place in the algorithm of management are yet unclear, and current transplant guidelines by the Kidney Disease Improving Global Outcomes (KDIGO) and European Association of Urology (EAU) have not included renal scintigraphy as a tool for functional assessment post-transplant. However, the American College of Radiology (ACR) Appropriateness Use Criteria has acknowledged MAG3 as

a complementary tool (along with doppler ultrasonography) in the assessment of transplant dysfunction (Taffel *et al.*, 2017).

The various studies performed on DRS in the post-transplant period also markedly differ in terms of population demographics, end points, protocol and cut-off values, hindering meaningful comparisons and application in our local setting (Stevens *et al.*, 2001, Heaf and Iversen, 2000, Russell *et al.*, 2000 and Guignard, Mourad and Mariano-Goulart, 2011). Per our institutional protocol, DRS is routinely performed using MAG3 in all renal transplant recipients within 72 hours post-transplant.

Hence, this research aims to determine whether qualitative and quantitative parameters derived from DRS using MAG3 can be utilized to assess graft function. In addition to that, baseline DRS using MAG3 is also evaluated for its' short-term prognostic value by assessing the grafts' GFR at 1- and 3-months post-transplant. This research also aims to provide institutional data on the incidence and risk factors of DGF among our population. It is hoped that this study would rationalise the use of DRS in the post-transplant period and delineate useful parameters for reporting.

LITERATURE

REVIEW

2.1 OVERVIEW OF RENAL FAILURE AND RENAL REPLACEMENT THERAPIES

The kidney's primary function is the excretion of wastes and maintenance of electrolyte and acid-base homeostasis by means of glomerular filtration, tubular secretion and tubular reabsorption. In addition to that, the kidneys secrete erythropoietin for erythrogenesis, renin for blood pressure and osmolarity regulation, and play a role in calcium absorption via the activation of vitamin D (Koushanpour and Kriz, 2013).

Renal failure occurs when the kidney has lost its' ability to filter waste products resulting in the accumulation of toxic wastes. This is manifested clinically by oliguria/ anuria, fluid overload and anaemia, and biochemically by a reduction in GFR, acidosis and electrolyte imbalance. Renal failure can be broadly classified into acute and chronic kidney disease based on the onset of the disease (Ferenbach and Bonventre, 2016).

Acute kidney injury occurs due to an acute insult, e.g. sepsis, nephrotoxic drugs, hypovolemia and glomerulonephritis. Conversely, chronic kidney disease (CKD) is characterised by a gradual, progressive decline in the GFR. The major causes of CKD include diabetes mellitus and hypertension. Diabetes mellitus is the lead cause of CKD and end-stage renal disease (ESRD). Our local data supports this, whereby an overwhelming 65% of ESRD is attributed to diabetes mellitus (Malaysian Society of Nephrology, 2018).

CKD can be further classified based on its' aetiology, GFR, and albuminuria (Levin *et al.*, 2013). Based on the GFR, CKD can be classified into 5 stages (Table 2.1.1).

Table 2.1.1 Stages of chronic kidney disease based on GFR

Stages of Chronic Kidney Disease	**Glomerular Filtration Rate (ml/min/1.73m^2)**
Stage 1	≥90
Stage 2	60-89
Stage 3	30-59
Stage 4	15-29
Renal Failure	<15

(Source: Adapted from KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease (Levin *et al.*, 2013). Available at https://kdigo.org/guidelines/ckd-evaluation-and-management/. Accessed on 1st December 2020)

Worldwide, the prevalence of CKD is estimated to be 9.1% of the global population (Bikbov *et al.*, 2020). In Malaysia, the prevalence of CKD is 9.07%, whilst the prevalence of ESRD is at 0.36% of the total population (Ismail *et al.*, 2019). Patients with ESRD would require renal replacement therapies, which include haemodialysis, peritoneal dialysis and renal transplant. Globally, the selection of renal replacement therapy modalities varies widely based upon cost-effectiveness, access to healthcare, local health system and policies, availability of expertise and facilities, and cultural acceptance. Locally, haemodialysis remains the most common modality of renal replacement therapy, while renal transplant was the least common. The prevalence of haemodialysis was 1159 per million population (pmp), peritoneal dialysis was 127 pmp and renal transplant was 59 pmp in 2016 (Malaysian Society of Nephrology, 2018).

