AN INVESTIGATION OF THE PREDICTORS OF REJECTION AND INFECTION IN THE ELDERLY KIDNEY TRANSPLANT RECIPIENTS

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Declaration of originality

I, INJI M ALSHAER, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The incidence of end-stage renal disease (ESRD) increases with age. Transplantation is the treatment of choice for most patients with ESRD and improves both the survival and quality of life of the older transplant recipients when compared with dialysis. However, it is also associated with morbidity consequent to the necessary pharmacological manipulation of the immune system, including infection and malignancy. There is little evidence to inform any particular immunosuppression regimen in older kidney transplant recipients. A United States(US) cross-specialist working group recommended that future research directions include investigation of the critical immune mechanisms that change with age, the need for immunosuppressive strategies to vary by age and be based on measures of immune exhaustion, investigation of clinical or laboratory parameters that could guide IS in older adults and potential development of novel measures of immune status that could be more valuable or informative in older adults.

In this thesis, I describe the existing literature on infection and frailty in older kidney transplant recipients and confirm the existing evidence that older transplant recipients (> 60 years) are at significantly increased risk of viral infections, particularly cytomegalovirus (CMV), post-transplantation. This can lead to increased frequency of hospitalization, frailty, and increased mortality. I then identified clinical parameters that could predict outcomes and demonstrated that frailty before transplantation in this cohort (>60 years old at the time of transplantation) is associated with an increased risk of infections, particularly CMV, infection-related

hospitalization, and graft failure. Finally, I looked at laboratory parameters, in particular a ratio of Interleukin-10(IL-10+) and tumour necrosis factor-α(TNF-α+) cells within transitional B cells, which have been shown to predict transplant outcomes based on recent evidence. Transitional B cell IL-10/TNF-α ratio was not affected by age in this small cohort; however, a larger cohort is needed to study the association between frailty and infection with transitional B cell cytokines.

Impact statement

The findings in this work impact scientists, physicians, patients, and society. Throughout this work, I provide evidence that older kidney transplant recipients are at significantly increased risk of viral infections, particularly cytomegalovirus (CMV), post-transplantation. This can lead to increased frequency of hospitalization, frailty, and increased mortality. On the other hand, older kidney transplant recipients have a low risk of rejection compared to young recipients. These findings may bring assurance to transplant physicians in assessing fitness for transplantation in older recipients and reducing immunosuppression (IS) to mitigate these complications, improving outcomes for older patients with end-stage kidney disease.

Frailty is a physical biomarker of ageing, rather than just chronological age-predicted infection, particularly CMV infection and infection-related admissions in older kidney transplant recipients (KTR). Therefore, using frailty scoring as a risk assessment tool before kidney transplantation could help optimise frail KTR and stratify immunosuppression to reduce adverse infectious outcomes and re-admissions. From the scientific perspective, I did not find a difference in transitional B cells IL-10/TNF with age. Recent evidence suggests low IL-10/TNF ratio is associated with poor outcomes; therefore, this could potentially be used as a biomarker in older KTR to predict rejection or adverse graft outcomes, and for stratification of immunosuppression. The data from this analysis could be used for further work on a larger cohort of the B cell subsets in young and older KTR at three months post-transplantation.

These findings may bring comfort to older kidney transplant recipients and physicians looking after older KTR in identifying suitable recipients who would benefit from a personalised approach. I used 60 years as the cut-off age instead of 65 years, given established evidence that patients with ESRD start ageing early, especially if accompanied by other comorbidities.

This work also has a societal impact, especially in older patients with ESRD. Our data highlights the importance of assessing frailty before transplantation, which is subject to improvement and consequently can have a positive impact on outcomes. The emerging biomarkers I describe also carry huge promise in allowing the tailoring of immunosuppressive regimens safely to reduce adverse effects and increase the longevity of both graft and recipient.

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Abbreviations

AMR	Antibody-mediated rejection
ANZDATA	Australia and New Zealand Dialysis and Transplant
APC	Antigen Presenting Cells
AR	Acute Rejection
ARIC	Atherosclerosis Risk in Communities
ATG	Anti-Thymocyte Globulin
BCR	B cell receptor
Be-1-cells	Effector B cells 1
BKV	BK virus
BKVN	BK virus nephropathy
BMI	Body mass index
BPAR	Biopsy-proven acute rejection
C RF	Calculated reaction frequency
CD40L	CD40 ligand
CKD	Chronic Kidney disease
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CRP	C-reactive protein
CVD	Cardiovascular disease
DBD	Donation after brain death
DC	Dendritic cells

DCD	Donation after circulatory death
DGF	Delayed graft function
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DSA	Donor-specific anti-HLA antibodies
E GFR	Estimated glomerular filtration rate
EDTA	Ethylenediaminetetraacetic acid
EFS	Edmonton Frail Scale
ERA-EDTA	European Renal Association- European Dialysis Transplant Association
ESRD	End Stage Renal Disease
FAC-H	Forward scatter Height
FACS	Fluorescence activated cell sorting.
FBS	Foetal bovine serum
FI	Frailty index
FMO	Fluorescence minus one
FSC	Forward scatter
FSC-A	Forward scatter area
HIV	Human Immunodeficiency virus
HLA	Human leucocyte antigen
HR	Hazard ratios
HSV	Herpes Simplex Virus
IFN	Interferons
IFNγ	interferon gamma
IFTA	Interstitial fibrosis and tubular atrophy

IL	Interleukin
IL2RA	interleukin-2 receptor alpha subunit
IQR	Inter-quartile range
IS	Immunosuppression
KDOQI	Kidney Disease Outcomes Quality Initiative
KIDIGO	Kidney Disease Improving Global Outcomes
KM	Kaplan-Meier
KTOP	Kidney Transplantation in Older People
KTR	kidney transplant recipients
LPS	Lipopolysaccharides
MFI	Mean fluorescence intensity
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MMF	mycophenolate mofetil
MPA	Mycophenolic acid
NHSBT	National Health Service Blood and Transplant
ODT	Organ donation and transplantation
OPTN	Organ Procurement and Transplantation Network
PBMC	peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PMA	Phorbol 12-myristate 13-acetate
RRT	Renal Replacement Therapy
SAF	Standard Analysis Files
SALT	Swedish Screen Across the Lifespan Twin

SD	Standard deviation
SLE	Systemic lupus erythematosus
SSC	Side scatter
TCR	T cell receptor
TGFb-1	Transforming growth factor beta 1
TLRs	Toll-like receptors
TNF	Tumour Necrosis factor-α
TrBs	Transitional B cells
TTV	Torque Teno Virus
UK	United Kingdom
UNOS	United Network for Organ Sharing
US	United States
USA	United States of America
USRDS	US Renal Data System

CHAPTER 1. **INTRODUCTION**

1.1. Chronic Kidney disease (CKD)

1.1.A. Definition

CKD is defined as a condition of progressive structural and functional changes to the kidneys and is caused by many underlying conditions[1]. The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) and the international guideline group Kidney Disease Improving Global Outcomes (KIDIGO) developed a CKD classification to provide better care to patients. They use estimated glomerular filtration rate (eGFR) and albuminuria as the basis of their classification, which describes five CKD stages, with prognostication and risk based on the severity of eGFR decline and extent of proteinuria[2]. End-stage renal disease (ESRD) is defined as CKD stage 5 when eGFR <15 ml/min.

1.1.B. Prevalence

The prevalence of CKD increases with age. In the United Kingdom (UK), an estimated 13.5% of people aged between 65 and 74 years and just over a third of those over the age of 75 have CKD stage 3-5, with the median age of patients presenting with ESRD at around 65 years[3].

In high-income countries, the prevalence of chronic kidney disease is generally around 11%. Hypertension and Diabetes Mellitus (DM) continue to be the most common causes of chronic kidney disease in these countries[4]**.**

It is estimated that by 2036, there will be more than four million patients with CKD stage 3-5 in the UK, according to 2012 national population projections for England. This rise in prevalence reflects an ageing population, with 24% of the UK population projected to be 65 years or older by 2036, in addition to the rise in prevalence of the underlying leading causes of CKD such as DM and Hypertension [3].

1.1.C. Treatment of CKD: Transplantation

Kidney transplantation remains the optimum renal replacement therapy for patients with ESRD, conferring survival and quality of life benefits over dialysis for the majority of patients [5] [6] [7] [8]. There are now more than 35,000 people in the UK being monitored with a working kidney transplant, which is more than are currently receiving dialysis, and represents 55% of all UK patients receiving renal replacement therapy (RRT) [9].

However, not all those with ESRD have access to kidney transplantation as a treatment modality. According to the European Renal Association- European Dialysis Transplant Association (ERA-EDTA) registry annual report in 2012, the proportion of elderly patients (≥ 65 years) receiving kidney transplants ranges between 0-35 % in Europe [10]. There is evidence that over the last decades, the number of patients over 50 years receiving transplants has increased [11].

1.1.C.A. Comparison of kidney transplantation and dialysis as modalities of RRT

Superior survival post-transplantation compared to remaining on dialysis has been reported for several decades. In 1988, data from Norway demonstrated a significantly higher survival in patients more than 60 years old who received kidney transplants in comparison with those remaining on dialysis four years post-transplant, at 62% vs 13%, respectively[12]. Analysis of data extracted from the United States Renal Data System (USRDS) Standard Analysis Files (SAF) compared patients ≥75 years old who remained on dialysis with those receiving kidney transplants. Survival at 5 years post kidney transplant was significantly higher in KTR than in those remaining on the waiting list and in those remaining on dialysis (59.9% for living donor kidneys, 40.3% for deceased donor kidneys, 29.7% remaining on the waiting list, and 12.5 % for remaining on dialysis)[13]. The risk of death post-transplantation is highest in the first 3 months, after which the relative risk of death decreases, such that by one year post-transplant there is a survival benefit for the vast majority of patients compared to staying on the waiting list [14].

Elderly recipients (>65 years old) of deceased-donor kidney transplants require a longer time to achieve the survival benefit associated with transplantation, with time to equal survival with those remaining on the waiting list ranging from 470 to 521 days [15].

1.1.C.B. Causes of mortality post transplantation

Increasing age remains a major risk factor for mortality after kidney transplantation, with the risk of death increasing proportionally with age at the time of transplantation [16] [17]. The median risk of 4-year mortality ranges from 5.8% for recipients under 50 to 22% for those aged over 60, with the three most common causes of death for recipients aged 70 and over being cardiac-related (21.2%), infection (21.2%), and malignancy (20.2%) [16]., Death with functioning graft accounts for the largest proportion of allograft losses in the older age category[18].

In comparison to those aged 18–29 years, recipients aged over 65 have a seven-fold risk of death with a functioning graft, with cardiovascular disease (CVD) being the most frequent cause of death [19]. Death due to cardiovascular disease or malignancy post-transplant is reported in 71 % of kidney transplant recipients aged >60 years old [20]. Whilst many older patients have a history of cardiovascular morbidity including hypertension, diabetes, and hyperlipidaemia, immunosuppressive drugs, in particular corticosteroids and calcineurin inhibitors (CNIs), may aggravate CVD risk after kidney transplantation[21]. Infection and cancer are two other common causes of death in older recipients [22] [23].Death related to infection increases with recipient age, as shown in a large registry study of kidney transplant recipients registered in the USRDS and United Network for Organ Sharing

(UNOS) Renal Transplant Scientific Registries [24].

1.1.C.C. Graft survival

Both US and European registry data show relatively stable graft failure rates beyond the first year post-transplantation since the late 1980s [25] [26] [27]. Notably, short-term improvements in graft survival have decreased since 2000, while long-term attrition rates have remained unchanged [26]. However, this is despite considerable changes in donor and recipient demographics over the past few decades, with increasingly older donor kidneys being transplanted into progressively older recipients with greater comorbidities [26] [28] [29].

In the United States, graft survival at 10 years post-transplant in 2008-2011 was 53.6%, almost 10% higher than graft survival in 1996-1999 for kidneys from deceased donors [30]. Immunological factors such as chronic antibody-medicated rejection are the most common causes of graft failure. Subclinical inflammation in protocol biopsies was associated with higher subsequent rejection, development of DSA, and development of chronic inflammation leading to kidney transplant failure [31].

Recent data demonstrated an association between borderline rejection or inflammation within the first 4 months post-transplant and progression to late acute rejection, interstitial fibrosis, and tubular atrophy (IFTA), and subsequently worse graft survival at 7 years post-transplantation [32].

Graft survival among older recipients (>60 years old) was comparable with young recipients in one report despite higher mortality in older recipients [23] [20]. Norwegian data showed graft survival that was similar even in recipients >70 years with those aged 60-69 years and younger groups. [33].

Data from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry between 1997 and 2017 demonstrated the importance of acute rejection in the first 6 months post-transplant, and its association with a higher risk of graft loss or death with functioning graft due to cardiovascular disease of malignancy [34]. Graft failure increased mortality 3-fold in a study of 4743 KTR from the Canadian organ replacement registry [35].The association between older age and a lower risk of graft failure is potentially explained by immune senescence in older patients, with a lower rejection rate [36].

1.1.C.D. Complications post transplantation

1.1.C.D.1. Rejection

Rejection, especially acute rejection within the first 6 months, remains an important short-term complication and is associated with adverse long-term outcomes [34], hence efforts directed towards risk prediction and risk stratification for recipients. Rejection is reported less frequently in elderly kidney transplant recipients (>60 years old) than younger; 6% vs 34% in one report. In this report, recipients received equivalent amounts of immunosuppressive medications [23].In another report comparing recipients aged >60 years with a younger cohort, the rejection rate was 6% in older KTR compared with 24% in young KTR [20].

A multivariate analysis of United Network for Organ Sharing (UNOS) data reported decreasing frequency of rejection with age [24] [37] [38]; the corollary of this suggests that older recipients are at increased risk of infection and death from infectious causes compared to younger transplant recipients. Kidneys from older donors are associated with a higher rate of rejection. However, the effect of donor age on rejection is diminished when transplanted into older recipients [37] [38]. Furthermore, other data demonstrate that kidneys from older (>55 years) living donors to older recipients (>60) have a lower rejection rate than standard or extended criteria deceased donor kidneys [39].

In addition, the elderly are at lower risk of developing de novo donor-specific antibodies (DSA), a major risk factor for rejection and associated with a fall in graft survival to 40% at 10 years. Non-adherence, which is reported considerably less in elderly KTR, in addition to HLA-DRb1 mismatches, is the main risk factor for developing these DSAs [40]. The presence of complement-binding DSA one-year post-transplantation carries a higher risk of developing graft failure than non-complement-binding antibodies [41].

1.1.C.D.2. Infection post transplantation in the elderly

Data from the ERA/EDTA registry of patients who started renal replacement therapy published in 2015 demonstrated that infection-related mortality was 32-fold higher in transplant recipients and 82-fold higher in dialysis patients in comparison with the general population in Europe. This mortality due to infection was also reported to increase with age [42].

The frequency of infectious complications secondary to CMV and urinary tract infections is significantly greater in recipients over the age of 65 years compared to those under 65 [43].

A study in 2001 utilizing the United States Renal Data System (USRDS) database demonstrated an exponential increase in mortality related to infection in elderly vs younger ESRD patients. Furthermore, the increased risk of mortality related to infection with increasing age was observed linearly in patients on the waiting list [44].

- **Cytomegalovirus (CMV)**

CMV is one of the most ubiquitous viral pathogens in the general population and is considered a risk factor for adverse outcomes in kidney transplantation. Infection with CMV frequently happens early in life and is usually asymptomatic in healthy individuals. Post-primary infection, the CMV virus has the characteristic of lifelong latency where the virus genome persists, with no infectious virus in the host. In humans, the site of latency is peripheral blood mononuclear cells (PBMC). Primary CMV infection and CMV reactivation can cause symptomatic diseases such as hepatitis, encephalitis, and pneumonitis in immunocompromised individuals, including transplant recipients on immunosuppression [45] [46].

CMV seroconversion increases with age and is reported to be higher in females than males. The percentage of CMV seropositive Asian transplant recipients was reported to be 80 %, with a lower prevalence in African Americans at 71% and Caucasians at 56 % [47].

Older data reported higher graft failure and lower patient survival in adult Caucasian kidney transplant recipients with HLA mismatch who received a kidney from a CMV-

positive donor regardless of the recipient status [10]. CMV infection is also associated with atherosclerosis and chronic rejection post-organ transplantation [46].

- **BK viremia**

BK virus is a double-stranded DNA virus from the polyomavirus family. BK was isolated from the urine of a KTR who presented with a ureteric stricture for the first time in 1971. The virus is named BK after the patient in whom it was first identified [48].Almost two decades later, BK viral inclusions were identified in a transplant kidney biopsy of a patient who presented with clinical rejection [49], and BK nephropathy can resemble tubulo-interstitial nephritis on histology [50].

It is commonly acquired in childhood and becomes latent in uroepithelial and renal tubular epithelial cells after primary infection. In those who are immunosuppressed, the virus can become active, causing cell lysis and viruria before crossing peritubular capillaries and causing viraemia. This can subsequently lead to viral invasion of the graft and BK virus nephropathy (BKVN) [51]and can result in graft loss. BKVN is difficult to treat, generally requiring a reduction in immunosuppression, which increases the risk of subsequent rejection.

1.1.C.D.3. Cancer and transplantation

Developing cancer is one of the most worrying complications post-transplantations for recipients and clinicians. There is a higher risk of malignancy among solid organ recipients with overall double the risk of the general population [52] [53].The incidence of all cancers (except non-melanoma skin cancer and cancers commonly associated with end-stage disease) is higher after transplantation in comparison with

during the dialysis period. The dialysis period also carries a higher risk of cancer in comparison with the pre-dialysis period, which in turn is higher than the matched normal population. There is a particularly increased risk of known viral-associated cancers, which reach almost a 3-fold increase in risk [54] [55]. The cumulative incidence of developing cancer and death was 12% and 38% respectively 17 years post-transplant in a large Canadian kidney transplant cohort from the Canadian Organ Replacement Register database (CORR) [55].

In another analysis the risk of developing cancer was reported to be 3% in a large cohort of dialysis patients from the USA, Europe, Australia, and New Zealand registries over a follow up period of 2.2 to 2.9 years for most patients and 10 years for 1.6 % of the cohort. The increased risk of cancer was also evident in younger age groups [56].

The 5-year cumulative incidence of cancer among patients with end-stage renal disease after initiating dialysis therapy was reported to be 9.48% in a large study from USRDS of 88,676 people in 1996 and increased in the following years to 164,214 in 2009. In contrast to the above-mentioned study, the incidence was higher at 11.28 % for patients who were 65 or older at the dialysis initiation [57].

In addition, cancer mortality is higher in kidney transplant recipients as well as dialysis patients. Mortality risk increases steadily, with a 10-year cumulative incidence of cancer death post-kidney transplantation reported at 4.5%. However, the standard mortality rate in transplant patients was 2.7 (95% CI, 2.6-2.9), which is similar to that for dialysis patients. Cancer deaths caused by preexisting cancer contributed to 9.6% of total cancer deaths in transplant patients [58].

1.2. Immunosuppressive agents and age

Maintenance Immunosuppressive agents

Advances in immunosuppressive regimens used as induction and maintenance therapy, and as treatment for rejection, have contributed to the improvement in outcomes for KTR over the years [59]. Azathioprine and prednisolone were introduced in the 1960s. In the 1980s, ciclosporin was introduced, leading to a significant improvement in graft survival at 1 year, from 50% to 80% [60].

Due to chronic nephrotoxicity leading to long-term renal dysfunction caused by ciclosporin, tacrolimus replaced ciclosporin in 1994 [60] [61]. One year later, mycophenolate mofetil (MMF) replaced azathioprine after research in liver transplant recipients found a reduction in rejection rates[60].The Symphony trial published in 2007 concluded that use of low-dose CNIs, combined with an anti-proliferative resulted in the fewest rejection episodes, greatest graft survival, and was associated with the lowest risk of nephrotoxicity [61], prompting the inclusion of tacrolimus as a first-line CNI and mycophenolate mofetil (MMF) as a first-line anti-proliferative in guidelines developed by the Kidney Disease Improving Global Outcomes (KDIGO) consortium.

- **Tacrolimus**

Tacrolimus is a calcineurin inhibitor (CNI) and is lipophilic, mainly distributed in organs rich in fat such as adipose tissue and the kidneys. CNIs are not only nephrotoxic, contributing to chronic CNI-associated toxicity and chronic allograft

dysfunction [62], but can also cause hyperlipidaemia, hypertension, and diabetes, and thus lead to increased cardiovascular morbidity and mortality [63] [64] [65] [66] [67]. One study reported that a higher incidence of diabetes 6 months post-transplantation was observed in elderly KTR [68]. Although the pharmacokinetics of tacrolimus do not differ according to age, data shows that body composition, especially lean mass, is associated with changes in Tacrolimus levels rather than total body weight [64].

- **Mycophenolate mofetil (MMF)**

Mycophenolic acid (MPA) is the active form of MMF. MPA is extensively bound to albumin and the free fraction is responsible for the actions of MMF [69]. Renal impairment and the uraemic state decrease binding of MPA to albumin and thus increase the free MPA concentration. Low serum albumin also associated with an increase in the free MPA [70].

The rate of infection is related to the type of maintenance IS. In particular, mycophenolate mofetil (MMF) [71] [72] [73] is associated with a higher incidence of CMV infections with more severe CMV disease, and a higher incidence of polyoma virus nephropathy, especially in combination with tacrolimus [71] [73] [74].The overall level of immunosuppressive burden is also an important factor as recipients that receive depleting immunosuppressive (IS) induction therapy or additional IS because of rejection have a higher risk of developing CMV disease [75].

1.2.A. Pharmacokinetic changes associated with ageing

Physiological changes associated with age can influence the pharmacokinetics of immunosuppressive drugs. Key changes include:

-Stomach acidity decreases with age due to senile atrophy of the gastric mucosa [76]. The increase in stomach pH can potentially affect the bioavailability of immunosuppressive agents.

-Gastric motility and emptying are reduced in the elderly population [77] [78]. This reduction can subsequently influence the absorption of certain medications, especially reducing the absorption of medications that need a fast transit time.

- Body fat content increases with age, which may increase the bioavailability of lipophilic medications such as ciclosporin [79].

The above-mentioned changes are thought to have only a minor impact on the effects of immunosuppression in elderly KTR. However, pharmacodynamic changes have a more significant effect as discussed below.

In the past, there was little to no evidence of a difference in the metabolism of immunosuppressive medications with age [63].However, the increasing number of older transplant recipients has permitted larger trials to investigate this question. In one study CNI pharmacokinetics were studied in 2553 adult KTR, of which 393 were between 65 and 84 years of age. This trial demonstrated a decrease in the clearance of tacrolimus with age [68].

In another large trial looking at tacrolimus at induction, minimizing the exposure to tacrolimus was not associated with an increased risk of rejection and in fact, was associated with better graft survival and kidney function [61].

In a study comparing azathioprine versus MMF alongside standard treatment with prednisolone and cyclosporine, >60 KTR treated with MMF in the first-year post-transplant in both groups had more infection and hospitalization [80]. This may reflect changes in response to immunosuppressive medications in the elderly. UNOS data showed a lower rate of rejection in the elderly with a corollary of higher rates of infection and death. However, to the contrary MMF had a protective effect against infection-related mortality compared to Azathioprine [24].

1.2.B. Pharmacodynamic changes associated with ageing

Pharmacodynamic changes associated with age are less convincing, especially concerning IS agents.

The proportion of fat versus muscle mass increases with age. Thus, the distribution of lipophilic drugs and half-life elimination increases with age. However, there is no convincing data relating this mechanism to immunosuppressive regimens [63].

Reduction in hepatic blood flow and hepatic mass with age which affects CYP3A substrates reduces the metabolism of certain drugs [63] [81].

Tissue receptors especially in the nervous system also change with age, which may influence drug responsiveness and increase adverse reactions. Increased neurotoxicity to CNI with age is an example of this [82].

1.3. Frailty and infection in Kidney Transplant Recipients

1.3.A. Definition of Frailty

Frailty is described as a condition of increasing vulnerability to health problems and reduced resilience to stressors. Regardless of the different tools used to assess frailty in the literature and practice, frail individuals experience a reduction in physical function and an increase in adverse health outcomes such as falls, hospitalizations, and mortality associated with the disease. The Clinical Frailty Scale (CFS) is a commonly used tool for evaluating frailty. It categorises individuals into different levels of frailty based on their overall health, functional capacity, comorbidities, and cognitive function. The scale ranges from 1 (very fit) to 9 (terminally ill), with the intermediate scores indicating various degrees of frailty. [83] [84]. The concept of prefrailty has gained attention in the literature, defined as a condition that exists between robustness and frailty, contributing to the progression of frailty [85]. Greater chronological age and the presence of chronic disease are two major factors increasing predisposition to frailty [86]. The probability of being referred and subsequently receiving a cadaveric kidney transplant decreases with increasing age at the time of starting dialysis. In a large study in Glasgow of 1692 RRT patients, it was found that <4% of patients aged ≥65 years at the start of renal replacement therapy received a cadaveric kidney transplant within 5 years of listing [87]. Being old and waiting for a kidney transplant whilst receiving dialysis will likely increase

frailty. The KDIGO guidelines emphasize the importance of assessing frailty at transplant assessment but do not consider it alone as a contraindication for transplantation. Risk stratifying patients according to frailty may help in counselling them, managing expectations, and defining a strategy for improving outcomes [88].

1.3.B. Measurement of Frailty

Several methods can be used to measure frailty. The Physical frailty or phenotype, frailty index, and the Tilburg Frailty Indicator were the most common methods used to assess the prevalence of frailty in 62 countries in a recent systematic review [89]. The Physical model of frailty focuses on the physical features of frailty whilst the frailty index investigates frailty in greater depth, taking into account a wide range of medical, psychological, and functional factors [90].

1.3.B.A. Frailty index (FI)

The frailty index relies on counting deficits or conditions which accumulate with age. The variables or deficits adopted by researchers vary from 30 to 70 items. These items are from three basic criteria; they are biological, accumulate with age, and are not present early in life [91]. The frailty index is then calculated as the number of these variables or deficits (the presence of a variable scores 1, otherwise it is 0) divided by the total number of variables. An example of a calculated Frailty index of 36 items is shown in Appendix 1. A score of ≤0.08 is considered as non-frail, whilst a

score ≥0.25 is considered frail, and between 0.08 to 0.25 is considered pre-frail [91]. Some studies have used different cut-offs for this score.

1.3.B.B. Physical frailty

Fried and colleagues analysed data from the Cardiovascular Health Study. This was a prospective observational study of individuals aged ≥ 65 years, not designed for frailty assessment. This frailty assessment tool excluded individuals with cognitive impairment. However, the Fried phenotype, which adopted sarcopenia as a sign of frailty was validated as a predictor of several outcomes including falls, hospitalization, and death [92].

In the Fried phenotype, individuals are characterized as frail if they have at least three of five criteria: unintentional weight loss or shrinking reported in the last year before assessment, exhaustion, weak grip strength, slow walking speed, and low physical activity (Shown in detail in Appendix 2 and abbreviated in Figure 1.1). Frail individuals score three of these 5 factors, while pre-frail score one or two, and non-frail score none of these factors [92].

Figure 1.1: Fried phenotype of frailty [89]

A. *Characteristics of Frailty*	B. *Cardiovascular Health Study Measure**
Shrinking: Weight loss (unintentional) Sarcopenia (loss of muscle mass)	Baseline: >10 lbs lost unintentionally in prior year
Weakness	Grip strength: lowest 20% (by gender, body mass index)
Poor endurance; Exhaustion	"Exhaustion" (self-report)
Slowness	Walking time/15 feet: slowest 20% (by gender, height)
Low activity	Kcals/week: lowest 20% males: <383 Kcals/week females: <270 Kcals/week
	C. *Presence of Frailty*
	Positive for frailty phenotype: ≥3 criteria present
	Intermediate or prefrail: 1 or 2 criteria present

It is important to realise that certain components of these five parameters may contribute differently to certain outcomes. For example, shrinking and poor grip strength alone were predictive of postoperative complications within 30 days of intra-abdominal surgery [93].

