LIVER TRANSPLANTATION IN PATIENTS OVER 60 YEARS IN JOHANNESBURG, SOUTH AFRICA: A RETROSPECTIVE REVIEW OF RISK FACTORS, SURVIVAL OUTCOMES AND IMMUNOSUPPRESSION.

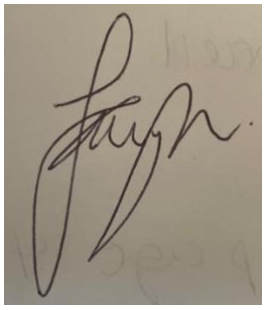
Ahmad Ismail Tayob

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Medicine (Internal Medicine)

Johannesburg, 2021

DECLARATION

I, Ahmad Ismail Tayob, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Signed on the 24th day of April 2022 in Johannesburg

For my parents,

Ismail and Zubeda Tayob

ABSTRACT

Background.

Evidence on how to tailor immunosuppression therapy to account for the unique risks and altered immune mechanisms in older liver transplant recipients is lacking.

Methods.

Data were collected from all patients aged >=60 years undergoing liver transplantation for the first time, between 2004 and 2019. We assessed survival outcomes and rejection rates, and identified demographic and clinical predictors of these outcomes. We compared survival outcomes and immunosuppression usage between an earlier and later transplant era, to establish whether there was an improvement in survival over time, and whether prescription patterns changed as evidence emerged that older patients may require less immunosuppression.

Results.

The median age of the patients was 64 years, and the most common cause of liver disease was alcoholic steatohepatitis and non-alcoholic steatohepatitis. The mean MELD scores were 16,7 (SD 6,9) and the mean DRI was 2.0 (SD 0,4). Patient survival rates at 1, 3, and 5 years were 75%, 69%, and 64%, respectively. Infections were the most common cause of death. 5% of patients developed ACR.

There was an increase in the risk of death for GFR categories 3a-5 unadjusted (HR 2.83 (95% CI 1.32-6.08)) and adjusted for sex, MELD score, and DRI (HR 3.47 (95% CI 1.547.84)) but no association between diabetes (HR 1.21 (95% CI 0.62-2.36)) or coronary artery disease (HR 1.46 (95% CI 0.70-3.02)) and recipient survival. Prednisone + tacrolimus + mycophenolate mofotil was the most common initial maintenance regimen. In the later

transplant era, the mean prednisone dose was lower (19.8 mg verses 13.2 mg, p=0.023) and dual immunosuppression was favoured over triple (p=0.070).

Conclusions.

Survival outcomes in older patients are impacted by renal impairment but not by coronary artery disease or diabetes mellitus. The average DRI is high due to organ shortages in South Africa, and this impacts survival outcomes. Immunosuppression prescribing patterns have adapted to account for altered immune mechanisms.

Abbreviations: MELD (Model for End Stage Liver Disease), DRI (Donor Risk Index), SD (Standard Deviation), GFR (Glomerular Filtration Rate), CI (confidence interval), HR (Hazrad Ratio)

ACKNOWLEDGEMENTS

This research report would not have been possible without the assistance of the following people. I would like to acknowledge

- Professor Adam Mahomed and Prof Jean Botha - *for their guidance and support as supervisors*
- Dr Bilal Bobat – *for guidance with selection of a topic and encouragement along the way*
- Dr Petra Gaylard – *for assistance and patience with statistical analysis*
- Dr Nabihah Tayob – *for advice on statistical analysis*
- Dr June Fabian and Heather Maher – *for assistance with navigation of this process and the database as well as their invaluable insights*
- Dr Charlene Fotso – *for assistance with collection of data*
- Dr Arpan Patel – *for reviewing the article for journal submission*

PRESENTATIONS ARISING FROM THIS PROJECT

1. Gastroenterology CPD Journal Club, 2020.

   Topic: Immunosenescence and the Gastro/Hepatologist

   Role: Presenter

   See Appendix J for CPD record of presentation

2. Transplantation Seminar at The Royal Free Hospital, London

   Topic: Living Donor Liver Transplantation from a HIV positive mother to her HIV negative child in South Africa

   Role: Organizer in co-ordination with Dr Nolitha Morare and Dr Dinesh Sharma at Royal Free Hospital

   See Appendix J for Seminar poster advertisement

INDEX

CHAPTER 1: PROTOCOL AND EXTENDED REVIEW OF THE LITERATURE

1. BACKGROUND

1.1. Introduction

1.2. Elderly Recipient Survival Outcomes

1.3. Model for End-stage Liver Disease

1.4. Donor Risk Index

1.5. Pre-operative Risk Stratification

1.5.1. Establishing Risks in the Elderly

1.5.2. Cardiac Risks in the Elderly

1.6. Immunosenescence, Immune Suppression Regimens and Acute Cellular Rejection

1.7. Immune Suppression Therapies

1.7.1. Mechanisms of Action

1.7.2. Induction

1.7.3. Maintenance

1.8. The South African Context

1.9. Summary

2. AIM

3. OBJECTIVES

3.1. Primary Objectives

3.2. Secondary Objectives

4. METHODOLOGY

4.1. Study Design

4.2. Data Collection

5. STATISTICAL ANALYSIS

6. ETHICS

7. TIMING

8. FUNDING

9. POTENTIAL LIMITATIONS

10. REFERENCES

CHAPTER 2: PROPOSED MANUSCRIPT

AUTHORSHIP

ABBREVIATIONS

ABSTRACT

MAIN BODY TEXT

Introduction

Materials and Methods

Results

Discussion

Conclusion **CHAPTER 3:**

APPENDIX

APPENDIX A: Data Collection sheet

APPENDIX B: WDGMC Adult Liver Transplant Protocol

APPENDIX C: Risk scores

APPENDIX D: Descriptive Results

APPENDIX E: Immunosuppression results

APPENDIX F: Patient and Graft survival results

APPENDIX G: Ethics clearance certificate

APPENDIX H: Plagiarism Declaration

APPENDIX I: Turnitin Assessment

APPENDIX J: Presentations arising from this project

APPENDIX K: Journal submission guidelines

LIST OF FIGURES

FIGURE 1: Kaplan-Meier curves depicting patient, graft and ACR-free survival amongst liver transplant recipients >= 60 years in Johannesburg, South Africa

FIGURE 2: Kaplan Meier curves depicting a comparison of survival rates between Era 1 (2004-2013) and Era 2 (2013-2019)

LIST OF TABLES

TABLE 1a: Demographic data and causes of end stage liver disease for transplant recipients >= 60 years at Wits Donald Gordon Medical Centre, Johannesburg.

TABLE 1b: Model for end stage liver disease (MELD) scores and Donor Risk Index (DRI) scores at the time of transplant for 91 transplant recipients >= 60 years.

TABLE 2: The incidence of pre liver transplant diabetes mellitus, kidney disease and cardiac disease amongst 91 recipients >= 60 years in Johannesburg, South Africa.

TABLE 3: Survival rates amongst 91 liver transplant recipients at WITS Donald Gordon Medical Centre in Johannesburg, South Africa

TABLE 4: The impact of pre-liver transplant diabetes mellitus, renal dysfunction and coronary artery disease on survival outcomes amongst LT recipients >= 60 years in Johannesburg, South Africa

TABLE 5: Choice of immunosuppressant and number of immunosuppressants taken concurrently at 1 and 3 months post-liver transplant with comparison made between an earlier and later transplant era.

1. **BACKGROUND**

1.1 Introduction

Since the first liver transplant (LT) performed in 1963, the upper limit of age for donors and recipients has been controversial. Initially recipient age over 60 was contraindicated but this limit gradually increased over the years. In 2015 the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines suggested a multidisciplinary evaluation for candidates over 65 with no upper age limit prescribed(1). These recommendations were on the basis of a number of single center reviews in the early 2000's showing equivalent outcomes. Those that evaluated risk factors in the older cohorts emphasized the importance of careful candidate selection with a thorough pre – transplant work up and optimization of co-morbidities. The introduction of the model for end stage liver disease (MELD) scoring system in 2002 and the Donor Risk Index (DRI) aided in stratifying patients urgency and risks for transplant(2)(3). The newer agents used for immunosuppression assisted in markedly improving posttransplant survival, graft rejection and complications of immunosuppression itself.

1.2 Elderly Recipient Survival Outcomes

A number of large database analyses in Europe and America as well as single center reviews to evaluate elderly recipient survival outcomes inform current practice and influence transplant allocation policies(4). A group from Yale University reviewed 5 year survival rates amongst 35,686 recipients over the age of fifty in the United Network for Organ Sharing (UNOS) database between 2002 and 2011 (5). Whereas survival rates in the reference group (50-54 years old) was 72.2%, the 60-64, 64-69 and 70+ age groups had rates of

69.5%, 65% and 57,5% respectively. They thus concluded that overall survival in the older cohort was worse. Su et al reviewed the same database over the same period but used a novel metric to establish a 'survival benefit' calculated as the difference between posttransplantation and waitlist life expectancies at 5 years(6). As older patients have a lower waitlist life expectancy, the 5 year survival benefit was found to be comparable.

One of the earlier reviews on elderly LT recipient outcomes was done by Collins et al in Wisconsin, USA(7). Between 1984 to 1991 survival rates for patients over 60 years old was 47.6% at 5 years. In comparison in their 'second era' from 1991 to 1997 survival was 58.1%. It was still markedly lower ($p=0.042$) than their young cohort which was 82.3% but this illustrates the trend *towards* significantly improved outcomes. Of note, malignancy was the most common cause of death in the older cohort suggesting that the earlier immune suppression strategies in the era prior to calcineurin inhibitors (CNI's) played a major role in these poorer outcomes.

The following three single center studies suggest that carefully selected patients over 60 have similar survival outcomes compared to younger patients.

- A retrospective review at Cleveland Clinic included all transplants performed between 2004 and 2010; 223 recipients over 60 years old and 515 under 60. One year mortality was 11.2% for the elderly cohort compared with 9.7% in the younger(8).

- Aduen et al did a case-control study between 1998 and 2004 comparing 2 groups of 45 patients, one group over 70 and the other under 60. Both were matched for MELD score, gender and cause of end-stage liver disease. They found no difference in five year mortality or graft survival amongst these patients(9).

- At King's College in London, a database review from 1988 to 2003 divided patients into three groups; over 65 (n=77), 60-64 (n=137) and 18-59 (n=202). The group with the best survival outcome at 5 years was those aged 60-64 (80% survival verses 78% in the young cohort and 73% in the patients 65 and older)(10). Of note, patients over 65 had on average a lower MELD score and all candidates over 60 had an extensive cardiac work up including echocardiography and coronary angiography, highlighting that with careful patient selection, elderly recipients should not be denied liver transplantation.

1.3 Model for End-stage Liver Disease (MELD) Score

In the 80's and early 90's, organ's were allocated on the basis of the level of care the candidate required as well as chronological time from listing. This was amenable to manipulation and patients could be kept in an Intensive Care setting till transplant. From 1996, the Child-Pugh-Turcotte (CPT) score was introduced which incorporated 3 objective (serum albumin, serum bilirubin and prothrombin time) and 2 subjective (encephalopathy and ascites) parameters with the idea that sicker patients would be prioritized(11). The Model for End-stage Liver Disease (MELD) score which was developed by researchers at the Mayo Clinic incorporates need for dialysis, serum creatinine, serum bilirubin, international normalized ratio (INR) and in 2016 was updated to include serum sodium(12). It was widely adopted from 2002 onwards as although on meta-analyses both CPT and MELD equally predict 3 month mortality in end stage liver disease MELD scoring was less subjective, included serum creatinine - an important factor in predicting patient survival and allowed for finer stratification. After it's introduction, there was a significant improvement in wait list mortality and a reduction in wait list times, without an adverse impact on posttransplant outcomes(2). Thus MELD-Na has replaced CPT scoring in clinical practice.

The UKELD score was developed around a similar time to the MELD-Na score but among a slightly different demographic in the United Kingdom (UK) (13). It uses the same parameters as the MELD score but different units and co-efficients and was found to better predict waitlist mortality in the UK population as compared to MELD(14). Currently, in clinical practice in the UK, a value of above 49 is used as an inclusion criteria for waitlisting for transplantation.

In a UNOS database review it was found that older patients with higher MELD scores tended to either die or become too ill to undergo transplantation(6). Older patients did not have the physiological reserves to compensate at high MELD scores as compared to younger patients. For this reason as well as more conservative patient selection, a meta-analysis of elderly LT reported a significantly lower average MELD score in older recipients at transplant(15). The current MELD scoring system is a 'sickest first' policy which allows for older recipients to be allocated donor livers but if allocation policies are changed to models that prioritize total life span or survival years, older patients will be heavily disadvantaged (4).

1.4 Donor Risk Index (DRI)

Despite the MELD score being an accurate predictor of 3 month mortality without transplant, in isolation it is only a weak predictor of post-transplant outcomes which has numerous other variables such as donor factors, surgical factors and post-transplant complications.

In order to better stratify post-transplant outcomes and with the increasing number of extended criteria donors, a Donor Risk Index (DRI) was developed which combines 7 donor characteristics that were independently identified as risk factors(3). These include donor age over 40, donation after cardiac death, the use of split or partial grafts, black race, shorter

height, cause of death as a cerebrovascular accident and the time for organ harvest. The impact of donor age is heavily weighted and is in keeping with other series that found it's impact to be significant(16)

Evaluation of the DRI in Brazil amongst 252 liver transplants with an average age of 54 and average DRI of 1.4 found that the DRI did not predict mortality at 30 days. However other scores like BAR (balance of risk), SOFT (survival outcomes following liver transplant) and PSOFT (preallocation survival outcomes following liver transplant) were found to have good prognostic value amongst their cohort at one month (17). These scores have not been evaluated in the South African context.

Aloaia et al in 2009 proposed a novel metric to be used as an alternative to the DRI, termed the Older Recipient Prognostic Score (ORPS). It was developed to determine posttransplant outcomes specifically in elderly recipients using 5 variables (both donor and recipient related) that they identified as independent risk factors for poor survival using the UNOS database namely; recipient mechanical ventilation, diabetes mellitus, serum creatinine greater than 140 umol/l, positive Hepatitis C serology and a combined donor and recipient age of 120 years(18). This scoring system has not been widely adopted nor evaluated in the South African context, and may be less applicable as Hepatitis C is not common in this region.

The concept of combining the donor and recipient age to establish risk was further investigated by Kostakis et al who used four combinations which incorporated these variables into the MELD and UKELD scoring systems: Donor age x MELD, Donor age x UKELD, (Donor age + Recipient age) x MELD, (Donor Age + Recipient Age) x UKELD (19). The one that was found to have the best prognostic value for recipient survival at 1 year was the DR-MELD score [(donor age + recipient age) x MELD score] with a score of more than 2345 being an independent risk factor for worse recipient survival within the first year

posttransplant. This was published in 2021 and no other center has replicated a similar finding. Currently, in contrast to kidney transplants no age matching policy exists for liver transplants.

1.5 Pre-operative Risk Stratification

1.5.1 Establishing Risks in the Elderly

The importance of an extensive pre-transplant workup to risk stratify elderly transplant recipients has been emphasized in a number of studies. In a review article published in the Journal of Hepatology in 2018, specific attention was paid to cardiovascular disease and malignancy risk. A thorough cardiac assessment by a cardiologist including an electrocardiogram and echocardiogram and a malignancy screen including prostate markers, mammography and colonoscopy were advocated(4).