2.2 RENAL TRANSPLANTATION

Renal transplantation is the recommended treatment modality for patients with ESRD due to improved survival, enhanced quality of life and better cost-effectiveness compared to other renal replacement modalities (Chadban *et al.*, 2020).

Worldwide, transplant rates differ between nations whereby the highest rates are seen in Nordic, and some European countries of up to 57–72% of ESRD patients whilst lower rates of less than 10% are seen in Asian and Eastern European countries (Robinson *et al.*, 2016). New transplant rates are low in Malaysia and have primarily remained static at 3-5 pmp annually from 2007-2016 (Malaysian Society of Nephrology, 2018).

Renal transplant grafts can generally be divided into cadaveric and living grafts. Prior to renal transplant, potential transplant recipients undergo extensive medical and psychosocial evaluation. Amongst factors taken into consideration are the patient's age, co-morbidities, duration of ESRD, primary renal disease, urological anatomy and abnormalities, cardiac status and probability of adherence to post-transplant management and follow up (Chadban *et al.*, 2020). Eligible potential recipients are either placed on the national transplant waiting list (for cadaveric grafts) or matched for a living related (e.g. sibling, parent, offspring) or non-related (e.g. spouse) donor graft.

Potential living donors are evaluated for pre-donation renal function and anatomy, co-morbidities, blood group and HLA compatibility, cardiac status and psychosocial state.

The process of renal transplantation begins with a nephrectomy from the donor. The renal graft, along with its' renal artery, vein and ureter, is harvested from the donor, then flushed and placed in cold preservation fluid. It is then transported in ice to the

transplant centre. Due to technical reasons, the graft is typically placed in the right iliac fossa of the recipient. The graft's renal artery is typically anastomosed to the recipient's internal or external iliac artery, and the graft's renal vein is anastomosed to the recipient's external iliac vein. The graft's ureter is then usually directly anastomosed to the recipient's urinary bladder (Barry, 2007).

Rigorous peri-operative management significantly improves the outcome of the graft (Taber *et al.*, 2013). Medical management begins with induction therapy of the recipient with immune-suppressive medications before surgery to reduce the risk of rejection. This is accomplished by inhibiting the recipient's T cell response to the graft antigen. Commonly used induction agents include interleukin-2 receptor α chain (e.g. basiliximab) and lymphocyte depleting agents (e.g. anti-thymocyte globulin). In the immediate postoperative period, maintenance immune-suppressive medications are initiated. These are usually a combination of calcineurin inhibitors (e.g. tacrolimus, cyclosporin A), anti-proliferative agents (e.g. mycophenolate mofetil) and corticosteroids (Kidney Disease: Improving Global Outcomes (KDIGO) Work Group, 2009). The patient is discharged with these medications with frequent follow up to monitor therapeutic drug levels, medication side effects and graft function.

Our local data shows that graft survival is fairly good, particularly amongst live grafts. Living donor graft survivals were 94%, 92% and 86% for 1 year, 3 years and 5 years post-transplant, whereas cadaveric graft survivals were 84% and 71% for 1 year and 5 years post-transplant (Malaysian Society of Nephrology, 2018).

2.3 COMPLICATIONS OF RENAL TRANSPLANTATION

Complications of renal transplant can be categorised into surgical and medical complications.

Surgical complications are as follows:

- urological complications (e.g. urinary leakage, ureteric fistula, ureteric stricture and vesicoureteric reflux),
- vascular complications (e.g. renal artery and renal vein stenosis),
- lymphocele,
- perirenal haematoma and
- wound infection (Breda *et al.*, 2020).

Medical complications are as follows:

- immunological complications (e.g. hyperacute rejection, acute T cell antibody-mediated rejection),
- acute tubular necrosis,
- calcineurin-inhibitor nephropathy,
- new-onset diabetes after transplantation and
- post-transplant lymphoproliferative disorder.