1.3.B.C. Clinical Frailty Scale (Rockwood scale)

In our renal unit, we use the Clinical Frailty Scale which is a clinical assessment of frailty developed from the Canadian Study of Health and Ageing. The clinical frailty scale is also known as Rockwood scale [83] [84]. There are nine scores in this frailty measurement, ranging from a score of 1 (indicating greatest fitness and least frailty)

to a score of 9 (terminally ill) (Figure 1.2). This tool is found to be practical, easy to use, and predictive of mortality in elderly cohorts (>65 years) and correlates with previously validated tools such as the frailty index [83].

Figure 1.2: The 9-point Clinical Frailty Scale [87]

There are other many tools in the literature for measuring frailty which mostly focus on physical activities as mentioned above. On the other hand, the Tilburg Frailty Indicator is a self-reporting questionnaire covering not only physical aspects of health but also social and psychological aspects (Appendix 3) [94].

1.3.B.D. Edmonton Frail Scale

The Edmonton Frail Scale (EFS) is a frailty index that assesses nine items (cognition, health status in general, independence at the functional level, social support, nutrition status, medications, mood, incontinence, and functional performance), giving a total score of 17 points. A score from 0-4 is classified as non-frail, 5-6 is vulnerable, 7-8 is frail, 9 to 10 is moderately frail and more than 10 is extremely frail [95]. EFS has been validated in elderly community cohorts (>65 years old) by geriatric specialists [96]. At recruitment for the ongoing Kidney Transplantation in Older People (KTOP) study, there was an association between psychosocial domains of EFS and worse patient experience and quality of life [97].

1.3.B.E. Conclusion

In summary, the Clinical Frailty Scale is a user-friendly tool for easily assessing frailty. The Clinical Frailty Scale does not only include physical features but also considers cognitive impairment (which is excluded in the Fried tool), comorbidities, and disabilities. The Rockwood Frailty Index on the other hand is based on a comprehensive tool that studies up to 70 clinical conditions to calculate a frailty score. However, this is time-consuming and requires physicians to fill out a list of variables including between 30-70 different factors [83]. There is a difference in the concept of frailty in the two major frailty assessment tools in the literature; whilst the Fried frailty tool relies on the biological causes of frailty, the Frailty index on the other hand depends on the accumulation of risks with time [98].

1.3.C. Prevalence of Frailty

The prevalence of frailty reported in the literature varies according to the studied populations and the tools used to assess frailty.

1.3.C.A. General population

In a systematic review published in 2021, frailty was reported in 12% of the general population aged ≥50 years using the physical activity tool, whilst 24% were classified as frail based on the Rockwood frailty index. For the age group 60-69 years, the prevalence of frailty was 12 % and 23 % for the physical frailty and Rockwood frailty index, respectively. A pre-frailty condition scoring between frail and non-frail was reported in 46 & 49% of those assessed by physical frailty and frailty index, respectively [89].

The prevalence of frailty and pre-frailty increases with age. Data from the UK Biobank included 493,737 individuals aged 37-73 years between 2006-2010 who were assessed for frailty using the Fried phenotype tool. The prevalence of frailty and pre-frailty increased with age but overall was 3% & 38 % for frailty and pre-frailty respectively, whilst 59 % were non-frail [99].

1.3.C.B. Chronic kidney disease (CKD) populations

In a systematic review of frailty in CKD patients, the prevalence of frailty, measured mostly by the Fried phenotype or FI, increased with a progressive decline in kidney

function. In one study, 7% of individuals with an estimated GFR of 49 ml/min were frail, whilst the prevalence of frailty was much higher at 42% in a smaller cohort of patients with an estimated GFR of 27 ml/min [86].

1.3.C.C. Dialysis populations

Among dialysis patients, the prevalence of frailty ranges between 14-73% in the literature. The very wide range of prevalence reported in this systematic review may be due to several reasons. Most studies used the Fried and modified Fried phenotype. However, interpretation of the five items in Fried frailty assessment tools varies in these studies. Furthermore, some studies replaced hand grip and speed with a questionnaire, which likely overestimated prevalence in dialysis patients [86].

For example, using the Fried tool the prevalence of intermediate frailty (also called pre-frailty) and frailty were reported at 32% and 41.8 % respectively in haemodialysis patients. In this study, the average age of intermediately frail and frail haemodialysis patients was 62.9 and 62.1 years respectively compared to 55.5 in the non-frail haemodialysis patients [100]. In another study frailty measured by the Clinical Frailty Scale in incident dialysis patients (average age 63 years) was associated with mortality. For each point increase in frailty score, the HR for mortality was 1.22 (95% confidence interval, 1.04 to 1.43; P=0.02) [101].

1.3.D. Risk factors for frailty

1.3.D.A. Chronological age

Frailty is also reported in younger age groups but increases with chronological age [99, 102] [103].Frailty prevalence, measured by frailty index (FI ≥0.21), was 10.3% in those ≤55 years, 14.4% in the 55–64-year-old group, and 19.2% in the 65–75-year-old age group, as reported in a large UK and Swedish population study (405123 individuals from UK Biobank and 43641 individuals from the Swedish Screen Across the Lifespan Twin (SALT) study). Age groups scored differently in the frailty assessment; the older cohort scored high in the cardiometabolic, sensory, and musculoskeletal items, whilst the younger cohort scored higher in pain and mental well-being [103]. In the US, increasing age also correlated with increasing frailty in a very large study of 4,987 participants selected from the atherosclerosis Risk in Communities (ARIC) Study cohort (mean age 78 years in the frail compared to 74 years in the non-frail group). In this study, frailty was assessed using the Fried phenotype tool [104]. However, Aging is a complex process that varies significantly among individuals. [105]. In 2000, Professor Franceschi coined the term "inflamm-aging" to describe a chronic low-grade inflammatory state that intensifies with age, reflecting a diminished ability to cope with stressors, which can lead to age-related diseases and conditions [106]. A key feature of this phenomenon is the increase in pro-inflammatory mediators and the dysregulation of the immune response, resulting in a persistent, low-level inflammation in older adults. Consequently, inflamm-aging is believed to play a role in the development and progression of age-related diseases.[107]. Targeting inflamm-aging may pave the way for innovative

interventions that could prevent or delay these diseases and enhance overall health outcomes for the elderly.

1.3.D.B. Chronic kidney disease

Frail individuals have a higher prevalence of chronic kidney disease. The prevalence of CKD (eGFR <60 ml/min) in frail individuals was found to be 45-77 % in the above-mentioned study of individuals from the (ARIC) study [104].

1.3.D.C. Dialysis

Frailty risk (using the Fried definition) in haemodialysis patients is high and associated with peripheral vascular and cardiac disease. Within this group, those with high serum albumin and those of black ethnicity had lower frailty rates than white haemodialysis patients. In one haemodialysis cohort, the rate of frailty was 78% in the slowness component of the Fried frailty phenotype, while 56% were frail in the poor grip component [108].

1.3.D.D. Diabetes Mellitus

The presence of diabetes mellitus, a common cause of CKD and ESKD, is similarly associated with frailty [104] [109] [102].The prevalence of frailty in old (50–90-year-old) diabetic individuals was reported to be 28 %, some 5-10 % higher than in age-matched non-diabetic individuals [110].

1.3.D.E. Frailty and sex

The prevalence of frailty is reported to be higher in females than in males [89] [104]. In the UK, in a younger age group (<65 years), the prevalence of frailty is, 11.7 % vs 9.9 % in females and males, respectively [111]. However, data from the US, found the prevalence of frailty in females to be twice as high than in males in both the under and over 65 year olds[102]. Frail individuals were also more likely to be female in the ARIC study (64.8% vs 55 % for women and men respectively) [104].

1.3.D.F. Other risk factors

The common risk factors for frailty among all age groups are smoking, obesity, low alcohol intake, poor educational status, and low income, as well as anaemia, cardiovascular disease, raised CRP, and a higher BMI [99] [104] [111] [102]. CMV seropositivity and the gut microbiome are additional factors linked to inflamm-aging and frailty [112]. CMV seropositivity alters the immune profile, resulting in the secretion of pro-inflammatory cytokines. Additionally, aging is associated with increased gut permeability, which elevates the levels of circulating bacterial toxins, contributing to inflammation and tissue damage [112].

1.3.E. Mortality and Frailty

Increasing age and presence of chronic disease are associated with increased mortality related to frailty. Mortality is reported to be higher among frail older (>65 years) diabetic patients regardless of the other comorbidities [113], whilst frailty was associated with increased mortality among dialysis patients[1], with the risk of

mortality in frail haemodialysis patients measured by the Fried tool reported to be 2.6-fold higher in comparison to non-frail subjects regardless of age, sex or comorbidities [100]. Hospitalization and mortality were also reported to be higher in older (>65 years) stage 4 and 5 CKD non-dialysis frailer individuals, based on the Frailty index classification [114]. Frailty was also associated with increased hospitalization and falls among dialysis patients [86] [115].

1.3.F. Frailty and Kidney Transplantation

The prevalence of frailty among KTR before transplantation was 17% in a recent systematic review, with most studies using the Fried phenotype [116]. In one longitudinal study of 537 kidney transplant recipients' frailty and intermediate frailty were associated with a 2.17- and 1.49-fold increase in mortality respectively post kidney transplantation, in all age groups. Frailty was measured at the time of admission for kidney transplantation and was based on the Fried phenotype with patients classified as non-frail, intermediate frailty, and frail. Outcomes were adjusted for recipient, donor, and transplant risk factors [117]. Re-hospitalizations after kidney transplantation were also higher in frail recipients [86]. A longitudinal study of 383 kidney transplant recipients showed an increase in the early readmission rate within the first 30 days by 1.6-fold in frail recipients measured by the Fried frailty tool, adjusted for other recipient factors [118].

1.3.G. Frailty and Infection post Kidney Transplantation

Data from the US Renal Data System (USRDS) database highlighted that the risk of death due to infection rises exponentially with kidney transplant recipient age [119]. Overall infectious complications were higher in recipients receiving Anti-Thymocyte Globulin (ATG) compared with Basiliximab (IL2RA) as induction therapy (77 % vs 56% respectively) in a study of 145 elderly kidney transplant recipients (> 65-year-old). Specifically, CMV infection was significantly more common following ATG induction (in 24 % vs 4% of patients) and with a higher median peak viral load in those who underwent ATG induction (4759 vs 1942 copies /ml).In addition, overall infection risk was significantly higher in elderly recipients in comparison with a matched cohort of younger recipients, and viral infections were reported in 44 % of elderly recipients compared with 32 % of younger recipients [120].

Frailty has been linked to an increase in infection-related hospitalizations among non-transplant individuals [103]. In women aged 70-79, CMV infection was found to be associated with frailty, with the impact of chronic infection being amplified by elevated IL-6 levels. However, data on the relationship between infection post-transplant and frailty is limited. [115] [121].

1.3.H. Frailty and Delayed Graft Function

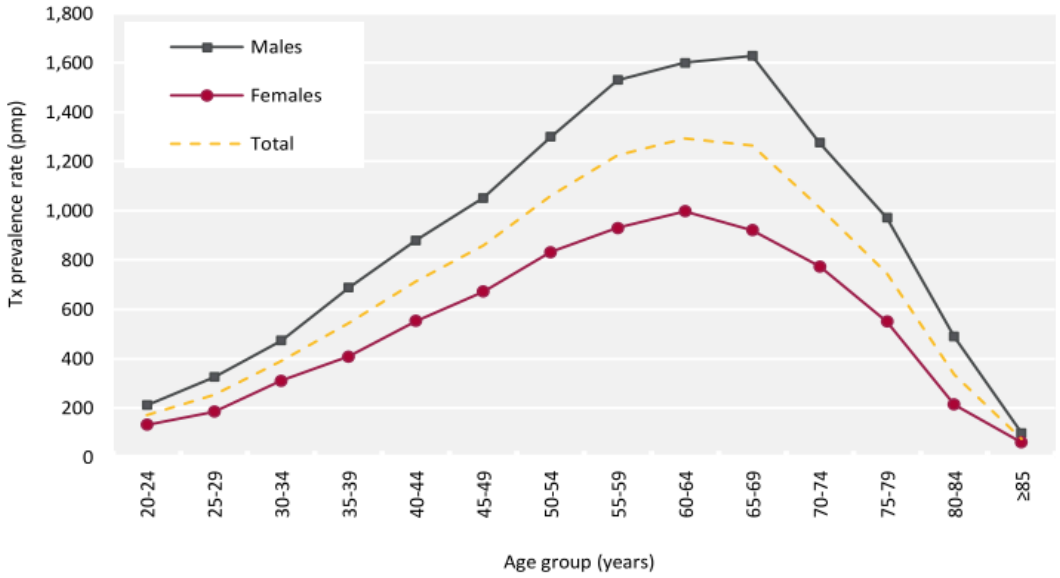
Delayed graft function (DGF) refers to the requirement for dialysis in the first week following kidney transplantation, excluding dialysis for hyperkalaemia within the first

24 hours of surgery. DGF is associated with increased rejection rates and adverse short and long-term outcomes [122], with a higher rate of mortality with a functioning graft among recipients who experienced DGF[123] [124]. The rate of DGF was 23% in an analysis of USRDS data for first adult cadaveric transplants over the period 1998 to 2004 [123]. DGF is associated with frailty before transplantation[116] [125]. In another study, frailty was assessed by the Fried tool and was performed immediately before transplantation in 183 patients with ESRD The incidence of DGF in frail kidney transplant recipients was found to be double that in non-frail KTRs (30% vs 15 % respectively), and frailty was an independent risk factor for DGF after adjusting for risk factors, and irrespective of KTR age [125].

DGF irrespective of age was reported to be 31% in an Italian study of 452 kidney transplant recipients aged more than 60 years at the time of cadaveric kidney transplantation [126].

The prevalence rate of adult kidney transplants per million population in the UK as of the end of 2021 according to age group and sex is shown in Figure 1.3 below [127], this shows that the greatest proportion of prevalent transplant patients is>60 years old. DGF rate is as high as 55 % in DCD recipients, and 31% in DBD recipients [128]. Since elderly kidney transplant recipients are more likely to receive DCD rather than DBD kidneys, the risk of DGF in elderly KTRs is likely to be higher.

Figure 1.3: Prevalence rate of adult kidney transplant 31/12/2021 by age group and sex – per million population [120].



1.3.I. Transplantation in elderly recipients

Patient survival was reported at 98.7% and 89% at one and 5 years in an Italian cohort of elderly (>60-year-old) cadaveric kidney transplant recipients. In this study, the most common cause of death was cardiovascular accidents (32%), sepsis (25%), and neoplasms (11%). In this study, recipient age was a significant risk factor for survival post-transplantation (HR=1.083, 95% CI: 1.021-1.15, P=.008) [126]. Data from the UNOS/OPTN database reported the outcome of 26,721 elderly first KTR (>65) with a documented functional status. The functional status was classified as the following: total assistance requirement, moderate assistance (needing some help but capable of self-care), and no assistance required. Patient survival at 3 years post-transplantation was significantly higher in KTR who received live donor kidneys

(90.1%) compared to deceased donors (83 %). In both groups, mortality was higher for total assistance than moderate assistance compared with no assistance [129].

1.4. Ageing

Despite efforts to understand ageing, no single theory explains the underlying driving mechanism fully. The accumulation of damage at molecular, cellular, and tissue damage dominates ageing models [130].

1.4.A. Immunosenescence

Immunosenescence is a term used to describe the changes in the immune system observed with age [131]. A reduction of the immune response function with age contributes to a reduction in the ability to fight infection and respond to vaccination [132] [133]. Gender and infection with CMV are among the factors that influence immunosenescence [132].

Cellular senescence is a state in which cells lose their ability to divide and function properly. This process can occur in response to various stressors such as DNA damage, telomere shortening, oxidative stress, and oncogenic signals. Senescent cells are characterized by a distinct phenotype, including changes in gene expression, cell morphology, and altered metabolic activity [134]. Senescent cells are involved in the production of inflammatory mediators, and their accumulation is associated with or may contribute to frailty [131].

Immunosenescence is manifested as described in the literature by impaired T cell IL-2 production and Th1 immunity [135], reduced CD28 signalling and immune synapse formation [136], and attenuated immunoglobulin class switching leading to reduced antibody responses [137].

Several other changes are reported in the immune system with age. A reduction in the number of naïve T cells in the peripheral blood and likely in lymph nodes has been described. As a result, the diversity of the naïve T cell receptor (TCR) repertoire is also reported to be reduced. These changes explain the reduction of the ability of the immune system in the elderly to handle any pathogen exposure for the first time. Thus, there is a decrease in the diversity of memory T cell subsets both CD4+ and CD8+, and their functional integrity despite the increase in the total number of memory T cells [138] [139]. This change compromises the ability of the immune system to resist reinfection and persistent infection [133].

Older data reported significant changes on T cell surface markers with CMV infection similar to changes that occur with age. One possible explanation for this is the increase in CMV infection prevalent with age, therefore it was suggested that CMV status should be taken into consideration when investigating the change in T cells with age [140]. Some studies use TTV (Torque Teno virus) viral load as an indicator of immune system function and strength. TTV viral load increases in patients on immunosuppression.

1.4.A.A. Torque Teno Virus (TTV) and immune function

Torque Teno Virus is a small circular single-stranded DNA anellovirus (AV) that is not known to cause pathology and has an extremely high prevalence in humans.

TTV is detected in 90% of tested individuals. Therefore, viral load in the peripheral blood can be studied as a reflection of the immune system status of the host [141]. Young females were found to have lower TTV load in comparison with males in the age group 20-30 years, but this difference by sex was not observed in other age groups [142]. Data showed individuals with higher TTV viral load are associated with positive CMV serostatus in young and middle-aged groups. However, this association was not observed in older individuals. The association was explained by persistent CMV infection impairing the antiviral effect against simultaneous infection with TTV [132]. TTV viremia was reported to be inversely related to markers of immune system competency, such as the total level of CD8+57+ T lymphocytes [143]. Measurement of the peripheral blood level of TTV has been hypothesized to be useful in guiding immunosuppression and monitoring alloreactivity. A study of 113 transplant recipients found that acute biopsy-proven rejection was associated with lower TTV titre measured retrospectively from stored peripheral blood taken at the time of biopsy. A low level of TTV was found one month before histological changes of rejection, opening the door for its use as a marker of under-immunosuppression and subsequent rejection. In this study, the risk of rejection was reduced by 10 % per log level of TTV [144]. Other studies have reported a 4-fold decrease in the TTV viral load in kidney transplant recipients who developed antibody-mediated rejection compared with recipients without rejection [141]. Older recipients have been found to have a higher level of TTV titre [141] [144]. Healthy old people (>60 years) have also been found to have a higher TTV viral load [132].

1.5. Immune system in relation to regulatory B cells

The immune system is a sophisticated and highly regulated defence system that protects the body from external pathogens but prevents attacks directed against self-antigens [145]. The immune system has two systems of responding to pathogens-innate and adaptive, which both interact [146].

1.5.A. Innate immunity

This is the immediate defence in humans, and also in the simplest animals, and is not antigen specific. It includes macrophages, monocytes, neutrophils, cytokines, complement activation, and acute phase proteins [146].

1.5.B. Adaptive immunity

The main feature of the adaptive immune system is that it is antigen-specific and is dependent on B and T lymphocytes [145] [146]. The adaptive response takes time to develop but creates immune memory following exposure which makes subsequent reactions on re-exposure to the same antigen faster [146].

1.5.B.A. Lymphocytes:

B lymphocytes have a surface receptor consisting of immunoglobulins (or antibodies). T lymphocytes have T-cell receptors (TCR) recognizing antigen presented on HLA molecules by antigen-presenting cells. These receptors on T and B lymphocytes recognize non-self while avoiding significant self-reactivity [145].

Following antigen exposure, lymphocytes proliferate and differentiate. B lymphocytes ultimately become antibody producers. This is called the humoral immune response,

while T cells are either helper CD4+ T cells (coordinating the appropriate response, recognizing class II HLA on antigen-presenting cells) or cytotoxic CD8+T cells (producing cytokines and directly killing target cells). B and T cells are collectively called the cellular immune response. In addition, CD4+ T cells help B cells in their role of producing antibodies, as well as producing cytokines. However, the participation of B cells in the cellular immune response is less clear, with evolving data regards this matter [145] [147].

However, following the activation of the immune response, regulation should be there to switch it off and this is achieved by B and T regulatory cells. Most of the differentiated antigen-specific lymphocytes involved die following activation against non-self-antigen, but some persist in producing life-long immune memory. Following repeat exposure, the process of activating the immune system occurs more rapidly [145].

Collaboration between the innate and adaptive arms of the immune system

The phagocytes of the innate immune system are responsible for capturing foreign antigens and processing them into peptides, to be presented on HLA molecules to T cells. These cells are called antigen-presenting cells (APC), and dendritic cells (DC) are the most common type of APC [145].

1.5.B.A.1. B cells

In addition to producing antibodies, B cells are also shown to act as antigen-presenting cells to T cells which subsequently proliferate and differentiate [148]. Thus, B cell depletion therapy has been used to treat many T cell-mediated autoimmune diseases like multiple sclerosis, which suggests that B cells modulate T cell inflammatory responses in addition to producing autoantibodies in this condition [149] [150]. B cells are capable of secreting cytokines in response to certain stimuli, which include T cells themselves, which subsequently can be modulated by these secreted cytokines [112] [150].

B cell phenotypes

Regulatory B cells, suppress effector B and T cell function. They mainly produce anti-inflammatory Interleukin 10(IL-10) cytokine or Transforming growth factor beta 1 (TGFb-1), which have been shown to modulate the T cell inflammatory immune response [150] [151].

Effector B cells: there are two effector B cells, classified by different proinflammatory cytokine production. Effector B cells 1 (Be-1-cells) are capable of producing interferon-gamma (IFNγ), IL-12, and tumour necrosis factor-alpha (TNF-α) cytokines, while effector B cells 2 (Be-2-cells) secrete IL-2, IL-4, TNF-a, and IL-6 [150]. Elevation in the production of proinflammatory cytokine IL-8 was reported in association with diabetes mellitus patients [152].

a) Regulatory B cells

Regulatory B cells or B 10 cells are B cells capable of producing IL10 with no specific phenotypic features [153]. B 10 cells represent 0.6 % of blood B cells [154].

IL10-producing B cell subsets were named as regulatory B cells due to their role in suppressing inflammatory responses in experimental autoimmune conditions [155]. Any type of B cells (mature or immature) and plasma cells can differentiate into regulatory B cells as a response to certain stimuli such as CD40 ligation [156] [157] [158].

In addition to CD40 ligation and B cell receptor (BCR) stimulation, there is another B cell regulatory stimulation pathway via Toll-like receptors (TLRs)[159]. These TLRs recognize a variety of pathogens including viruses [160]. Figure 1.4 shows the effect of regulatory B cells, as defined by mouse models.

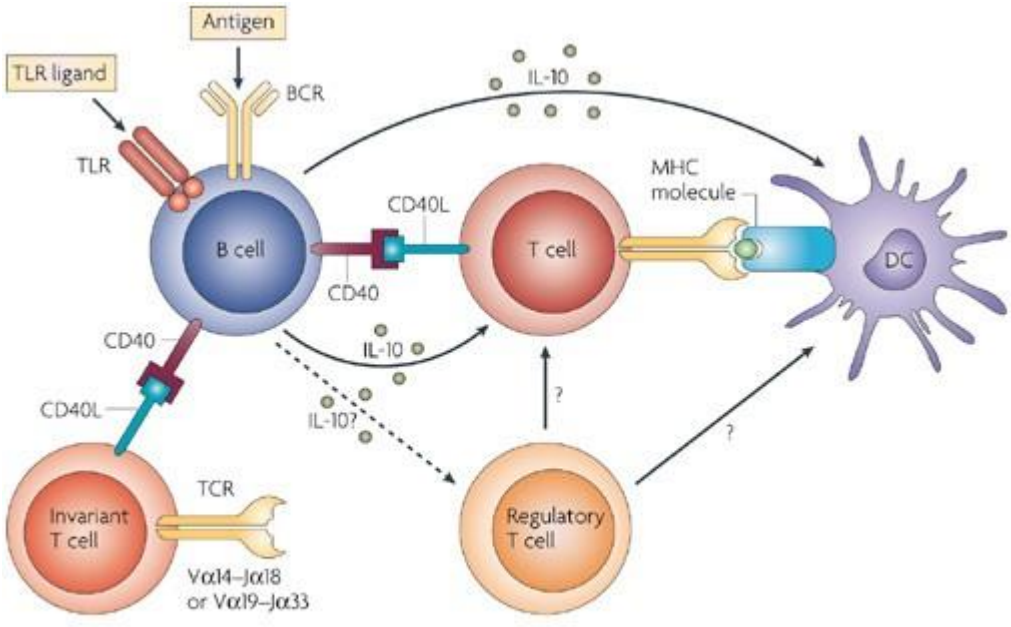


Figure 1.4: B-cell-derived interleukin-10: stimuli and effects. Figure taken from [152] This figure shows B cell from immunized mice.

B cells were stimulated to produce IL-10 via:
-B cell receptor (BCR) ligation by antigen
-CD40 ligation by CD4+ and T-cell receptor (TCR)
-Toll-like receptor (TLR) ligation
Effect of B cell production of IL10:
Inhibit pathology via:
-Direct effect on the CD4+ effector T cells
- Reduction in immune priming by innate cells
-Augment activation of regulatory T-cell populations

Polymorphism leading to increase production of IL-10 was associated with an increased risk of developing SLE in a large Chinese study [161]. With this understanding, the administration of anti-IL-10 monoclonal antibodies in active and steroid-resistant SLE shows promising outcomes [162].

There are several regulatory B cell subsets identified in animal models as well as in humans [163]. Data showed CD19+ CD24hi CD38hi transitional B cells (TrBs) suppressed the T helper 1 cell differentiation via IL 10 production upon stimulation of CD40 in humans [164] [165]; CD19+CD24hi CD27+ cells contribute to the majority of B 10 cells. B 10 cells were capable of negatively regulating monocytes also via IL10 secretion [166]. Further studies characterized the CD19+CD24hiCD27+ subset as memory B cells, and CD19 +CD27-CD24+CD38+ as naïve B cells [154].

A poor response to CD 40 stimulation by the regulatory B cells and subsequent reduction of the anti-inflammatory effect of IL10 was found in systemic lupus erythematosus (SLE) patients [164]. Animal data confirmed this role of regulatory B cells or IL -10 producing B cells in T cell-driven autoimmune disease [157]; and T

cell-mediated inflammatory responses [167] [168]. Additionally, in diabetes mellitus patients, B cells were found to have altered Toll-like receptor function and produce minimal amounts of IL-10 [152]. Lack of IL-10 production by B cells was also reported in patients with multiple sclerosis [169].

The key trait of the regulatory B cell is IL-10 production in addition to other cytokines. IL10 is an anti-inflammatory cytokine. However, IL10 has other functions including promoting T cell activation, epithelial repair, and favouring plasma cell differentiation to antibody-producing cells which play a significant role in certain autoimmune diseases like SLE [170] [171]. Proinflammatory cytokine production and TNF-a, in addition to the production of anti-inflammatory cytokines such as IL10, were studied in Transitional B cells, memory, and naïve B cells, for a better understanding of the role of the regulatory B cells [154].

Although B regs are known by their IL-10 expression, which is considered a signature cytokine, B cells from healthy individuals after in vitro stimulation also co-express TNF-a. The expression of these pro-inflammatory cytokines (TNF-a) differs among different B cell subsets in contrast to IL-10 expression on B cells which varies between 10-15 % in transitional, Naïve and Memory B cells. Therefore, IL-10/ TNF-an expression has been used as a reflection of the relative regulatory activity [154].

The IL-10/TNF-a ratio in transitional B cells was higher than in memory and naïve B cells. IL-10/TNF-a ratio rather than absolute levels of IL10 alone was associated with suppression of T cell proinflammatory cytokine expression. Therefore, this ratio and their potency in suppressing T cell activation and cytokine production has been used to define the regulatory B cells [154].