1.5.2 Renal dysfunction at liver transplant

In 1996 Brown et al reviewed the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) liver transplantation database (20). Patients who were in fulminant hepatic failure with renal dysfunction, defined as a creatinine level of over 141 umol/L, had a survival rate at 1 year of 50% verses 83% in those without renal dysfunction (p=0.04). However, patients with cirrhosis and renal dysfunction but who were not in fulminant hepatic failure had similar survival rates at 1 year of 86% and 89% respectively.

In contrast, Gonwa et al. found that preoperative renal dysfunction was an independent risk factor for survival in all liver transplant recipients (21). In comparison with patients with a serum creatinine level less than 88 umol/L, patients with serum creatinine level more than 176 umol/L had a 58% greater risk of death, and patients on dialysis had a 77% greater risk of death.

Lafayette et al also found that among 115 liver transplant recipients, patients with a pretransplant serum creatinine level more than 88 umol/L had a higher mortality rate (32% versus 6%) than patients with pretransplant creatinine values less than 88 umol/L (22). In fact, an elevated pretransplant serum creatinine value was the strongest predictor of both the development of acute kidney injury (AKI) requiring dialysis and subsequent death. Other studies have made similar findings (23).

With specific reference to an older cohort, a group in Spain retrospectively compared 72 patients between 66-70 years old with 314 patients 65 and under. Pre-LT (liver transplant) diabetes, hypertension and cardiovascular disease were all analyzed but pre-LT renal insufficiency was found to be the only statistically significant risk factor on multivariate analysis(24) .

In general, renal dysfunction at the time of liver transplantation has been accepted as a risk factor for mortality since the 90's (25). This informed the MELD scoring system and as a result serum creatinine was included to risk stratify patients. Apart from short and long term patient survival, there is also an increase in costs, post transplant sepsis, the need for dialysis following transplant and the duration of ICU stay.

1.5.3 Cardiac Risks at Liver Transplant

In contrast at Cleveland Clinic, pre-LT cardiovascular disease especially coronary artery disease was found to be the most statistically significant independent risk factor for poor outcomes in the elderly recipients (8).

To further delineate specific cardiac risks, Główczyńska et al in 2018 reviewed 160 patients with a mean age of 49 who underwent pre-transplant consultations by a designated cardiologist at the First Department of Cardiology at the Medical University of Warsaw (26). In a multivariate analysis of patient characteristics, the risk of death in long-term post-

livertransplantation follow-up was most strongly associated with 3 variables: history of prior CAD (HR 4.70), angiographically confirmed coronary stenosis (HR 7.78), and left ventricular EF below 50% (HR 10.71).

A scientific statement from the American Heart Association (AHA) and the American College of Cardiology (ACC) in 2012 recommended screening for CAD in individuals with more than 3 cardiovascular risk factors. In such patients, stress testing is recommended as an initial screening tool, to be followed by cardiac catheterization when the findings are positive (27).

However, despite this recommendation, pre-operative non-invasive tests like Dobuatime Stress Echocardiography and Single-Photon Emission Computed Tomography (SPECT) have been found to correlate poorly with the presence of CAD in liver transplant candidates (28)(29). As a non-invasive option, there may be a growing role for CT coronary angiography especially in patients in poor clinical condition (30)(31). In contrast to the AHA, the 2009 European Society of Cardiology guidelines recommended pre-LT coronary angiography routinely (32).

In 2021, in the Journal of Hepatology, Rachwan et al published a CAD LT score based on a retrospective review of 1634 stress echocardiography and 1266 cardiac catheterization findings for pre LT workup(33). Patients at the single centre at Indiana University were all assessed by a single interventional cardiologist who decided upon pre-transplant coronary angiography based on a host of traditional cardiac risk factors. In the scoring algorithm that was developed (see appendix B) a patient with a score of more than or equal to 9 should proceed directly to catheterization, and for those with a score of 7-8, cardiac catheterization was at the discretion of the treating physician. Of relevance, patients aged 60-70 were automatically allocated 8 points (independent of other comorbidities) and those over 70 are allocated 10 points. This would imply that cardiac catheterization would be recommended almost routinely in the over 60 age group.

Similarly, Pang et al reviewed 122 patients between 2006 and 2013 in Singapore and found that age over 65 by itself was a significant predictor of CAD as evidenced by their coronary angiography findings pre-transplant(34).

1.6 Immunosenescence, Immune Suppression (IS) Regimens and Acute Cellular Rejection

“Immunosenescence is the process of progressive dysfunction of the immune system that increases the susceptibility of the elderly to infection, autoimmune disease, and cancer, contributing significantly to morbidity and mortality of the elderly. Immunosenescence changes result in both quantitative and qualitative modifications of specific cellular subpopulations rather than a global deterioration of the immune system, as previously thought. ”(35)

Immunosenescence has particular relevance in transplant medicine with regards to immune suppression strategies and graft rejection, risks of malignancy and risks of infection. With impaired immunity on the basis of a smaller population of naïve T cells, impaired memory cells, increased susceptibility to immune suppression and altered cytokine profiles, a trend towards lower rates of acute rejection has been noted in older cohorts(9,10,24). However, infections that occurred in the post-operative period tend to be more severe. In addition, higher rates of malignancy compared to the general population, and as a cause of death, compared to younger cohorts, have been documented(36).

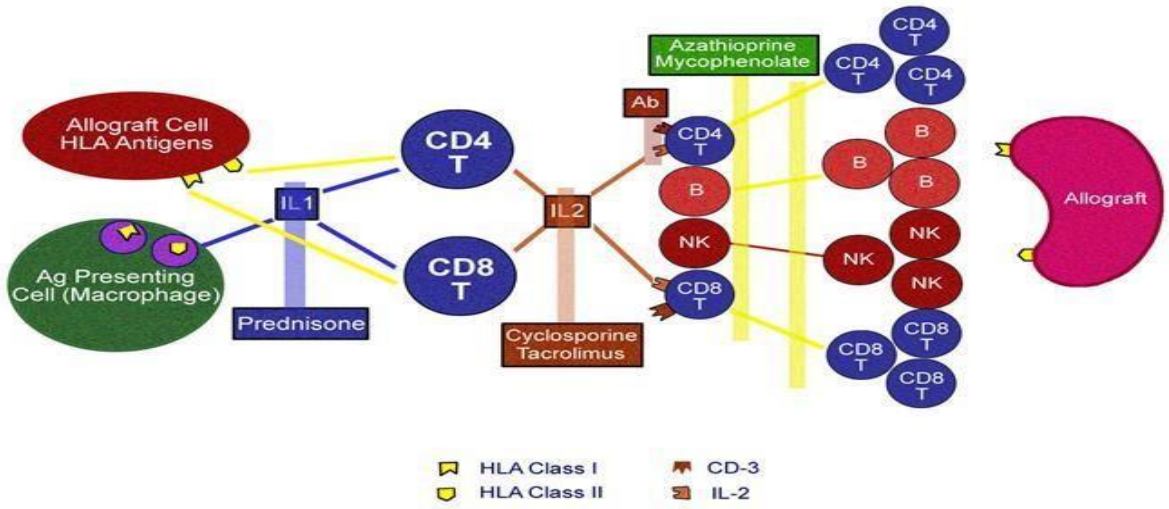
Based on this combination of less acute rejection, more severe infections, impaired drug clearance and higher rates of malignancy; in the last 5 to 10 years there has been a focus on reducing or minimizing IS therapy in this special population. Dual or triple therapy is not required and slow withdrawal can be considered with advancing age(4)

1.7 Immunosuppression Therapies

The introduction of effective targeted immunosuppression therapies in the late nineties revolutionized liver transplants and transformed it into the treatment of choice for end stage liver disease.

1.7.1 Mechanisms of Action

Diagram: Mechanisms of Action for Commonly Used Immunosuppressants



The above diagram by David A Hatch illustrates immune mechanisms of immunosuppressants. Corticosteroids inhibit production of interleukin-1. Macrolides (cyclosporine, tacrolimus, sirolimus) inhibit production of or use of interleukin-2, thus inhibiting stimulation of a clone of cytotoxic T lymphocytes directed against specific human lymphocyte antigen types. Antimetabolites (mycophenolate mofetil, azathioprine) inhibit purine production, thus impairing cell proliferation.

1.7.2 Induction

There has been a gradual increase in the use of induction agents in the last fifteen years. This has been done to reduce immunosuppression toxicity by minimizing CNI and steroid use. Among induction agents, IL-2 receptor (CD25) monoclonal antibodies (daclizumab and basiliximab) have been the ones most commonly used(1) Most centres use induction agents when CNI avoidance is desired, or as the treatment for steroid resistant rejection. In the United States, only 10% of de novo liver transplant patients receive induction therapy with Anti Thymocyte Globulin or IL2 receptor antagonists (37). In Germany, less than half of the transplant centres use these drugs for induction therapy and of those that do, they are only used in select patients (38).

A meta-analysis of 12 randomized controlled trials (RCT), involving 3251 liver transplant recipients investigated the effect of induction with monoclonal antibodies on biopsy-proven acute rejection episodes (BPAR) (39) and found that using IL-2 receptor antagonists resulted in a significant reduction in BPAR (23.1% vs. 28.1% without; relative risk [RR] 0.82, 95% CI [0.68–0.99]; p = 0.04), although there was no significant effect on mortality or graft loss.

Neuberger and colleagues in the RESPECT trial investigated three different protocols: (i) standard dose TAC (trough >10 ng/mL) plus steroids; (ii) low dose TAC (trough <8 ng/mL) plus steroids and MMF; (iii) IL2-RA induction, MMF plus delayed low dose TAC (started 5 days post-transplant) and steroids (40). Patients receiving IL-2RA induction had significant preservation of renal function without differences in graft rejection at 1-year post-LT. There was no difference in GFR or ACR between patients receiving immediate, low-dose tacrolimus with MMF and steroids compared with immediate standard-dose tacrolimus and steroids alone. Other RCT's have also provided evidence that IL-2 receptor antagonists allow for delay of CNI introduction or reduction without increasing the risk of acute rejection (41).

In current German practice, between 50% and 100% of de novo patients receive an antiproliferative medication, usually MMF, within the first four weeks post-transplant (38).

1.7.3 Maintenance

A survey of 15 liver transplant centers across North America in 2018 revealed wide variability in current immunosuppression strategies for maintenance. In the early post transplant period most centers used triple therapy; steroids, an antimetabolite (mycophenolate mofetil) and a calcineurin inhibitor (tacrolimus), however maintenance therapy and minimization strategies as well as use in special populations such as the elderly varied(42). A consensus statement issued by the International Liver Transplantation Society in 2018 makes no mention of special recommendations for immunosuppression in the elderly(43).

A single-center retrospective evaluation of 143 adult liver transplant recipients in Chicago from 2013 to 2018 compared acute rejection, infection, malignancy and survival outcomes amongst recipients over 60 (mean age 65) to those under 60 (mean age 49) on the same immunosuppression regimen (44). Patients received methylprednisolone induction and were maintained on tacrolimus, mycophenolate mofetil (MMF), and a prednisone taper. Mean tacrolimus levels and the duration of MMF and steroids were comparable. Both groups had a similar incidence of first rejection within 1 year (19.2% in the elderly group vs. 23.1% in the younger group, $p = 0.57$) and there were no statistical differences in terms of infection, malignancy, or patient survival.

An Italian group compared the combination of Tacrolimus and MMF verses Tacrolimus and Everolimus between two matched groups of 178 patients each with a mean age of 51 (45). The group on Everolimus had a decreased incidence of treated biopsy proven acute rejection episodes (6 patients vs 13 patients). They also noted a significant decreased risk of de novo malignancy amongst those on Everolimus (1,7% verses 6,7%). However, EVR was associated with more adverse events and drug discontinuations most frequently due to hyperlipidemia, thrombocytopenia, edema, and oral sores.

In earlier use of EVR between 2005-2008, 70% of patients developed an adverse event to the drug however dose adjustments resolved most issues and only 16% needed discontinuation(46). No local review of the side effect profile and tolerance of Everolimus amongst transplant recipients has been published.

1.8 The South African Experience

Currently, two centers perform liver transplants in South Africa; Groote Schuur Hospital in Cape Town and Wits Donald Gordon Medical Centre (WDGMC) in Johannesburg. WDGMC is a private academic hospital within the Faculty of Health Sciences at The University of Witwatersrand that supplements public hospitals in training specialists and subspecialists. Owing to a collaboration with the provincial government's health department, public sector patients are evaluated and if suitable referred for transplant(47).

Between 1988 and 1998, 44 adults underwent liver transplantation at Groote Schuur Hospital, however age over 60 was at that time a contra-indication for transplant. 1 and 5 year survival rates were 72% and 61% respectively(48). WDGMC performed liver transplants on 275 patients between 2004 and 2016. The commonest aetiologies for ESLD requiring transplant were primary sclerosing cholangitis followed by non-alcoholic and alcoholic steatohepatitis, which contrasts with first world countries where hepatitis C virus and hepatocellular carcinoma are the leading causes. Median age of the recipients was 51, median MELD score was 17 and the median Donor Risk Index was 1.61. Overall survival outcomes at 1 and 5 years was 81.7% and 70% respectively(49). Despite age over 60 being identified as an *independent risk factor* at WDGMC, 91 patients over 60 were transplanted. No local review has focused specifically on outcomes amongst the elderly liver transplant recipients. There is also no local data on immunosuppression in this cohort.

1.9 Summary

In the South African context where both donor shortages and resource constraints are limiting factors, it is especially important to evaluate the outcomes amongst this cohort to make informed decisions on transplant candidacy. By establishing significant independent risk factors and trends in immunosuppressant therapies, local transplant units will be able to tailor selection and treatment.

2. Aim

The aim of this study was to assess survival outcomes and rejection rates, and identify demographic and clinical predictors of these outcomes. In addition, the aim is to identify whether survival outcomes have improved over time and whether prescription patterns have changed.

3. Objectives

3.1 Primary Objectives

- To determine mortality outcomes amongst elderly liver transplant recipients

Mortality will be assessed at

- 1 year
- 3 year
- 5 year

3.2 Secondary Objectives

- To evaluate if the following specific pre-transplant co-morbidities predict a poorer outcome
- Diabetes

- Pre-existing ischaemic (coronary artery disease) and non-ischaemic (cardiomyopathy, arrhythmia, valvular heart disease, other causes of heart failure) cardiac disease
- Lower GFR
- To describe the Donor Risk Index and MELD scores at transplant in this cohort
- To describe the immune suppression regimens and dosages used according to age
- To determine the rates of acute cellular rejection (within 90 days of transplant)

4. Methodology

4.1 Study Design

The study will be a single center retrospective observational study. Consecutive sampling will be applied as all liver transplant recipients aged 60 and older undergoing transplant surgery at the Transplant Unit at the Wits Donald Gordon Medical Centre over a 15 year period from August 2004 to February 2019 will be included. The closing date for sampling allows for a minimum of one year follow up at the time of submission of the protocol for this study.

The cohort will include a total of 91 patients.

Exclusion criteria

- Age under 60

4.2 Data Collection

The study data will be sourced using a retrospective database hosted on a secure, webbased application called Research Electronic Data Capture (REDCap). Clinical records

at the Wits Donald Gordon Medical Centre Transplant Unit will be reviewed where necessary.