Medical complications can be further characterised based on the time of onset. As this research focuses on the early post-transplant period, of particular interest are hyperacute and acute rejections as well as acute tubular necrosis. In the rare hyperacute rejection, major ABO or HLA incompatibility results in the recipient's pre-existing antibodies attacking the graft's antigen and inevitably results in graft failure. Acute

rejection can be further classified pathologically into acute T-cell mediated rejection or acute antibody-mediated rejection based on the Banff Classification of Allograft Pathology (Roufosse *et al.*, 2018). The incidence of acute rejections is reducing in trend thanks to newer immune-suppressive medications. In the 1990s, the rate of acute rejections was reported to be 32.5% (Pallardó Mateu *et al.*, 2004), reducing to 14.9% in the mid-2000s (Salcedo-Herrera *et al.*, 2019). Patients with acute rejection episodes tend to have worse renal function and lower graft survival, and this worsens with increasing number of rejection episodes.

Acute tubular necrosis (ATN) usually occurs by 48-72 hours to a week post-transplant. The initiating event in ATN is tissue hypoxia, which results in renal tubular epithelial cell injury and production of chemokines and cytokines, leading to activation of the inflammatory cascade. This explains why ATN is more common in cadaveric grafts compared to live grafts, as cadaveric grafts typically have a prolonged time interval between organ harvest and tissue reperfusion. In addition to that, haemodynamic changes due to the altered balance in production and response to vasoconstrictors/ vasodilators lead to an increase in renal vascular resistance and a further drop in GFR (Basile, Anderson and Sutton, 2011).

2.4 DELAYED GRAFT FUNCTION

Delayed graft function (DGF) is essentially the failure of the graft to function optimally in the early post-transplant setting. DGF has been reported to be an important prognostic indicator of short and long term graft outcomes and contributes to an extended duration of hospitalisation, increased patient morbidity and is associated with higher rates of graft loss (Gill *et al.*, 2016). DGF has also been associated with a higher risk of developing acute rejection compared with grafts that demonstrated immediate graft function (Wu *et al.*, 2015).

There are multiple proposed definitions of DGF, encompassing creatinine and dialysis-based definitions that include failure of serum creatinine to fall >10% on 3 consecutive days in the first week, elevated serum creatinine at post-op day 7 or 10 of >2.5mg/dL (221µmol/L), creatinine reduction ratio (ratio of creatinine of day 1 over creatinine of day 2 post-transplant) <30% and dialysis requirements within the first 7 or 10 days post-transplant. A study by Mallon *et al.*, 2013 showed similar prognostic ability for graft outcome irrespective of the definitions used. However, the authors conclude that the definition of dialysis requirement within the first week post-transplant to be the most practical with added clinical significance in terms of reflection of the duration of hospitalization. Hence, this definition was chosen for the current study.

The incidence of DGF varies significantly between study populations but is generally more common in cadaveric grafts. The incidence ranges from 1.6% to 13.7% amongst grafts from live donors (Hellegering *et al.*, 2013 and Park *et al.*, 2012); and from 2% to 50% amongst grafts from cadaveric donors (Perico *et al.*, 2004).

The pathophysiology of DGF is an interplay of ischaemic-reperfusion injury and immune-modulated injury.

Upon procurement of the graft, a period of ischaemia ensues, which affects renal tubular epithelial cells, endothelial cells and vascular smooth muscles. In the renal tubular epithelial cells, hypoxia triggers anaerobic glycolysis and cellular acidosis. Immersion of the graft in cold preservation fluid aims to hamper this process. Once adenosine triphosphate (ATP) is depleted intracellularly, there is derangement of the Na^{+}/K^{+} ATPase pump, which leads to cellular oedema. In addition to that, mitochondria within the epithelial cells produce reactive oxygen species (ROS). These processes lead to epithelial cell injury. In the vascular endothelial cells, the production of ROS causes endothelial membrane injury and fibrin deposition. Hypoxia also causes the vascular smooth muscles to vasoconstrict (Siedlecki, Irish and Brennan, 2011).