Regulatory B cells and tolerance

A higher percentage of naïve cells and transitional B cells, along with increased IL-10 production was reported in tolerant patients (a rare group of patients with stable kidney function after withdrawal of immunosuppressive agents) in comparison with patients on IS who had stable kidney function [172].

The B cell phenotype was examined in tolerant patients and compared with patients with stable kidney function who remained on IS. There was no difference in the B cell phenotype between the two groups, however, the frequency of transitional (CD20+CD24hiCD38hi) and naïve (CD20+CD24loCD38lo) B cells were higher in the tolerant patients. Differentiated plasma cells (CD19+CD38+CD138+) were lower in tolerant patients. Memory cells were similar in the two groups. Furthermore, upon in vitro stimulation, B cells from tolerant patients produced more IL-10 than patients with stable function on IS or healthy individuals [173].

Increased transitional B cell IL-10 production was also observed in tolerant patients in comparison with stable KTR or healthy individuals despite no changes in the B cell subset numbers [174].

In another study, tolerant patients and healthy individuals were found to have lower CD86 expression on transitional B cells and higher IL-10 production upon CD40 ligation compared with patients with chronic rejection. Data also shows the downregulation of CD86 by IL-10 was found to inhibit T cell proliferation and TNF production, suggesting that IL10 production leads to CD86 downregulation and may have contributed to the development of tolerance in transplantation [158].

Similarly, IL-10 produced by transitional B cells after CD40 ligation was observed to be at higher levels in tolerant patients than healthy individuals [175].

Regulatory B cells and transplantation

A significant rise in acute rejection was reported when a B cell-depleting agent treatment (Rituximab) was used as induction therapy in comparison with anti-CD25 antibody daclizumab supports the role of regulatory B cells in preventing rejection [176].

In contrast, combining Rituximab with IV immunoglobulin in highly sensitized patients was not associated with increased risk of rejection in a small trial [177]. In another trial of rituximab as induction, transitional B cells recovered at 12 months post-transplant, however, the memory cells remained low. In this trial, there was no increased risk of rejection, which confirms the possible role of transitional B cells in improving transplant outcomes [178].

However, B cell depletion has age-dependent effects in murine skin transplant recipients. B cell depletion was found to enhance immune regulation and thus skin graft survival in aged mice and the opposite effect in young mice [179].

Transitional B cells with a low IL-10/TNF-a ratio was found to be low in kidney transplant recipients at the time of rejection in comparison with patients with stable graft function or graft dysfunction not due to rejection or in healthy individuals. This reduction in IL-10/TNF-a ratio was not observed in the total B cell populations. Furthermore, low IL-10/TNF-a ratio in transitional B cells was associated with worse graft outcome at 3 years [154].

Higher frequencies of transitional B cells were reported to be protective for rejection [180]. A multicentre observational study published in 2021 confirmed this association. The frequency of transitional B cells and Transitional B-1 cells (CD19+CD24hi CD38 hi) were lower in patients with rejection than patients without rejection, at 3- and 6-months post-transplant and to a lesser extent also at 12 months [181]. Recent data showed a 2.2-fold reduction in transitional B cells and 4-fold reduction in transitional B-1 cells at three months post-transplant in patients with borderline rejection within the first four months post-transplant who subsequently developed acute rejection in comparison with KTR with borderline rejection who did not progress to acute rejection [32].

The same study also confirmed that IL-10/TNF ratios of transitional B cells and Transitional B-1 cells were lower in patients with T cell-mediated rejection. This low IL-10/TNF ratio was observed mainly at 3 months post-transplant. On further analysis at three months post-transplant, the IL-10/TNF ratio of the transitional B-1 cells was found to be the strongest predictor of rejection in a logistic regression model analysis in the first-year post-transplant at an average time of 8.0 ± 2.3 months [181]. Moreover, graft survival and kidney function were worse in patients with low IL-10/TNF of transitional cells [181].

Recent data demonstrated patients with borderline rejection and low IL-10/TNF-a ratio of transitional B-1 cells at three months were associated with late rejection and development of IFTA and graft failure at 7 years. In contrast, patients with borderline rejection and high IL-10/TNF-a ratios had similar outcomes as patients with no rejection on protocol biopsies within four months post-transplant [32].

1.6. Summary

Kidney transplantation provides the optimal form of renal replacement therapy for the majority of people with end-stage renal disease (ESRD). Compared to dialysis, kidney allograft transplantation offers substantial survival and quality-of-life benefits for ESRD patients and is a highly cost-effective treatment [182] [183] [184] [185] [186]. However, older age at the time of receiving a kidney transplant is the major risk factor for mortality after kidney transplantation [16] [17]. Most deaths among older KTR are with functioning grafts [18]. Cardiovascular disease, infection, and cancer are the most common causes of death in older recipients [16] [22] [23].

In terms of infection-related complications, cytomegalovirus (CMV) is one of the most common opportunistic infections that affects the outcome of kidney transplantation and is associated with allograft rejection and increased mortality [187] [188] [189] [190] [191].

Standard clinical practice for post-transplantation immunosuppressive therapy has not fundamentally changed since the late 1990s when the highly successful combination of tacrolimus and mycophenolate was introduced for the prevention of acute rejection [192] [193] [194] [195]. Although the transplant community has focused on the early withdrawal of steroid therapy and the introduction of inhibitors of the mammalian target of rapamycin, this has not translated into graft survival benefits [196]. As a result, this leaves an evidence gap concerning the optimal IS regimen, especially among elderly renal transplant recipients who are more susceptible to post-transplant infections such as CMV, and malignancy.

Theoretically, older kidney allograft recipients may not require the same level of IS as given per standard protocols, but caution needs to be exercised to ensure that IS for elderly KTR is sufficient to avoid the risk of rejection, as acute rejection (AR) in older KTR can be more severe than in younger recipients [197] [198] and deaths related to AR are more common in older adults [199]. Therefore, there is a fine balance, and targeted clinical trials in the elderly are necessary to develop optimal post-transplant IS management.

There is a clear underrepresentation of elderly recipients in clinical studies on kidney transplantation [200], with up to 20% of clinical trials excluding recipients over the age of 65 years [201]. This is in contrast to the fact that nearly a third of all kidney transplant waiting list patients are over the age of 60 in the UK [202], and about a quarter in the US [203]. Given the changing demographics of both donors and recipients, in particular, increasingly older donor kidneys are transplanted into progressively older recipients with increasing comorbidities [26] [28] [29] [204], transplant clinical trials up to now have not reflected these populations and how best to manage their post-transplant IS therapy.

A major limitation of IS minimisation trials has been the absence of recipient stratification that can identify the recipients that would most benefit from personalised IS [205]. Acute rejection is one of the strongest risk factors for death [206], therefore any minimisation of IS should be clinically guided by a biomarker that has a high negative predictive value for acute rejection. The current diagnosis of renal allograft dysfunction mainly relies on clinical monitoring, including measurement of serum creatinine and proteinuria. However, elevation of serum creatinine typically occurs when the kidney has undergone a substantial amount of

injury/inflammation [207] [208], resulting in recipients sustaining progressive subclinical renal injury that remains undetected. The use of serial protocol allograft biopsies to monitor for subclinical rejection and guide IS management has limited applicability. Not only are biopsies invasive, but they are limited by sampling errors, with around 25% biopsies yielding an inadequate specimen and multiple samples potentially needed to increase diagnostic accuracy [209]. Although therapeutic drug monitoring of immunosuppressive drugs is used as a surrogate for adequate IS, there is no element of stratified personalisation with this approach.

A proactive and biomarker-guided minimization strategy after transplantation can potentially reduce IS drug-associated morbidity and potentially lead to significant improvements in long-term graft and patient survival. Frailty before transplantation is a useful tool and predictive of certain outcomes like length of stay in hospital post-transplant [116] and subsequent morbidity and mortality associated with this stay. Frailty can then be used as a tool for risk stratifying KTR and a guide for minimising immunosuppression. Recently, there has been increasing evidence of an association between Transitional B cell IL-10/TNF ratios and graft outcome. Using this biomarker could add more support in achieving a more personalized IS in elderly KTR.

1.7. Aim and hypotheses

This project aims to investigate the effect of kidney transplant recipients' age and frailty at the time of transplantation on outcome, with a particular focus on infection risk.

Specifically, I aim to:

- Confirm previous evidence of increased infection risk (particularly CMV viremia) and reduced rejection risk in older KTR (in this work > 60 years) in comparison with matched younger KTR.
- Investigate whether frailty before transplantation in the older KTR predicts outcomes, particularly CMV viremia, hospitalization, and mortality.
- Investigate whether B cell subsets and their IL-10/TNF ratios differ between old (>60) and younger KTR or according to frailty before transplantation at three months post-transplantation.

My hypotheses are:

1. Older KTRs have a higher risk of opportunistic infections (particularly CMV viremia)

2. Older KTRs have a lower risk of rejection compared to younger ones.

3. Frailty in older KTR could predict outcomes better than chronological age, in particular infection, hospitalization, and mortality risk.

4. Older or frail KTR have different B cell subpopulations and IL-10/TNF ratios of these subsets which may influence alloimmune response or vulnerability to opportunistic infection.

CHAPTER 2. MATERIALS AND METHODS

2.1. The impact of standard immune suppression on the immunological and infective complications in older kidney transplant recipients

2.1.A. Study design and participants

This was a single-centre retrospective cohort study of first-time kidney-only transplants performed between 1st April 2009 to 1st April 2019. Older recipients were defined as adults aged >60 years at transplantation, based on an age of 60 being cut off for differing immunological outcomes and survival benefits in other studies [210] [211]. We compared clinically-defined outcomes (see below) in the older cohort to contemporaneous transplant controls matched for the number of human leucocyte antigen (HLA) donor-recipient mismatches (at HLA-A, B and DR loci, 0-6), HLA calculated reaction frequency(cRF)(0%, 0-85% or >85%) and CMV serostatus (Recipient- or Recipient+), across a range of younger age groups at the time of transplantation, subdivided into 18-34, 35-49, and 50-60 years of age. HLA antibody reaction frequency (or cRF) is calculated by comparison of unacceptable HLA specificities with HLA types of donors of identical ABO blood groups in a pool of 10,000 donors on the NHSBT database. Recipients with cRF ≥85% were classified as highly sensitized. We included only first transplants and recipients who had recorded blood results for at least 12 months post-transplantation, and whose grafts

survived for at least three months. Follow-up commenced at the time of transplantation and ended on January 31st, 2022. Kidney transplant biopsies were performed only for clinical indications.

Data for this observational cohort study were obtained via the Royal Free Hospital electronic records system and from the UK Transplant Registry of the Organ Donation and Transplant Directorate of National Health Service Blood and Transplant (NHSBT). Data collection was done as a service improvement audit.

2.1.B. Immunosuppression protocol

All patients received standard immunosuppression using induction therapy with Basiliximab (20mg) given on the day of surgery and on the fourth post-operative day. Standard maintenance immunosuppression consisted of tacrolimus (started at 0.15mg/kg/day, with target trough levels of 8-12ng/ml in the first 3 months, 6-8ng/ml in months 4-12 and 4-8ng/ml after the 1st year), mycophenolate mofetil (MMF) (at 1g twice daily for the first month, reduced to 750mg twice daily between months 3-12 and then tapered at 12 months to 500mg twice daily) and early steroid withdrawal (methylprednisolone 40mg once daily for the first 3 days, followed by prednisolone 20mg for the next 7 days, reduced to 5mg for one more week, then stopped). Importantly, prophylactic therapy for CMV prevention is not instituted at our centre regardless of donor or recipient CMV status. Active monitoring of CMV viraemia by twice-weekly plasma CMV DNA polymerase chain reaction (PCR) is employed for the first three months, then according to clinical indication. Anti-viral therapy (valganciclovir) is initiated if the detection of virus DNA is above a threshold of 200 IU/ml and 3000 IU/ml in CMV naïve and CMV seropositive recipients, respectively.

Recipients who were CMV IgG positive and who were treated with anti-thymocyte globulin therapy for steroid-resistant biopsy-proven acute rejection (BPAR) received universal prophylaxis with valganciclovir for 180 days followed by CMV PCR monitoring every 2 weeks for a minimum of 3 months. All patients received co-trimoxazole prophylaxis for three months, and valaciclovir prophylaxis is administered for 1 month to recipients who are Herpes Simplex Virus (HSV) IgG negative. BKV DNA monitoring was carried out on blood on a monthly basis in the first year.

2.1.C. Survey

An online survey was sent to the other 22 UK renal transplant centres to ascertain the variation in use of immunosuppression in older kidney transplant recipients:

Transplant unit survey:

Immunosuppression in older renal transplant recipients (>65 years old)

Your transplant unit's use of immunosuppression

1. What is your induction regimen for renal transplantation at your unit? (select all that apply)

- ☐ Campath
- ☐ Simulect
- ☐ ATG
- ☐ MMF
- ☐ Tacrolimus
- ☐ Methylprednisolone
- ☐ Ciclosporin

☐ Azathioprine
Other (please specify) --

2. If you have selected use of more than one monoclonal/polyclonal antibody, please explain when each is indicated?

3. What is your target calcineurin inhibitor levels post transplantation? (e.g. TAC, 0-3 months, target level 8-12 ng/ml; 3-12 months, target 6-8; over 12 months target 4-8)

4. What is your target MMF doses post transplantation?

5. Do you have any other target levels or doses for other immunosuppressive drugs post transplantation?

Modifying immunosuppression in older recipients

6. Do you change the immunosuppression (IS) regimen for the older transplant recipients at your transplant unit (at induction or post transplantation)?

Yes

No - skip to next page.

7. Do you change the IS regimen at induction for older transplant recipients?
If yes, how?

If no, skip to next question.

8. Do you change their IS regimen post transplantation?
If yes how? how?

If no, skip to next question

Expert opinion

9. If your unit does not change the IS regimen for older recipients, do you think older transplant recipients should have a modified IS regimen?

Yes

No

10. Do you think a trial into reducing overall immunosuppression burden in older recipients is required?

Yes

No

2.1.D. Outcome variables

Outcome measures included graft loss, graft loss censored for death, the occurrence of the first CMV viraemia within the first 6 months, patient death, occurrence of biopsy-proven acute rejection (BPAR), new-onset non-skin malignancy, and development of donor-specific anti-HLA antibodies (DSA), by one year after transplantation. The incidence of CMV viremia was based on CMV DNA PCR, using a threshold of 200 IU/ml and 3000 IU/ml in CMV naïve and CMV seropositive recipients, respectively. Acute humoral and cellular rejections were defined according to the Banff classification [201]. Screening for anti-HLA antibodies was performed by flow cytometry using the xMAP (Luminex) platform, utilizing LAB Screen Mixed Bead kits (One Lambda, West Hills CA, USA). Positive samples were tested on HLA Class I and Class II single antigen kits to define antibody specificities. A baseline means fluorescence intensity (MFI) cut-off of ≥2000 was used to report pre-transplant unacceptable antigens to NHS organ donation and transplantation (ODT); any donor-specific antibodies (DSA) ≥ 500MFI were considered to be significant post-transplantation. We also assessed graft and patient survival. Graft

survival was defined as the time from transplantation to graft failure (earliest of return to dialysis, graft nephrectomy, or re-transplantation), with censoring for death with a functioning graft or at last follow-up evaluation. Patient survival was defined as the time from transplantation to patient death.

Finally, we also collected data on BK virus infection, defined as a plasma BK virus level above 100 copies/ml or evidence of BK nephropathy on biopsy; Delayed graft function (DGF), defined as the need for dialysis within 7 days after transplantation and renal function using estimated glomerular filtration rate (eGFR) which was determined using the Modification of Diet in Renal Disease equation[202].

2.1.E. Statistical Analysis

Continuous data are expressed as mean ± standard deviation (SD) or median and inter-quartile range (IQR) according to their distribution. Categorical data are expressed as percentages, with frequencies compared using Pearson’s c2 test. Analyses for multiple groups were completed using ANOVA for normally distributed variables or the Kruskal–Walli's test for data with a non-parametric distribution.

Time to death, graft loss, first CMV viraemia, first malignancy, and first episode of BPAR were plotted using Kaplan-Meier (KM) survival curves. Associations between age group and outcomes were assessed using Cox proportional hazards regression with adjusted models used to control for potential confounding. Confounders considered in multivariable analyses included (i) recipient factors (sex, race, primary renal disease, body mass index, smoking history, and length of dialysis;) (ii) donor factors (age, sex, race, and deceased or live status); and (iii) transplant factors (cold ischemia time). Finally, a further sensitivity analysis was conducted to address the

impact of the matching strategy by additionally including CMV serostatus, cRF, and HLA mismatch [212] [213] [34] [214] [215] [216]. Hazard ratios (HRs) and 95% confidence intervals were calculated for each variable. Associations were judged to be significant where the 95% confidence interval for the coefficient did not include unity. Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA).

2.2. Study Approval

Data for this observational cohort study were obtained via the Royal Free Hospital electronic records system and from the UK Transplant Registry of the Organ Donation and Transplant Directorate of National Health Service Blood and Transplant (NHSBT). Data collection was done as a service improvement audit.

2.3. Frailty and infections in older kidney transplant recipients

2.3.A. Study design and participants

This was a single-centre retrospective cohort study of older (≥60 years old) deceased and living donation kidney-only transplant recipients performed between 1st April 2009 to 1st April 2021. Only kidney transplant recipients with a frailty score documented within one year before transplantation on our database and who had a functioning transplant beyond three months were included. We compared the outcome of two groups, those considered non-frail who had a frailty score of 1-3, and

the frail group who had a frailty score of 4-6, based on the clinical frailty scale. None of the severely frail (score ≥7) in our cohort (≥60) were listed to receive kidney transplants. Follow-up commenced from the time of transplantation and ended in October 2022. Kidney transplant biopsies were performed only for clinical indications.

Data for this observational cohort study were obtained via the Royal Free Hospital electronic records system and from the UK Transplant Registry of the Organ Donation and Transplant Directorate of National Health Service Blood and Transplant (NHSBT). Data collection was done as a service improvement audit.

2.3.B. Immunosuppression protocol

Patients received induction therapy with Basiliximab, (20mg given on the day of surgery and the fourth post-operative day). Standard maintenance immunosuppression is as mentioned in the protocol above.

2.3.C. Outcome variables

Outcome measures assessed included graft loss, (not censored for death because all graft loss happened while recipients were alive), the occurrence of the first CMV viraemia within the first 6 months, patient death, and the occurrence of biopsy-proven acute rejection (BPAR).

The incidence of CMV viremia was based on CMV DNA PCR, using a threshold of 200 IU/ml and 3000 IU/ml in CMV naïve and CMV seropositive recipients, respectively. Acute humoral and cellular rejections were defined according to the Banff classification.

We also assessed graft and patient survival. Graft survival was defined as the time from transplantation to graft failure (earliest of return to dialysis, graft nephrectomy, or re-transplantation), with censoring for the last follow-up evaluation. Patient survival was defined as the time from transplantation to patient death, censored to graft loss.

Finally, we also collected data on BK virus infection, defined as a plasma BK virus level above 100 copies/ml or evidence of BK nephropathy on biopsy; Delayed graft function (DGF), defined as the need for dialysis within 14 days after transplantation and renal function using estimated glomerular filtration rate (eGFR) which was determined using the Modification of Diet in Renal Disease equation.

2.3.D. Statistical Analysis

Continuous data are expressed as mean ± standard deviation (SD) or median and inter-quartile range (IQR) according to their distribution. Categorical data are expressed as percentages, with frequencies compared using Pearson's c2 test. Time to death, graft loss, and first CMV viraemia, were plotted using Kaplan-Meier (KM) survival curves. Associations between frailty groups and outcomes were assessed using Cox proportional hazards regression with adjusted models used to control for potential confounding. Confounders considered in multivariable analyses included (i) recipient factors (sex, race, primary renal disease, body mass index,

smoking history;) (ii) donor factors (age, sex, race, and deceased or live status); and (iii) transplant factors (cold ischemia time). Hazard ratios (HRs) and 95% confidence intervals were calculated for each variable. Associations were judged to be significant where the 95% confidence interval for the coefficient did not include unity. Finally, I investigated the predictors of developing CMV infection using logistic regression.

Statistical analyses were performed using SPSS version 29 (SPSS Inc., Chicago, IL, USA).

2.4. Materials and methods used to pertain to investigation reported in Chapter 5

2.4.A. Materials

2.4.A.A. General equipment

- Sodium heparin-containing vacutainer tubes.
- 50 mL falcon tubes.
- 10 mL serological pipets.
- 3 mL sterile pipets
- Round-bottom 96-well culture plates.
- 5 mL round-bottom polystyrene tubes
- Cryovial tubes
- Flow cytometer
- Freezing container (Mr Frosty; ThermoFisher, cat #5100-001)

2.4.A.B. Buffer and reagents

- RPMI 1640 culture medium with L-glutamine.
- Foetal bovine serum (FBS)
- Complete medium: RPMI 1640 culture medium, 10 % FBS, and 100 IU/mL penicillin and streptomycin.
- Phosphate-buffered saline (PBS), with MgCl2 and CaCl2, sterile-filtered, PH 6.9-7.1.
- Trypan blue (0.4%; Sigma Aldrich, cat ≠93595)
- Staining buffer: 2 % FBS and 0.01 % sodium azide in PBS.
- Lymphoprep (density: 1.077 ±0.001 g/ml).
- CpGC ODN 2395 (Oligodeoxyribonucleotides containing CpG motifs)
- Human mega CD40L.
- PIB cocktail: 50 ng/mL phorbol 12-myristate 13-acetate (PMA), 250 ng/mL ionomycin, and 5 micro g /mL Brefeldin A in complete medium.
- Zombie red fixable viability: dye to distinguish the viable from non-viable cells. Zombie red dye was then diluted in PBS 1 micro-L:1ml.
- Monoclonal antibodies fluorescently conjugated for surface markers, TNF, and IL10 detection:

Anti-human antibodies were used:

BUV737-IgD (clone: IA6-2)

BUV 395-CD19 (Clone: HIB19) from BD Biosciences.

PE/Cyanine 7-CD27 (Clone O323, isotype: Mouse IgG1, k)

Brilliant Violet 650 TM-CD38 (Clone: HB-7, isotype: Mouse IgG1, k)

PE-IL10 (Clone: JES-19F1, isotype: Rat IgG2a, k)

FITC-CD24 (Clone: ML5, isotype: Mouse IgG2a, k)

Brilliant Violet 421 TM -TNF-a (Clone: Mab11, isotype: Mouse IgG1, k) from Bio legend.

- Permeabilization and fixation solution from BD biosciences
- Ultrapure DNase/RNase-Free distilled water, PH 6-8
- Anti-Mouse Ig, K/ compensation beads from BD biosciences
- Anti-Rat and anti-hamster Ig, K/ compensation beads from BD bioscience
- Arc beads for Zombie red dye.
- Negative control beads
- DMSO (Dimethyl Sulfoxide)

2.4.B. Methods

2.4.B.A. Peripheral blood mononuclear cell (PBMC) isolation

PBMC isolation was done via density centrifugation as per this protocol:

A 30 ml venous blood was collected via peripheral venipuncture in Ethylenediaminetetraacetic acid (EDTA) coated tubes from each patient. Blood was diluted with sterile PBS in a 1:1 ratio. The diluted blood was then layered gently on top of 15 ml Lymphoprep in a 50 ml falcon tube with a sterile pipette with a ratio of 1:3 lymphoprep to blood.

The mix was spun at 1850 revolutions per minute (RPM) with no brake for 30 minutes. The PBMC layer at the interphase was carefully removed using a 3 ml pipette then resuspended in PBS (total volume 50 ml) and centrifuged for 10 minutes

at 1350 RPM (break on). Cells were washed once with PBS and then pelleted cells were resuspended in 10 ml PBS for counting. A 50 ul of suspended cells were diluted 1:1 with trypan blue and the number of cells was counted with a haemocytometer. Cells were pelleted by centrifugation and either resuspended in the appropriate media for culture or frozen as below for future analysis.

2.4.B.B. PBMC freezing and defrosting

A 1 ml of freezing media (90 % foetal bovine serum with 10 % dimethyl sulfoxide (DSMO) was added to the cell pallet at a concentration of 8-10 X10 6 cells /ml and transferred to cryovial tubes on ice. Cells were then placed in a freezing container (Mr Frosty) at -80 °C to achieve a rate of cooling of -1 C per minute. Cells were stored at – 80 °C until subsequent analysis. In some experiments, cells were cultured straight away and in other experiments, frozen cells were used after unfreezing. To defrost cells, cryovials were submerged in a 37 °C water bath for 1 minute. Cell suspensions were diluted immediately in 50 ml PBS, washed twice, and then PBMCs were counted and resuspended as above for subsequent use and analysis. Cell cultures were undertaken in round bottom 96-well culture plates with PBMCs at a starting concentration of 1-1.5 million cells suspended in 100 micro-L of complete medium per well. For each patient, two wells were designed for the stimulation and two wells were control samples.

In the wells containing stimulated cells, another 100 micro-L of stimuli (see below) were added, producing a final volume of 200 micro-L. In the control wells, another 100 micro-L of the complete medium was added to reach the same total volume of

200 Micro L/well. The 96-well plates were left in the incubator at 37 degrees for 72 hours. In the last 5 hours, the supernatant was collected from the wells, and cells were suspended in 100 micro-L of PIB cocktail (50 ng/mL phorbol 12-myristate 13-acetate (PMA), 250 ng/mL ionomycin, and 5 micro g /mL Brefeldin A in complete medium.) then returned in the incubator.

2.4.B.C. Cell stimulation

CpGC ODN 2395 (1 Micro M/mL) and mega (CD40 Ligand) CD 40L (1 micro g/mL) were added to complete medium to stimulate cell IL-10 production l in culture [217].

2.4.B.D. FACS staining

Each experiment contained wells for live dead stain only, unstained cells, and Fluorescence minus one (FMO) for the cell surface markers. One of the stimulated cells' well and one of the unstimulated cells well were treated as FMOs for IL10 and TNF. The 96-well culture plate was centrifuged at 600 X g at 4 degrees for 5 minutes then cells washed twice in PBS. The cells (except for the unstained controlled) were suspended in 50 micro-L of Zombie red dye solution (1 micro-L zombie red dye in 1000 micro–L PBS). The well was then incubated for 20 minutes at room temperature in the dark.

The cells were then washed twice with staining buffer with centrifuging at 800 x g at 4 degrees for 3 minutes after each wash.

The cells then were suspended in 100 micro-L of staining buffer containing the surface marker monoclonal antibodies at a concentration of 5 micro-L per well. The cells were then incubated for 30 minutes at 4 degrees in the dark.

The plate was then washed twice with staining buffer followed by centrifuging at 800X g at 4 degrees for 3 minutes after each wash.

For intracellular staining, each well was then resuspended with 100 micro-L intracellular fixation solution and incubated for 10 minutes in the dark at 4 degrees. Wells were washed twice in the staining buffer with centrifuging at 800 X g at 4 degrees for 3 minutes after each wash. Finally, the cells were washed twice with permeabilization buffer with centrifuging at 800 X g at 4 degrees after each wash.

The cells then were re-suspended with 50 Micro L permeabilization solution and incubated for 5 minutes at 4 degrees in the dark, followed by the addition of 5 micro-L of anti-IL10 and 5 micro-L of anti-TNF-a The wells were incubated for 40 minutes at 4 degrees in the dark.

The cells were washed twice with staining buffer with centrifuging at 800 X g at 4°C for 3 minutes after each wash, and finally resuspended in 200 micro-L staining buffer and transferred to 5 ml round-bottom polystyrene tubes for immediate analysis by

or stored at 4 °C in the dark for analysis the following day.

FACS was undertaken on the LSR Fortessa flow cytometer (BD Biosciences). Analysis was undertaken using Flow Jo v 10 software (https://www.flowjo.com). Gates were determined using a fluorescence minus one (FMO) strategy unless otherwise specified.