- Demographic information
- age at transplant
- gender
- ethnicity
- Existing pre-transplant co-morbid conditions
- Diabetes
- Pre-existing cardiac disease
- Coronary artery disease
- Ejection Fraction
- Rhythm
- Diastolic Function
- GFR as defined by CKD-EPI formula
- Aetiology of end stage liver disease
- MELD score
- at time of transplant surgery
- Donor Details to calculate Donor Risk Index
- Age
- Race
- Height
- Cause of Death
- Donation after cardiac death
- Type of graft – split/partial or whole
- Follow up
- Date of transplant
- Date of last follow up

- The reason for loss to follow up (if known)
- Immunosuppression
- Immunosuppression drugs and dosages used
- Development of acute cellular rejection (within 90 days)
- Mortality data in recipients that have demised
- Age at death
- Time from transplant to death
- Cause of death

5. Statistical Analysis

Data analysis will be conducted using Statistics version 13.5 (Tibco), a software package developed by StatSoft. Guidance from an experienced biostatistician provided by WITS University will be sought.

In order to determine whether there was an improvement in survival over time, based on the premise that initial experiences and new emerging data would have informed updated practice with regards to patient selection and post-transplant immune suppression strategies, the survival analysis will be divided into two eras. These will be based on equivalent numbers in the first era as the second.

The continuous variables will be presented as means, medians, range and standard deviations

The categorical variables will be presented as percentages

The co-morbid risk factors associated with post transplant outcomes will be presented as odds ratios

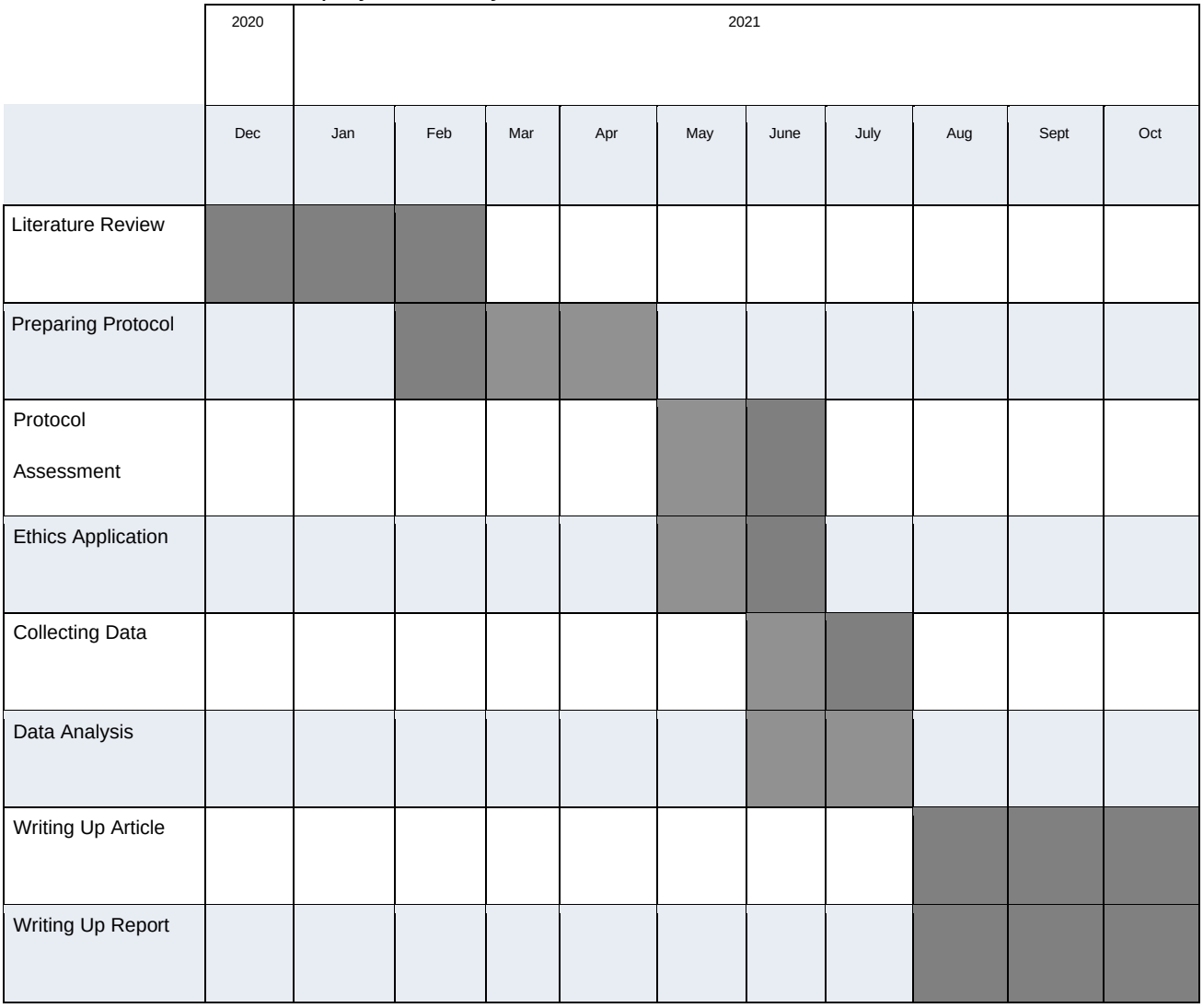
The post transplant outcomes will be analysed by box and whisker plots, line plots and survival curves from serial data collection at 1, 3 and 5 years.

6. Ethics

The protocol for the study will be submitted for assessment to the Human Ethics Research Committee of The University of the Witwatersrand. Permission to access the WDGMC Transplant Unit database and records will be obtained from the Head of the Transplant unit, as well as the clinical director and CEO at the institution.

7. Timing

A Gantt chart illustrates the projected study timeframe.



8. Funding

The primary investigator will cover basic costs related to printing and the statistical software.

No additional funding will be required by the hospital or participants.

9. Potential Limitations

- Database Limitations

  Any information that has not been captured in the database or in the clinical records may be difficult to find in retrospect.

- Population Limitations

  By virtue of the study being limited to a single center with the majority of recipients from the private sector, the sample may not be representative of the larger South African demographic and disease profile as a whole.

- Study Limitations

  Graft outcomes are not differentiated from patient survival outcomes.
  Frailty and functional status is not assessed.

10. References

1. Burra P, Burroughs A, Graziadei I, Pirenne J, Valdecasas JC, Muiesan P, et al. EASL Clinical Practice Guidelines: Liver transplantation. J Hepatol [Internet]. 2016;64(2):433–85. Available from: http://dx.doi.org/10.1016/j.jhep.2015.10.006
2. Kanwal F, Dulai GS, Spiegel BMR, Yee HF, Gralnek IM. A comparison of liver transplantation outcomes in the pre- vs. post-MELD eras. Aliment Pharmacol Ther. 2005;21(2):169–77.
3. Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, et al. Characteristics associated with liver graft failure: The concept of a donor risk index. Am J Transplant. 2006;6(4):783–90.
4. Durand F, Levitsky J, Cauchy F, Gilgenkrantz H, Soubrane O, Francoz C. Age and liver transplantation. J Hepatol [Internet]. 2019;70(4):745–58. Available from: https://doi.org/10.1016/j.jhep.2018.12.009
5. Malinis MF, Chen S, Allore HG, Quagliarello VJ. Outcomes among older adult liver transplantation recipients in the model of end stage liver disease (meld) era. Ann Transplant. 2014;19(1):478–87.
6. Su F, Yu L, Berry K, Liou IW, Landis CS, Rayhill SC, et al. Aging of Liver Transplant Registrants and Recipients: Trends and Impact on Waitlist Outcomes, Post-Transplantation Outcomes, and Transplant-Related Survival Benefit. Gastroenterology. 2016;150(2):441-453.e6.
7. Collins BH, Pirsch JD, Becker YT, Hanaway MJ, Van Der Werf WJ, D'Alessandro AM, et al. Long-term results of liver transplantation in patients 60 years of age and older. Transplantation. 2000;70(5):780–3.

8. Sonny A, Kelly D, Hammel JP, Albeldawi M, Zein N, Cywinski JB. Predictors of poor outcome among older liver transplant recipients. Clin Transplant. 2015;29(3):197–203.
9. Aduen JF, Sujay B, Dickson RC, Heckman MG, Hewitt WR, Stapelfeldt WH, et al. Outcomes after liver transplant in patients aged 70 years or older compared with those younger than 60 years. Mayo Clin Proc. 2009;84(11):973–8.
10. Cross T. Liver Transplantation in Patients Over 60 And 65 Years: Evaluation of Long-Term Outcomes and Survival. Liver Transplant. 2007;13(5):767–8.
11. Singal AK, Kamath PS. Model for End-stage Liver Disease. J Clin Exp Hepatol [Internet]. 2013;3(1):50–60. Available from: http://dx.doi.org/10.1016/j.jceh.2012.11.002
12. Asrani SK, Kim WR. Model for End-Stage Liver Disease: End of the First Decade. Clin Liver Dis. 2011;15(4):685–98.
13. AROORI S, Gupta P, Gunson B, Nightingale P, Isaac J, Muiesan P, et al. Comparison of Model for End-Stage Liver Disease (Meld) & United Kingdom End-Stage Liver Disease (Ukeld) Scores As a Predictor for Death on the Liver Transplant Waiting List and As a Predictor of Mortality Following Liver Transplantation. In: Transplantation Journal. 2010. p. 15.
14. Neuberger JM. MD Calc [Internet]. Available from: https://www.mdcalc.com/united-kingdom-model-end-stage-liver-diseaseukeld#creator-insights
15. Gómez Gavara C, Esposito F, Gurusamy K, Salloum C, Lahat E, Feray C, et al. Liver transplantation in elderly patients: a systematic review and first metaanalysis. Hpb [Internet]. 2019;21(1):14–25. Available from: https://doi.org/10.1016/j.hpb.2018.07.025

16. Rana A, Hardy MA, Halazun KJ, Woodland DC, Ratner LE, Samstein B, et al. Survival Outcomes Following Liver Transplantation (SOFT) score: A novel method to predict patient survival following liver transplantation. Am J Transplant. 2008;8(12):2537–46.
17. Silveira F, Silveira FP, de Freitas ACT, Coelho JCU, Ramos EJB, Macri MM, et al. Liver transplantation: Survival and indexes of donor-recipient matching. Rev Assoc Med Bras. 2021;67(5):690–5.
18. Aloia T, Knight R, Gaber O, Ghobria M, Goss J. Analysis of Liver Transplant Outcomes for United Network for Organ Sharing Recipients 60 Years Old or Older Identifies Multiple Model for End-Stage Liver Disease–Independent Prognostic Factors. Liver Transplant. 2010;13(5):950–9.
19. Kostakis ID, Iype S, Nasralla D, Davidson BR, Imber C, Sharma D, et al. Combining donor and recipient age with preoperative meld and ukeld scores for predicting survival after liver transplantation. Exp Clin Transplant. 2021;19(6):570–9.
20. Brown RSJ, Lombardero M, Lake JR. Outcome of patients with renal insufficiency undrgoing liver or liver-kidney transplantation. Transplantation. 1996;62(12):1788–93.
21. Gonwa TA, McBride MA, Anderson K, Mai ML, Wadei H, Ahsan N. Continued influence of preoperative renal function on outcome of orthotopic liver transplant (OLTX) in the US: Where will MELD lead us? Am J Transplant. 2006;6(11):2651–9.
22. Lafayette R, Pare G, Schmid C, King A, Rohrer R, Nasraway S. Pretransplant renal dysfunction predicts poorer outcome in liver transplantation. Clin Nephrol. 1997;48:159–64.

23. Markmann JF, Markmann JW, Markmann DA, Bacquerizo A, Singer J, Holt CD, et al. PREOPERATIVE FACTORS ASSOCIATED WITH OUTCOME AND THEIR IMPACT ON RESOURCE USE IN 1148 CONSECUTIVE PRIMARY LIVER TRANSPLANTS. Transplantation. 2001;72(6):1113–22.

24. Bilbao I, Dopazo C, Lazaro JL, Castells L, Escartin A, Lopez I, et al. Our experience in liver transplantation in patients over 65 yr of age. Clin Transplant. 2008;22(1):82–8.

25. Weber ML, Ibrahim HN, Lake JR. Renal dysfunction in liver transplant recipients Evaluation of the critical issues. Liver Transplant. 2012;18(11):1290–301.

26. Główczyńska R, Galas M, Witkowska A, Ołdakowska-Jedynak U, Raszeja-Wyszomirska J, Krasuski K, et al. The Pre-Transplant Profile of Cardiovascular Risk Factors and Its Impact on Long-Term Mortality After Liver Transplantation. Ann Transplant. 2018;23:591–7.

27. Lentine KL, Costa SP, Weir MR, Robb JF, Fleisher LA, Kasiske BL, et al. Cardiac disease evaluation and management among kidney and liver transplantation candidates: A scientific statement from the American Heart Association and the American College of Cardiology Foundation. J Am Coll Cardiol [Internet]. 2012;60(5):434–80. Available from: http://dx.doi.org/10.1016/j.jacc.2012.05.008

28. Nicolau-Raducu R, Gitman M, Ganier D, Loss GE, Cohen AJ, Patel H, et al. Adverse Cardiac Events After Orthotopic Liver Transplantation: A CrossSectional Study in 389 Consecutive Patients. Liver Transplant. 2015;21(1):13–
21.

29. Davidson CJ, Gheorghiade M, Flaherty JD, Elliot MD, Reddy SP, Wang NC, et al. Predictive value of stress myocardial perfusion imaging in liver transplant candidates. Am J Cardiol. 2002;89(3):359–60.

30. Chae WY, Hwang S, Yoon YI, Kang MC, Moon DB, Song GW, et al. Clinical value of preoperative coronary risk assessment by computed tomographic arteriography prior to adult living donor liver transplantation. Transplant Proc [Internet]. 2012;44(2):415–7. Available from: http://dx.doi.org/10.1016/j.transproceed.2012.01.056

31. Löffler AI, Gonzalez JA, Sundararaman SK, Mathew RC, Norton PT, Hagspiel KD, et al. Coronary Computed Tomography Angiography Demonstrates a High Burden of Coronary Artery Disease Despite Low-Risk Nuclear Studies in Pre–Liver Transplant Evaluation. Liver Transplant. 2020;26(11):1398–408.

32. Poldermans D, Bax JJ, Boersma E, De Hert S, Eeckhout E, Fowkes G, et al. Guidelines for pre-operative cardiac risk assessment and perioperative cardiac management in non-cardiac surgery. Eur Heart J. 2009;30(22):2769–812.

33. Rachwan RJ, Kutkut I, Timsina LR, Chaaya RGB, El-am EA, Sabra M, et al. CAD-LT score effectively predicts risk of signi fi cant coronary artery disease in liver transplant candidates. J Hepatol [Internet]. 2021;75(1):142–9. Available from: http://dx.doi.org/10.1016/j.jhep.2021.01.008

34. Pang NQ, Kow WCA, Law JH, Pan LTT, Lim BLK, Wong CCR, et al. Role of Coronary Angiography in Pre–Liver Transplantation Cardiac Evaluation: Experience From an Asian Transplant Institution. Transplant Proc [Internet]. 2017;49(8):1797–805. Available from: http://dx.doi.org/10.1016/j.transproceed.2017.04.021

35. Martins PNA. Immunosenescence and Immune Response in Organ Transplantation. 2014.

36. Zhou J, Hu Z, Zhang Q, Li Z, Xiang J, Yan S, et al. Spectrum of de novo cancers and predictors in liver transplantation: Analysis of the scientific registry of transplant recipients database. PLoS One. 2016;11(5):1–14.