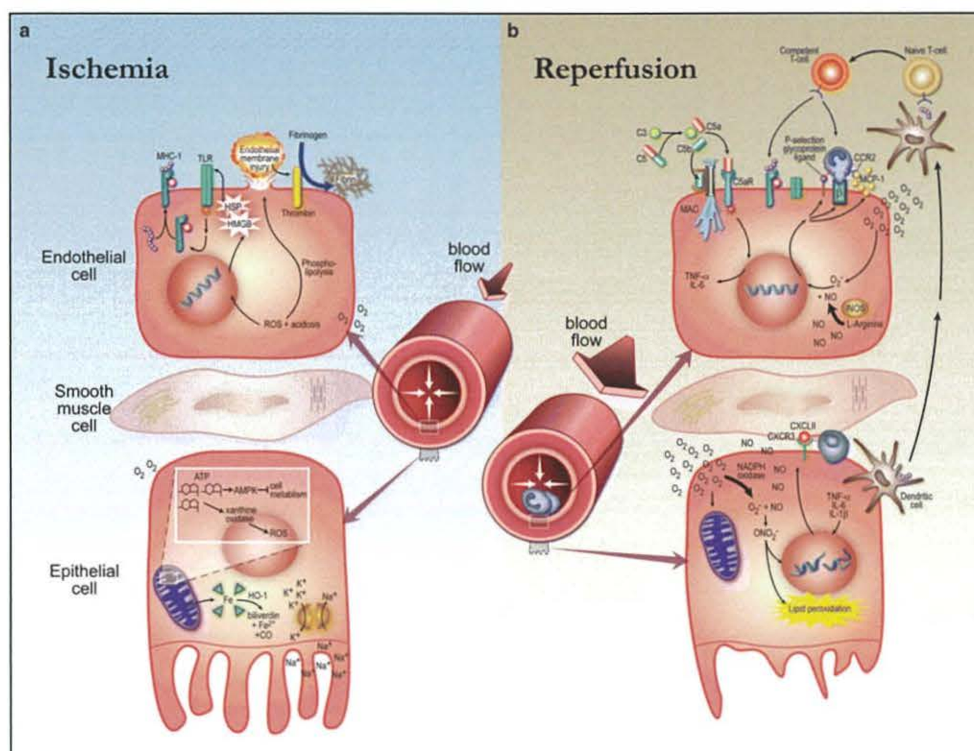

Figure 2.4.1 Pathophysiology of delayed graft function
[Source: Delayed Graft Function in the Kidney Transplant. (Siedlecki, Irish and Brennan, 2011)]

Upon anastomosis and reperfusion of the graft, there is a restoration of aerobic metabolism. However, rapid generation of ROS in tubular epithelial cells leads to lipid peroxidation and cellular apoptosis. Inflammatory chemokines and cytokines released as a response to injury activate the inflammatory cascade, which prolongs and worsens tissue damage. Damaged vascular endothelial cells become activated, secrete chemokines and express adhesion molecules. This causes chemo-attraction of neutrophils and adherence/ plugging of neutrophils on the endothelial cell surface (Perico *et al.*, 2004).

Time is of the essence in renal transplantation, as it is related to tissue hypoxia and hence graft survival. Three clinical parameters, cold ischaemia time, warm ischaemia and anastomotic time, have been widely used to measure the amount of time taken in different phases of the transplant. Prolonged cold and warm ischaemia times have been associated with an increased risk of delayed graft function (Kamińska *et al.*, 2016).