2.4.B.E. Compensation preparation:

Compensation beads using Anti-Mouse IgK, with negative control compensation beads were used for CD19, CD24, CD38, IgD, CD27, and TNF-α as per the manufacturer's instructions.

Anti-Rat IgK with negative control compensation beads were used for IL-10 as per the manufacturer's instructions.

Arc beads were used for the Zombie red dye as per the manufacturer's instructions.

2.4.B.F. Flow cytometry acquisition – gating strategy

Lymphocytes were gated on forward and side scatter, then doublets and dead cells were excluded. The cells were then gated on the CD19 population (Figure 2.2)

Within the CD 19+ population, cells were gated using the CD24 and CD 38 expression or CD24 and CD27 to:

- total CD24+ CD 38 +, CD24 hi CD38 hi subset, CD24 inter CD 38 inter.

- CD 27 hi CD 27 +.

Then for each of these subsets, IL10 and TNF + populations were identified.

Unstimulated sample and fluorescence-minus-one (FMO) controls were used to get gating thresholds

Example of CD24 FMO and CD38 FMO are shown in Figure 2.1:

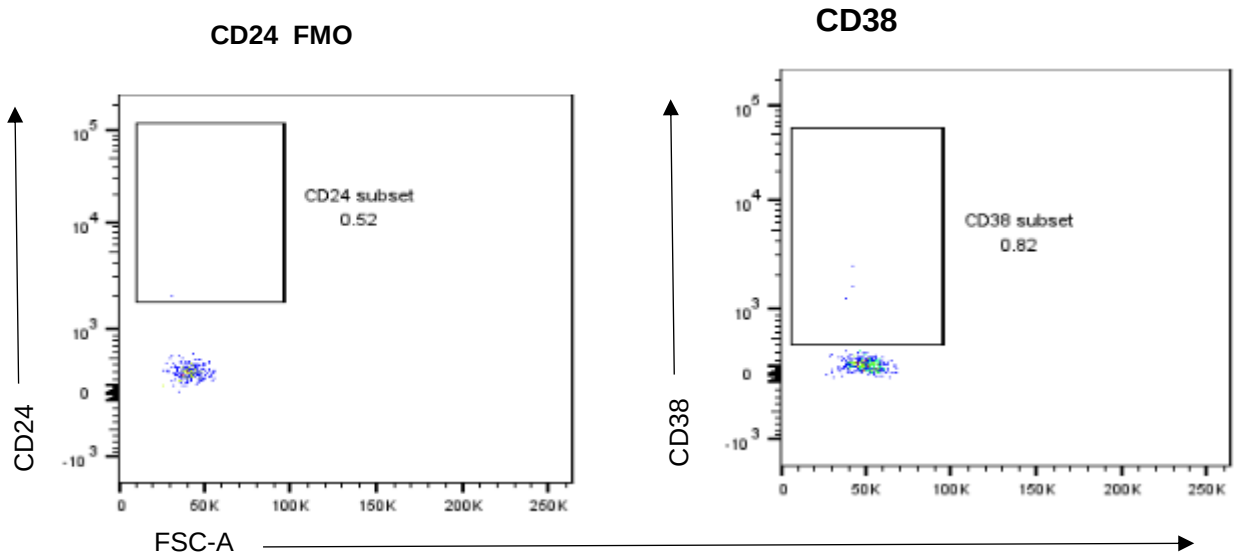


Figure 2.1: CD24 fluorescence -minus-one (FMO)(left) and CD38 fluorescence(right).

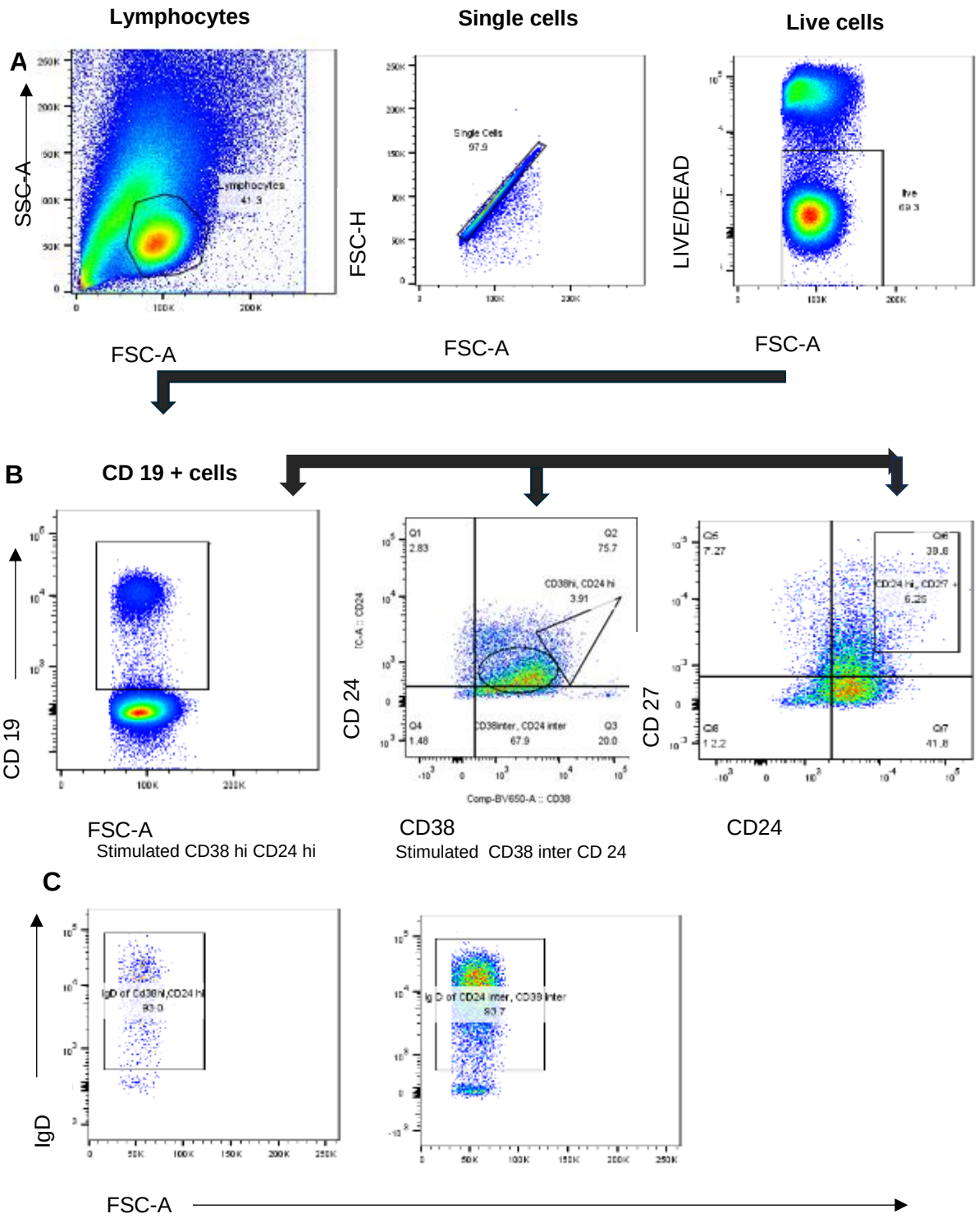
2.4.B.G. Gating used during FACS analysis of B cells subsets

Gates for IL-10 and TNF were determined by fluorescence minus one (FMO)

Figure 2.2 shows the gating strategy, Lymphocytes were gated on an FSC-SSC plot; single cells were gated on an FCS-A-FSC-H plot, live cells were gated on an FSC-viability dye plot. CD19 expression was determined by CD19 gating; CD24 and CD38 expression and different subsets were determined by CD24 and CD38 gating, CD24 and CD 27 expression were determined by CD 24 and CD 27gating, IgD of different subsets was determined by IgD gating.

Figure 2.3 demonstrates the gating for IL-10 and TNF in stimulated B cell subsets.

Figure 2.2 (next page): Gating strategy for ex vivo of CD24 hi CD38 hi and CD24 hi CD27+ B cell subsets. (a) PBMC sample is gated on lymphocytes, then single cells are included then only live cells are included. (b) B cells expressing CD19 are identified. CD24 and CD38 markers are used to identify CD24hiCD38hi, CD24 inter CD38inter, while CD27 and CD24 markers are used to identify CD24hi CD27+ B cells. (c) Expression of IgD in these B cells subsets used to validate gates for these subsets.


Lymphocytes
Single cells
Live cells
A
SSC-A
FSC-A
Lymphocytes
41.3
FSC-H
FSC-A
Single Cells
97.9
LIVE/DEAD
FSC-A
live
69.3
B
CD 19 + cells
CD 19
FSC-A
Stimulated CD38 hi CD24 hi
CD 24
CD38
Stimulated CD38 inter CD 24
Q1
2.83
Q2
75.7
CD38hi, CD24 hi
3.91
Q4
1.48
CD38inter, CD24 inter
67.9
Q3
20.0
Comp-BV650-A :: CD38
CD 27
CD24
Q5
7.27
Q6
39.8
CD24 hi, CD27 +
6.26
Q8
12.2
Q7
41.8
C
IgD
IgD of Cd38hi,CD24 hi
IgD of CD24 inter, CD38 inter
FSC-A

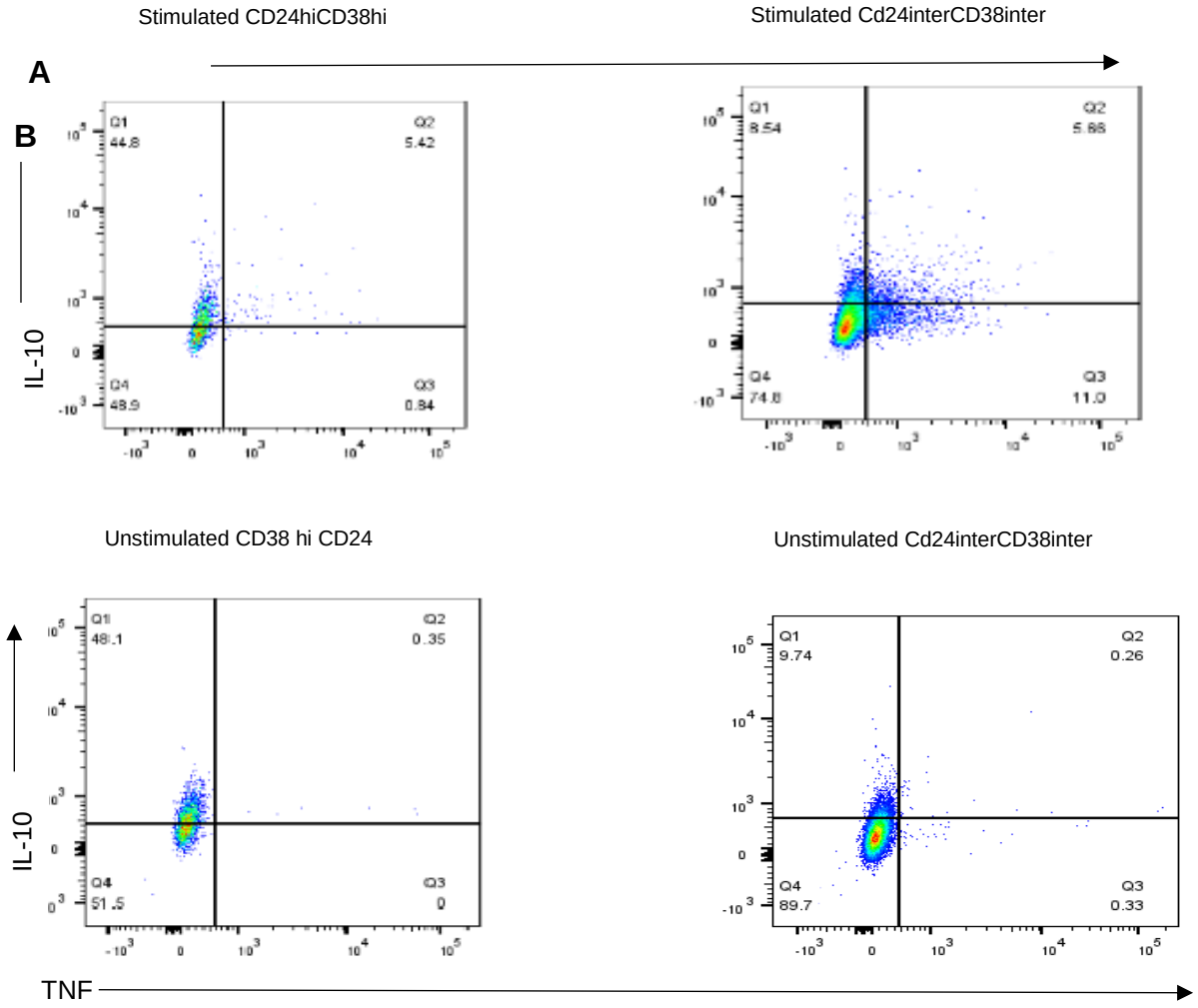


Figure 2.3: IL-10 and TNF intracellular staining post-stimulation in vitro. (a)Flow cytometry dot plots show IL-10+ and TNF + and dual IL-10 + & TNF+ B cells in the CD38hi CD24hi, and CD 38 inter, and CD 24 inter subsets following 72 hours stimulation. (b) unstimulated sample of B cells subsets to indicate true staining. MFOs for IL-10 and TNF are used to inform IL-10 + and TNF+ gate placement.

A summary of all B subsets and their IL-10 and TNF expressions are shown in appendix 5.

2.5. B cells subsets and their IL-10/TNF ratio in older kidney recipients

2.5.A. Study design and participants of the B cell phenotype

Adult (>18 years of age) kidney transplant recipients at three months of transplantation were recruited from the Nephrology Outpatient Department of the Royal Free Hospital, London. All patients were on maintenance immunosuppression at the time of recruitment. Demographic and clinical data were recorded. PBMCs were isolated and stimulated with CpGC and mega CD 40L for 72 hours. B cell subsets and intracellular staining for IL-10, TNF-α, and IL-10/TNF-α ratio were compared between young and old KTR (≥60). We also compared clinically defined outcomes (see below) in the older cohort to the young age group at the time of transplantation.

All patients provided written informed consent before participation in the study (Royal Free Hospital Research and Development Committee study identification number NREC 05/Q0508/6).

Follow-up commenced at the time of transplantation and ended in January 2023. Kidney transplant biopsies were performed only for clinical indications.

2.5.B. Immunosuppression protocol

All patients received standard immunosuppression using induction therapy with Basiliximab (20mg) given on the day of surgery and the fourth postoperative day. Standard maintenance immunosuppression is as mentioned above.

2.5.C. Outcome variables

Outcome measures included the occurrence of the first CMV viraemia within the first 6 months, the occurrence of biopsy-proven acute rejection (BPAR), and the development of donor-specific anti-HLA antibodies (DSA), by three months after transplantation. The incidence of CMV viraemia was based on CMV DNA PCR, using a threshold of 200 IU/ml and 3000 IU/ml in CMV naïve and CMV seropositive recipients, respectively. Acute humoral and cellular rejections were defined according to the Banff classification. [202] Screening for anti-HLA antibodies was performed by flow cytometry using the xMAP (Luminex) platform, utilizing LAB Screen Mixed Bead kits (One Lambda, West Hills CA, USA). Positive samples were tested on HLA Class I and Class II single antigen kits to define antibody specificities. A baseline means fluorescence intensity (MFI) cut-off of ≥2000 was used to report pre-transplant unacceptable antigens to NHS organ donation and transplantation (ODT); any donor-specific antibodies (DSA) ≥ 500MFI were considered to be significant post-transplantation.

Finally, we also collected data on renal function using an estimated glomerular filtration rate(eGFR) which was determined using the Modification of Diet in Renal Disease equation [218].

2.5.D. Statistical Analysis

Continuous data are expressed as mean ± standard deviation (SD) or median and inter-quartile range (IQR) according to their distribution. Categorical data are expressed as percentages, with frequencies compared using Pearson's c2 test. Analyses for multiple groups were completed using ANOVA for normally distributed variables or the Kruskal–Walli's test for data with a non-parametric distribution. Mann-Whitney U Test used in two groups median comparisons. Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA).

2.5.E. Study Approval

The Royal Free Hospital Research and Development committee approved the study (identification number NREC 05/Q0508/6), and all subjects provided written consent before enrolment.

Data regards the subjects were obtained via the Royal Free Hospital electronic records system and from the UK Transplant Registry of the Organ Donation and Transplant Directorate of National Health Service Blood and Transplant (NHSBT). All the tests were done for clinical reasons.

CHAPTER 3. THE IMPACT OF STANDARD IMMUNOSUPPRESSION REGIMENS ON IMMUNOLOGICAL AND INFECTIVE COMPLICATIONS IN OLDER KIDNEY TRANSPLANT RECIPIENTS

3.1. Introduction

Kidney transplantation is an effective treatment for end-stage kidney disease, the incidence of which increases with age. It improves both survival and quality of life and is economically beneficial [173] [174] [175] [176] [177]. There are increasing numbers of older patients undergoing kidney transplantation. Transplantation is associated with morbidity consequent to the necessary pharmacological manipulation of the immune system, including infection and malignancy. Data have demonstrated a decreased risk of rejection with age [24] [37] [38], while the most common causes of death among older KTR are cardiovascular disease followed by infection and malignancy [16] [19]. There are likely synergistic effects of both immunosenescence and immunosuppressive therapy that result in older transplant recipients succumbing to more post-transplant infections and malignancies [119] [219]. Therefore, in theory, older kidney allograft recipients may not require the same level of immunosuppressive as younger patients. While there is evidence for age-related immunosenescence increasing the risk of infective complications, few centres enforce age-specific immunosuppression adjustments. In our transplant unit, all transplant patients of any

age or immunological risk receive the same immunosuppression protocol and prophylaxis. CMV prophylaxis is not routinely given, regardless of donor or recipient CMV status. Thus, CMV viremia rates can be considered as a true reflection of the level of immunosuppression and could provide an evidence base to personalize the delivered immunosuppression.

3.2. Aims

i. To investigate the rates of infection (particularly CMV viremia) among older KTR (>60 years) compared with younger age groups
ii. To investigate the rate of rejection among older KTR compared with younger age groups

3.3. Brief Methods

This was a retrospective observational study. I investigated 148 kidney transplants performed in our centre between April 2009 and March 2019 in recipients aged > 60 years. They were compared to 272 younger recipients, matched for degree of HLA sensitization, and number of HLA-mismatches, and divided into three groups according to their respective age at transplantation (18-34, 35-49 & 50-60 years). Recorded outcome measures included the incidence of biopsy-proven acute rejection (BPAR), development of donor-specific anti-HLA antibodies (DSA), and occurrence of CMV viraemia. Statistical analyses were performed using SPSS version 26(SPSS Inc., Chicago, IL, USA).

3.4. Results

3.4.A. Baseline characteristics of participants

One hundred and forty-eight recipients aged older than 60 years receiving their first kidney transplant were identified. The number of kidney transplants performed in older patients remained relatively stable over the study period in our centre (Figure 3.1), averaging 24% of our total cadaveric transplant activity in 2009-2014 and 27% in 2015-2019.

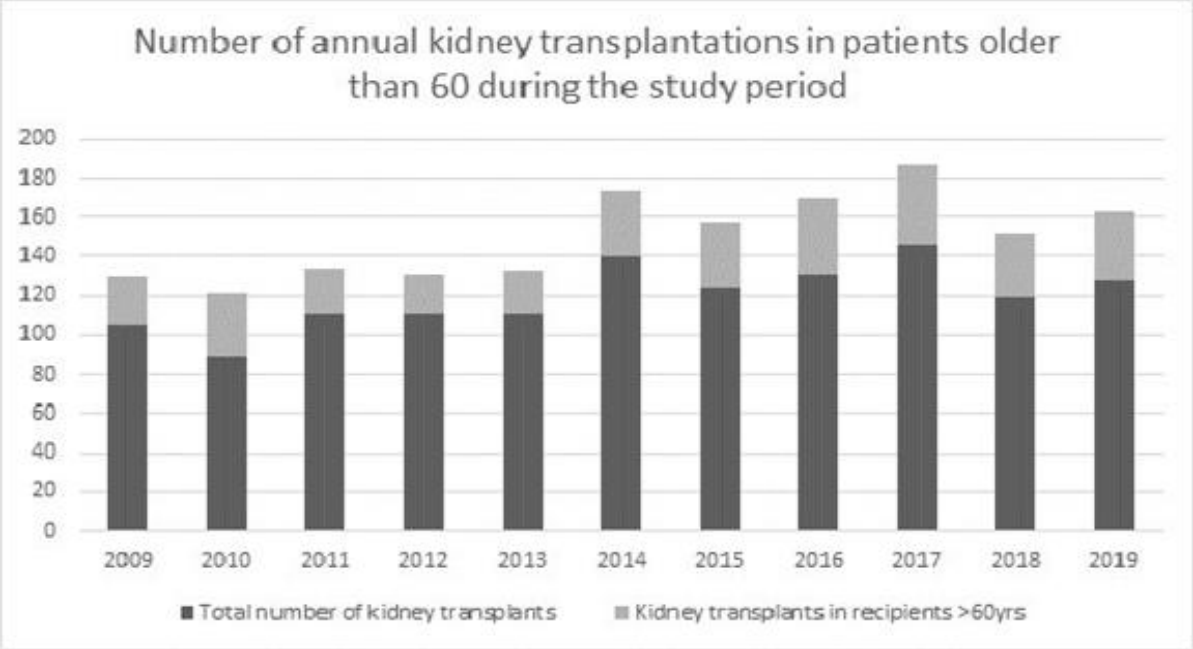


Figure 3.1: Graph showing the proportions of kidney transplants in recipients over 60 years of age according to year during the study period.

The patients from the older transplant group were each matched to two control first-time kidney transplant recipients under the age of 60(a total of 272 patients), selected from at least two of three age groups(18-34, 35-49, 50-60 years) identified

from across each contemporaneous transplant year (Table 3.1), and matched by the total number of HLA-A, -B and -DR mismatches, cRF level and recipient CMV serostatus.

Table 3.1: Demographic and clinical characteristics of transplant recipients

Variables	KTR age (18-34yrs) (n=71)	KTR age (35-49yrs) (n=90)	KTR age (50-60yrs) (n=111)	KTR age (>60yrs) (n=148)	P value *
Recipient age, years Median (IQR)	28 (24-31)	42 (39-46)	55 (62-58)	68 (65-71)	<0.001
Male recipient gender, n(%)	35 (50)	49 (49)	70 (63)	101 (68)	0.03
Recipient ethnicity, n(%)					
White	29 (41)	35 (39)	48 (43)	84 (57)	
Asian	22 (30)	26 (29)	32 (29)	42 (28)	0.03
Black	20 (29)	29 (32)	31 (28)	22 (15)	
Cause of ESRD, n (%)					
DM	3 (4)	6 (7)	21 (19)	31 (21)	
HTN	4 (6)	11 (12)	12 (11)	22 (15)	
IgA nephropathy	10 (14)	17 (19)	7 (6)	9 (6)	<0.001
Urological	9 (13)	4 (4)	6 (5)	7 (6)	
Congenital	12 (17)	10 (11)	3 (3)	5 (3)	
Vasculitis	8 (11)	10 (11)	10 (9)	5 (3)	
ADPKD	1 (1)	8 (9)	9 (8)	11 (7)	
Others or unknown	24 (34)	24 (27)	43 (39)	58 (39)	
BMI, kg/m^2 mean (SD)	24 (4.7)	26 (4.7)	27 (3.8)	26 4.1)	<0.001
HLA-A, -B, and -DR mismatches, n (%)					
0-2	22 (31)	22 (24)	19 (17)	34 (23)	0.20
3-4	43 (61)	53 (59)	81 (73)	94 (63)	
5-6	6 (8)	15 (17)	11 (10)	20 (14)	
Donor-recipient CMV IgG serostatus, n (%)					
Pos-pos	30 (42)	48 (53)	47 (42)	72 (48)	
Pos-neg	3 (4)	9 (10)	10 (9)	12 (8)	<0.001
Neg-pos	18 (25)	19 (21)	42 (38)	56 (38)	
Neg-neg	20 (28)	9 (10)	11 (10)	8 (5)	
Unknown to positive	0	5	1 (1)	0	
Recipients' current history of smoking, n (%)	10 (14%)	15(17%)	14 (13%)	15 (10%)	0.312
Recipients' Previous history of smoking, n (%)	24 (34%)	34 (38%)	42 (38%)	77 (52%)	0.055
Pre-emptive transplantation, n (%)	24 (34)	28 (31)	28 (25)	40 (37)	0.60
Median dialysis duration, days (IQR)	456 (191-913)	775 (463-1669)	878 (394-1744)	943 (465-1682)	0.29
Sensitization at transplantation, n (%)					
0-0	49 (69)	56 (62)	70 (63)	111 (75)	
1-70	21 (29)	24 (27)	33 (30)	33 (22)	0.08
70-85	1 (2)	4 (4)	3 (3)	3 (2)	
>85	0(0)	6(7)	5(5)	1(1)	

CMV titer, median (IQR) in those with detected viraemia	1492 (428-2938)	4582 (1329-7991)	1172 (471-5020)	3251 (618-6925)	0.06
DGF, n (%)	17 (24)	20 (22)	45 (40)	45 (30)	0.02
Median time to graft ,months,loss (IQR) in those with graft loss	49(17-86)	61(31-81)	48(22-75)	49(40-84)	0.698
Median length of follow-up, months (IQR)	87 (55-119)	100 (56-123)	66 (48-113)	65 (47-95)	0.005

Continuous variables are shown as either mean (SD) or median (IQR) and categorical variables as an absolute value (percentage). ADPKD, autosomal dominant polycystic kidney disease; ATG, anti-thymocyte globulin; BMI, body mass index; CMV, cytomegalovirus; DBD, donation after brain death; DCD, donation after circulatory death; DGF; delayed graft function, DM, diabetes mellitus; DSA, donor-specific antibody; ESRD, end-stage renal disease; HLA, human leucocyte antigen; HTN, hypertension; LD, live-donor.
The Kruskal-Walli's test was used for continuous variables and the Pearson Chi2 test was used for categorical variables.

There were statistically significant differences in donor age between the groups, with older recipients having received kidneys from older donors, which is as expected from the UK National Allocation Scheme where deceased-donor kidneys are allocated according to a points-based scoring system via a computer algorithm that prioritizes waiting time, HLA match, and donor-recipient age match [220]. Additionally, although there were roughly similar proportions of recipients who received kidneys donated after brain death (DBD) among the four age groups, a higher proportion of older recipients (50-60 years) received kidneys donated after circulatory death (DCD) while a higher proportion of younger recipients received kidneys from live donors (Table 3.2).

Table 3.2: Demographic and clinical characteristics of transplant donors

Variable	Recipient age: 18-34yrs (n=71)	Recipient age: 35-49yrs (n=90)	Recipient age: 50-60yrs (n=111)	Recipient age >60yrs (n=148)	p value
Donor age, years median (IQR)	41 (30-51)	45 (37-53)	52 (45-60)	60 (50-68)	<0.001
Male donor gender, n (%)	45 (63)	53 (59)	60 (54)	94 (63)	0.43
Donor ethnicity, n (%) **White** **Asian** **Black** **unknown**	47 (66) 8 (11) 9 (13) 7 (10)	54 (60) 11 (12) 8 (9) 17 (19)	81 (72) 12 (11) 5 (5) 13 (12)	114 (77) 11 (7) 8 (6) 15 (10)	0.16
Donor status, n (%) **LD** **DBD** **DCD**	35 (49) 28 (40) 8 (11)	32 (35) 36 (40) 22 (25)	21 (19) 46 (41) 44 (40)	41 (28) 59 (39) 48 (33)	<0.001
KDPI, % Mean (SD)	20 (29)	15(27)	36 (34)	42 (42)	<0.001
KDRI, Median (IQR)	1.02 (0.84-1.16)	0.92 (0.74-1.26)	1.14 (0.95-1.31)	1.48 (1.24-1.77)	<0.001
CIT, mins Median (IQR)	336 (135-744)	601 (185-887)	616 (431-843)	637 (225-864)	0.11

Continuous variables are shown as mean (SD) and categorical variables as absolute value (percentage). CIT, cold ischemic time; CMV, cytomegalovirus; DBD, donation after brain death; DCD, donation after circulatory death; DSA, donor-specific antibody; ESRD, end-stage renal disease; HLA, human leucocyte antigen; KDPI, kidney donor profile index; KDRI, Kidney Donor Risk Index; LD, live-donor. The Kruskal-Walli's test was used for continuous variables and the Pearson Chi^2 test was used for categorical variables.