37. Turner AP, Knechtle SJ. Induction immunosuppression in liver transplantation: A review. Transpl Int. 2013;26(7):673–83.

38. Herzer K, Strassburg CP, Braun F, Engelmann C, Guba M, Lehner F, Nadalin S, Pascher A, Scherer MN, Schnitzbauer AA, Zimmermann T, Nashan B SM. Selection and use of immunosuppressive therapies after liver transplantation: current German practice. 2016.

39. Wang XF, Li JD, Peng Y, Dai Y, Shi G, Xu W. Interleukin-2 receptor antagonists in liver transplantation: A meta-analysis of randomized trials. Transplant Proc [Internet]. 2010;42(10):4567–72. Available from: http://dx.doi.org/10.1016/j.transproceed.2010.09.169

40. Neuberger JM, Mamelok RD, Neuhaus P, Pirenne J, Samuel D, Isoniemi H, et al. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: The “ReSpECT” study. Am J Transplant. 2009;9(2):327–36.

41. Calmus Y, Kamar N, Gugenheim J, Duvoux C, Ducerf C, Wolf P, et al. Assessing renal function with daclizumab induction and delayed tacrolimus introduction in liver transplant recipients. Transplantation. 2010;89(12):1504–10.

42. Hussaini T, Turgeon RD, Partovi N, Erb SR, Scudamore CH, Yoshida EM. Immunosuppression practices in liver transplantation: A survey of North American centers. Exp Clin Transplant. 2018;16(5):550–3.

43. Charlton M, Levitsky J, Aqel B, O’Grady J, Hemibach J, Rinella M, et al. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. Transplantation.

2018;102(5):727–43.

44. Almalki B, Kane C, Cunningham K, D'Agostino C, Novak A, Kapugi M, et al. Evaluation of rejection, infection, and malignancy outcomes in elderly liver transplant recipients receiving a similar level of immunosuppression compared to a younger group. Transpl Immunol [Internet]. 2021;69(August):101485. Available from: https://doi.org/10.1016/j.trim.2021.101485

45. De Simone P, Carrai P, Coletti L, Ghinolfi D, Petruccelli S, Precisi A, et al. Everolimus vs Mycophenolate Mofetil in Combination With Tacrolimus: A Propensity Score-matched Analysis in Liver Transplantation. Transplant Proc. 2018;50(10):3615–20.

46. Vallin M, Guillaud O, Morard I, Gagnieu MC, Mentha G, Adham M, et al. Tolerability of everolimus-based immunosuppression in maintenance liver transplant recipients. Clin Transplant. 2011;25(4):660–9.

47. Tager S, Etheredge HR, Fabian J, Botha JF. Reimagining liver transplantation in South Africa: A model for justice, equity and capacity building - the Wits Donald Gordon Medical Centre experience. South African Med J. 2019;109(2):84–8.

48. Botha J., Spearman C., Millar AJ., Michell L, Gordon P, Lopez T, et al. Ten years of liver transplantation at Groote Schuur hospital. South African Med J [Internet]. 2000;90(9 I):880–3. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L30763334%5Cnhttp://jq6am9xs3s.search.serialssolutions.com/?sid=EMBASE&issn=00382469&id=doi:&atitle=Ten+years+of+liver+transplantation+at+Groote+Schuur+hospital&stitle=S.+Afr.+Med.+J

49. Song E, Fabian J, Boshoff PE, Maher H, Gaylard P, Bentley A, et al. Adult liver transplantation in Johannesburg, South Africa (2004-2016): Balancing good outcomes, constrained resources and limited donors. South African Med J. 2018;108(11):929–36.

CHAPTER 2: PROPOSED MANUSCRIPT

The following article has been submitted to *Transplantation* for publication. The format is in line with guidelines as set out by the target journal (Appendix K)

Transplantation is the official journal of the International Liver Transplantation Society and The Transplantation Society.

LIVER TRANSPLANTATION AMONG OLDER RECIPIENTS IN JOHANNESBURG, SOUTH AFRICA: RISK FACTORS, OUTCOMES AND IMMUNOSUPPRESSION

Ahmad I. Tayob[1] , MBChB, Dip HIV (SA), FCP (SA)

Adam Mahomed[1,2], MBBCh, FCP (SA), Cert Gastroenterology (SA)

Jean F. Botha[2,3], MBBCh, FCS (SA)

Bilal Bobat[2], MBChB, FCP (SA), Cert Gastroenterology (SA)

1. Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, 2193
2. Wits Donald Gordon Medical Centre, University of the Witwatersrand, Johannesburg, South Africa, 2193
3. Department of Surgery, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, 2193

Keywords: liver transplant, elderly, Immunosenescence, immunosuppression, survival, risk factors

Word Count: Abstract = 250 words. Main body text (excluding headings and tables) = 3750 words

Address correspondence to Ahmad Tayob. Physical Address: 24 Killarney Hills, 6 10th Street, Killarney, Johannesburg, 2193. Email: ahmadtayob@gmail.com.

Telephone: +27726520425.

AUTHOR CONTRIBUTIONS

A. I. T. participated in the research design, data acquisition, data analysis, writing the paper, and critical revision. A.M. and J.F.B. participated in research design, data analysis, and critical revision. B.B. participated in research design and critical revisions.

The authors declare that they have no conflicts of interest. No funding was received for this study.

ABBREVIATIONS

ACR	Acute Cellular Rejection

BPAR	Biopsy proven acute rejection
CAD	Coronary Artery Disease
CI	Confidence Interval
CNI	Calcineurin Inhibitor
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CPT	Child Pugh Turcotte
DRI	Donor Risk Index
ESLD	End Stage Liver Disease
GFR	Glomerular Filtration Rate
HR	Hazard Ratio
IS	Immunosuppression
LT	Liver transplantation
MELD	Model for end-stage liver disease
MMF	Mycophenolate Mofetil
WDGMC	Wits Donald Gordon Medical Centre

ABSTRACT

Background.

The goal of this study was to evaluate outcomes in older patients undergoing liver transplantation. We assessed rejection rates, graft and patient survival, and demographic and clinical predictors of these outcomes.

Methods.

Data were collected from all patients aged >=60 years, undergoing liver transplantation for the first time, between 2004 and 2019. We compared survival outcomes and immunosuppression usage between an earlier and later transplant era to establish whether there was an improvement in survival over time, and whether immunosuppression prescription patterns changed as evidence emerged that older patients may require less therapy.

Results.

The median age of the patients was 64 years, and the most common cause of liver disease was ASH/NASH. The mean MELD scores were 16,7 (SD 6,9) and the mean DRI was 2.0 (SD 0,4). Patient survival rates at 1, 3, and 5 years were 75%, 69%, and 64%, respectively. Infections were the most common cause of death. 5% of patients developed ACR.

There was an increase in the risk of death for GFR categories 3a-5 unadjusted (HR 2.83 (95% CI 1.32-6.08)) and adjusted for sex, MELD score, and DRI (HR 3.47 (95% CI 1.547.84)) but no association between diabetes (HR 1.21 (95% CI 0.62-2.36)) or coronary artery disease (HR 1.46 (95% CI 0.70-3.02)) and recipient survival. Prednisone + tacrolimus + mycophenolate mofotil was the most common initial maintenance regimen. In the later transplant era, the mean prednisone dose was lower (19.8 mg verses 13.2 mg, p=0.023) and dual immunosuppression was favoured over triple (p=0.070).

Conclusions.

Survival outcomes in older patients are impacted by renal impairment but not by coronary artery disease or diabetes mellitus. The average DRI is high due to organ shortages in our setting, and this impacts survival outcomes. Immunosuppression prescribing patterns have adapted to account for altered immunity in old patients.

MAIN BODY

Introduction

The goal of this study was to evaluate outcomes of patients 60 years or older receiving liver transplants at WITS Donald Gordon Medical Centre in Johannesburg, South Africa. We aimed to assess rates of rejection, graft survival, and patient survival, as well as demographic and clinical predictors of these outcomes. WDGMC is one of only two liver transplant units in sub-Saharan Africa and performs the highest volume of transplants in this region annually. A retrospective review of all transplants undertaken since the establishment of the unit in 2004, was conducted in 2016, which established 4 significant independent risk factors; age over 60, biliary leaks, hepatic artery thrombosis and long theatre times (1). In the South African context, donor shortages due to a lack of awareness or cultural beliefs and resource constraints are limiting factors (2). Thus, it is especially important to evaluate the outcomes among the older cohort of recipients to make informed decisions regarding transplant candidacy. By establishing predictors of poor outcomes and evaluating how immunosuppressant therapies are used in this population, local transplant units will be able to tailor selection, post-transplant immunosuppression regimens, and doses.

There has been interest in recent years on the impact that Immunosenescence plays in the management of the older transplant recipient. With impaired immunity on the basis of a smaller population of naïve T cells, impaired memory cells, increased susceptibility to immune suppression, and altered cytokine profiles, a trend towards lower rates of acute rejection has been noted in older cohorts(8–10). However, infections that occur in the postoperative period are more severe and higher rates of malignancy compared to the general population, and as a cause of death compared to younger cohorts have been documented(11). Based on this combination of less acute rejection, more severe infections, impaired drug clearance, and higher rates of malignancy, in the last 5–10 years, there has been a focus on reducing or minimizing IS therapy in this special population (4). We thought

it would be valuable to evaluate whether in recent years lower doses of IS or different immunosuppression agents were used among older recipients and whether this has had an impact on survival outcomes. In order to do so, we evaluated survival outcomes and compared IS usage in an earlier transplant era between 2004 and 2013 and a later era between 2013 and 2019. We also reviewed the incidence of ACR and the cause of death amongst our older cohort as we were interested as to whether severe infections and posttransplant malignancy played a role in their mortality.

Materials and methods

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (Medical) (M200663) and was conducted in accordance with the principles of the Declaration of Helsinki of 1996. Patient records were anonymized prior to analysis. Data were collected from 91 first liver transplants in recipients aged 60 years and older carried out at WDGMC from 1 Aug 2004, to 28 Feb 2019 (with at least 1 year of follow-up). Clinical and biochemical data were extracted from the WDGMC Adult Liver Transplant Research Electronic Data Capture (REDCap) database.

Pre-transplant variables analyzed included demographic data (age at transplant, sex, and ethnicity), etiology of end-stage liver disease or indication for liver transplant, and MELD and DRI scores. The pre-transplant comorbidities analyzed were diabetes mellitus, cardiac disease, and renal dysfunction. Pre-LT diabetic screening was routinely undertaken with a HBA1C and fasting glucose and pre-LT creatinine levels were used to calculate the GFR according to the CKD-EPI formula. Specific markers of cardiac disease were based on specialist cardiologist reports during the pre-LT work up including ECG findings of rhythm disturbances, echocardiographic findings of systolic and diastolic function, and presence of coronary artery disease at routine coronary angiography.

Post-transplant variables analyzed were the choice and number of immunosuppressant agents, including doses of prednisone and MMF. These were evaluated at induction, discharge, and 1 and 3 months post-transplantation. These time frames were used to give an overview of the induction and early maintenance regimen selection and coincided with the 3 month period of acute rejections being evaluated. To evaluate whether there was a trend in IS usage over time, the IS regimens were evaluated in two eras: Era 1 was the earlier transplant period between August 2004 and June 2013, and Era 2 extended from July 2013 to February 2019.

At follow-up, the rates of ACR within 3 months, graft survival, and patient survival were assessed. These outcomes were also compared between the two transplant periods.

Sample size

Sample size estimation was based on the key research question, namely, the effect of risk factors on patient survival. As a starting point, we assumed equally sized risk factor groups, accrual and final follow-up periods of 7y and 1y respectively, and survival of 49% at 8 years (12). The detection of a hazard ratio (HR) of at least 2.0 with 80% power at the 5% significance level required a minimum sample size of 86 patients. The detection of a HR of 1.5 requires a sample size of 152 patients. Given our sample size, a HR of 2.0 was achievable.

Methodology

In order to determine whether there was an improvement in survival over time, based on the premise that initial experiences and new emerging data would have informed updated practice with regards to patient selection and post-transplant immune suppression

strategies, the survival analysis will be divided into two eras. These will be based on equivalent numbers in the first era as the second.

Patient, graft, and ACR-free survival rates were estimated using the Kaplan-Meier method. The association between immunosuppressive agents, number of agents, and transplant era was determined by the chi-squared test (Fisher's exact test was used for 2 × 2 tables or where the assumptions of the chi-squared test were not met). The mean doses were compared using the independent sample t-test. The effect of each risk factor on patient and graft survival was assessed using Cox proportional hazards regression, unadjusted and adjusted for sex, MELD, and DRI. Data analysis was performed using SAS version 9.4 for Windows. A significance level of 5% was used.

The continuous variables will be presented as means, medians, range and standard deviations. The categorical variables will be presented as percentages. The co-morbid risk factors associated with post transplant outcomes will be presented as hazard ratios.

Results

The median age was 64 years, with only 12% of the patients aged over 70 years. The majority of recipients were white men. The most common indication for liver transplant was ASH/NASH (48%) followed by cholestasis (22%). Malignancy accounted for only 11% of indications for transplant, and viral hepatitis accounted for only 6%.

At the time of transplantation, the mean MELD score was 16.7 (SD 6.9), with the majority of patients having a MELD score between 15 and 29. 42% of the patients received grafts with a DRI of more than 2.0. The mean DRI score across the cohort was 2.0 (SD 0,4).

Table 1

(a) Demographic data and causes of end stage liver disease for transplant recipients 60 years or older at Wits Donald Gordon Medical Centre, Johannesburg.

Age at transplant	60-64y	57
	65-69y	31
	70-74y	12
Sex	Male	75
	Female	25
Ethnicity	White	87
	Black African	5
	Other	8
Cause of ESLD / Indication for transplant	ASH/NASH	48
	Cholestatic	22
	Other	11
	Malignancy	9
	Hepatitis C	5
	Metabolic disease	3
	Hepatitis B	1

(b) Model for end stage liver disease (MELD) scores and Donor Risk Index (DRI) scores at the time of transplant for 91 transplant recipients 60 years or older.

MELD score	<15	41
	15-29	56
	30-34	2
	>35	1
MELD score	mean (SD)	16.7 (6.9)
DRI	1.41-1.60	11
	1.61-1.80	15
	1.81-2.00	32
	>2.00	42
DRI	mean (SD)	2.0 (0.4)

Data are presented as percentages. ASH, alcoholic steatohepatitis. NASH, non alcoholic steatohepatitis. ESLD: end-stage liver disease. SD, standard deviation.

30% of the patients had diabetes. 80% of patients had a GFR of 60 or more. The remaining 10% of patients had a GFR between 45 and 59, and 9% had a GFR of 30-44.

During routine pre-transplant coronary angiography, 24% of the patients were found to have coronary artery disease of varying degrees, and 76% had normal coronary arteries. Percutaneous coronary intervention was performed, as indicated. All patients had a normal ejection fraction and sinus rhythm; however, 18% had grade 1 diastolic dysfunction.

Table 2
The incidence of pre liver transplant diabetes mellitus, kidney disease and cardiac disease amongst 91 recipients 60 years or older in Johannesburg, South Africa.