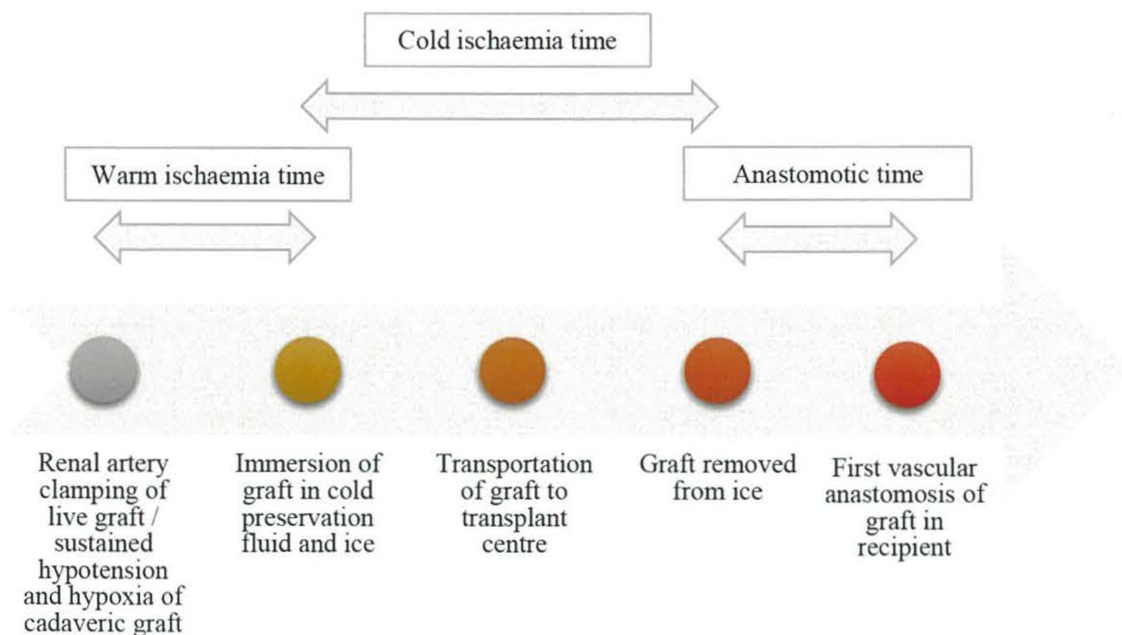

Figure 2.4.2 Definitions of cold, warm ischaemia and anastomotic time

Warm ischaemia time is defined as the time taken from the cessation of graft perfusion from the donor's circulation until immersion in cold preservation fluid. Cold ischaemia time is defined as the time taken from the onset when the graft is immersed in cold preservation fluid until the graft is removed from ice. Anastomotic time is the time when the graft is removed from ice to reperfusion, i.e. vascular anastomosis in the recipient.

Other factors found to increase the risk of DGF are cadaveric grafts (vs live grafts), advanced donor age, HLA mismatch, recipient obesity and prolonged dialysis vintage (duration of pre-transplant dialysis).

2.5 MODALITIES TO ASSESS RENAL GRAFT AND ITS FUNCTION

Modalities commonly used to monitor graft function include urine output, serum creatinine, and ultrasonography. For the assessment of graft pathology, the gold standard is graft biopsy.

Urine output is a simple clinical tool to evaluate early graft function. The timing and amount of urine produced in the postoperative period have been reported to be associated with graft outcomes. However, the amount of urine produced is also dependent on intravenous fluids and diuretics administered in the peri and postoperative periods, as well as the type of graft. Live grafts tend to produce more urine initially compared to cadaveric grafts due to activation of the renin-angiotensin-aldosterone axis in the latter as a result of a longer cold ischaemia time (Kim *et al.*, 2019). In addition to that, polyuria is common in the post-transplant period with significant variation, stabilising only at 1-month post-transplant. The reported average urine produced are 10.06±5.89L at 24 hours and 5.45±3.05L at 48 hours post-transplant (Khosroshahi *et al.*, 2007). Due to multiple confounding variables mentioned above, the cut-off value of appropriate amount of urine has yet to be established.

Serum creatinine is a convenient and widely available tool used in the postoperative period. Failure of serum creatinine level to reduce post-transplant often reflect poor renal function. The creatinine reduction ratio (percentage of reduction of serum creatinine by day 2 post-transplant compared to day 1) <30% is predictive of patients with DGF (Govani *et al.*, 2002). However, there are limitations in the usage of serum creatinine as creatinine metabolism differs from each individual depending on muscle mass, age and gender. The interpretation of serum creatinine in the post-transplant setting is made even more challenging when calcineurin inhibitors (resulting