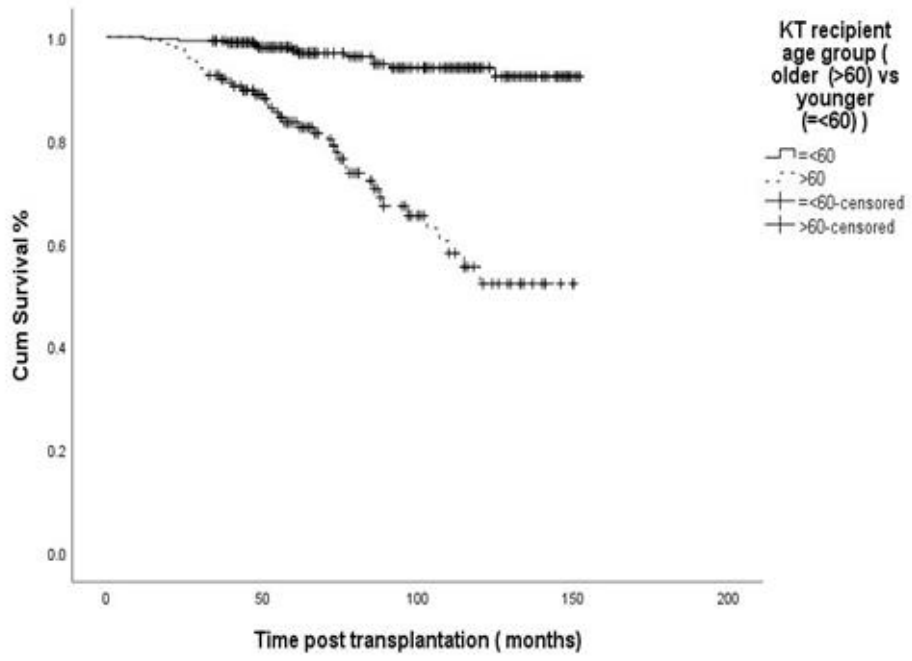
The median follow-up duration was 87, 100, 66, and 65 months for the 18-34, 35-49, 50-60, and over 60-year-old age groups respectively (p=0.005) (Table 3.1). A total of 290 patients (69%) needed dialysis before transplantation and the median time on dialysis for the different age groups was not significantly different, (Table 3.1). Diabetes and hypertension were the main causes of ESRD in the oldest recipients, and congenital and IgA nephropathies were the most common causes among the younger age group. Transplantation from CMV seropositive donors into seronegative recipients (CMV D+ R-) occurred in 4%, 10%, 9%, and 8% in the age groups 18-34,

35-49, 50-60, and >60, respectively. The majority of patients received Basiliximab induction therapy except for 6 patients (two in the 50-60 age group and four in the over 60-year age group) who received alternative induction agents as part of the 3C study [221] and one who received ATG for a positive flow cytometric cross match.

3.4.B. Patient and graft outcomes

Overall, there were 52 deaths (12% of the total cohort) with 40 (27%) and 12 (4%) in the over and under 60 age groups respectively, (Figure 3.2 a), of which 13 (9%) and 9 (3%) were deaths with a functioning graft. Univariate analysis showed that patient survival was significantly better in all the age groups compared to the >60 years (taken as reference) (Figure 3.2 b and Table 3.3). These hazards changed little with multivariate analysis controlling for potentially confounding recipient and donor factors, although the Wald test in the adjusted models did not completely support rejecting the null hypothesis, this likely represents inadequate power to adjust for multiple variables (Table 3.3).

(a)



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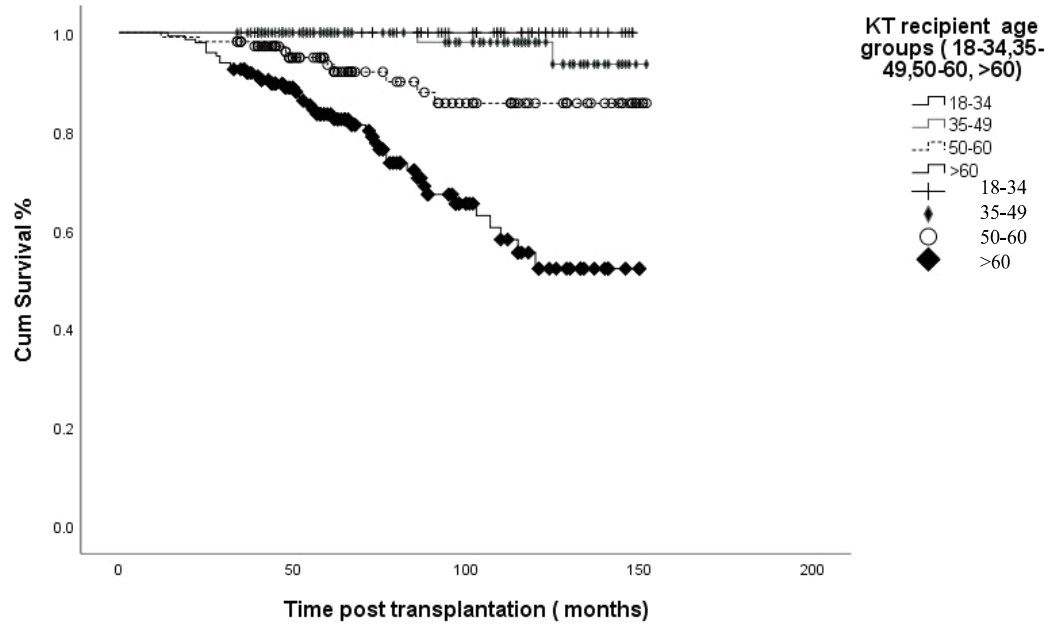


Figure 3.2: Kaplan–Meier curves showing (a) overall patient survival in recipients aged under 60 years old and those over 60 years old (p<0.0001, log rank test) and (b) survival after kidney transplantation in each age group 18-34,35-49, 50-59 and over 60 years old (p<0.0001, log-rank test).

Table 3.3: Cox regression model for different patient outcomes

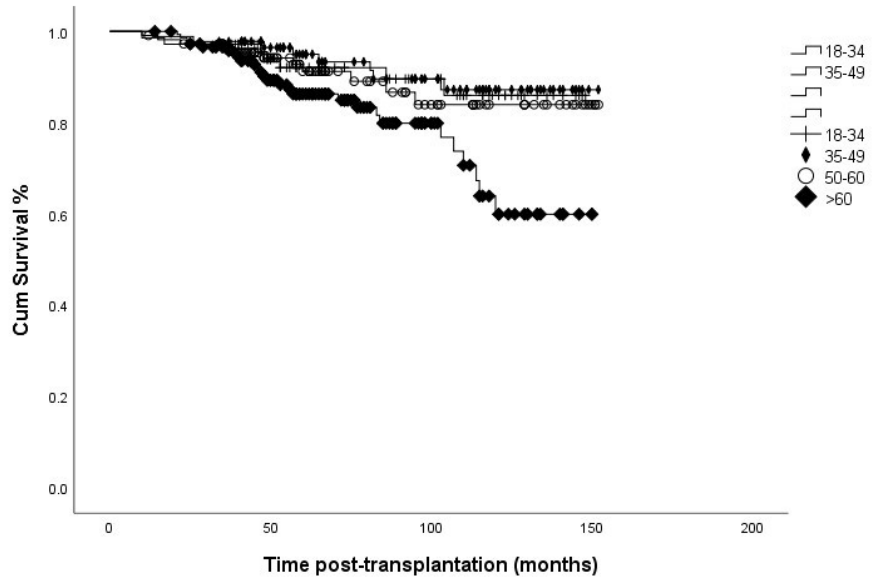
Recipient outcome per Age group (years)	**Univariate HR (95% CI)**	**Multivariable HR (95% CI) ***	**multivariable HR (95% CI) ****
Graft loss 18-34 35-49 50-60 >60	 Reference 0.84 (0.30-2.32) 1.22(0.47-3.15) 2.38(1.03-5.48) Wald P=0.02	 Reference 0.49(0.13-1.76) 0.67(0.20-2.23) 1.33(0.48-3.66) Wald P =0.24	 Reference 0.35(0.09-1.37) 0.46(0.13-1.62) 0.88(0.30-2.59 Wald P =0.29
Graft loss censored to patient loss 18-34 35-49 50-60 >60	 Reference 0.95(0.35-2.55) 1.88(0.78-4.55) 4.01(1.81-8.90) Wald P =0.00	 Reference 0.49(0.14-1.71) 1.02(0.35-2.97)1.54(0.58-1.05) Wald P =0.17	 Reference 0.45(0.12-1.64) 0.85(0.28-2.56)1.31(0.48-3.59) Wald P =0.28
CMV infection within 6 months post KT 18-34 35-49 50-60 >60	 Reference 1.82(0.966-3.438) 2.55(1.394-4.574) 2.66(1.497-4.745) Wald P 0.005	 Reference 1.28(0.63-2.60) 1.95(0.99-3.86) 2.09(1.06-4.12) Wald P 0.09	 Reference 1.47(0.70-3.09) 1.89(0.94-3.82) 2.33(1.16-4.68) Wald P 0.09
Patient death 18-34 35-49 50-60 >60	 0.00(0.00-3.87E+145) 0.05(0.01-0.23) 0.29(0.14-0.59) Reference Wald P =0.00	 0.00(0.00-2.50E+175) 0.00(0.00-7.67E+160) 0.295(0.20-1.30) Reference Wald P =0.58	 0.00(0.00-3.46E+176) 0.00(0.00-2.69E+168)0.94 0.51(0.20-1.28) Reference Wald P =0.57
Rejection 18-34 35-49 50-60 >60	 Reference 0.40(0.17-0.92) 0.32(0.14-0.75) 0.50(0.24-1.04) Wald P =0.03	 Reference 0.30(.10-0.86) 0.18(0.05-0.59) 0.30(0.11-0.81) Wald P =0.01	 Reference 0.22(0.06-0.74) 0.09(0.02-0.37) 0.23(0.07-0.67) Wald P =0.002
Malignancy 18-34 35-49 50-60 >60	 Reference 2.29(0.46-11.35) 1.53(0.28-8.39) 7.26(1.69-31.10) Wald P =0.001	 Reference 3.305(0.30-35.39) 0.00(0.00-2.52E+192) 11.68(1.4-97.35) Wald P 0.05	 Reference 3.99(0.33-47.86) 0.00(0.00-3.10E+128) 10.47(1.14-96.01) Wald P 0.15

*Recipient gender and ethnicity, Donor age, gender and ethnicity, presence of DM, current and previous smoking, recipient BMI and dialysis prior to transplant.
** Recipient gender and ethnicity, Donor age, gender and ethnicity, presence of DM, current and previous smoking, recipient BMI, and dialysis prior to transplant, CIT cRF, total MM, graft type donor-recipient CMV status

The two older recipient groups experienced the highest rates of DGF (40 % and 30 % in the 50-60 and > 60 age groups respectively).

Graft survival is shown in Figures 3.3a-b. During follow-up, the 50-60 age cohort experienced almost double the proportion of graft losses compared to the 18-34- and 35–49-year-old recipients. Death-censored graft survival at 5 years was 90.1%, 90%, 85%, and 69% for the 18-34, 35-49, 50-60, and > 60-year-old recipient groups, respectively.

(a)



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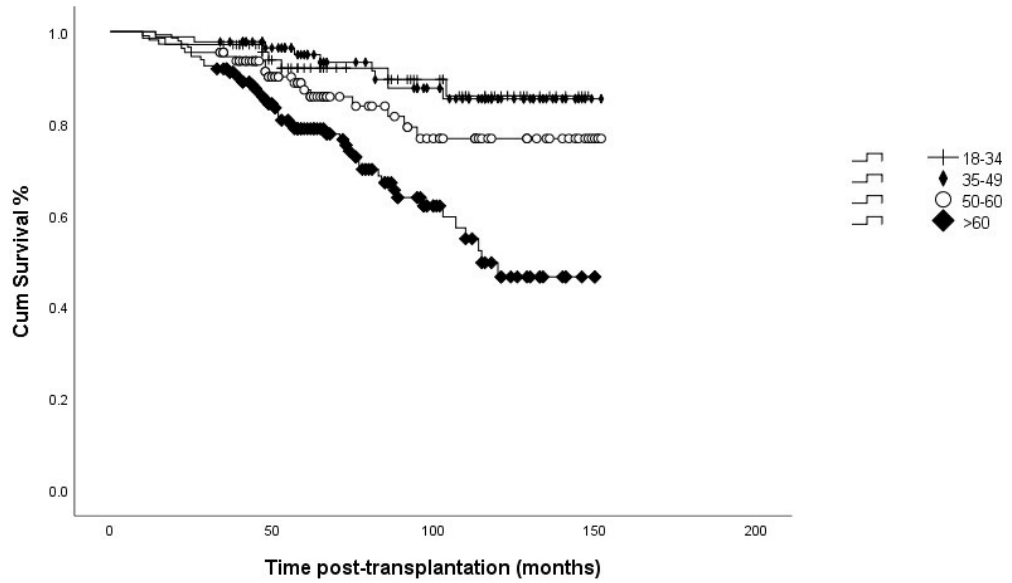


Figure 3.3: Kaplan–Meier curves showing (a) overall graft survival rates at the time of study completion following kidney transplantation in recipients age group, 18-34,35-49,50-59, and =>60, p=0.015 and (b) overall death-censored graft survival rates at the time of study completion following kidney transplantation in recipients age group, 18-34,35-49,50-59, and =>60, p<0.001, log-rank test.

Univariate analysis demonstrated a significant impact of age on graft survival, with an HR of 4.01(1.81-8.90) for the over-60-year cohort. However, this association was substantially diluted when the additional recipient and donor factors were considered in the multivariate model, including recipient gender, ethnicity, diabetes, BMI, smoking history, and dialysis before transplantation as well as donor age, gender, and ethnicity (Table 3.3).

Univariate analysis showed that the risk of BPAR was substantially lower in all age groups compared to the youngest cohort. Kaplan-Meier curve (Figure 3.4) showed a significant reduction in rejection-free survival among the younger kidney transplant recipients (18-34 age group) in comparison with all other groups (p<0.026, log-rank test). This risk was further reduced when additional recipient and donor factors were included in the multivariate model (Table 3.3). In the over-60-year-old group, the unadjusted HR was 0.5 (0.24-1.04) which decreased to 0.23 (0.07-0.67) in the complete multivariable model (Table 3.3). The emergence of de novo class I & II DSAs or an increase in the level of preexistent antibodies occurred in similar proportions across the four age groups but these were performed at various time points during follow up making comparison difficult (data not shown).

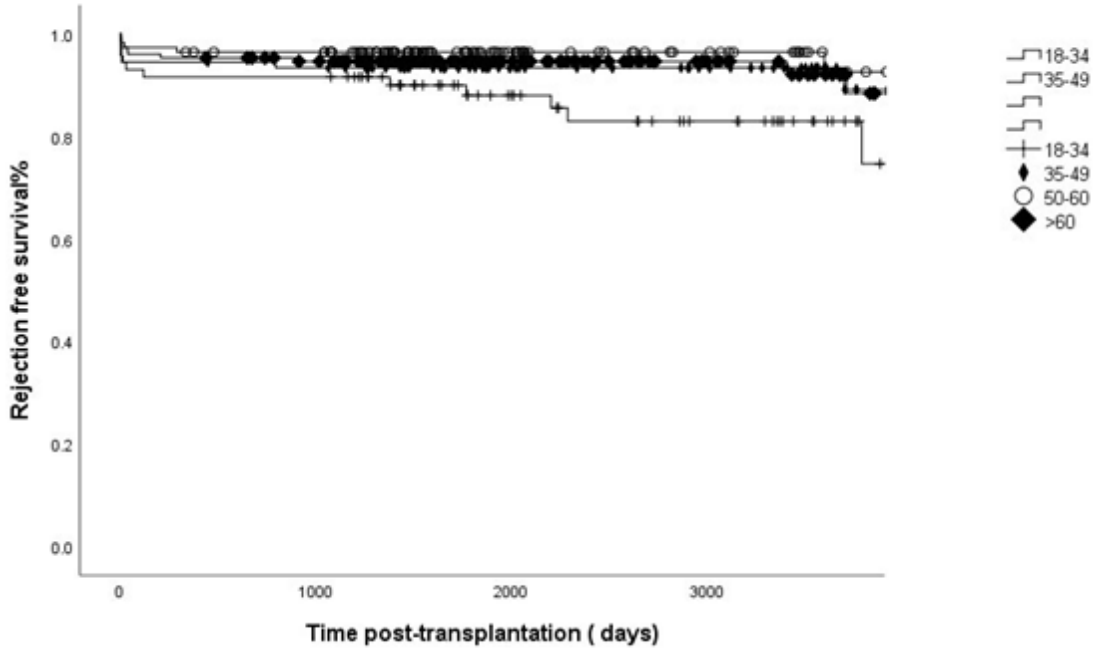


Figure 3.4: Kaplan-Meier curves showing rejection-free survival after transplantation in recipients age group, 18-34,35-49,50-59, and =>60, ($p<0.026$, log-rank test).

The median eGFRs for the patients with surviving grafts from the four recipient age groups are depicted in Figure 3.5. Although there was a significantly better GFR in the younger age group at one year, by 5- & 10 years following transplantation, there was no difference in graft function across the different age groups in the patients with surviving grafts.

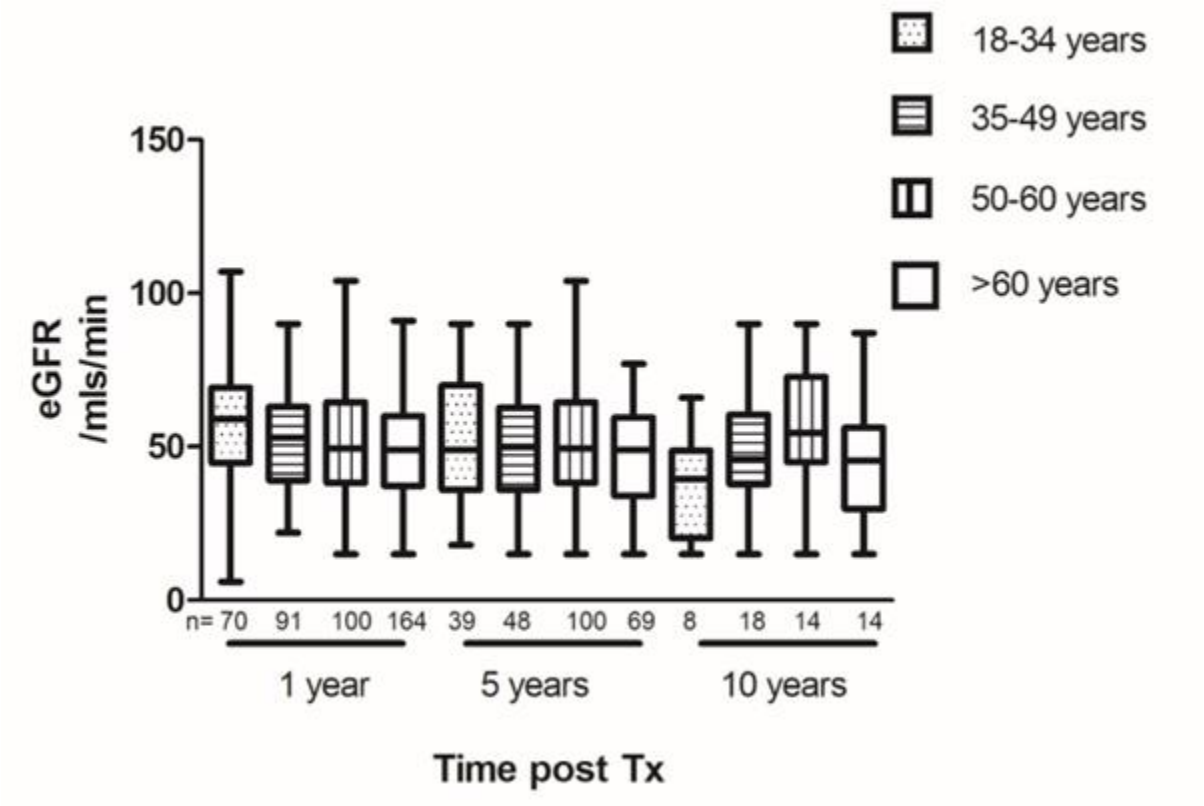


Figure 3.5: Box and Whisker plot (showing min and max range) of MDRD eGFR in each age group cohort at 1, 5 and 10 years after transplantation. Although the 1-year eGFR was statistically better in the younger cohort (p=0.002, one way ANOVA), there was no difference at 5 or 10 years.

3.4.C. Infectious complications

With regards to CMV infection, 44% of patients from the two older age groups (50-60 and >60) developed at least one episode of CMV viraemia in the first six months after kidney transplantation compared to 19% and 33% in the 18-34yr and 35-49yr groups respectively($p<0.003$) (Figure 3.6). There was no difference in the median time to developing CMV in the four age groups and although the median peak CMV viral load was almost double in the >60-year-old age compared to the youngest

cohort, this did not reach statistical significance (Figure 3.7).

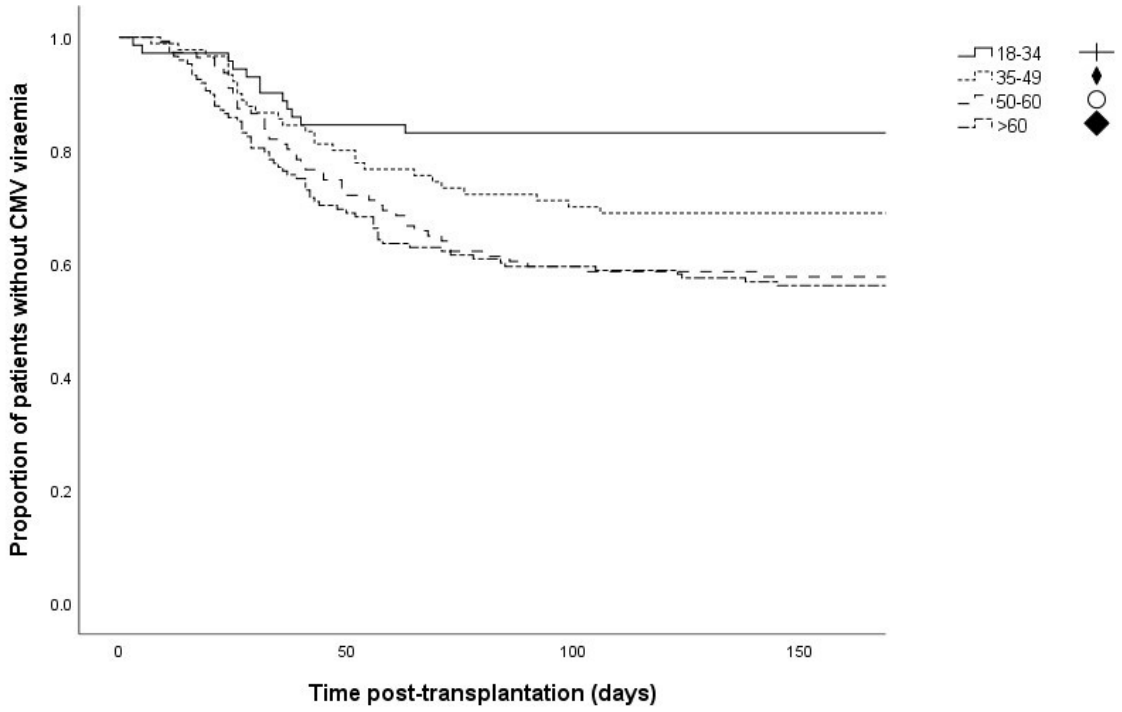
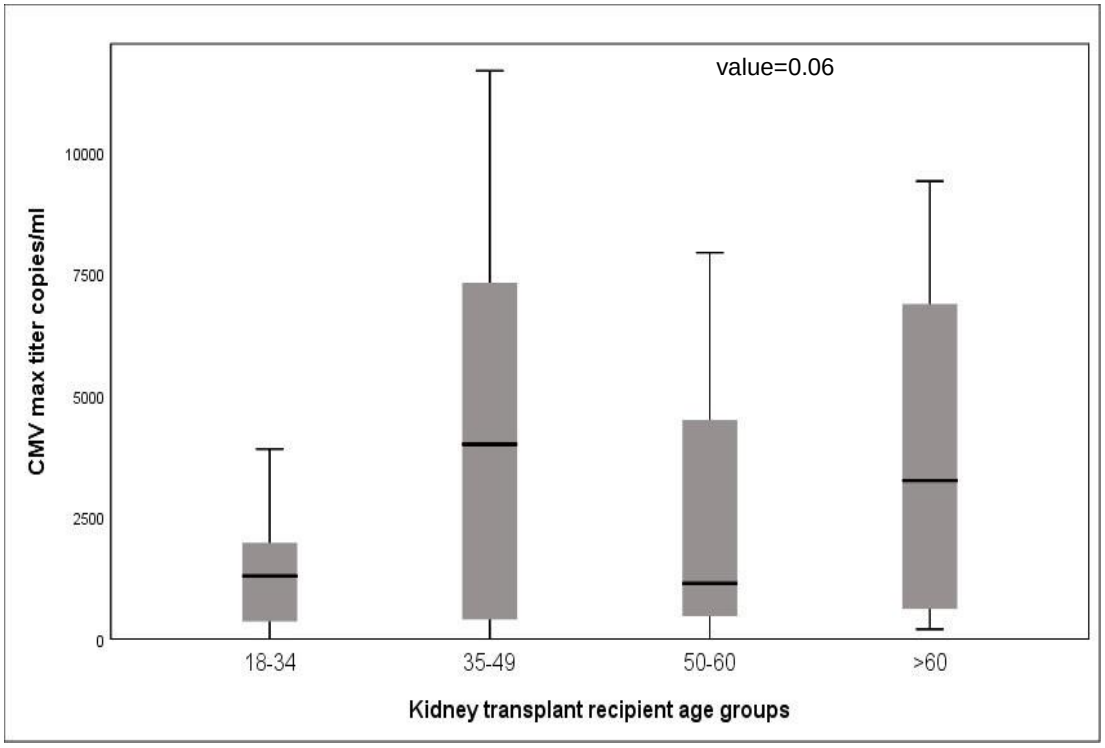


Figure 3.6: Kaplan-Meier curves for proportion of patients without CMV viraemia within the first 6 months after transplantation in recipients age group, 18-34,35-49,50-59, and =>60, ($p<0.003$, log-rank test).

Figure 3.7: Peak CMV viraemia in recipients testing positive post-transplant, according to age groups, 18-34,35-49,50-59, and =>60, p value=0.063(one way ANOVA)



Recipient aged above 60 was associated with more than two-fold increased risk of CMV viraemia (HR=2.67; 95% CI=1.50-4.75). The multivariate analysis demonstrated some attenuation of the hazard ratio suggesting some of this risk may be confounded by donor and recipient factors other than their biological age. Although again, the Wald test in the adjusted models did not completely support rejecting the null hypothesis, this represents inadequate power to adjust for multiple variables, and the 95% CI for the oldest group still suggested greater risk(Table 3.3). The rates of BK infection at any time post kidney transplantation among the four age groups were not significantly different, but these were not always tested at standard times or consistently across the groups, making a time-dependent analysis more difficult (data not shown).