Diabetes	No	70
	Yes	30
GFR (KDIGO category)	G1	24
	G2	56
	G3a	10
	G3b	9
Ejection Fraction	Above 50%	100
Diastolic Dysfunction	Grade 1	18
	None	82
ECG Rhythm	Sinus Rhythm	100
Coronary Artery Disease	No	76
	Yes	24

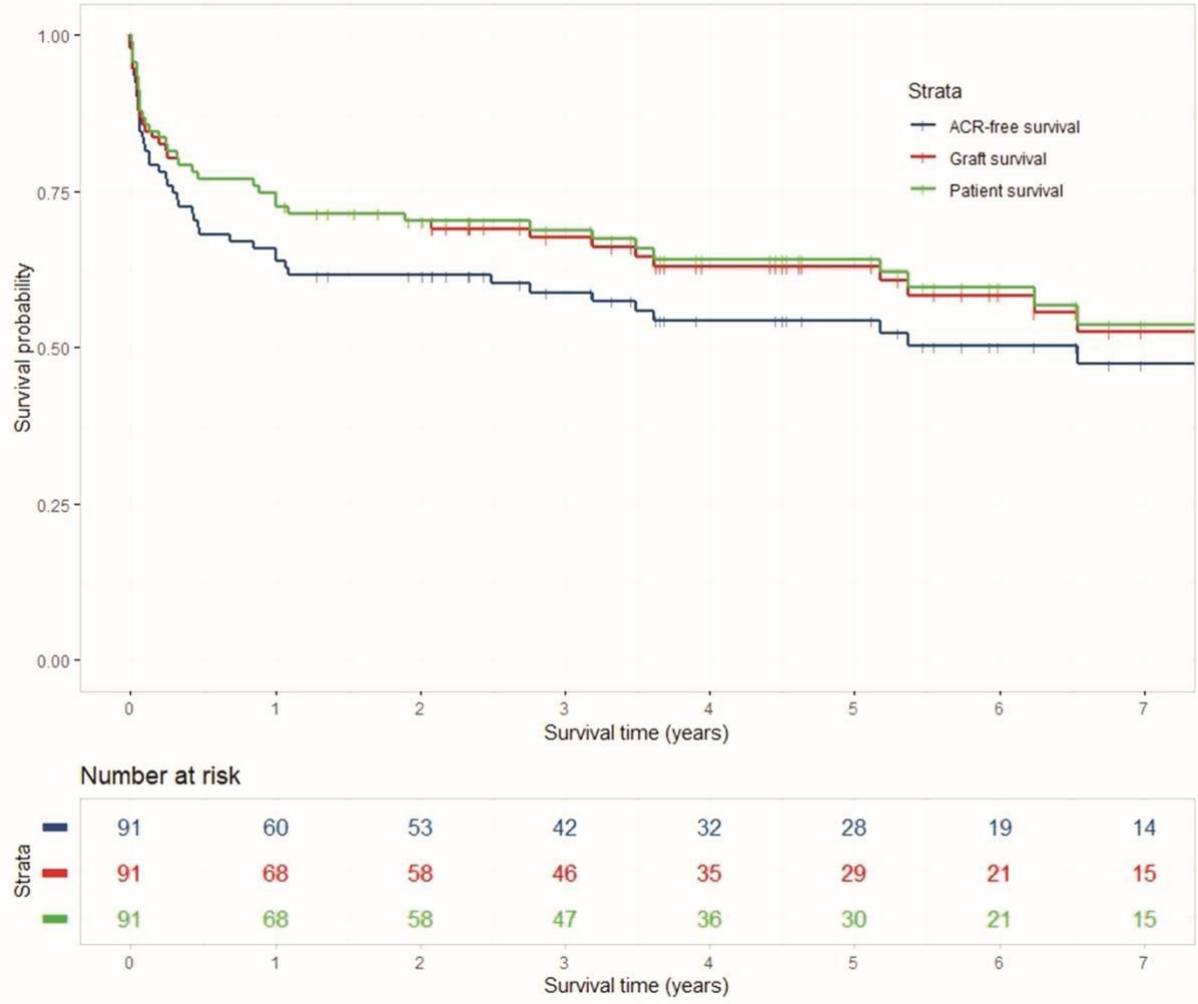
Data are presented as %. KDIGO, Kidney Disease Improving Global Outcomes

The median follow-up duration was 3.8 years. Patient survival rates at 1, 3, and 5 years were 75%, 69%, and 64%, respectively. Graft survival rates were similar. Of the patients with a known cause of death, infections were the most common cause (n=8), followed by multi-organ failure (n=3), primary non-function of the graft (n=2), cardiopulmonary complications (n=2), surgical complications (n=2), and neurological complications (n=1).

Of the 91 patients evaluated, five (5%) developed acute cellular rejection within the first 3 months, four of which were empirically treated and one that was biopsy-proven.

Table 3 Survival rates among 91 liver transplant recipients at WITS Donald Gordon Medical Centre in Johannesburg, South Africa		
Estimated patient survival (%, 95% CI)	1 year	75 (64-82)
	3 years	69 (58-77)
	5 years	64 (53-73)
Estimated graft survival (%, 95% CI)	1 year	75 (64-82)
	3 years	68 (57-76)
	5 years	63 (52-72)
Estimated ACR-free survival (%, 95% CI)	90 days	77 (67-84)
	1 year	66 (55-75)

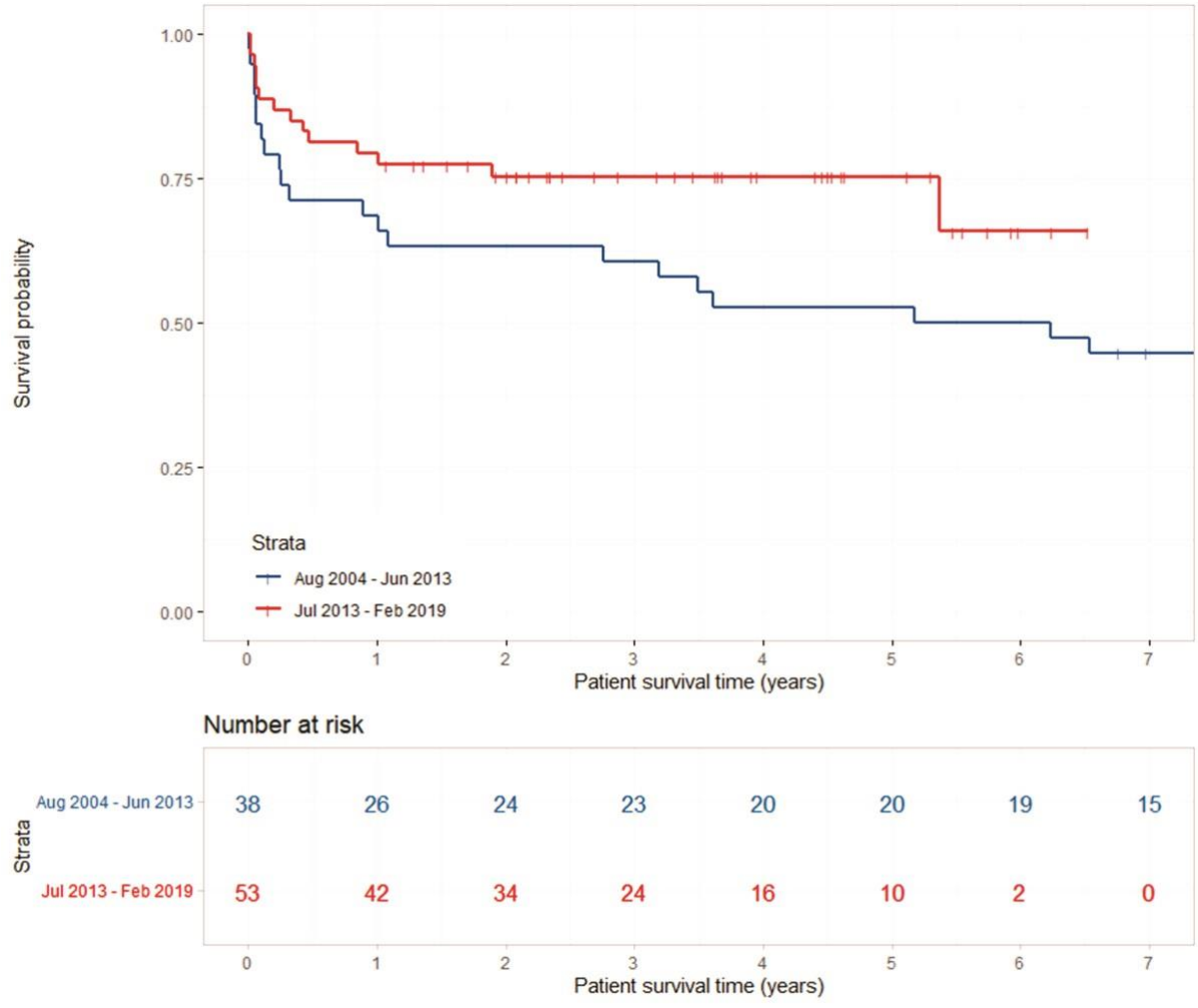
Figure 1. Kaplan-Meier curves depicting patient, graft and ACR-free survival among liver transplant recipients 60 years or older in Johannesburg, South Africa



ACR, acute cellular rejection

Kaplan Meier curves reflecting the difference between survival outcomes in the earlier and later years are depicted in Figure 2. Survival outcomes between the 2 eras did not reach statistical significance for a difference in patient survival (HR for era 2 vs. era 1: 0.54 (95% CI 0.27-1.08)), graft survival (HR 0.59 (95% CI 0.30-1.16)), or ACR-free survival (HR 0.75 (95% CI 0.41-1.38)). Non-significance may be impacted by the small sample size and other confounders.

Figure 2. Kaplan Meier curves depicting a comparison of survival rates between Era 1 (2004-2013) and Era 2 (2013-2019)



There was no significant association between diabetes and recipient survival, either unadjusted (HR 1.21 (95% CI 0.62-2.36)) or adjusted for sex, MELD score, and DRI (HR 1.17 (95% CI 0.59-2.31)). There was a significant increase in the risk of death for GFR categories 3a-5 (vs 1-2) unadjusted (HR 2.83 (95% CI 1.32-6.08)) and adjusted for sex, MELD score, and DRI (HR 3.47 (95% CI 1.54-7.84)). There was no significant association between coronary artery disease and recipient survival, either unadjusted (HR, 1.46 (95% CI 0.70-3.02)) or adjusted for sex, MELD score, and DRI (HR, 1.33 (95% CI 0.60-2.95)).

Table 4
The impact of pre-liver transplant diabetes mellitus, renal dysfunction and coronary artery disease on survival outcomes amongst LT recipients 60 years or older in Johannesburg, South Africa

Characteristic	n	p-value	HR for death	95% CI for HR	
Diabetes (unadjusted)	88	0,58	1,21	0,62	2,36
Diabetes (adjusted)	88	0,65	1,17	0,59	2,31
Gender (Female vs Male)		0,37	0,68	0,30	1,57
MELD score		0,53	0,98	0,94	1,04
DRI		0,10	1,83	0,89	3,76
GFR (3a-5 vs 1-2) (unadjusted)	86	0,0076	2,83	1,32	6,08
GFR (3a-5 vs 1-2) (adjusted)	86	0,0027	3,47	1,54	7,84
Gender (Female vs Male)		0,32	0,64	0,27	1,55
MELD score		0,08	0,95	0,90	1,01
DRI		0,06	2,06	0,97	4,39
Coronary Artery Disease (unadjusted)	77	0,31	1,46	0,70	3,02
Coronary Artery Disease (adjusted)	77	0,48	1,33	0,60	2,95
Gender (Female vs Male)		0,32	0,58	0,20	1,69
MELD score		0,16	0,96	0,90	1,02
DRI		0,018	2,42	1,17	5,02

All patients received induction with solumedrol, and 94% received solumedrol as the only induction agent. In Era 1, basiliximab was used as a second induction agent in 3 patients, and daclizumab was used as a second agent in 2 patients. During Era 2, none of the patients received a second induction agent. In Era 1, 30% received 0.5 g of solumedrol and 70% received 1.0 g as compared to Era 2 in which all patients received a standard dose of 1.0 g.

Discharge immunosuppression data were missing in more than 30% of cases and thus could not be reported.

At *1 month*, the majority of patients were on triple immunosuppression with prednisone, tacrolimus, and MMF. There were no significant changes in the number or choice of immunosuppressants between Era 1 and Era 2 but the mean daily prednisone doses in Era 1 was 19,8 mg and in Era 2 were significantly lower at 13,2 mg (p=0.023).

At *3 months*, more patients were on dual as opposed to triple IS in Era 2 (this approached significance p=0.070). In addition, fewer patients were still on prednisone in Era 2 (this approached significance p=0.10). There was a significant decrease in the use of MMF between Era 1 and Era 2 (91% vs. 65%, p=0,031). Mean prednisone doses at 3 months were not significantly different.

Table 5
Choice of immunosuppressant and number of immunosuppressants taken concurrently at 1 and 3 months post-liver transplant with comparison made between an earlier and later transplant era.

Characteristic	**Category**	Overall		Aug 2004 - Jun 2013		Jul 2013 - Feb 2019		p-value for comparison of transplant eras
1m Immunosuppression (n=62)		**n**	**%**	**n**	**%**	**n**	**%**	
	Tacrolimus	55	89	20	87	35	90	>0.99
	Prednisone	53	85	23	100	39	100	>0.99
	MMF	48	77	21	91	27	69	0,067
	Everolimus	10	16	3	13	7	18	0,73
	Cyclospori n	2	3	1	4	1	3	>0.99
Number of 1m immunosuppressants (n=62)	1	1	2	0	0	1	3	0,67
	2	18	29	5	22	13	33	
	3	41	66	17	74	24	62	
	4	2	3	1	4	1	3	
1m mean daily prednisone dose (n=53)		18,5		19,8		13,2		0,023
1m mean daily MMF dose (n=48)		1,9		1,9		1,9		0,95
3m Immunosuppression (n=60)	Tacrolimus	51	85	19	83	32	86	0,72
	Prednisone	39	65	18	78	21	57	0,10
	MMF	45	75	21	91	24	65	0,031
	Everolimus	15	25	4	17	11	30	0,37
	Cyclospori n	3	5	2	9	1	3	0,55
	1	1	2	0	0	1	3	0,070
	2	27	45	7	30	20	54	
Number of 3m immunisuppressants (n=60)	3	30	50	14	61	16	43	
	4	2	3	2	9	0	0	
3m mean daily prednisone dose (n=39)		11,8		13,7		10,8		0,33
3m mean daily MMF dose (n=45)		1,8		1,7		1,8		0,42

Discussion

We found that pre-LT renal impairment has a significant impact on survival outcomes, but that coronary artery disease and diabetes have no significant impact. Survival outcomes

were not significantly different between transplant eras, however prescription patterns have changed between transplant eras, likely on the basis of initial experience and emerging data highlighting the role of Immunosenescence in older patients. The small sample size and other contributors like the high DRI and impact of pre-LT renal impairment on survival outcomes may have limited significant improvement over time. A significantly lower prednisone dose has been used in the later era, and a trend towards favouring dual over triple IS approached significance. We found a low incidence of ACR overall and found severe infections to be the most common cause of death whereas malignancy did not account for any deaths.