in nephrotoxicity) and corticosteroids (resulting in catabolism) usage can affect serum creatinine (Poggio, Batty and Flechner, 2007). Cases of raised creatinine levels in situations devoid of renal parenchymal pathology have been reported in ureteral obstruction, lymphocele (causing a mass effect to urinary outflow), hypovolemia, infection and hyperosmolarity (Khater and Khauli, 2013). Changes in serum creatinine could also lag behind by 24 hours after a deterioration of renal function. Renal scintigraphy has shown to detect renal functional change 24-48 hours before the change in serum creatinine becomes apparent (Heaf and Iversen, 2000 and Yazici *et al.*, 2013). An experimental study on mice induced with renal ischaemia-reperfusion injury found that significant impairment of renal function has been observed by DRS within 5 hours of injury when only a mild increment in serum urea and creatinine is seen (Roberts *et al.*, 2007).

Ultrasonography of the graft is a helpful screening tool for the early evaluation of graft perfusion and common surgical complications. Resistive and pulsatility indices obtained by Doppler ultrasound US and findings such as increased/decreased renal parenchymal echogenicity and diminished corticomedullary differentiation have been used as markers for graft dysfunction. However, ultrasonography is operator dependant, with its negative predictive value of only 17-50% (Brown *et al.*, 2000). Resistive indices might be normal despite poorly functioning grafts in the early postoperative period (Aschwanden *et al.*, 2009). One study compared resistive indices from Doppler ultrasonography with renal scintigraphy found that renal scintigraphy was superior to ultrasonography in differentiating between well-functioning and reduced function grafts (Yazici *et al.*, 2013). In addition to that, another study showed that renal scintigraphy was superior to ultrasonography for the detection of biopsy-proven acute rejection (Lee *et al.*, 2018).

Other imaging modalities which are under investigation are contrast-enhanced ultrasonography, diffusion-weighted magnetic resonance imaging (MRI) and blood oxygen level-dependent (BOLD) MRI. However, they are not yet widely available, and robust data on these modalities are still lacking.

Graft biopsy is obtained in patients with DGF if presence of a persistent lack of clinical improvement (usually by 7-10 days), with the main objective being the exclusion of acute graft rejection. However, graft biopsy is not without complications; hence the decision for biopsy should be made judiciously. Information on graft function is a pivotal factor in guiding clinician's decision for biopsy. A large cohort study of 2514 biopsies showed that major complications occurred in 1.9% of biopsies, defined by the need for hospitalisation or active intervention (Morgan *et al.*, 2016). The risk of minor complications such as haematuria, haematoma (not requiring intervention) and arteriovenous fistula (AVF) was estimated to be 17%. Post biopsy, the pathology samples are then reported based on the Banff classification. The Banff classification uses a scoring system of various pathology findings such as interstitial inflammation, tubulitis and intimal arteritis to divide grafts into normal graft, acute antibody-mediated rejection, acute T-cell mediated rejection, suspicious for T cell-mediated rejection (borderline changes) and others (e.g. calcineurin-inhibitor nephrotoxicity, BK virus nephropathy, etc.) (Roufosse *et al.*, 2018). Although graft biopsy can provide information on the underlying pathological process of the graft, it does not provide any functional information, and the severity of graft pathology does not always correlate with the severity of graft injury when compared to transcript levels of acute kidney injury genes (Obeidat *et al.*, 2011).

2.6 POST-TRANSPLANT DYNAMIC RENAL SCINTIGRAPHY

It is clear from the preceding section that urine output, serum creatinine and ultrasonography may not reliably provide an accurate picture of renal graft function, particularly in the post-operative period. As dynamic renal scintigraphy (DRS) allows direct visualisation of renal graft perfusion and function, it could be a valuable adjunct to complement the information from other modalities.

DRS can generally be divided into three phases: flow phase (reflecting graft perfusion), excretion phase (transit of tracer from the nephrons into the pelvicalyceal system) and clearance phase (washout of tracer from the pelvicalyceal system into the urinary bladder).