3.4.D. Malignancy

The most common malignancy reported was skin cancer (32%) for the whole cohort, with a new onset rate of 3% vs 15% in the 18-34 and >60 age groups, respectively. The time to develop either skin, hematological or solid organ malignancy in the four age groups is shown in Figure 3.8. Developing malignancy was significantly more common for recipients over 60 years HR 7.26(1.69-31.10; Table 3.3) versus the youngest age group. This effect persisted in the multivariate analysis after adjustment for recipient and donor variables.

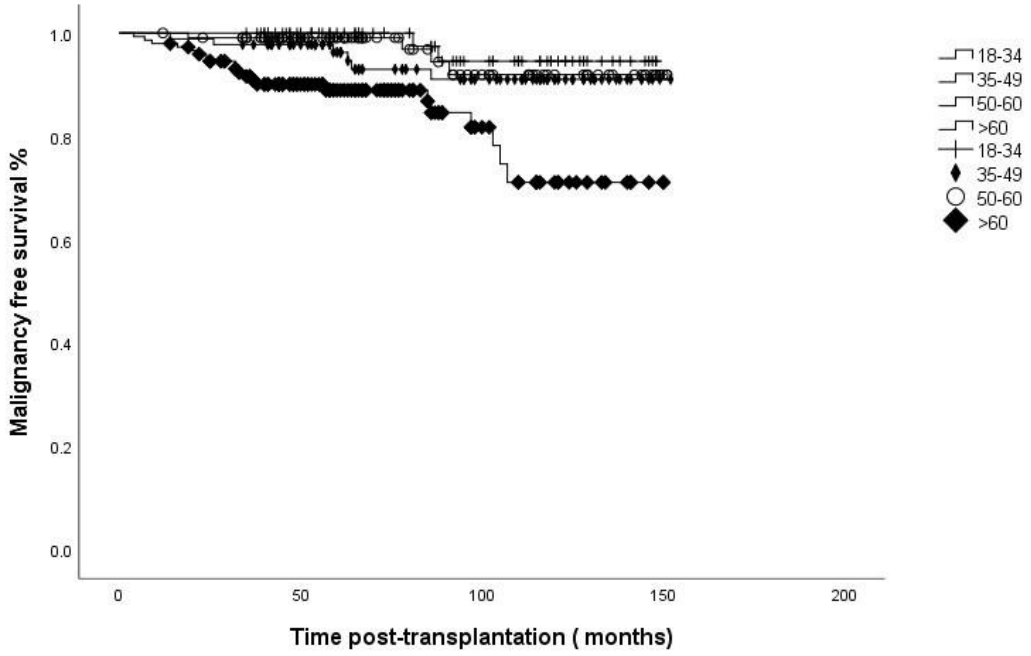


Figure 3.8. Kaplan-Meier curves for death-censored malignancy-free survival after transplantation in recipients age group, 18-34,35-49,50-59, and =>60, ($p<0.001$, log-rank test).

3.4.E. UK transplant centers survey

Seventeen out of twenty-two (77%) UK renal transplant centers responded to our survey. 12 centers reported the use of Basiliximab as an induction agent, while 5 of 17 (29.4%) also used anti-thymocyte globulin, and 7 centers (41.2%) also incorporated alemtuzumab into their IS protocol. Tacrolimus was universally used, but with significant variability in target trough levels across units, ranging from 5–14ng/ml in the first three months, and then 4–9ng/ml thereafter. Mycophenolate mofetil (MMF) was administered at a dose of 1.5g/day by 3 units (17.6%), while 14 (82.4%) centers used 2g/day.

Immunosuppression was modified for older recipients by only 8 (47.1%) centers, with a high degree of variability in the strategy employed, including the avoidance or reduction in dose of an induction agent, avoidance of maintenance steroids, or reducing the dosage of MMF or tacrolimus. Transplant clinicians from 10 (58.8%) responding centers agreed that more robust evidence was required for tailoring IS in the elderly transplant population and that a randomized trial was needed to investigate the outcomes of reduced immunosuppression in this cohort.

3.5. Discussion

More than half of the deaths in older kidney transplant recipients are attributed to infection and malignancies[222]. Older subjects have decreased responses to vaccination and there is increased progression of diseases associated with chronic inflammation and augmented rates of infection[223]. The innate and adaptive immune systems already dysregulated by age are further disrupted by immunosuppressive

medications, contributing to increased morbidity and mortality in kidney transplant recipients. Responses to bacterial infections are impaired in older transplant patients when compared to younger patients with identical regimens of induction and post-transplant immunosuppressive therapy [224]. CMV infection in older kidney transplant recipients is associated with higher mortality and is one of the risk factors for delayed graft function and the requirement for haemodialysis post-transplantation in cadaveric transplant recipients[225] [226] [43]. Here I have demonstrated increased risks of CMV viraemia and malignancies in our older patient population treated with similar immunosuppressive regimens. This supports a more harmful impact of such standard immunosuppressive strategies in older rather than younger subjects.

In this study, I found that younger kidney transplant recipients experienced more rejection episodes in comparison with older kidney recipients. This is in keeping with previously reported data[227] [210]. In part, this may be related to issues with drug compliance, which may be poorer in the younger age cohorts. In addition, there may be the impact from a lower frequency of pre-existing anti-HLA antibodies, both non-donor and donor-specific antibodies in the older recipients. Moreover, de novo class II DSAs are found less frequently in older as compared to younger recipients [228].The regression of B cell kinetics[229]and the innate immune system in the elderly is a possible explanation for this (see Chapter 5). While the presence of class II DSAs is associated with a higher rate of antibody-mediated rejection (AMR) in all transplant recipients[230], the rate of AMR was found to be lower in older recipients[229], reflecting the overall reduced antibody response in older patients.

Lower rates of cRF percentages were similarly found in the UNOS data set in older recipients [231] which is although older patients would have been expected to have been exposed to more sensitizing events such as pregnancy, illnesses, and possibly

blood transfusions during their lifetimes. Most likely, subdued sensitization stems from the ageing immune system although exactly when this occurs is difficult to ascertain due to paucity of available data. However, there was no difference in the development of de novo DSA among the different age groups, with the caveat that, the study included only first kidney transplants, and the matching strategy included finding patients with similar cRF which might explain the lack of difference in development of de novo DSA.

Older patients are also found to have a reduced response to interleukin-2 (IL-2) [64] [232]. It is therefore possible that the use of tacrolimus may be less effective in this patient group and that the exposure to deleterious side effects associated with CNIs are unnecessary. Using a mammalian target of rapamycin inhibitors like everolimus or sirolimus as a substitute for CNIs in older transplant patients is an attractive substitute and studies have shown that there is potential to improve outcomes post-transplantation in older patients [187] [233]. A recent phase III trial was in favour of an everolimus-facilitated tacrolimus minimization regimen of immunosuppression[234]and a study into the pharmacokinetics of this did not show any differences between younger and older kidney transplant recipients [235].The use of Basiliximab induction therapy may also be of less benefit in the older population due to an impeded response to IL-2. What these data suggest, is that as we transplant an increasing number of older patients, age-specific immunosuppression protocols could lead to improved patient and graft outcomes with reduced infections and malignancies without an increase in rejection in the ageing immune system. This study is distinctive in that we sought to match older patients to younger patients based on their HLA mismatch, cRF levels, and CMV serostatus, aiming to reduce bias in alloimmune responsiveness. Our transplant unit

is also unique in that all transplant patients receive the same immunosuppression protocol and pre-emptive CMV therapy is not given regardless of donor or recipient CMV status.

The increased risk of infection and subsequent morbidity and mortality post-kidney transplant in old kidney transplant recipients is likely to be due in part to the burden of immunosuppression. Therefore, especially during the COVID-19 global pandemic, there is an increasing need to establish guidelines on the usage of immunosuppression in older patients. In turn, such guidelines need to be informed by a robust evidence base, in the form of a clinical trial of modified immunosuppression in older recipients.

CHAPTER 4. FRAILTY AND INFECTIONS IN KIDNEY TRANSPLANT RECIPENTS

4.1. Introduction

Despite the lack of a precise definition, frailty is a syndrome of reduced reserve or resilience to conditions or environments that prone individuals to adverse outcomes. Resilience is not only related to physical, physiological, or health factors but also mental [91] [88] [236]Frailty index and physical frailty (Fried tool) are the most used frailty tools in the literature [86]. The Fried tool focuses on the physical features of frailty whilst the frailty index is a more detailed approach that considers a wide range of medical, psychological, and functional factors [87]. The clinical frailty scale which used in this work is among several tools used to measure frailty. The clinical frailty scale was predictive of mortality in ageing Canadian population after 5 years of follow-up [91]. There is evidence that pre-transplant frailty correlates with poorer outcomes post-transplant. Frailty and mortality related to frailty increase with chronological age and the presence of chronic disease [97, 100] [101] [107]. Data showed an increased risk of hospitalization among frail KTR post-transplant [83] [112]. The KDIGO working group suggested referring frail kidney transplant candidates to rehabilitation for optimization before transplantation [85].

4.2. Aims

-To investigate whether frailty before transplantation in the older KTR predicts patient and graft mortality

-To investigate whether frailty before transplantation in the older KTR predict infection-related hospitalization

-To investigate whether frailty before transplantation in the older KTR predicts opportunistic infection, particularly CMV infection

4.3. Brief Methods

This is a retrospective observational work. We investigated 101 kidney transplants performed in our centre between April 2009 and March 2019 in recipients aged > 60 years. They were divided into two groups according to clinical frailty scale before transplantation: Frail KTR who scored ≥ 4 and non-frail KTR scored 1-3. In our unit, clinicians and transplant coordinators are encouraged to document the Clinical Frailty Scale for all potential transplant recipients during the assessment clinic. Recently, the recording of frailty has improved significantly for most patients, although this data collection began before the push to record it for everyone. Recorded outcome measures included incidence of infection-related hospitalization, patient mortality, graft mortality, and occurrence of CMV viremia. Statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA).

4.4. Results

4.4.A. Study participants Frailty scores

Three hundred and sixty-one kidney transplant recipients (KTR) aged 60 years or older who received deceased or living donation kidneys between 2009 and 2021 were identified. One hundred and ninety-one recipients were 60 to 65 years old at

the time of transplant, while one hundred and six were 65- to 70 years old, and only 64 were aged 70 and above at the time of transplant. Ten kidney transplant recipients were excluded due to graft or patient death within the first three months. Only 120 recipients had a documented frailty score at the time of transplantation. Of these 120 recipients, 101 had a documented frailty score within one year before transplant (Figure 4.1). I therefore studied these one hundred and one kidney transplant recipients. The basic characteristics before transplant are mentioned in Table 4.1 and the distribution of frailty scores is shown in Figure 4.2. Most KTRs in my work had a frailty score of 3 (45%) followed by a score of 4 (27%). Recipients were then divided into two groups according to their recorded frailty score, 65 KTR were in the non-frail group with a frailty score of 1-3 while 36 KTR were in the frail group with a frailty score of 4-6.

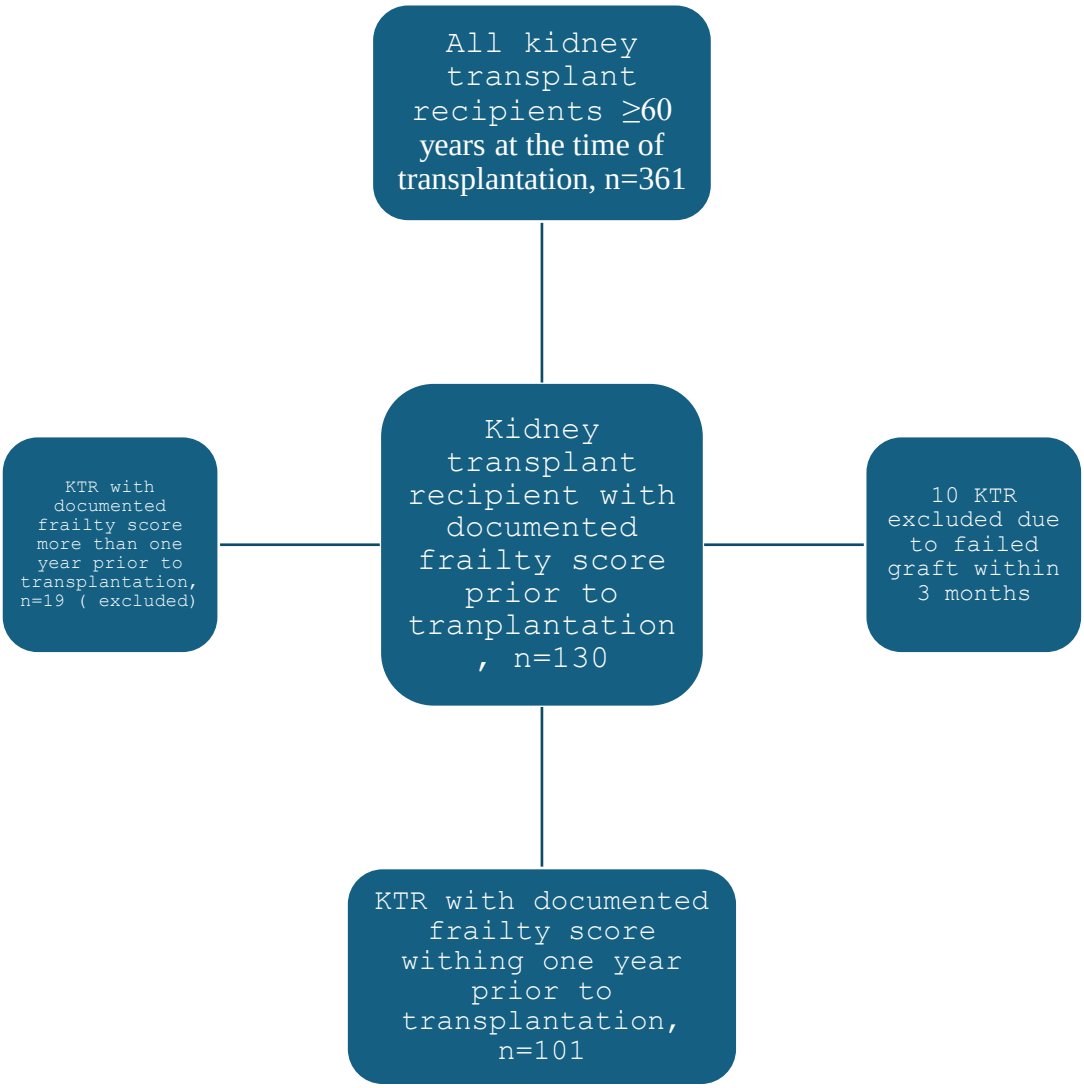


Figure 4.1:Flow chart of patients recruited to the study.

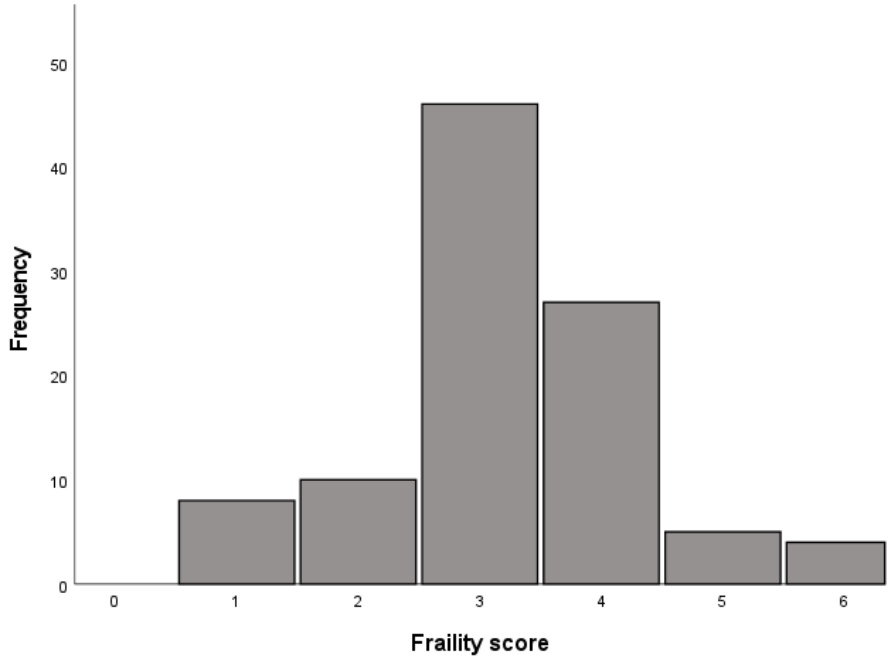


Figure 4.2: Distribution of Frailty scores in the study population.

4.4.B. Characteristics of the study population

4.4.B.A. Recipient details (Table 4.1)

The median age for all study participants was 64 (range 62-68.5) with no significant difference between the two groups. The majority of KTR were male and non-white with no statistical difference between the two groups. There were more diabetic patients in the frail group although again this was not statistically different (55 % vs 40%), p=0.13.

Transplantation from CMV seropositive donors into seronegative recipients (CMV D+ R-) occurred in 17% and 6 % of the non-frail and frail groups, respectively. There

were more participants on dialysis before transplantation in the frail group, (97%) compared to the non-frail group (83%) p =0.03. However, the median time spent on dialysis was not statistically different between the two groups. The frail group had lower serum albumin levels at the time of transplant, p =0.05. All patients received Basiliximab induction therapy. This study included a higher proportion of non-white recipients, with 57% overall and 64% within the frail group.

Table 4.1: Demographic and clinical characteristics of the transplant recipients

Variable	Total N=101	Non frail N=65	Frail N=36	p-Value
Recipient age, years Median (IQR)	64(62-68.5)	66 (62-69)	64 (61.2-67.7)	0.24
Male recipient gender (%)	67(66)	46 (71)	21(58)	0.20
White recipient ethnicity, n (%)	44(43)	31(47)	13 (36)	0.26
DM Cause of ESRD, n (%)	46 (45)	26(40)	20 (55)	0.13
BMI, kg/m2 mean (SD)	26.4(3.7)	26.9 (3.7)	25.9 (3.8)	0.36
HLA-A, -B, and -DR mismatches, n (%) **0-2** **3-4** **5-6**	 28 (28) 54 (53) 19 (19)	 16(25) 37(57) 12(18)	 12(33.5) 17(47) 7(19.5)	 0.59
Donor-recipient CMV IgG serostatus, n (%) **Pos-post** **Pos-neg** **Neg-post** **Neg-neg**	 39 (39) 13 (13) 41 (40) 8 (8)	 22 (34) 11 (17) 27 (41) 5 (8)	 17(47) 2(6) 14(39) 3(8)	 0.32
Recipients previous history of smoking, n (%)	59 (58)	37(57)	22(61)	0.68
Recipients current history of smoking, n (%)	15(15)	7(11)	8(22)	0.12
Dialysis prior to transplantation, n (%)	89 (88)	54(83)	35(97)	**0.03**
Previous transplant (%)	9 (9)	4 (5)	5 (13)	0.19
Positive pre-transplant myocardial perfusion scan (MPS)	22(22)	14 (22)	8 (22)	0.35
Time on dialysis in years, median (IQR)	2.73(1.1, 4.6)	2.99 (1.25, 4.5)	2.4 (1.11, 4.7)	0.38
Albumin, mean (SD)	38.3(5.3)	39 (5.4)	36.9(4.9)	**0.05**

Continuous variables are shown as either mean (SD) or median (IQR) and categorical variables as absolute value (percentage). BMI, body mass index; CMV, cytomegalovirus; DM, diabetes mellitus; HLA, MPS, Myocardial perfusion scan; The Kruskal-Walli's test was used for continuous variables and the Pearson c^2 test was used for categorical variables.

4.4.B.B. Donor details

The donor characteristics are summarised in Table 4.2. The UK National Allocation Scheme uses a computer algorithm to allocate deceased donor kidneys according to a points-based scoring system that prioritizes waiting time, HLA match, and donor-recipient age match[220]. Therefore, older recipients will be offered older donor kidneys and in keeping with this, our study found that older recipients received a kidney from older donors but there was no difference between the two frailty groups (Table 4.2). Most of the donors were male. There were more white ethnicity donors in the non-frail recipient group than in the frail group, 91% vs 83 % respectively, p=0.05 (Table 4.2). Additionally, most of the kidneys were donated after brain death (DBD) with similar proportions of recipients who received kidneys donated after brain death (DBD) among the two age groups. The two groups received a similar percentage of expanded criteria donor ECD. kidneys (Table 4.2). Accepting organs from ECDs is not without risk, however, accepting organs from ECDs does increase organ supply. The ECD definition was designed by the Organ Procurement and Transplantation Network (OPTN) and UNOS in 2002 to help with the best decision-making in organ acceptance. ECDs were defined as organs from brain-dead donors aged ≥60 years old, or 50-59-year-old donors with two of these criteria: history of hypertension, creatinine ≥133 mmol/L, or a cerebrovascular accident as the cause of death [237].

The median follow-up duration was 2.8 and 3.5 years for the non-frail and frail, groups respectively (p=0.45) (Table 4.2).

Table 4.2: Demographic and clinical characteristics of transplant donors

Variable	Total N=101	Non frail N=65	Frail N=36	p-Value
Donor age, years median (IQR)	58 (48-67)	58 (49-67)	57.5 (45-67)	0.94
Male donor gender, n (%)	56 (55)	35 (54)	21(58)	0.66
Donor ethnicity, n (%) White	89(88)	59 (91)	30 (83)	0.05
Donor status, n (%) **LD** **DBD** **DCD**	 16 (16) 58 (57) 27 (27)	 11 (17) 34 (52) 20 (31)	 5 (14) 24 (67) 7 (19)	0.35
ECD, n (%)	52 (51)	33 (51)	19 (53)	0.84
CIT, hrs Median (IQR)	9.8(7.3-13.6)	9.5 (7.3-13.4)	9.9 (7.9-14)	0.94
Anastomosis time (min) Median (IQR)	35(30-41)	34 (30-39.2)	36(31-42)	0.18
Follow up time in years, median (IQR)	3 (2-4.3)	2.85 (1.99-4.11)	3.5 (2-4.6)	0.36

Continuous variables are shown as mean (SD) and categorical variables as absolute value (percentage). CIT, cold ischemic time; DBD, donation after brain death; DCD, donation after circulatory death; KDRI, Kidney Donor Risk Index; LD, live-donor. * Pearson Chi-Square Kruskal-Wallis

4.4.C. Infectious complications post-transplantation

4.4.C.A. CMV infection

In this cohort of elderly KTR, 41% developed at least one episode of CMV viremia in the first six months after transplantation (Table 4.3). This result is consistent with our previous results in Chapter 3. However, when analysing CMV infection according to frailty, there was a higher percentage of CMV infection in the frail group (55%) compared with the non-frail group (34%), P value =0.03 (Table 4.3). The proportion of patients without CMV viremia within the first 6 months after transplantation was 66 % and 44 % in the non-frail and frail groups respectively, (p=0.02, log-rank test) (Figure 4.3). Using univariate analysis, frail recipients demonstrated a more than

two-fold increased risk of CMV viraemia (HR=1.97; 95% CI= 1.07-3.62) p=0.02 (Table 4.4).

When using multivariate analysis to understand confounding factors associated with frailty and risk of CMV infection, two models were run including the following variables.

The first included the Recipient's male gender, age, and white ethnicity, the Donor's age, male gender, and white ethnicity, presence of DM, current and previous smoking, the recipient's BMI, and dialysis before transplant.

The second included all the above and CMV serostatus, immune mismatch, and cold ischaemic time. In both models, frailty was associated with a more than twofold increased risk of developing CMV viremia (2.07(CL 1.04-4.11) p=0.03 and 2.15 (1.01-4.55) p=0.04 for models 1 and 2 respectively).

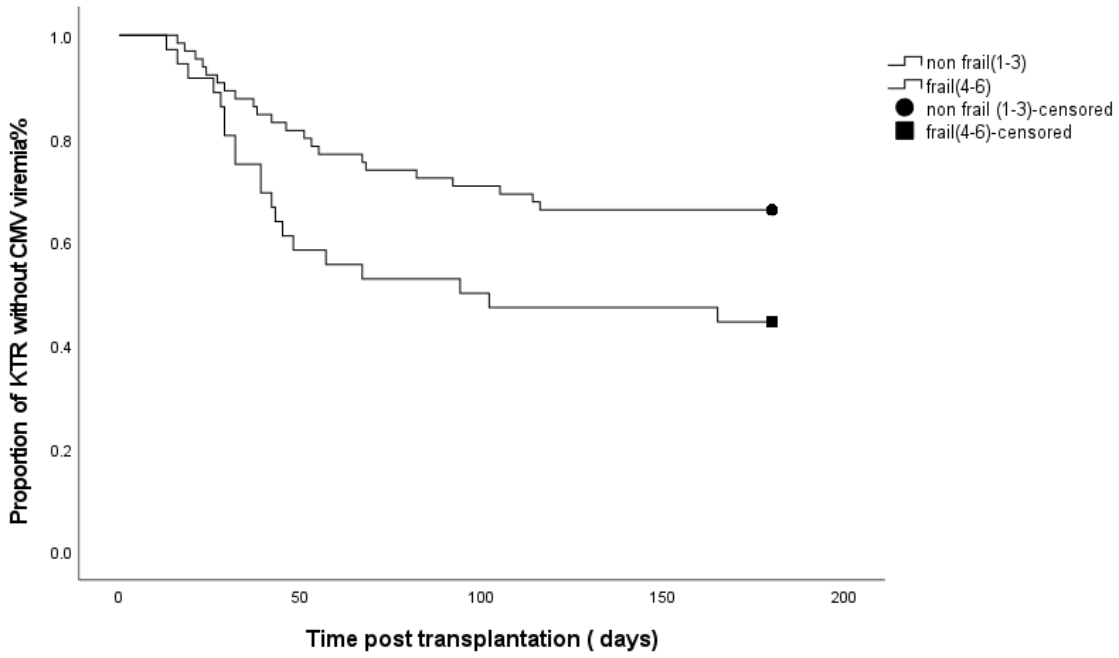


Figure 4.3: Kaplan-Meier curves for proportion of patients without CMV viraemia within the first 6 months after transplantation in frail (44 %) and non-frail (66%) KTR, (p<0.02, log-rank test).

Table 4.3: Comparison of transplant outcomes according to frailty status

Variable	Total N=101	Non frail N=65	Frail N=36	p-Value
Last GFR (ml/min), median (IQR)	51 (35.5-61.5)	50.5(34-59.7)	52.5(35.7-66.5)	0.36
Infection related readmission with 30 days post transplantation, n (%)	60(59)	41 (63)	19 (52)	0.3
Infection related readmission with 12 months post transplantation, n (%)	36(36)	19 (29)	17 (47)	0.07
No Infection related readmission with 30 days post transplantation, mean (SEM)	0.29 (0.05)	0.26(0.05)	0.36(0.1)	0.23
No Infection related readmission with 12 months post transplantation, mean (SEM)	1.56 (1.76)	1.26(0.16)	2.11(0.37)	0.01
Any malignancy (%)	**5 (5)**	3 (4)	2 (5)	0.82
BK viremia/nephropathy, n (%)	20 (20)	13(20)	7 (19)	0.94
CMV viremia within 6 months post-transplant, n (%)	42 (41)	22 (34)	20 (55)	0.03
DGF, n (%)	7 (7)	4 (6)	3 (8)	0.68
BPAR, n (%)	5(5)	3(4)	2(5)	0.83
KTR death	10 (10)	7 (11)	3 (8)	0.69
Follow up time in years, median (IQR)	3 (2-4.3)	2.85 (1.99-4.11)	3.5 (2-4.6)	0.45
Days of admission post-transplant, mean (SEM)	9.37 (0.82)	8.37(0.48)	11.19(2.13)	0.66

BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; DGF; delayed graft function, DSA, donor-specific antibody.
The Kruskal-Walli's test was used for continuous variables and the Pearson c^2 test was used for categorical variables.

Table 4.4: Multivariable Cox regression model for different patient outcomes.