Despite a 2012 survey indicating overall awareness around organ donation in metropolitan populations in sub-Saharan Africa, a shortage of organ donors persists and leads to a poorer quality of grafts (3). The impact of donor age and graft quality on survival outcomes is more pronounced in the older cohort (4)(5). Older patients are also more likely to die or drop off transplant lists at higher MELD scores so waiting for good quality donors is not always possible (5,6). As such we evaluated the Donor Risk Index and MELD scores at transplant. The average DRI among this cohort of recipients was 2.0, which confers a higher relative risk to graft survival and reflects the poorer quality of grafts available in a country with significant organ shortages (2). It is also likely to reflect a selection bias where older recipients are allocated “extended criteria donors”. In contrast, the average DRI for all transplant recipients at the WDGMC between 2004 and 2016 was 1.61 (1). Among 9882 deceased donor liver grafts in the US between 2002 and 2003, only 504 (5%) had DRI scores above 2, in contrast to this cohort, where 42% of DRI scores were above 2. Within the Eurotransplant region, the average DRI score was 1.71 at database review between 2003 and 2007(13). Individual factors influencing the high DRI in our cohort were not reviewed in this paper; however, the score is significantly influenced by donor age and the impact of advanced donor age as a prognosticator of post-transplant grafts, and patient

outcomes have been well described (5)(4). Thus the impact of the high DRI must be taken into consideration when comparing survival outcomes in our cohort to international centres.

A large meta-analysis of liver transplants in the elderly found that 5 studies displayed a significantly lower MELD in the older cohort and 6 studies did not establish a difference (6). The mean MELD score of 16.7 was on par with the mean reported in 2016 across all recipients at WDGMC and fell within the range of 12,2 to 18,4 reported as average scores among the five studies with significantly lower MELD scores internationally. This may reflect a circumspect approach to candidate selection in older patients, especially in light of the above-average DRI in our setting and given that increasing MELD scores are associated with higher 5 year mortality among patients over 50 years (14). It may also reflect that older patients with higher MELD scores did not make it to transplant as they tolerate higher scores poorly and are more likely to die or drop off the transplant list (5).

Given the high DRI and intermediate MELD scores in this cohort, future studies should evaluate the DR-MELD score [(donor age + recipient age) × MELD], which has shown prognostic significance (15).

The most common causes of ESLD or indications for liver transplant among our cohort contrast with the UK and US, where HCC and hepatitis C are more common, followed by ASH/NASH (16)(10). This reflects a higher burden in the developed world of chronic hepatitis C with progression to HCC and may change with the advent of direct-acting antiviral therapy. Although South Africa has a high prevalence of chronic hepatitis B, this is primarily in the public sector, where education and referral for evaluation of transplant once HCC has developed may need to be optimized.

Overall 1 year survival rates were lower than those in developed nations; a center in Spain and another in the UK reported 82%, and a French center reported 84% for cohorts 65 and older (10) (7); however, these were high-volume centers with older patient populations and

lower average DRI. At 5 years, survival is comparable to that of the US; a 2014 UNOS database review reported 5 year survival rates of 69,5%, 65% and 57,5% for age groups 6064, 65-69 and over 70, respectively (14)(17). However, the Mayo Clinic reported a higher 5 year survival rate for recipients aged 60-69 at 77,2% (18).

The finding that malignancy is not a cause of death in this cohort is in contrast to developed countries where de novo and recurrent malignancy accounted for a significant proportion of deaths (9) (16). This is likely due to fewer patients with HCC as an indication for transplant but may also reflect a shorter median follow-up (3,8 years) or possibly, benefits of increasing the use of everolimus as has been shown elsewhere (19).

In general, renal dysfunction at the time of liver transplantation has been accepted as a risk factor for mortality since the 90's. Apart from short and long term patient survival, there is also an increase in costs, post transplant sepsis, the need for dialysis following transplant and the duration of ICU stay. In contrast, coronary artery disease has not been clearly established as a risk factor as variable findings have been made in different cohorts. A scientific statement from the American Heart Association (AHA) and the American College of Cardiology (ACC) in 2012 recommended screening for CAD pre-LT in individuals with more than three cardiovascular risk factors. In contrast to AHA, the 2009 European Society of Cardiology guidelines routinely recommend pre-LT coronary angiography (7). Given the conflicting recommendations, we specifically evaluated coronary artery disease to establish it's impact on survival outcomes in our cohort. We routinely do pre-LT coronary angiography and PCI as indicated among older patients so evaluating whether coronary artery disease still impacted their outcomes was of interest.

Our findings corroborated those of Bilbao et al. who retrospectively compared 72 patients between 66-70 years old with 314 patients aged 65 and under. Pre-LT (liver transplant) diabetes, hypertension, and cardiovascular disease were all analyzed, but pre-LT renal

insufficiency was found to be the only statistically significant risk factor on multivariate analysis(10).

However, our findings conflicted with Sonny et al at Cleveland Clinic where CAD was found to have a significant impact on post-transplant mortality at 30 days, 1 year and last follow up (odds ratio of 7.3 at 1 year, 95% CI 1.8-29.4). Of their cohort of 223 patients over 65, only 4% had evidence of CAD during pre-transplant workup as compared to 24% in our cohort. The higher incidence of CAD is likely due to the demographic of our patients which were predominantly white males with ASH/NASH (48%) as compared to their cohort which was predominantly HCC (39%) and Hepatitis C (24%). Patients with ASH/NASH have a higher prevalence of systemic comorbidities including a higher BMI, diabetes mellitus and systemic cardiovascular disease. Given the multisystemic metabolic issues that these patients face it may be expected that their post-transplant survival outcomes would be worse as compared to Hepatitis C Virus and Hepatocellular Carcinoma. However Wong et al in a review of the UNOS database from 2002 to 2012 found the opposite to be true (20). Despite having a higher BMI and a higher prevalence of diabetes and cardiac disease, patients with NASH had better post-liver transplantation survival compared to patients with HCV (HR 0.69, 95% CI 0.63–0.77) or HCC. Patients with ASH also had superior survival outcomes.

Induction using monoclonal antibodies has shown benefits in terms of preserving GFR without negatively impacting acute cellular rejection (21), although this has not been assessed specifically in an older cohort who may require less IS (22). Given our finding that pre-LT renal insufficiency is a significant risk factor for posttransplant mortality, using monoclonal antibodies or antithymocyte globulin (ATG) to delay CNI introduction or reduction may be considered in specific cases. The current usage in our setting is limited to underlying autoimmune hepatitis or rejection.

In the more recent era, at 3 months, fewer patients were on triple IS (p=0,07, approaching significance). This reflect a trends towards weaning to dual IS more quickly as seen elsewhere (23)(6). Factors influencing the decrease in use of MMF at 3 months in the later era, such as CMV and other infections, as well as the increasing use of everolimus, need to be evaluated further.

A consensus statement issued by the International Liver Transplantation Society in 2018 did not mention any special recommendations for immunosuppression in the elderly(24). A survey of 15 liver transplant centers across North America in 2018 revealed a wide variability in current immunosuppression strategies for maintenance. In the early post-transplant period, most centers use triple therapy with steroids, an antimetabolite (MMF), and a calcineurin inhibitor (tacrolimus)(25). This aligns with our current most common initial maintenance strategy.

At our centre, despite a lower dose of prednisone used at 1 month (p=0,023) and a trend towards dual IS at 3 months, rates of ACR were low (5%). To our knowledge, no other study has documented the successful usage of lower prednisone dosages in an older cohort, and prospective randomized trials evaluating adapted immunosuppressive protocols are so far not available (22). A single-center retrospective evaluation of 143 adult liver transplant recipients in Chicago from 2013 to 2018 compared acute rejection, infection, malignancy, and survival outcomes among recipients aged > 60 years to those aged < 60 years on the same immunosuppression regimen (23). The patients received methylprednisolone induction and were maintained on tacrolimus, mycophenolate mofetil (MMF), and prednisone taper. Both groups had a similar incidence of first rejection within one year (19.2% in the elderly group vs. 23.1% in the younger group, p = 0.57), and there were no statistical differences in terms of infection, malignancy, or patient survival.

At meta-analysis, two studies analyzing the incidence of acute rejection showed a significantly lower incidence in the elderly group, whereas five did not establish a significance (6). The Scientific Registry of Transplant Recipients (SRTR) database has shown that the rate of rejection in LT recipients aged 34 years or younger is over 50% higher than that in recipients aged over 65 years. (26). Tullius and Milford (27) observed a steady decrease in acute rejection with increasing recipient age in kidney transplant recipients. Similarly, Cross et al. found lower ACR rates in LT recipients in an older cohort than in younger cohorts at the same center between 1998 and 2003 (43% vs. 61%, p=0,0016). A more recent publication by Almalki et al. at a single center in Chicago between 2013 and 2018 found ACR rates among a cohort (n=68) with a mean age of 65 to be 11,5% with a time to rejection of less than 100 days (23). Consistent with these findings, low rates of ACR have been shown here (5%).

Despite this, due to a lack of prospective data, older patients are often treated with the same IS regimen (10) (28). This may lead to over-immunosuppression in elderly recipients, resulting in an increased rate of infections and malignancies without any positive impact on acute rejection (29)(30)(31).

An Italian group compared the combination of tacrolimus and MMF with tacrolimus and everolimus between two matched groups of 178 patients, each with a mean age of 51 years (32). The everolimus group had a decreased incidence of biopsy-proven acute rejection episodes (6 patients vs. 13 patients). They also noted a significantly decreased risk of de novo malignancy among patients on everolimus (1,7% verses 6,7%). However, EVR was associated with more adverse events and drug discontinuation, most frequently due to hyperlipidemia, thrombocytopenia, edema, and oral sores. In earlier use of EVR between 2005-2008, 70% of patients developed an adverse event to the drug; however, dose adjustments resolved most issues, and only 16% needed discontinuation(33). No local review of the side-effect profile and tolerance of everolimus among transplant recipients has

been published. Further evaluation of rates of ACR and rates of de novo malignancy amongst patients on Everolimus at WDGMC may be beneficial.

Study limitations

The study was conducted at a single center and in a retrospective manner with a limited cohort of 91 patients, impacting the statistical significance. Discharge immunosuppression data were missing and could not be commented upon. For patients who died at follow-up at home, cause of death was not recorded. However, its strength resides in the relative consistency of clinical practice, including the selection process, transplant surgical approach, and pre-transplant and post-transplant multidisciplinary management, as defined by protocols in our transplant program. In this study, we did not directly assess the quality of life of the elderly LT recipients. Although the selection process requires the functional capability of all elderly candidates, their quality of life after LT requires further evaluation.

Conclusion

Although age has previously been identified as an independent risk factor at WDGMC, carefully selected older LT recipients have survival outcomes comparable to international standards, despite a poor availability of donor organs in our setting resulting in a higher average DRI. Efforts to improve organ donation should be enhanced to afford older recipients better grafts which in turn would improve survival outcomes. Pre-LT renal insufficiency has a significant impact on survival outcomes among older LT recipients, and strategies to minimize the risk of this condition should be considered. Of note, as compared to some other international centres, CAD did not have an impact on survival. IS prescribing patterns have adapted to accommodate for Immunosenescence over the years but further prospective studies are required to establish clearer guidelines for IS in the elderly.

References

1. Song E, Fabian J, Boshoff PE, Maher H, Gaylard P, Bentley A, et al. Adult liver transplantation in Johannesburg, South Africa (2004-2016): Balancing good outcomes, constrained resources and limited donors. South African Med J. 2018;108(11):929–36.
2. Tager S, Etheredge HR, Fabian J, Botha JF. Reimagining liver transplantation in South Africa: A model for justice, equity and capacity building - the Wits Donald Gordon Medical Centre experience. South African Med J. 2019;109(2):84–8.
3. Etheredge HR, Turner RE, Kahn D. Public attitudes to organ donation among a sample of urban-dwelling South African adults: A 2012 study. Clin Transplant. 2013;27(5):684–92.
4. Durand F, Levitsky J, Cauchy F, Gilgenkrantz H, Soubrane O, Francoz C. Age and liver transplantation. J Hepatol [Internet]. 2019;70(4):745–58. Available from: https://doi.org/10.1016/j.jhep.2018.12.009
5. Su F, Yu L, Berry K, Liou IW, Landis CS, Rayhill SC, et al. Aging of Liver Transplant Registrants and Recipients: Trends and Impact on Waitlist Outcomes, Post-Transplantation Outcomes, and Transplant-Related Survival Benefit. Gastroenterology. 2016;150(2):441-453.e6.
6. Gómez Gavara C, Esposito F, Gurusamy K, Salloum C, Lahat E, Feray C, et al. Liver transplantation in elderly patients: a systematic review and first metaanalysis. Hpb [Internet]. 2019;21(1):14–25. Available from: https://doi.org/10.1016/j.hpb.2018.07.025

7. Poldermans D, Bax JJ, Boersma E, De Hert S, Eeckhout E, Fowkes G, et al. Guidelines for pre-operative cardiac risk assessment and perioperative cardiac management in non-cardiac surgery. Eur Heart J. 2009;30(22):2769–812.
8. Aduen JF, Sujay B, Dickson RC, Heckman MG, Hewitt WR, Stapelfeldt WH, et al. Outcomes after liver transplant in patients aged 70 years or older compared with those younger than 60 years. Mayo Clin Proc. 2009;84(11):973–8.
9. Cross T. Liver Transplantation in Patients Over 60 And 65 Years: Evaluation of Long-Term Outcomes and Survival. Liver Transplant. 2007;13(5):767–8.
10. Bilbao I, Dopazo C, Lazaro JL, Castells L, Escartin A, Lopez I, et al. Our experience in liver transplantation in patients over 65 yr of age. Clin Transplant. 2008;22(1):82–8.
11. Zhou J, Hu Z, Zhang Q, Li Z, Xiang J, Yan S, et al. Spectrum of de novo cancers and predictors in liver transplantation: Analysis of the scientific registry of transplant recipients database. PLoS One. 2016;11(5):1–14.
12. WDGMC. Registry of Transplant Recipients Annual Report: Adult Liver Transplantation December 2019. 2019.
13. Blok JJ, Braat AE, Adam R, Burroughs AK, Putter H, Kooreman NG, et al. Validation of the Donor Risk Index in Orthotopic Liver Transplantation Within the Eurotransplant Region. Liver Transplant. 2012;18:113–20.
14. Malinis MF, Chen S, Allore HG, Quagliarello VJ. Outcomes among older adult liver transplantation recipients in the model of end stage liver disease (meld) era. Ann Transplant. 2014;19(1):478–87.
15. Kostakis ID, Iype S, Nasralla D, Davidson BR, Imber C, Sharma D, et al. Combining donor and recipient age with preoperative meld and ukeld scores for predicting survival after liver transplantation. Exp Clin Transplant. 2021;19(6):570–9.

16. Sonny A, Kelly D, Hammel JP, Albeldawi M, Zein N, Cywinski JB. Predictors of poor outcome among older liver transplant recipients. Clin Transplant. 2015;29(3):197–203.

17. Kalra A, Kriss M, Francis P, Norvell JP. CON: Patients of Advanced Age Should Not Routinely Undergo Liver Transplantation. Clin Liver Dis. 2019;14(2):70–3.

18. Mousa OY, Nguyen JH, Ma Y, Rawal B, Musto KR, Dougherty MK, et al. Evolving Role of Liver Transplantation in Elderly Recipients. Liver Transplant. 2019;25:1363–74.

19. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. Transplantation. 2005;80(7):883–9.

20. Wong RJ, Chou C, Bonham CA, Concepcion W, Esquivel CO, Ahmed A. Improved survival outcomes in patients with non-alcoholic steatohepatitis and alcoholic liver disease following liver transplantation: An analysis of 2002-2012 United Network for Organ Sharing data. Clin Transplant. 2014;28(6):713–21.