Baseline DRS is defined as a DRS performed early post-transplant, ideally within the first 24 hours. However, the patient's post-operative stabilization usually takes precedence. Most studies on baseline DRS are performed from 24-96 hours post-transplantation, with the majority of studies performing DRS within the first 48-72 hours (Volkan-Salanci and Erbas, 2021, Kim *et al.*, 2019 and Benjamens *et al.*, 2018). In terms of practicality, the timing of baseline DRS is influenced by factors such as patient's condition and stability for transportation, availability of on-call imaging services and logistics. In the study's location, it is an institutional protocol for all renal transplant recipients to undergo a baseline DRS within 72 hours post-transplant.

The commonly used radiotracers for DRS are Technetium-99m-mercaptoacetyltriglycine (^{99m}Tc-MAG3) and Technetium-99m-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA). ^{99m}Tc-DTPA is cleared by glomerular filtration, whereas ^{99m}Tc-MAG3 is secreted actively into the renal tubules. ^{99m}Tc-MAG3 is the

recommended radiotracer for patients with impaired renal function due to its higher plasma extraction rate (Taylor *et al.*, 2018). This is because tubular secretion is responsible for up to 80% of renal plasma clearance. In this study's location, ^{99m}Tc-MAG3 was used for DRS.

Various qualitative and quantitative parameters derived from DRS have been described in the literature. Depending on the phases of the scintigraphy from which they are derived, parameters that have been studied amongst renal transplant patients can also be broadly divided into perfusion (flow) and parenchymal (excretion and washout) parameters.

Perfusion parameters examine the vascular flow of the radiotracer bolus to the graft. The most commonly used perfusion parameters are Hilson's perfusion index and Kirchner's kidney/ aorta ratio. Studies looking into these angiographic parameters often utilise a higher dose of the radiotracer (e.g. 10mCi compared to the standard 1-5mCi for renal scintigraphy); in order to minimise noise during the rapid frame rate of the flow phase. This is not routinely practised in most local nuclear medicine centres as it would incur higher radiation exposure to patients. These parameters are also susceptible to technical errors (e.g. fragmented bolus, dose extravasation), which can invalidate these indices.

Parenchymal parameters evaluate tracer extraction, excretion and clearance. Assessment of the shape of the renogram curve was initially proposed by Heaf and Iversen using a numerical scoring system from 0 to 5 whereby a higher score indicated worse tracer extraction and washout. They found that a score of ≥2 was found to be associated with DGF (Heaf and Iversen, 2000). Other studies using various modifications of the initial scoring system found that a worse renogram score was able to predict a

longer duration of DGF (Stan Benjamens *et al.*, 2018) and was associated with rates of acute rejection (Park *et al.*, 2013).

The tubular injury severity score (TISS) examines tracer extraction of the graft whereby adequate tracer extraction is postulated to reflect tubular integrity. It was first described in 1997 based on the assessment of a cortical uptake phase image (Tulchinsky *et al.*, 1997). This cortical uptake phase image is a compressed image of 75 seconds, taken after 1-minute post-injection of the radiotracer. This image is scored visually with a score of 1 to 6 based on the relative uptake of the graft versus the background and surrounding vessels, with a higher score indicating lower radiotracer uptake. The original investigators tested the TISS in patients with clinical graft dysfunction in the early postoperative period and found that graft recovery was seen in grafts with a score of < 4, while those with a score of 5 or 6 suffered from graft loss (Tulchinsky *et al.*, 1997). A modification of this score using a *cortical uptake phase* image of 150 seconds in DRS performed within 72 hours post-transplant found that a score of ≥ 4 significantly increased the risk of transplant failure at 6 months (Guignard, Mourad and Mariano-Goulart, 2011).

The background-corrected ratio of radiotracer counts in the graft at 20 minutes versus 3 minutes (R20:3) is a measure of cortical tracer retention. Good functioning grafts are able to eliminate the tracer promptly from the parenchyma into the pelvicalyceal system with minimal tracer activity remaining by 20 minutes of the study. However, poorly functioning grafts would demonstrate delayed tracer excretion, and higher cortical graft counts at 20 minutes resulting in higher R20:3 values. R20:3 has been found to correlate with clinically assessed severity of acute tubular necrosis and acute rejection using a cut-off value of 0.8 using ^{99m}Tc DTPA (Li *et al.*, 1994). Another study using ^{99m}Tc DTPA found that a cut-off value of 0.85 had a sensitivity of 93% and

a specificity of 73% for the diagnosis of acute rejection (Yazici *et al.*, 2013). However, a study using ^{99m}Tc MAG3 data on cadaveric grafts found that a cut-off value of 1.4 was more useful in predicting cadaveric graft survival (Russell *et al.*, 2000).