Recipient outcome per frailty group	Univariate HR (95% CI)	Multivariable HR (95% CI) *	Multivariable HR (95% CI) **
CMV viremia within the first 6 months post-transplant Frailty score	1.46(1.09-1.94)0.009	1.44(1.03-2.01)0.03	1.44(1.01-2.05)0.04
CMV viremia within the first 6 months post-transplant Non frail frail	Reference 1.97(CI 1.07-3.62) p=0.02	Reference 2.07(CL 1.04-4.11) p=0.03	Reference 2.15 (1.01-4.55) p=0.04
Rejection Non frail frail	Reference 1.18(0.19-7.06) p=0.85	Reference 0.92(0.12-6.73) p=0.93	Reference 1.04(0.08-12.2) p=0.97
Graft failure Non frail frail	Reference 6.39(1.32-30.8) p=0.02	Reference 7.41(1.3-42) p=0.02	Reference 9.2 (1.07-79.3) p=0.04
KTR survival censored to graft loss Non frail frail	Reference 0.67(0.17-2.6), P=0.56	Reference 0.22 (0.02-2.48) p=0.22	Reference 0.01(0-3.5) p=0.13

*Recipient male gender, age and white ethnicity, Donor age, male gender and white ethnicity, presence of DM, current and previous smoking, recipient BMI and dialysis prior to transplant.
** Recipient male gender, R age and white ethnicity, Donor age, male gender and white ethnicity, presence of DM, current and previous smoking, recipient BMI, and dialysis prior to transplant, CIT, total HLA MM group, graft type donor-recipient CMV status.

4.4.C.B. Predictor of CMV infection

I tested if frailty pre-transplant predicts CMV viremia post-transplant. I adjusted for the predictors of CMV reported in the previous study in our Logistic regression model (Table 4.5 and Figure 4.4): recipient gender, kidney type, primary renal function, CMV donor status, and total mismatch [238].

The risk of developing CMV does increase with the recipient's age. Logistic regression shows the risk of developing CMV during the first 6 months was higher in KTR from CMV-positive donors as shown in Table 6 which is consistent with what is described in the literature[238]. Logistic regression also showed that frailty impacts

risk for CMV, as the frail group had an almost 3-fold higher risk of developing CMV than the non-frail group (OR=2.99, 95 % CI=1.03-8.66, P value=0.04).

Table 4.5: Results of logistic regression analysis of developing CMV post transplantation.

Variable	Coeff(B)	S.E	Odds Ratio (Exp (B))	95% CL Upper lower	P-value
Frailty Frail group	1.09	0.54	2.99	1.03-8.66	0.04
KTR age	0.11	0.05	1.12	1.01-1.24	0.03
KTR gender Male	0.33	0.53	1.39	0.48-4.02	0.53
KTR ethnicity White	-1.04	0.62	0.095	0.10-1.20	0.09
Donor age	0.02	0.02	1.02	0.98-1.07	0.22
Donor ethnicity White	-0.88	1.06	0.41	0.05-3.32	0.4
Total HLA	-0.01	0.17	0.98	0.7-1.38	0.95
CMV positive donor	1.45	0.52	4.28	1.51-12.09	0.006
R. DM	-0.69	0.61	0.5	0.15-1.67	0.26
LRD	Ref				
DBD	1.43	0.94	4.18	0.65-26.7	0.13
DCD	1.8	1.01	6.09	0.84-44	0.07

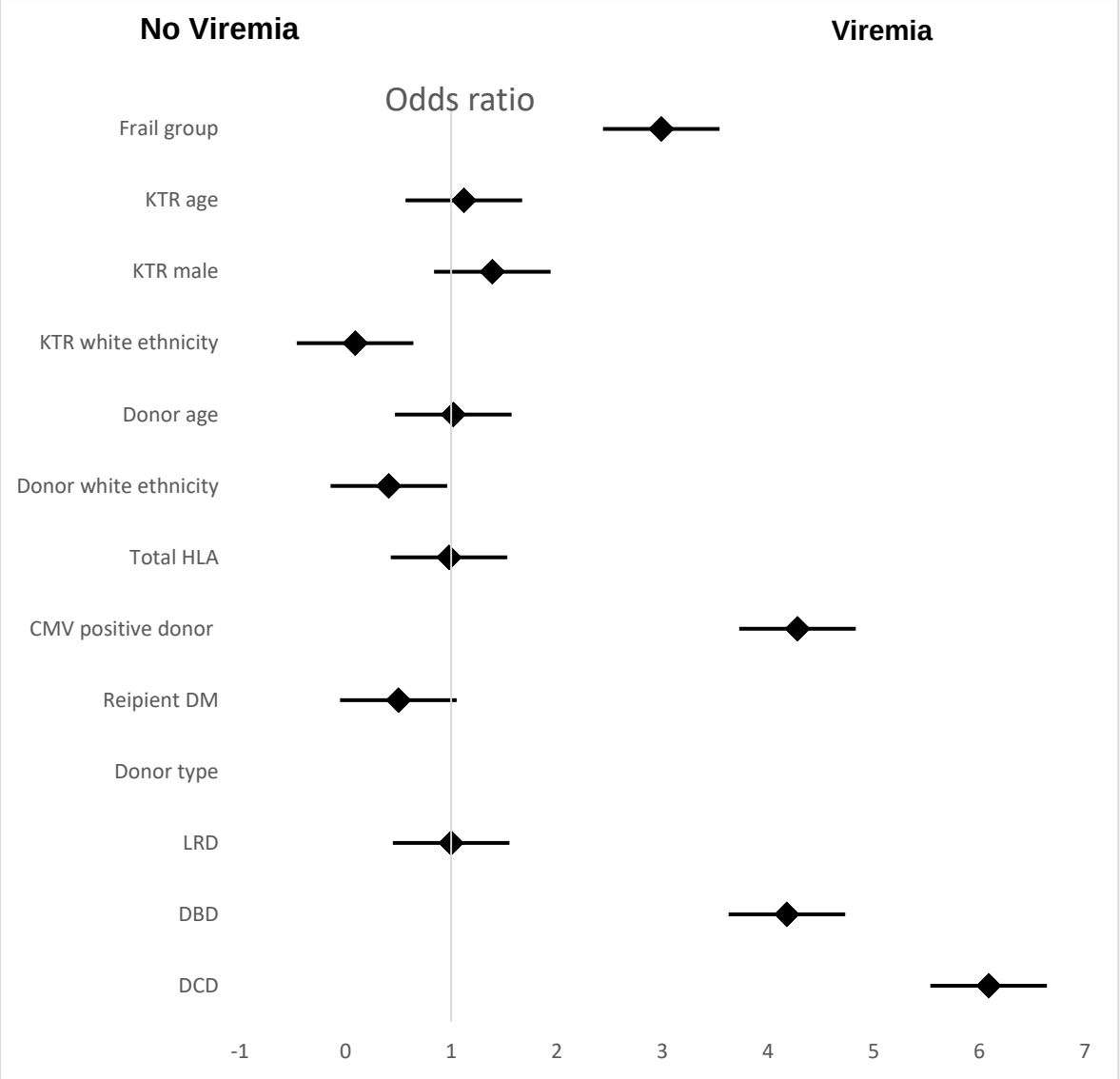


Figure 4.4: Independent predictors of developing CMV viremia by Multivariate analysis.

4.4.D. BK virus (BKV) infection

Since BKV and CMV infections do not always, or indeed often, occur in the same patients, I also analysed the incidence of BKV infection concerning frailty in this cohort.

The rates of BKV infection at any time post kidney transplantation among the frail and non-frail groups were not significantly different (20 % and 19% respectively) (Table 4.3), but these were not always tested at standard times or consistently across the groups, making a time-dependent analysis more difficult. Additionally, the means of testing for BKV infection varied over the time course of the study. Finding urine BK virus, or decoy cells, used to be the diagnostic method until 2018 when it was replaced by BKV PCR on whole blood. Therefore, accurate rates of assessment of BKV infection among our cohort were not possible.

4.4.E. Infection related Hospital Readmission rates and frailty

The overall percentage of KTR requiring readmission to the hospital due to infections was higher, but not statistically different, in the frail group compared with the non-frail group in the first 12 months (47% vs 29 % respectively, p=0.07) Similarly, the infection-related admissions were not significantly higher in the non-frail compared to frail groups in the first 30 days post-transplantation (63 % vs 52% respectively, P=0.3) (Figure 4.5). The mean length of hospital stay for the transplant operation admission for the frail group was 11 days and for the non-frail group was 8.37 (Table 4.2).

However, the mean number of infection-related admissions was higher in the frail vs non-frail group (2.11 vs 1.26 respectively, p=0.01 for the first-year post-transplantation and 0.37 vs 0.26 respectively, p=0.23 for the first-month post-transplantation) (Figure 4.6).

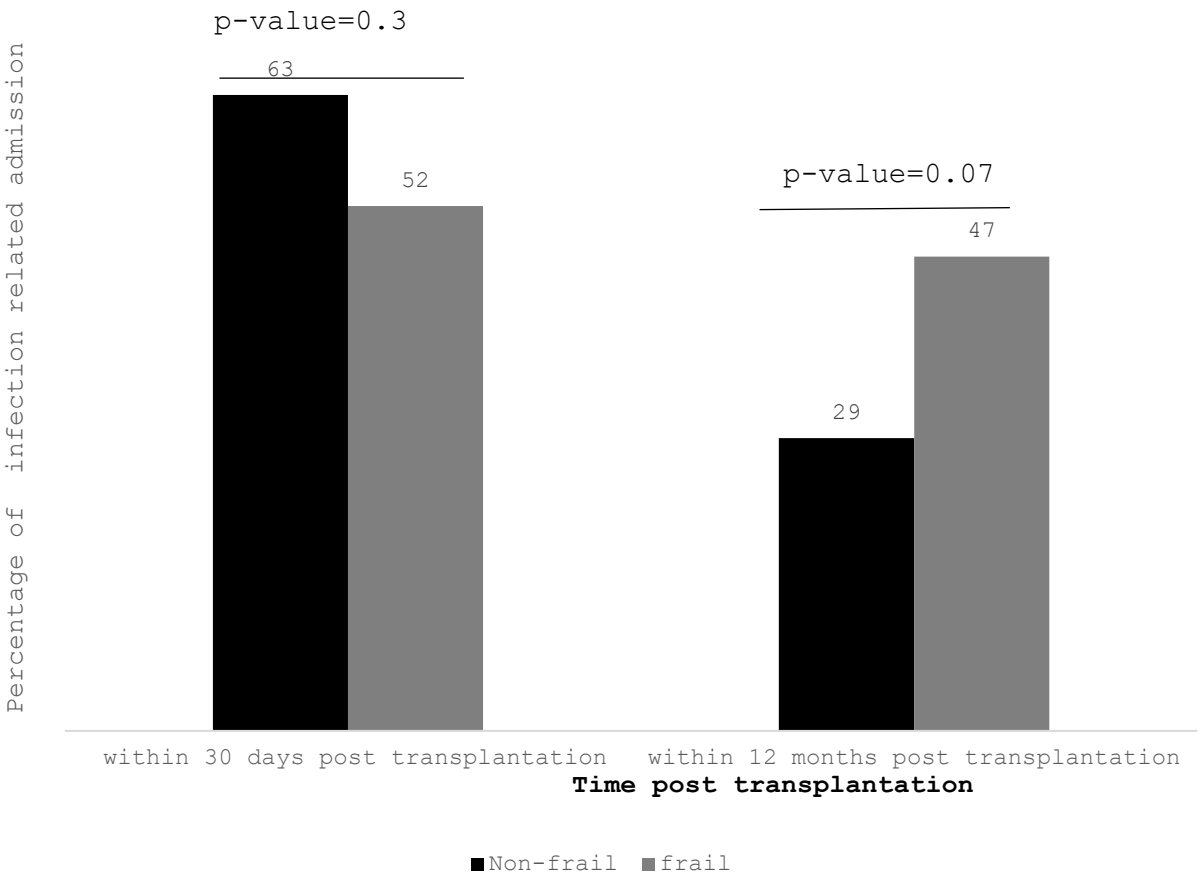


Figure 4.5: Percentage of infection related admissions in the first 30 days and 12 months post transplantation in the frail and non-frail groups.

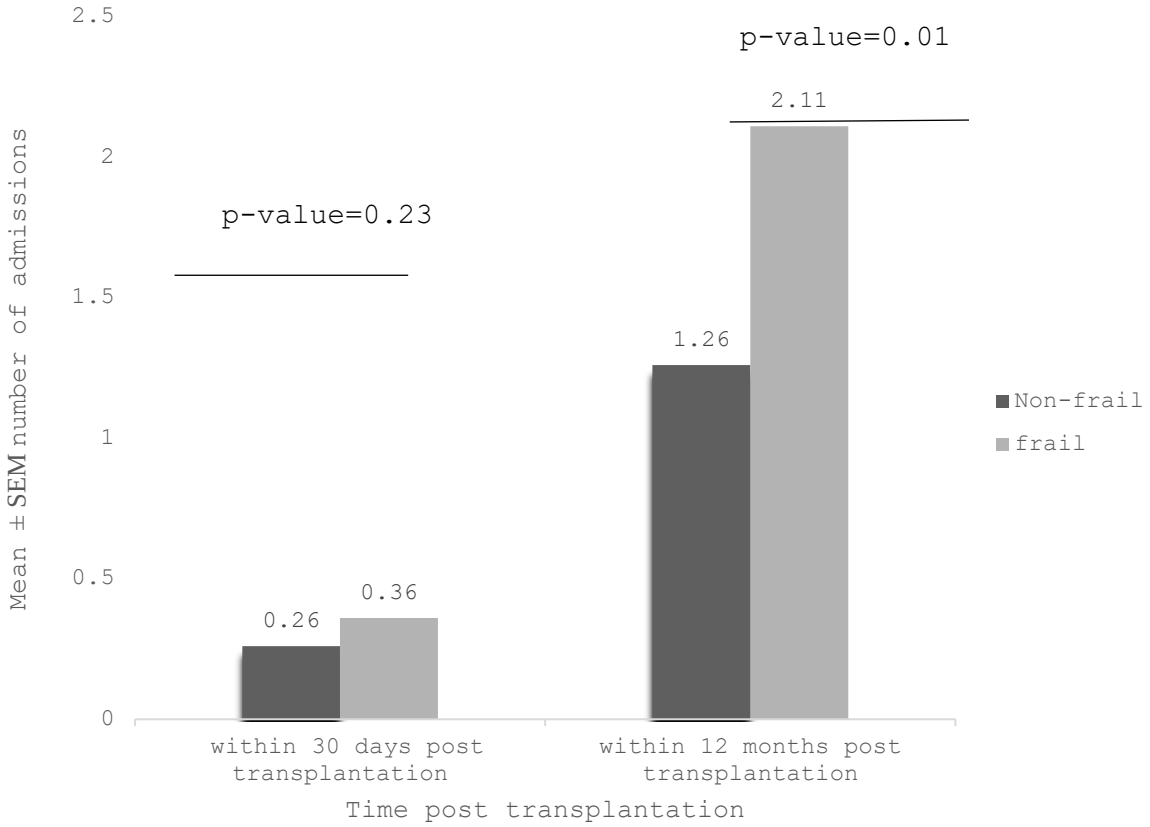


Figure 4.6: Mean number of infection related admissions in the first 30 days and 12 months post transplantation in the frail and non-frail groups.

In our cohort, the overall incidence of infection requiring hospital admission (a marker of severity) was 36 %. However, the incidence of infections in the frail group was higher than non-frail (55% vs 34%, p=0.07).

4.4.F. Patient and graft outcomes

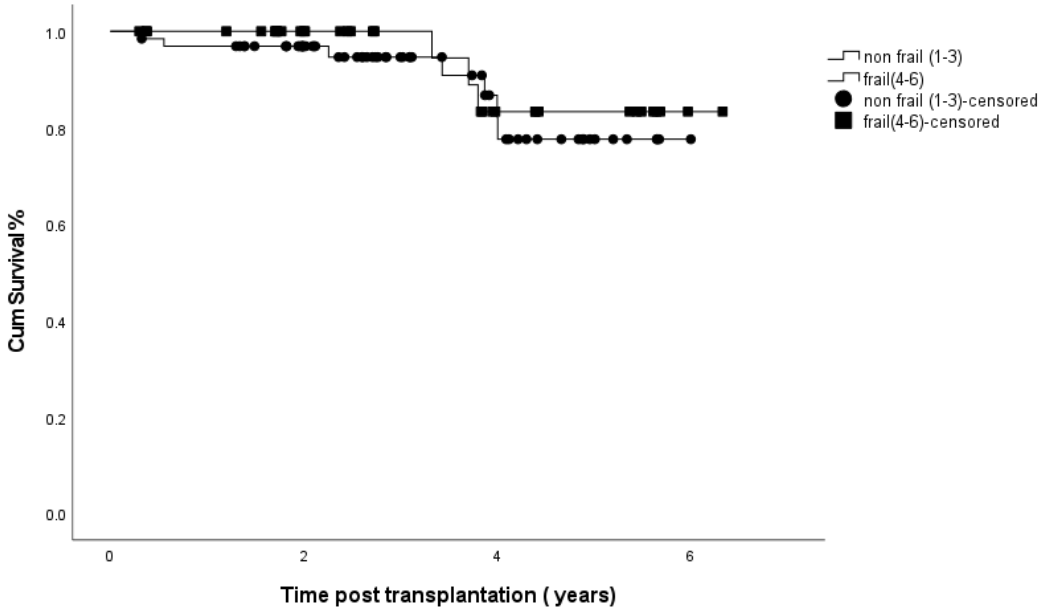
Overall, there were 10 deaths (10% of the total cohort) with 11% and 8% in the non-frail and frail groups respectively, all deaths were with a functioning graft. There was

no statistically significant difference in the mortality between the two groups, the survival rate in the non-frail cohort was 89.2% compared with the frail group was 91.7%, log Rank p =0.60 (Figure 4.7a). Survival was higher among the 60-65 age group in comparison with the≥ 65 age group, Log Rank (Mantle cox) p =0.01. (Figure 4.7b).

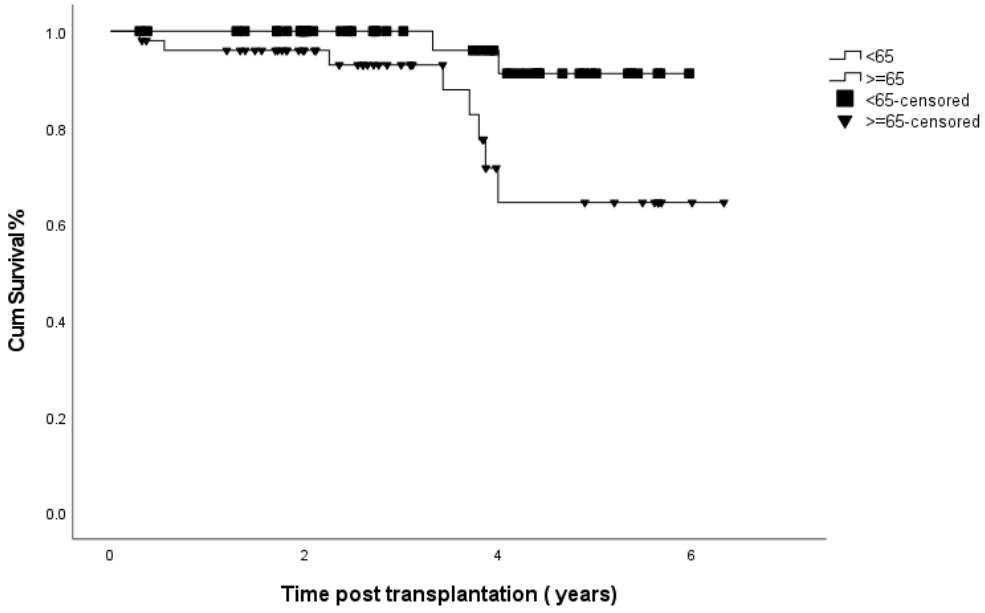
Univariate analysis showed that KTR survival was not significantly different between the frail and non-frail groups (taken as reference) (Table 4.4). These hazards changed little with multivariate analysis controlling for potentially confounding recipient and donor factors but were not statistically significant.

No deaths were reported in KTRs scoring 1,2, 5 and 6. There were 6 deaths in KTRs scoring 3 and 3 deaths in KTRs scoring 4.

(a)



(b)



Age group	Survival rate	Log Rank (Mantle cox)
<65 (n=51)	95.9%	
≥65(n=50)	84.3%	0.014

Figure 4.7:(a) Kaplan Meier: Overall survival rate in frail and non-frail KTR, (b)Kaplan Meier: survival rate of 60-65 age group vs≥65 age group.

Pearson correlation of frailty score before transplantation and mortality post-transplant was found to be weakly positive and statistically non-significant (r=0.02, p=0.82).

4.4.G. Graft survival

During follow-up, the frail KTR experienced almost triple the proportion of graft losses compared to the non-frail recipients. Graft survival at the end of follow-up was 96.9% and 80.6%, for non-frail and frail KTR groups respectively, with Log Rank (Mantle cox) = 0.008(Figure 4.8). Univariate analysis demonstrated a significant impact of frailty on graft survival, with an HR of 6.39 (1.32-30.8) p=0.02 for the frail cohort (Table 4.4). However, this association became even more significant when additional recipient and donor factors were considered in the multivariate model, including recipient gender, ethnicity, diabetes, BMI, smoking history, and dialysis before transplantation as well as donor age, gender, and ethnicity (Table 4.4), reaching a HR of 9.2 (1.07-79.3) p=0.04. The cause of graft failure was not documented.

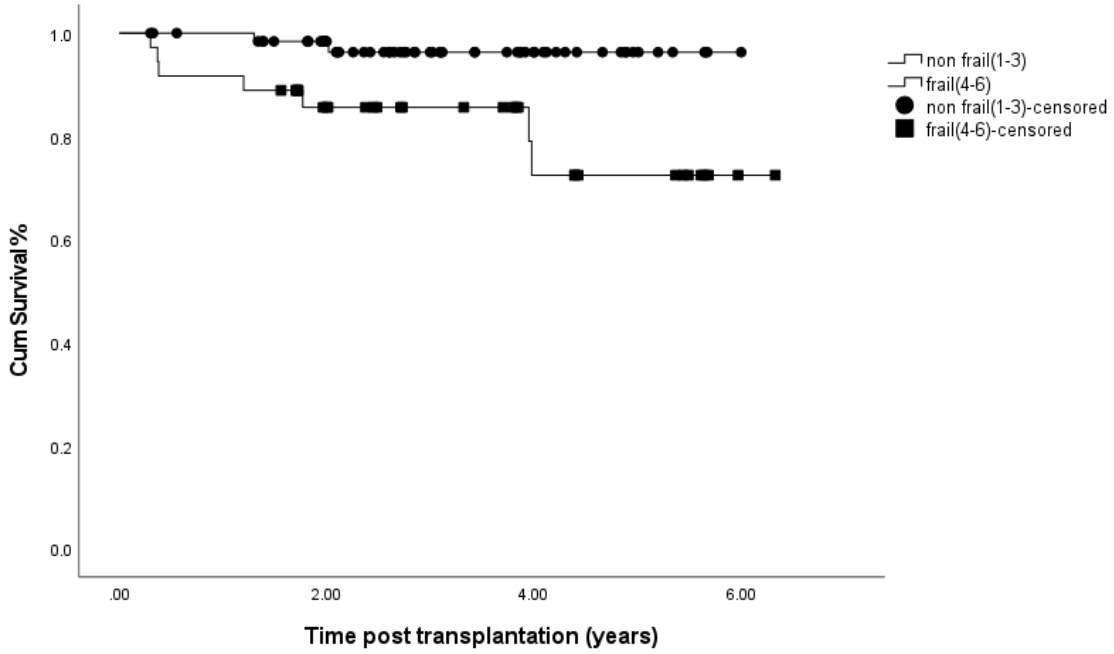


Figure 4.8: Kaplan Meier survival graphs showing overall graft survival rates in frail and non-frail KTR.

4.4.H. Other outcomes

DGF was observed similarly in 6% and 8% in the non-frail and frail groups, respectively. Of the KTR graft survivals, GFR at the end of follow-up was not different among the two groups, the estimated median GFR at the end of follow-up was 52.5 ml/min in the frail group compared to 50.3 ml/min in the non-frail group. (Table 4.3).

4.5. Discussion

These data show higher mortality among older KTR, (> 65 years old compared with 60–65 years old). This finding is consistent with previous reports. In elderly

recipients, aged 60 to 74 years, the cumulative survival rate increases steadily with age with an estimated 4.3-year excess improvement in the life span in the 60-64 age group, 2.8 years for the 65 to 69 age group, and 1 year for 70 to 74 age group [5]. The Clinical Frailty Scale score predicted mortality in a cohort of 10,263 Canadians aged 65 and older, and these findings were correlated with the frailty index tool. [91]. The clinical Frailty scale was used to assess frailty in the pre-dialysis cohort, and it was an independent predictor in 283 pre-dialysis patients average age of 70 years[239]. Frailty measures by Fried phenotype were associated with mortality post-transplantation especially if accompanied by depressive symptoms in 773 KT candidates from multiple centres in the US [240].

Frailty did predict mortality in HIV (human immunodeficiency virus) positive patients who received liver transplants[241], however, in this study, frailty did not predict mortality post-transplantation. There are a few potential explanations for this finding. Firstly, the two groups, frail and non-frail, were all over 60 years old with similar co-morbidities, including cardiac history, DM, smoking, and BMI. In addition, we had a short follow-up time. Secondly, in this work, the majority of KTRs scored 3 or 4, and death was only reported in these two scores. This might contribute to the lack of a significant difference in mortality. Thirdly, a change in frailty post-transplantation could have impacted on the survival outcome. Improvement in the frailty scores three months post kidney transplantation, despite an initial deterioration was reported in 349 kidney transplant recipients with a mean age of 53.3 ± 14.2. In this study, the frailty was assessed by the Fried tool. Interestingly, of those who reported improvement in frailty, hand grip improved in 47%, weight loss in 15%, 55% physical activity, 25% exhaustion, and 19% in walk speed[242]. The potential improvement in frailty post-transplantation could subsequently contribute to the survival benefit in the

frail group to reach the non-frail group survival rates. Finally, in this work, I included KTRs who had recorded frailty scores anytime within 1 year of transplantation which is potentially subject to change. Between transplant assessment to KT (median time 1.1 year), 22% become frailer and 24 % become less frail in a study of 569 KTR with an average age of 51.7 years assessed by Fried frailty. Worsening frailty was associated with a 2-fold increase in mortality irrespective of other factors[243]. Poor hand grip and low physical activity (from Fried phenotype) were reported in frail kidney transplant candidates and associated with a more than 2-fold increased risk of mortality post transplantation[244].

Frailty assessment, in term of gait speed, did predict respiratory complications post liver transplantation in one study[245]. Increased length of stay post-transplantation was associated with increased mortality in frail and non-frail recipients [246].

To date, Frailty scale scores have not been reported in mortality outcome predictions for kidney transplant recipients. Initial data of the ongoing KTOP study demonstrated a prevalence of frailty of 15.8% measured by the Edmonton Frail Scale (EFS) in the patients on the waiting list while 20.1% were vulnerable (or moderately frail). The outcome of this trial is awaited, and it will be interesting to know what the impact of frailty on post-transplantation outcomes will be in addition to any dynamic changes in frailty post-transplantation [247]. The immune response to the common bacterial antigen Lipopolysaccharides (LPS) was studied in frail and non-frail old adults (>74 years). The stimulation of PBMC by LPS was suppressed in frail old adults in comparison with age-matched non-frail subjects. These stimulated PBMC produced higher levels of IL6 in the frail than non-frail subjects. This supports the link between inflammatory change and immunosenescence in the old frail individuals which contribute to the subsequent increased risk of infection in frail old adults [248].

In this study, I demonstrated this link between increased infection in frail old adults and showed higher risk of early CMV infection in frail kidney transplant recipients. In another study, CMV infection was associated with prevalent frailty and increased IL6 production. This suggests a relationship between CMV infection and inflammatory states that may contribute to frailty [121].