21. Neuberger JM, Mamelok RD, Neuhaus P, Pirenne J, Samuel D, Isoniemi H, et al. Delayed introduction of reduced-dose tacrolimus, and renal function in liver transplantation: The “ReSpECT” study. Am J Transplant. 2009;9(2):327–36.

22. Heinbokel T, Hock K, Liu G, Edtinger K, Elkhal A, Tullius SG. Impact of immunosenescence on transplant outcome. Transpl Int. 2013;26(3):242–53.

23. Almalki B, Kane C, Cunningham K, D’Agostino C, Novak A, Kapugi M, et al. Evaluation of rejection, infection, and malignancy outcomes in elderly liver transplant recipients receiving a similar level of immunosuppression compared to a younger group. Transpl Immunol [Internet]. 2021;69(August):101485.

Available from: https://doi.org/10.1016/j.trim.2021.101485

24. Charlton M, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, et al. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. Transplantation. 2018;102(5):727–43.

25. Hussaini T, Turgeon RD, Partovi N, Erb SR, Scudamore CH, Yoshida EM. Immunosuppression practices in liver transplantation: A survey of North American centers. Exp Clin Transplant. 2018;16(5):550–3.

26. Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, Noreen SM, et al. OPTN/SRTR 2017 Annual Data Report: Liver. Am J Transplant. 2019;19:184–283.

27. Tullius S, Milford E. Kidney allocation and the aging immune response. N Engl J Med. 2011;364:1369 – 1370.

28. Naylor K, Li G, Vallejo AN, Lee W-W, Koetz K, Bryl E, et al. The Influence of Age on T Cell Generation and TCR Diversity. J Immunol. 2005;174(11):7446–52.

29. Watt KDS, Pedersen RA, Kremers WK, Heimbach JK, Gores GJ. Long-term Probability of and Mortality from De-Novo Malignancy after Liver Transplantation. 2017;137(6):2010–7.

30. Karim A, Farrugia D, Cheshire J, Mahboob S, Begaj I, Ray D, et al. Recipient age and risk for mortality after kidney transplantation in England. Transplantation. 2014;97(8):832–8.

31. Sutherland AI, Ijzermans JNM, Forsythe JLR, Dor FJMF. Kidney and liver transplantation in the elderly. Br J Surg. 2016;103(2):e62–72.

32. De Simone P, Carrai P, Coletti L, Ghinolfi D, Petruccelli S, Precisi A, et al.

Everolimus vs Mycophenolate Mofetil in Combination With Tacrolimus: A Propensity Score-matched Analysis in Liver Transplantation. Transplant Proc. 2018;50(10):3615–20.

33. Vallin M, Guillaud O, Morard I, Gagnieu MC, Mentha G, Adham M, et al. Tolerability of everolimus-based immunosuppression in maintenance liver transplant recipients. Clin Transplant. 2011;25(4):660–9.

CHAPTER 3: APPENDIX

APPENDIX A: Data Collection sheet

DATA COLLECTION SHEET: LIVER TRANSPLANTS IN PATIENTS OVER 60 AT WITS DGMC

Study Number

Demographic Data	Age at transplant	60-64	
		65-69	
		70 and above	
	Gender	Male	
		Female	
	Ethnicity	Caucasian	
		Black	
		Asian	
		Coloured	
		Other	
Pre-transplant comorb	Diabetes Mellitus		
	Heart Disease		
		Coronary Artery Disease	
		Ejection Fraction	
		Rhythm	Less than 40%
			40-49%
			50% and above
		Diastolic Function	Sinus Rhythm
	Kidney Disease		Arrythmia
			Grade 1
		Glomerular Filtration Rate (GFR)	Grade 2
			Grade 3
			>=90
			60-89
			45-59
			30-44
			15-29
			<15

Aetiology of recipient liver disease				
MELD Score at time of transplant				
Donor Details	Donor Risk Index			
	Age			
			<40	
			40-49	
			50-59	
			60-60	
			70+	
			Black	
		Race	Non-Black	
		Height	Yes	
			No	
		cardiac death	Whole	
			Split	
		Type of graft		
Follow Up	Date of last follow up		Daclizumab	
	Reason for loss to follow up		Basiliximab	
Immunosuppression	Induction		Corticosteroids	
			Dose	
			Corticosteroids	
			Dose	
			Cyclosporine	
		Maintenance	Dose	
	(DC,1m,3m)		Tacrolimus	
			Dose	
			Sirolimus	
			Dose	
			Mycophenolate Mofetil	
			Dose	
			Azathioprine	
			Dose	
			Everolimus	
			Dose	

		Sirolimus	
Mortality		Dose	
	acute cellular rejection	None	
		Empirically treated	
		Biopsy proven	
	Age at death		
	Cause of Death		

APPENDIX B: WDGMC Adult Liver Transplant Protocol

Adult Liver Transplant Protocol

Pre-op:

- No patient to be accepted without prior discussion with ICU consultant a nursing shift leader (to ensure bed availability and intensivist cover)
- Inform Blood bank (5666) of transplant and of patient's Blood Group (find out from transplant co-ordinator)
- Admission bloods: FBC, U&E, CMP, random Glucose, LFT, PI/PTT, CRP, PCT, Fibrinogen, Compat
- Order 10units Packed cells (leukodepleted), 10units FFP, and 3 Mega-units Platelets as Red Label Blood stat (Trenstar 5999)
- CXR, ECG
- NPO
- Consent
- Get patients` Transplant File from transplant co-ordinator

Intra-op:

- Induction of immunosuppression with Zenepax 75mg IV STAT + Solumedrol 1g IV STAT on induction

Post-op:

- Often ventilated due to acidosis and high lactates. Start with BILEVEL ventilation (PEEP=10, TV=6-8ml/kg). Change to Spontaneous Assisted Breathing as soon as patient is awake and responsive. Extubate when metabolically and haemodynamically stable.
- Run 1L 5% D/W with 150ml 8,5% Sodabic (if acidotic), or Balsol (if not acidotic and normal potassium) at 100ml/hr
- Stress Ulcer prophylaxis with Nexiam 40 mg IV BD
- Morphine titration for pain (NO Perfalgan or NSAIDS)
- Dipeptivan 100mg IV BD over 4 hrs each
- Albusol 20% IV @ 10-20 ml/hr
- Fluid bolus (use Voluven) and/or Dobs if MAP <70 mmHg
- Hourly HGT and insulin infusion
- 4 hourly ABG

Start maintenance immunosuppression:

- Solu-Medrol 40mg IV QID – wean steroids over 5-6 days down to Prednisone/Methylprednisolone 40mg daily
- Prograf 1mg NGT/PO BD - Adjust Prograf dose daily to achieve Tacrolimus trough levels of 6-8.
- Cellcept 1g IV BD – change to oral Cellecept 1g PO BD
- Repeat Zenepax 75mg IV on D5

Prophylaxis:

- Valcyte 450mg PO BD (*not* NGT)
- Bactrim 1 tab PO dly
- Fluconazole 200mg IV/PO dly

APPENDIX C: Risk Scores

Table 6. The CAD-LT risk score to predict significant coronary artery disease in liver transplant candidates.

Points associated with each category of the predictors		Risk score associated with points total		
Factors	**Points**	**Points total**	**Estimate of risk**	**Risk category**
Age		-2	0.006	
<30	0	-1	0.007	
30-39	2	0	0.010	Low-Risk
40-49	4	1	0.013	
50-59	6	2	0.016	
60-70	8	3	0.021	
>70	10	4	0.028	
Sex		5	0.036	
Male	0	6	0.046	Intermediate-Risk
Female	-2	7	0.060	
Diabetes		8	0.077	
Yes	2	9	0.098	
No	0	10	0.124	
Hypertension		11	0.156	
Yes	2	12	0.195	
No	0	13	0.240	
Tobacco pack years		14	0.292	
0-20	0	15	0.350	
21-40	1	16	0.413	
>40	2	17	0.479	High-Risk
Family history of CAD†		18	0.546	
Yes	2	19	0.611	
No	0	20	0.672	
Personal history of CAD‡		21	0.728	
Yes	7	22	0.778	
No	0	23	0.820	
		24	0.856	
		25	0.886	

CAD-LT, coronary artery disease in liver transplantation.
†Defined as history of CAD in a first-degree family member.
‡Defined as history of percutaneous coronary intervention, coronary artery bypass grafting and/or myocardial infarction.

United Network for Organ Sharing and Organ Procurement and Transplantation Network model for end-stage liver disease score

Initial MELD score =

0.957 × loge(creatinine in mg/dL) + 0. 378 × loge(bilirubin in mg/dL) + 1.120 x loge(INR) + 0.643

A value of 1.0 is used for any laboratory result less than 1.0.

For candidates with renal dysfunction (creatinine ≥4.0mg/dL) or those who have received two or more dialysis sessions or a single 24 hour session of continuous veno-venous haemodialysis in the past week, a creatinine value of 4.0mg/dL is used in the above formula.

The calculated score is rounded off to the tenth decimal place and then multiplied by 10 to give the initial MELD score. The maximum score is 40.

If the initial MELD score greater than 11, the next formula, accounts for serum sodium level and is used to calculate the final MELD score:

MELD = MELD(initial) + 1.32×(137-sodium) – [0.033×MELD(initial)×(137-sodium)]

A value of 125 is used for any sodium value less than 125 mmol/L and 137 for any sodium result above 137mmol/L.

APPENDIX D: Descriptive Results

Characteristic	Category	n	%
n		**91**	
Year of transplant	2006	2	2
	2007	3	3
	2008	4	4
	2009	7	8
	2010	5	5
	2011	6	7
	2012	7	8
	2013	6	7
	2014	9	10
	2015	10	11
	2016	11	12
	2017	10	11
	2018	10	11
	2019	1	1
Transplant era	Aug 2004 - Jun 2013	38	42
	Jul 2013 - Feb 2019	53	58
Age at transplant	60-64y	52	57
	65-69y	28	31
	70-74y	11	12
Age (years)	median (IQR)	64 (62-66)	
Gender	Male	68	75
	Female	23	25
Ethnicity	White	79	87
	Black African	5	5
	Other	7	8
Cause of ESLD	ASH/NASH	44	48
	Cholestatic	20	22

	Other	10	11
	Malignancy	8	9
	Hepatitis C	5	5
	Metabolic disease	3	3
	Hepatitis B	1	1
MELD score	<15	37	41
	15-29	51	56
	30-34	2	2
	>35	1	1
MELD score	mean (SD)	16.7 (6.9)	
DRI	1.41-1.60	10	11
	1.61-1.80	13	15
	1.81-2.00	28	32
	>2.00	37	42
	Unknown	3	
DRI	mean (SD)	2.0 (0.4)	
Diabetes	No	64	70
	Yes	27	30
GFR (KDIGO category)	G1	21	24
	G2	49	56
	G3a	9	10
	G3b	8	9
	Unknown	4	
Ejection Fraction	Above 50%	88	100
	Unknown	3	
Diastolic Dysfunction	Grade 1	16	18
	None	71	82
	Unknown	4	
ECG Rhythm	Sinus Rhythm	88	100
	Unknown	3	

Coronary Artery Disease	No	61	76
	Yes	19	24
	Unknown	11	
Induction immunosuppression (n=91 alive at induction)	Known	86	95
	Unknown	5	5
Induction Immunosuppression (n=86)	Solumed	86	100
	Simulect 20 mg	3	3
	Zenapax	2	2
	MMF	1	1
Number of induction immunisuppressants (n=86)	1	81	94
	2	4	5
	3	1	1
Solumed daily dose (n=86)	0,5	10	11
	1	76	84
Discharge immunosuppression (n=79 alive at discharge)	Known	50	63
	Unknown	29	37
1m immunosuppression (n=77 alive w functioning graft at 1m)	Known	62	81
	Unknown	15	19
1m Immunosuppression (n=62)	Tacrolimus	55	89
	Prednisone	53	85
	MMF	48	77
	Everolimus	10	16
	Cyclosporin	2	3
	Sirolimus	0	0
	Aza	0	0
Number of 1m immunisuppressants (n=62)	1	1	2
	2	18	29
	3	41	66
	4	2	3
1m daily prednisone dose (n=53)	2,5	1	2
	5,0	4	8
	7,5	1	2

	10	12	23
	15	7	13
	20	16	30
	25	1	2
	30	7	13
	40	3	6
	50	1	2
1m daily MMF dose (n=48)	1	9	19
	1,5	1	2
	2	35	73
	2,5	1	2
	3	1	2
	4	1	2
3m immunosuppression (n=74 alive w functioning graft at 3m)	Known	60	76
	Unknown	14	18
3m Immunosuppression (n=60)	Tacrolimus	51	85
	Prednisone	39	65
	MMF	45	75
	Everolimus	15	25
	Cyclosporin	3	5
	Sirolimus	1	2
	Aza	0	0
Number of 3m immunisuppressants (n=60)	1	1	2
	2	27	45
	3	30	50
	4	2	3
3m daily prednisone dose (n=39)	2,5	1	3
	4	1	3
	5	12	31
	7,5	2	5
	10	8	21
	15	6	15
	20	5	13
	30	1	3
	40	2	5
3m daily MMF dose (n=45)	0,5	1	2
	0,75	1	2
	1	9	20
	1,5	3	7
	2	28	62

	2,5	2	4
	3	1	2
ACR in first 3m	Deceased within 3m - Biopsy done, no ACR	2	2
	Deceased within 3m - Bx done, ACR present	1	1
	Deceased within 3m - no bx, no PM	8	9
	No	75	83
	Yes	4	4
	Unknown	1	
Estimated patient survival (%, 95% CI)	1 year	75 (64-82)	
	3 years	69 (58-77)	
	5 years	64 (53-73)	
Estimated graft survival (%, 95% CI)	1 year	75 (64-82)	
	3 years	68 (57-76)	
	5 years	63 (52-72)	
Estimated ACR-free survival (%, 95% CI)	90 days	77 (67-84)	
	1 year	66 (55-75)	
Cause of death (n=39)	Infection/ sepsis	8	
	Multiple organ failure	3	
	Primary nonfunction of graft	2	
	Cardiopulmonary	2	
	Surgical complications other that infection	2	
	Central nervous system	1	
	Unknown	21	

APPENDIX E: Immunosuppression results

Characteristic	Category	Overall		Aug 2004 - Jun 2013		Jul 2013 - Feb 2019		p-value for comparison of transplant eras
		n	%	n	%	n	%	
n		91		38		53		
Induction immunosuppression (n=91 alive at induction)	Known	86	95	33	87	53	100	
	Unknown	5	5	5	13			
Induction Immunosuppression (n=86)	Solumed	86	100	33	100	53	100	>0.99
	Simulect 20 mg	3	3	3	9	0	0	0,053
	Zenapax	2	2	2	6	0	0	0,14
	MMF	1	1	1	3	0	0	0,38
Number of induction immunisuppressants (n=86)	1	81	94	28	85	53	100	0,0068
	2	4	5	4	12			
	3	1	1	1	3			
Solumed daily dose (n=86)	0,5	10	11	10	30			
	1	76	84	23	70	53	100	
Mean daily solumed dose (n=86)		0,94		0,95		1,00		0,0007
Discharge immunosuppression (n=79 alive at discharge)	Known	50	63	16	52	35	73	
	Unknown	29	37	15	48	13	27	
1m immunosuppression (n=77 alive w functioning graft at 1m)	Known	62	81	23	74	39	85	
	Unknown	15	19	8	26	7	15	
1m Immunosuppression (n=62)	Tacrolimus	55	89	20	87	35	90	>0.99
	Prednisone	53	85	23	100	39	100	>0.99
	MMF	48	77	21	91	27	69	0,067
	Everolimus	10	16	3	13	7	18	0,73
	Cyclosporin	2	3	1	4	1	3	>0.99
Number of 1m immunisuppressants (n=62)	1	1	2	0	0	1	3	0,67
	2	18	29	5	22	13	33	
	3	41	66	17	74	24	62	
	4	2	3	1	4	1	3	
1m mean daily prednisone dose (n=53)		18,5		19,8		13,2		0,023
1m mean daily MMF dose (n=48)		1,9		1,9		1,9		0,95
	Known	60	76	23	79	37	82	