Unfortunately, other reported parameters which have been observed by other researchers to correlate with graft function require alteration in institutional acquisition protocol or additional software for analysis. These include the tubular function slope, which requires analysis of the gradient of the renogram curve, mean transit time requires deconvolution analysis of the renogram, uptake capacity requires imaging of the urinary bladder/ urine bag (in catheterised patients), estimated renal plasma flow (ERPF) requires plasma sampling and the elimination index requires urine collection and measurement of radiotracer counts in urine. Table 2.6.1 lists the various parameters which have been studied in renal transplant recipients.

Despite the wealth of research on post-transplant DRS, there are wide variations in terms of type and dose of radiotracer, imaging procedure, regions of interests (ROI), parameter cut-offs, study end-points, timing, peri-operative management and population demographics. In terms of demographics, studies that have been published thus far are predominantly evaluating cadaveric graft function. The prevalence of cadaveric grafts being studied are 85%, 68%, 71% and 96%, respectively (Stevens *et al.*, 2001, Heaf and Iversen, 2000, Russell *et al.*, 2000 and Guignard, Mourad and Mariano-Goulart, 2011). In our local setting, 11.0 - 47.2% of renal transplants were derived from cadaveric donors from 2012-2016 (Malaysian Society of Nephrology, 2018).

Table 2.6.1 Common parameters in post-transplant renal scintigraphy

Perfusion Parameters	
Hilson's Perfusion Index	Quantitative
Kirchner's Kidney/Aorta Ratio	Quantitative
Parenchymal Parameters	
Renogram curve	Qualitative
Tubular injury severity score (TISS)	Qualitative
R20:3	Quantitative
Tubular function slope	Quantitative
Uptake Capacity	Quantitative
Mean transit time	Quantitative
Elimination index	Quantitative
Estimated renal plasma flow (ERPF)	Quantitative

Hence data from previous studies cannot be extrapolated to our local setting. The choice of parameters and cut-off values need to be determined in each institution. This study examines qualitative parameters (renogram curve and TISS score) and the quantitative parameter R20:3.

DRS has also been used in evaluating transplant complications such as urinary obstruction and urinary leaks. Other nuclear medicine techniques that are used in assessing renal transplants include ^{51}Cr- ethylenediaminetetraacetic acid (EDTA) for calculation of glomerular filtration rate, ^{99m}Tc dimercaptosuccinic acid (DMSA) in infection/ scarring imaging and ^{18}F-fluorodeoxyglucose (FDG) Positron Emission Tomography-Computed Tomography (PET-CT) in imaging of graft rejection. (Volkan-Salanci and Erbas, 2021).

2.7 MANAGEMENT OF DELAYED GRAFT FUNCTION

The management of DGF is mainly supportive as no definitive treatment is available at the moment. Judicious fluid management to regulate blood pressure and graft perfusion, supportive dialysis, electrolyte management, and monitoring of plasma levels of calcineurin-inhibitors are the cornerstones of current DGF management. DGF can resolve resulting in cessation of dialysis dependence. The resolution of DGF depends on rapid treatment of its' underlying aetiology and graft biopsies are usually undertaken to exclude acute rejection. However, accurate timing of renal graft biopsy; balancing its benefit versus risk is also an essential component of DGF management.

There has also been emerging research on the adjustments and timing of initial immune-suppressive medications as well as novel therapies in the early postoperative period to reduce the risk/ duration of DGF. The use of everolimus with low dose calcineurin inhibitors, substituting calcineurin inhibitors altogether with sirolimus, and the use of monoclonal antibody alemtuzumab are examples of treatments undergoing evaluation (Nashan, Abbud-Filho and Citterio, 2016). Hence, accurate assessment of graft function is crucial to stratify patients for treatment when these new therapies are made available.