3m immunosuppression (n=74 alive w functioning graft at 3m)	Unknown	14	18	6	21	8	18	
3m Immunosuppression (n=60)	Tacrolimus	51	85	19	83	32	86	0,72
	Prednisone	39	65	18	78	21	57	0,10
	MMF	45	75	21	91	24	65	0,031
	Everolimus	15	25	4	17	11	30	0,37
	Cyclosporin	3	5	2	9	1	3	0,55
Number of 3m immunisuppressants (n=60)	1	1	2	0	0	1	3	0,070
	2	27	45	7	30	20	54	
	3	30	50	14	61	16	43	
	4	2	3	2	9	0	0	
3m mean daily prednisone dose (n=39)		11,8		13,7		10,8		0,33
3m mean daily MMF dose (n=45)		1,8		1,7		1,8		0,42

APPENDIX F: Patient and Graft survival results

Patient survival

Characteristic	n	p-value	HR for death	95% CI for HR	
Diabetes (unadjusted)	88	0,58	1,21	0,62	2,36
Diabetes (adjusted)	88	0,65	1,17	0,59	2,31
Gender (Female vs Male)		0,37	0,68	0,30	1,57
MELD score		0,53	0,98	0,94	1,04
DRI		0,10	1,83	0,89	3,76
GFR (3a-5 vs 1-2) (unadjusted)	86	0,0076	2,83	1,32	6,08
GFR (3a-5 vs 1-2) (adjusted)	86	0,0027	3,47	1,54	7,84
Gender (Female vs Male)		0,32	0,64	0,27	1,55
MELD score		0,08	0,95	0,90	1,01
DRI		0,06	2,06	0,97	4,39
Coronary Artery Disease (unadjusted)	77	0,31	1,46	0,70	3,02
Coronary Artery Disease (adjusted)	77	0,48	1,33	0,60	2,95
Gender (Female vs Male)		0,32	0,58	0,20	1,69
MELD score		0,16	0,96	0,90	1,02
DRI		0,018	2,42	1,17	5,02

Graft survival

Characteristic	n	p-value	HR for death	95% CI for HR	
Diabetes (unadjusted)	88	0,63	1,18	0,61	2,28
Diabetes (adjusted)	88	0,66	1,16	0,59	2,28
Gender (Female vs Male)		0,57	0,79	0,36	1,75
MELD score		0,60	0,99	0,94	1,04
DRI		0,17	1,65	0,80	3,38
GFR (3a-5 vs 1-2) (unadjusted)	86	0,0018	3,27	1,56	6,89
GFR (3a-5 vs 1-2) (adjusted)	86	0,0006	4,01	1,81	8,89

Gender (Female vs Male)		0,58	0,79	0,35	1,82
MELD score		0,08	0,95	0,90	1,01
DRI		0,12	1,82	0,85	3,88
Coronary Artery Disease (unadjusted)	77	0,35	1,41	0,69	2,92
Coronary Artery Disease (adjusted)	77	0,46	1,35	0,61	2,99
Gender (Female vs Male)		0,57	0,75	0,28	2,02
MELD score		0,22	0,96	0,91	1,02
DRI		0,042	2,10	1,03	4,29

APPENDIX G: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Al Tayob

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200663

NAME: (Principal Investigator)	Dr Al Tayob
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine Medical School University
PROJECT TITLE:	Liver transplantation in patients over 60 years in Johannesburg, South Africa: An retospective review of risk factors, survival outcomes and immunosuppression
DATE CONSIDERED:	2020/06/26
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professors J Botha & A Mahomed
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/09/07

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore reports and re-certification will be due early in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

APPENDIX H: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I AHMAD ISMAIL TAMOB (Student number: 307031) am a student registered for the degree of MM ED in the academic year 2021.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 16/12/2021

1

APPENDIX I: Turnitin Assessment

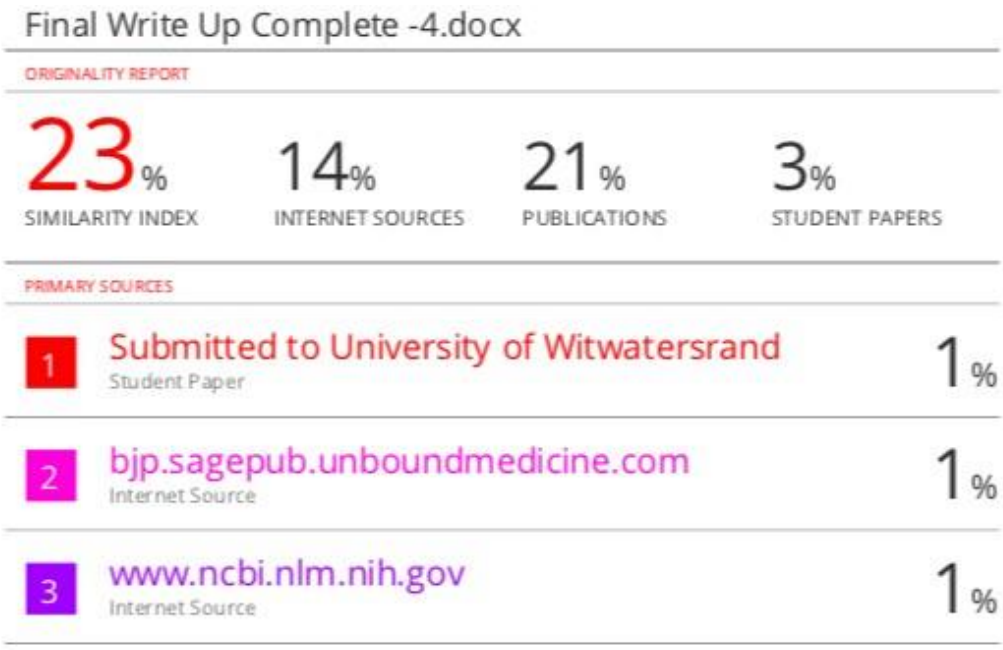
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Ahmad Tayob
Assignment title:	Draft Similarity Report
Submission title:	Final Write Up Complete -4.docx
File name:	Final_Write_Up_Complete_.docx
File size:	9.15M
Page count:	75
Word count:	14,120
Character count:	76,475
Submission date:	18-Feb-2022 02:35PM (UTC+0200)
Submission ID:	1765375489



Final Write Up Complete -4.docx

ORIGINALITY REPORT

23%	14%	21%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Witwatersrand Student Paper	1%
2	bjp.sagepub.unboundmedicine.com Internet Source	1%
3	www.ncbi.nlm.nih.gov Internet Source	1%

APPENDIX J: Presentations

ONLY ORIGINAL SIGNED ATTENDANCE REGISTERS WILL BE ACCEPTED

CONTINUING PROFESSIONAL DEVELOPMENT
WITS CPD OFFICE

CPD ATTENDANCE REGISTER COVER SHEET

Date of CPD Activity: ____10 June_____2020

Please note:

Please submit only original signed copies on a monthly basis attached to Form CPD 12 – this contains relevant information for the capture of points.

Provider Name of Company / Department	WDGMC	**Department / Division**	
Address	21 Eton Road, Parktown, 2193	**Responsible / contact person**	Kim Urban
Tel No.	011 356 6308	**Fax No.**	086 679 2228
Email	Kim.urban@mediclinic.co.za	**Accreditation No.**	
COMMENT		**Activity No.**	

NAME OF ACTIVITY: Gastroenterology CPD

Description "Do not go gently into the good night." Immunosenescence and the Gastro/Hepatologist.

PRESENTERS DETAILS: *Please Print Clearly*

PRESENTERS DETAILS	SAMDC NO / HPCSA NO	SIGNATURE	NO OF POINTS *(One) additional point per hour for presenting*	CONTACT TEL NO.
1. Dr A Tayob	MP0788848			072 652 0425
2.				
3.				
4.				
5.				

Return Address:
WITS CPD OFFICE, Suite 189 Private Bag X2600 Houghton 2041; 31 Princess of Wales Terrace, Parktown, 2193
Tel: 011 274 9274/5

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

APPENDIX K: guidelines for journal submission to *Transplantation*

MANUSCRIPT STUCTURE

□ **Cover Letter** (optional)

□ **Main Body Document:**

1. Title Page
2. Authorship Page
3. Abbreviations Page
4. Abstract (ifapplicable)
5. Main Body Text
6. Acknowledgments (ifapplicable) 7. References
8. Tables
9. Figure legend (ifapplicable)

□ **Figures** must be uploaded as separate in .tiff, .eps, or high- resolution PDF files.

□ **Supplemental Digital Content and/or Additional Files for Information Only** (if applicable) must be uploaded separately as a PDF file.

TITLE PAGE (required)

Title: This should be descriptive of the work; the title should not be a sentence.

Author listing: Include the full first name, middle initial(s), and family name of each author, each author's highest degree earned, and their affiliations. This should include the academic department(s) and institution(s) to which the work should be attributed.

Correspondence information: Full name, physical mailing address, and current email address for the corresponding author.

Clinical Trial Notation (if applicable): Provide the name of the trial registry and the registration number/identifier of the trial.

AUTHORSHIP PAGE

MANUSCRIPT CHECK: Prior to submitting, we recommend that you run your manuscript through the PaperPal Preflight service, which instantly checks your manuscript and helps you address the most common errors and omissions.

□ Arial or similar san serif typeface, 12-point font sizepreferred.

1. **Authorship:** Authorship statement is required for all submissions with more than 1 author. List each author's specific contributions to the work. For example, the areas of participation mightinclude:

- Participated in research design ○ Participated in the writing of the paper ○ Participated in the performance of the research
- Contributed new reagents or analytic tools ○ Participated in dataanalysis

An author may list more than 1 contribution, and more than 1 author may have contributed to the same aspect of the work. Each author's specific contributions to the work should be indicated; this information will be published with the manuscript. Please review ICMJE Recommendations for authorship at for further guidance.

2. **Disclosure:** Any actual or potential conflicts of interest by any of the author(s). If all of the author(s) have no conflict to disclose, please include the phrase, "The authors declare no conflicts of interest."
3. **Funding:** Disclosure of any funding received for this work, especially details of funding from any of the following organizations: National Institutes of Health, National Institute for Health Research, Wellcome Trust, Howard Hughes Medical Institute, and the Austrian Science Fund. For more information, please visit:http://www.wkopenhealth.com/inst-fund.php

ABBREVIATIONS PAGE

□ Authors are required to introduce all utilized acronyms and initialisms. These should be listed alphabetically.

□ Acronyms and initialisms should take the form: [term],[meaning] eg: DNA, deoxyribonucleic acid.

□ Terms should not be abbreviated unless they are used more than once in the document.

ABSTRACT (if applicable)

All abstracts are limited to 250 words. This is included in the overall manuscript word count. Abstracts for Overviews may be unstructured. Abstracts are not required for Letters, or Commentaries.

Abstracts for Articles and Short Reports must be structured and labeled as follows:

□ **Background:** The problem being addressed in the study.

□ **Methods:** How the study was performed.

□ **Results:** The salient results.

□ **Conclusions:** What the authors conclude from the results.

MAIN BODY TEXT

Introduction: A statement of the purpose of the work, the problem that stimulated it, and a brief summary of any relevant previously published investigations.

Materials and Methods: Detailed description of how the results were obtained. Avoid lengthy descriptions of well-known and previously published methods, cite the appropriate reference(s) instead. In-depth methods descriptions may be provided as Supplemental Digital Content and will appear in the online version only.

Guidelines: We expect studies to comply with and reference the appropriate international guidelines for creation and publication of scientific studies. Links to the following guidelines are provided on the home page of our submission website: https://www.editorialmanager.com/tpa/default.aspx
ARRIVE, **CONSORT**, **COREQ**, **ENTREQ**, **PRISMA**, **SAGER, STROBE**, **STREGA**, **TRIPOD**, **Uniform Clinical Trial Registration Policy**

Results: Raw or minimally reduced findings using the materials and methods described previously. The results should be concise, avoiding redundant tables and figures illustrating the same data.

Discussion: Interpretation, contextualization, and commentary on the results. Authors should use this section to argue the significance of their work with minimal restating of findings.

Do not include footnotes except in Authorship statements.

ACKNOWLEDGMENTS (if applicable)

This is intended for the public mention of individuals or groups who provided some type of support but do not merit co-authorship recognition under ICMJE guidelines. List funding sources on the Authorship page.

REFERENCES

Transplantation uses AMA (American Medical Association) style.

AMA Style General Guidelines:

□ References must be numbered and listed in the order in which they are cited in the text.

□ No more than 6 authors should be listed for a citation. If there are 7 or more authors, only the first 3 should be listed followed by ''et al.''

□ The title of the journal article must be included, followed by the National Library of Medicine accepted abbreviation of the journal name, the year of publication, the issue number, and page range. You can search for NLM abbreviations here: http://www.ncbi.nlm.nih.gov/nlmcatalog/journals □ Only published works and manuscripts that have been accepted for publication should be listed in the References.

TABLES

□ Each table must be typed and in an editable text file format. **DO NOT** upload tables as images.

□ Tables may be included in the main body text document, but do not upload tables more than once.

□ Each table should begin on a separate page.

□ Tables should not be embedded in the text sections, but must follow the References section or appear in a separate Word.doc file.

□ Tables should be numbered in sequence as they are referenced in the main text. Tables should have a concise, informative title, and data headings must succinctly describe the information in the column or row

□ Footnotes to tables must be made below the tables to which they refer, using superscriptletters.

□ Tables that have be published previously or are based on another source may require permission. See Permissions for more information.

FIGURE LEGENDS

□ Legends must be supplied for all figures at the end of the main text file or in a separate Word .docfile.

□ Typewritten and double spaced

□ Numbered to correspond with the appropriate figure.

FIGURES

Each figure should be individually uploaded separately as a figure file. These can be .tiff, .eps, or highresolution PDFs files types. PowerPoint .pptx, .jpg, and .png files are NOT acceptable. **Do not embed figures into the main body file.**

□ Each figure must be labeled with its figure number on the page it appears on in the figure file. Labeling the file name and/or description is not sufficient.

□ Figures should be uploaded in the highest resolution available. All figures must be at least 300 dots per inch (DPI) or higher.

□ Axis and labeling of these figures must be in black.

□ Monochrome images (such as line graphs, drawings, and diagrams) should be prepared at a resolution of 1200 dots per inch(DPI).

□ Halftones images (black/white or color) should be prepared at a resolution of 300 DPI.

□ Combination halftones (images containing both pictures and text labeling) should be prepared at 600DPI.

□ Sizing: Width for a 1-column figure is 228 pts; 2 column figure is 474 pts; Maximum height is 650pts.

□ Color images must be saved in the "CMYK" color space. Images saved in "RGB" color space are not acceptable for printing.

□ If an MS Office program is used to create artwork (Word, PowerPoint, Excel) then the file should be saved as a high- resolution PDF file before submission.