The incidence of opportunistic infections like CMV, which is the leading cause of opportunistic infection post-kidney transplantation, is 10 times higher in the first-year post-transplant compared to after the first year [249]. The most significant risk factors for developing opportunistic infections in KTR are CMV serostatus, recipient age, donor age, and class II HLA mismatch in addition to induction therapy with depleting agents [249].

The cumulative incidence of infection post-transplant in a large US database between 1999 and 2014 at one year was 53.7%[250]. In our cohort, the overall incidence of serious infections requiring hospitalization was greater in the frail patients compared with the non-frail ones.

The infection-related admissions in the first months were similar among the frail and non-frail. The mean number of infection-related admissions was significantly higher in the frail group in the first-year post-transplant. This finding is consistent with previous reports. Rehospitalization post-kidney transplant is higher in frail kidney transplant recipients [83]. However, the higher mean number of admissions within 30 days did not achieve statistically significant. Frail KTR did spend less time following discharge at home in the first 30 days post-transplantation to see a significant difference in the readmission rate within the first 30 days.

Risk factors for developing infections post-transplant were identified in many studies, however, to my knowledge patient frailty was never tested as a risk factor. In this study, I have identified that frail kidney transplant recipients have a higher risk of developing infection.

Multiple studies have been conducted to find an explanation for donor-specific hypo responsiveness, which may explain the reduced risk of rejection in the long-term KTR. Continuous antigenic exposure to the donor's kidney can cause exhaustion of donor-reactive cells. The changes in these T cells were marked in elderly recipients which is consistent with the evidence of a reduction in rejection rates in the elderly transplant recipients [251] [252]. In this study, the rejection rate was not different among the frail and non-frail recipients. However, the overall graft failure rate was significantly higher in the frail recipients. One potential explanation for the lack of difference in rejection rates could be related to the small number of rejection episodes in this small cohort. Additionally, in our unit, we do not perform protocol biopsies which could lessen the rate of subclinical rejection which may subsequently contribute to graft failure. A recent study demonstrated a higher risk of graft failure in the borderline rejection group [32]. The study did not record the cause of graft failure; however, factors such as CMV infection and multiple hospitalizations may contribute to the observed differences in graft failure rates between frail and non-frail patients, despite no differences in rejection or delayed graft function. Additionally, it's important to note that there were more non-white recipients in the frail group, which raises concerns about potential profiling of certain ethnicities. Consequently, these findings will be shared with the department for service improvement initiatives.

4.6. Conclusions

The high rates of CMV infection and low rates of rejection in frail KTRs are likely to be multifactorial and include the burden of immunosuppressive therapy. Infection post-transplant and hospitalization subsequently increases the risk of morbidity. Therefore, finding clinical predictors for infections could potentially be used as a target for optimization. Frailty scores before kidney transplantation predict CMV infection and infection-related admissions in elderly (>60) KTR in this study. Therefore, using frailty scoring as a risk assessment tool before kidney transplantation could potentially reduce adverse outcomes like infection and re-admissions. This could be achieved by optimizing frail KTR before transplantation. The KDIGO working group suggested referring frail kidney transplant candidates to rehabilitation for optimization before transplantation [85]. There is no available evidence for this strategy, however, prehabilitation in 18 kidney transplant candidates in a pilot study demonstrated improvement in physical activities and patient satisfaction. Furthermore, the length of hospital stays after transplantation in 5/18 who received kidney transplants during the study period was almost halved[253]. I think each unit should have more thorough investigations of their cohorts and the most contributing factors that could be optimized. For example, targeting weight loss and malnourishment in dialysis cohorts and aiming for normal serum albumin is one of my proposed strategies for optimization.

On the other hand, a more detailed frailty assessment may be required to accurately predict these risks.

Additionally, CMV prophylaxis and early reduction of immunosuppressive therapy in the frail group might lead to fewer infections and hospital admissions.

CHAPTER 5.B CELL SUBSETS AND THEIR REGULATORY ROLE IN OLDER KIDNEY RECEIPENTS

5.1. Introduction

Standard clinical practice for post-transplantation IS therapy has not fundamentally changed since the late 1990s when the highly successful combination of tacrolimus and mycophenolate was introduced for the prevention of acute rejection [183] [184] [185] [186]. Although the transplant community has focused on the early withdrawal of steroid therapy and the introduction of inhibitors of the mammalian target of rapamycin, this has not translated into graft survival benefits [187]. As a result, this leaves an evidence gap concerning the optimal IS regimen, especially among elderly renal transplant recipients who are more susceptible to post-transplant infections such as CMV and malignancies. As highlighted in Chapter 1, older kidney allograft recipients may not require the same level of IS as given per standard to younger patients, but caution needs to be exercised to ensure that IS for elderly kidney allograft recipients is sufficient to avoid the risk of rejection, as acute rejection(AR) in older transplant recipients can be more severe than in younger recipients especially for those recipients with older donors with poorer kidney reserve[189] [188] and deaths related to AR are more common in older adults [190]. Therefore, biomarkers informing of immune activity are crucial to risk stratifying KTR and provide a personalized medicine service to avoid undesired outcomes. Previous works had

shown a strong correlation between levels of regulatory B cells, producing low levels of IL-10/TNF-α ratio, at 3 months post-transplant, and with acute rejection, graft survival, and development of DSA [154] [181]. However, the impact of recipient age or frailty on these cell populations was not explored.

5.2. Aims

- To investigate whether transitional B cell IL-10/TNF-α ratio differs between the young and older KTR post-transplantation.
- To investigate whether frailty associates with transitional B cell IL-10/TNF-α ratio post-transplantation.
- To investigate whether transitional B cells IL-10/TNF-α ratio impacts CMV infection

5.3. Brief methods

PBMCs were isolated from 23 KTR three months post-transplantation and recruited randomly from the Nephrology Outpatient Department of the Royal Free Hospital. PBMCs were cultured with stimuli for 72 hours. The stimuli contained CpG ODN and CD40L. In the last 5 hours of the culture period, the supernatant was replaced with a mixture of PMA, Ionomycin, and Brefeldin, to optimally detect intracellular cytokines. The cells were then stained for surface markers CD19, CD24, CD38, IgD, and CD27. Then the cells were fixed and stained for intracellular cytokines IL-10 and TNF-α.

The expression of IL-10 and TNF-α was assessed in B cell subsets using FACS, and FlowJo software.

The patients' characteristics and outcomes were obtained from the electronic patients 'records. This cohort was divided into two groups according to age, <60 and > 60 years, in keeping with earlier work on outcomes related to age. IL-10/TNF ratios from B cell subsets were compared between the two different age groups as well as according to frailty and CMV infection.

5.4. Results

5.4.A. Baseline characteristics of participants

Recently, twenty-three KTRs were recruited from the Royal Free Hospital outpatient acute clinic, and peripheral blood was taken at three months post-transplant. The median age of the study participants was 57 years; 10 patients were ≥60 years old and 13 patients were younger than 60 years at the time of transplantation. The characteristics of both groups are summarized in Table 5.1. Major differences between the recipient groups apart from age, were the presence of diabetes, with 60 % of the older KTR group being diabetic and none of the young KTR and transplantation from CMV seropositive donors into seronegative recipients (CMV D+ R-) which occurred in 9 % of the whole cohort with 15.5 % of the younger cohort and none of the older cohort. All patients received Basiliximab induction therapy. The distribution of the study cohort according to age and frailty scale before transplantation is shown in Figure 5.1 and Figure 5.2, respectively. There were 11 KTR with frailty scores of 1 or 2, 6 with a frailty score of 3, 2 with a frailty score of 4, 1 with a frailty score of 7, and 3 with no documented frailty scores before

transplantation.

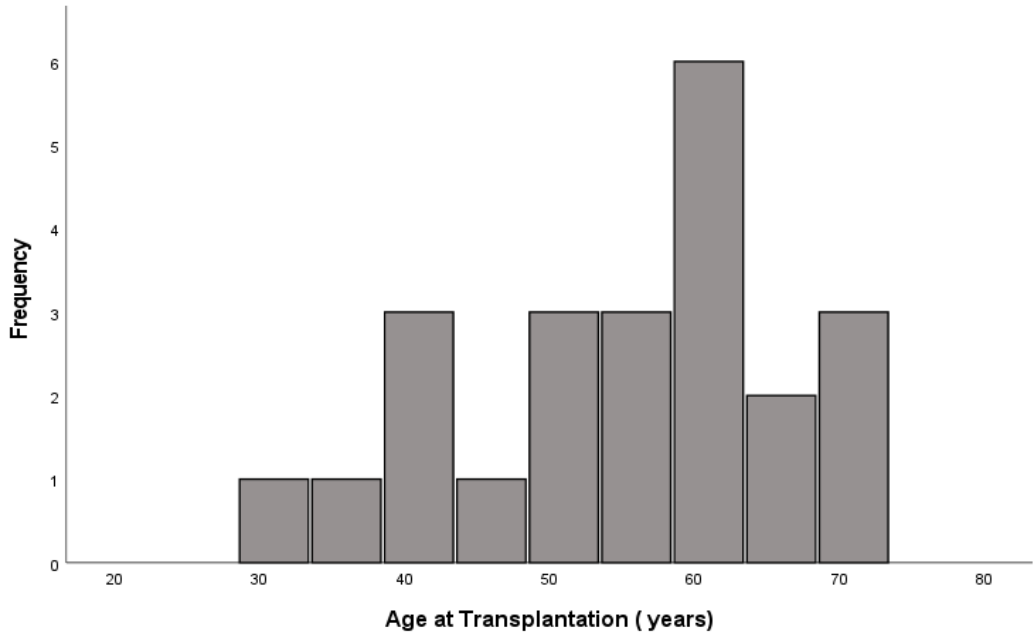


Figure 5.1: Distribution of age in the study population

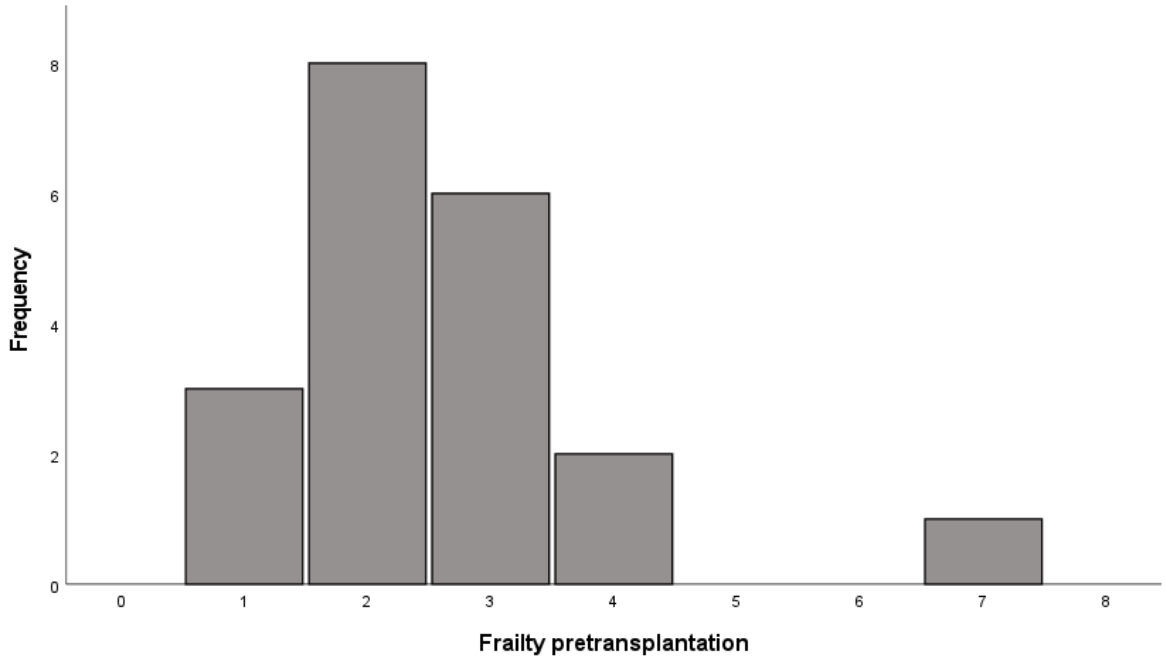


Figure 5.2: Distribution of Frailty scores in the study population

Table 5.1: Demographic and clinical characteristics of transplant recipients

Variable	Total (n=23)	Younger N=13	Older N=10	p-Value
Recipient age, years Median (IQR)	57 (45-63)	52(41-55)	63(63.5-70)	**<0.001**
Male recipient gender (%)	18 (78)	11 (84)	7 (70)	0.4
Recipient ethnicity, n (%) White	12 (52)	8 (61)	4 (40)	0.55
DM Cause of ESRD, n (%)	6 (26)	0 (0)	6 (60)	**0.001**
BMI, kg/m2 mean (SD)	25 (23-29)	26.6(23-29)	24 (22.6-28,7)	0.13
HLA-A, -B, and -DR mismatches, n (%) **0-2** **3-4** **5-6**	 7 (30) 11 (48) 5 (22)	 4 (31) 7 (54) 2 (15)	 3 (30) 4 (40) 3 (30)	0.67
Donor-recipient CMV IgG serostatus, n (%) **Pos-post** **Pos-neg** **Neg-post** **Neg-neg**	 9 (39) 2 (9) 8(35) 4(17)	 5 (38.5) 2 (15.5) 3 (23) 3 (23)	 4 (40) 0 (0) 5(50) 1(10)	0.35
Donor CMV IgG status(combined) (n) **Negative** **Positive**	 12 11	 6 7	 6 4	0.51
Recipients previous history of smoking, n (%)	13 (54)	9 (69)	4 (40)	0.16
Recipients current history of smoking, n (%)	1 (4)	1 (7)	0 (0)	0.37
Dialysis prior to transplantation, n (%)	18 (75)	11 (84)	7 (70)	0.4
Previous transplant (%)	3 (13)	2 (15)	1 (10)	0.74

Continuous variables are shown as either mean (SD) or median (IQR) and categorical variables as absolute value (percentage). ADPKD, autosomal dominant polycystic kidney disease; ATG, anti-thymocyte globulin; BMI, body mass index; CMV, cytomegalovirus; DBD, donation after brain death; DCD, donation after circulatory death; DGF; delayed graft function, DM, diabetes mellitus; DSA, donor-specific antibody; ESRD, end-stage renal disease; HLA, human leucocyte antigen; HTN, hypertension; LD, live-donor.
The Kruskal-Walli's test was used for continuous variables and the Pearson Chi^2 test was used for categorical variables.

There were also statistically significant differences in donor age between the groups, with older recipients having received kidneys from older donors, which is as expected from the UK National Allocation Scheme where deceased-donor kidneys are allocated according to a points-based scoring system via a computer algorithm that prioritizes waiting time, HLA match, and donor-recipient age match [202]. Most of the donors were male and there were more males in the older group than the younger group. There was no transplantation from live donors and most kidneys were donated after circulatory death (DCD)Table 5.2.

Table 5.2:Demographic and clinical characteristics of transplant donors

Variable	**Total N=23**	**Young N=13**	**old N=10**	**p-Value**
Donor age, years median (IQR)	50 (39-62)	43 (37-55)	65 (44-67)	**0.03**
Male donor gender, n (%)	13 (57)	5 (38)	8 (80)	**0.04**
Donor ethnicity, n (%) White	21 (91)	13 (100)	8 (80)	0.24
Donor status, n (%) **LD** **DBD** **DCD**	 0(0) 8 (35) 15 (65)	 0 (0) 5 (38) 8 (62)	 0 (0) 3 (30) 7 (70)	0.67
CIT, (min) Median (IQR)	641(565-903)	736 (577-921)	581 (345-724)	0.20
Anastomosis time (min) Median (IQR)	28 (26-35)	28 (25-33)	35(26-40)	0.18

Continuous variables are shown as mean (SD) and categorical variables as absolute value (percentage). CIT, cold ischemic time; CMV, cytomegalovirus; DBD, donation after brain death; DCD, donation after circulatory death; DSA, donor-specific antibody; ESRD, end-stage renal disease; HLA, human leucocyte antigen; KDPI, kidney donor profile index; KDRI, Kidney Donor Risk Index; LD, live-donor. The Kruskal-Walli's test was used for continuous variables and the Pearson Chi^2 test was used for categorical variables.

5.4.B. Outcomes

The glomerular filtration rate was lower in the elderly KTR group but not statistically significantly different from the young age group. There were similar percentages of patients in the two groups that developed at least one episode of CMV viremia in the first six months after kidney transplantation Table 5.3.

Table 5.3: Comparison of transplant outcomes according to age group

Variable	Total N=23	Younger N=13	Older N=10	p-Value
GFR at 3 mo. (ml/min), median (IQR)	43 (35-54)	47 (32-62)	40 (34-45)	0.2
GFR at 6 mo. (ml/min), median (IQR)	**48 (36-56)**	49 (34-62)	41.5 (36-50)	0.3
BK viremia/nephropathy, n (%)	3 (13)	2 (15)	1 (10)	0.70
CMV viremia within 6 months post-transplant, n (%)	11 (48)	6 (46)	5 (50)	0.85
DGF, n (%)	8(35)	4 (30)	4 (40)	0.64
C RF >85, n (%)	2 (8)	2 (15)	0	0.19
Rejection, n (%)	2 (9)	1 (7)	1 (10)	0.84
Days from tx to collection, mean (SD)	87.3(8.06)	87 (8.3)	87.9 (8.07)	0.79

B cell subpopulations or subsets were identified using the markers listed in Table 5.4.

Table 5.4: Markers of B cell subsets assessed in this study

B cell subsets	Phenotype	Reference
Transitional B cells	CD19+CD24hiCD38hi	[154] [254]
naïve B cells	CD19+CD24interCD38inter	[154] [254]
Memory B cells	CD19+CD24hiCD27+	[154] [254]

In this work, I used the following names for the B cells subsets and ns – not significant ($p>0.05$), *$p\leq0.05$.

5.4.B.A. B cells subsets in relation to KTR age at transplantation, frailty and CMV viremia

Transitional, naïve, and memory B cell proportions were compared in the young and old KTR age groups. The median percentage of transitional Bs in the young group was 2.51(IQR: 0.85-7.2) compared with 2.46 (IQR: 0.74-6.3) in the old group (p = 0.9). Similarly, there was no statistical difference in the proportions of naïve and memory B cells in the two age groups (Table 5.5). The comparison of different B cell subsets in the two age groups is shown in Figure 5.3.

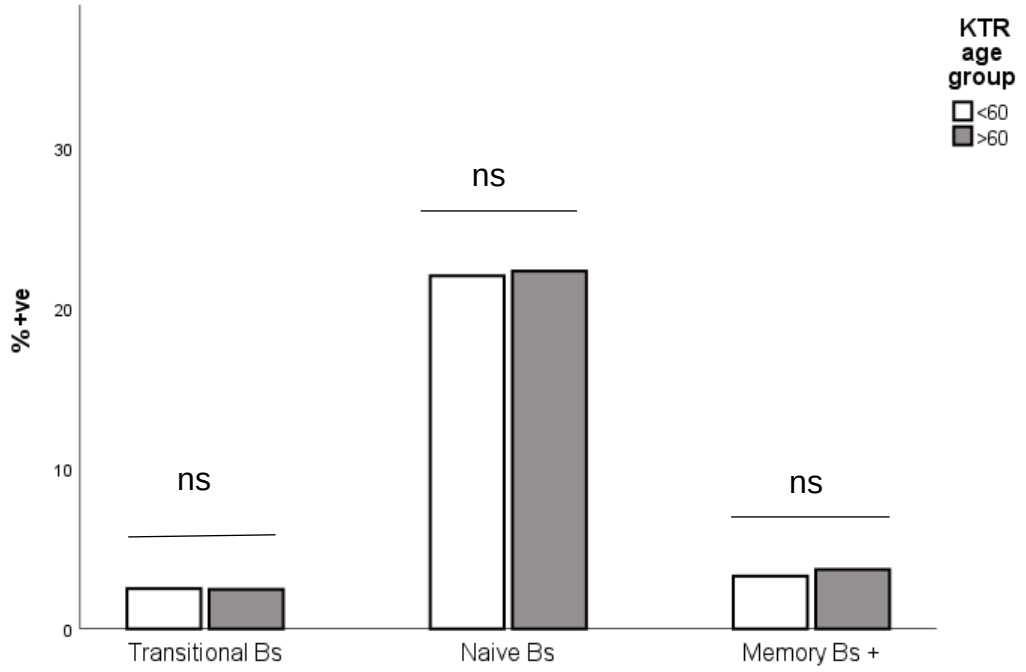


Figure 5.3: Proportions of transitional, naive and memory B cells in KTR patients aged younger (<60; n =13) or older (>60; n =10) than 60. Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

Table 5.5: Proportions of B cell subsets in KTR patients according to age at transplantation, frailty prior transplantation and development of CMV viremia post transplantation.

Parameters (n)	**B cells subsets (median (IQR))**		
	Transitional Bs	**Naïve Bs**	**Memory Bs**
KTR age:			
Young (<60) (n=13)	2.51(0.85-7.2)	22(11.2-48)	3.28(1.8-6.79)
Old (>60) (n=10)	2.46(0.74-6.3)	22.3(7.3-41)	3.69(2.7-6.8)
p value	0.9	0.7	0.49
Frailty score:			
Non frail (1 & 2) (n=11)	2.5 (0.98-6.3)	22(14-49.6)	4.73(2.1-7.9)
Moderately frail (3&4) (n=9)	3.1(1.18-8.9)	23.5(10.4-36)	3.6(2-6.7)
p value	0.62	0.62	0.71
CMV post transplantation:			
CMV viremia (11)	1.08(0.31-8.06)	14(7.48-24.6)	3.6(1.83-7.9)
No CMV viremia (12)	2.9 (1.53-5.8)	33.65 (19-48.8)	3.5(2.48-5.4)
P value	0.29	0.04	0.97

There were only three KTR with frailty score > 3 therefore comparison between non-frail (1-3) and frail (≥4) was challenging, therefore, the comparison was made between non-frail (Clinical frailty score of 1 & 2) and moderately frail (Clinical frailty score of 3&4) which is different than what was described earlier in chapter 4. The percentage of B subsets of moderately frail KTR and non-frail KTR are summarised in Table 5.5. The percentage of transitional, naïve, and memory B subsets (median (IQR)) were not different between non-frail and moderately frail KTR groups (Figure 5.4).

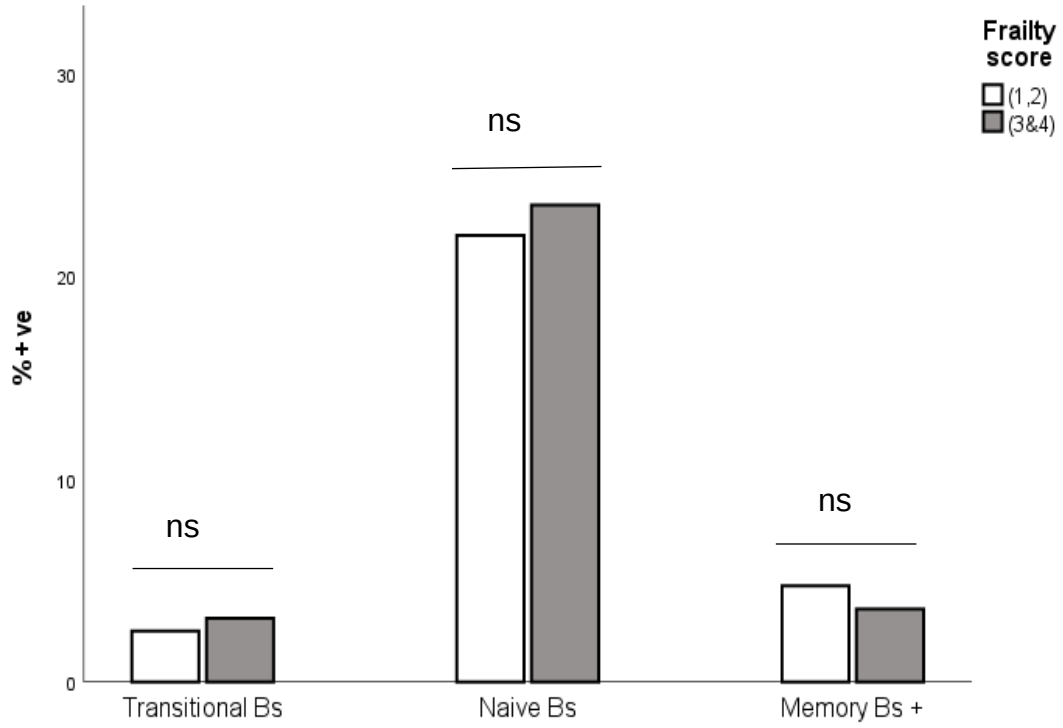


Figure 5.4: Proportions of transitional, naive and memory B cells in KTR patients with frailty score 1&2(n =11) or 3&4(n=9). Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

The median percentage of B subsets of KTR who developed CMV viremia and those who did not are summarised in Table 5.5. KTR who developed CMV viremia had lower percentages of naïve B subsets, median 14(7.48-24.6) compared with 33.65 (19-48.8) in KTR who did develop CMV viremia, p=0.04 Figure 5.5.

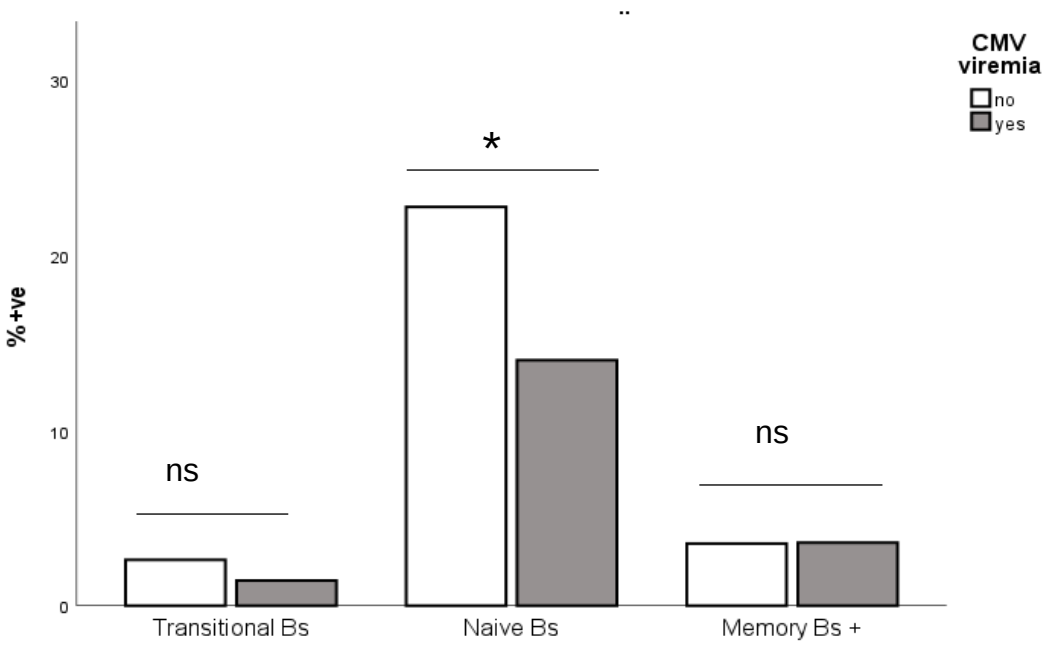


Figure 5.5: Proportions of IL-10 expressed on transitional, naive and memory B cells in KTR patients with CMV viremia (n =11) or with no CMV viremia (n=12). Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

5.4.B.B. IL-10 expression of different B cell subsets in relation to KTR age at transplantation, frailty and CMV viremia

The median percentage of transitional B cells expressing IL-10 was 69(34-81) in the younger group compared with the older one 74 (33-96), P 0.85(Table 5.6). Figure 5.6 shows the comparison of transitional, memory, and naïve B cells between the young and old KTR age groups.

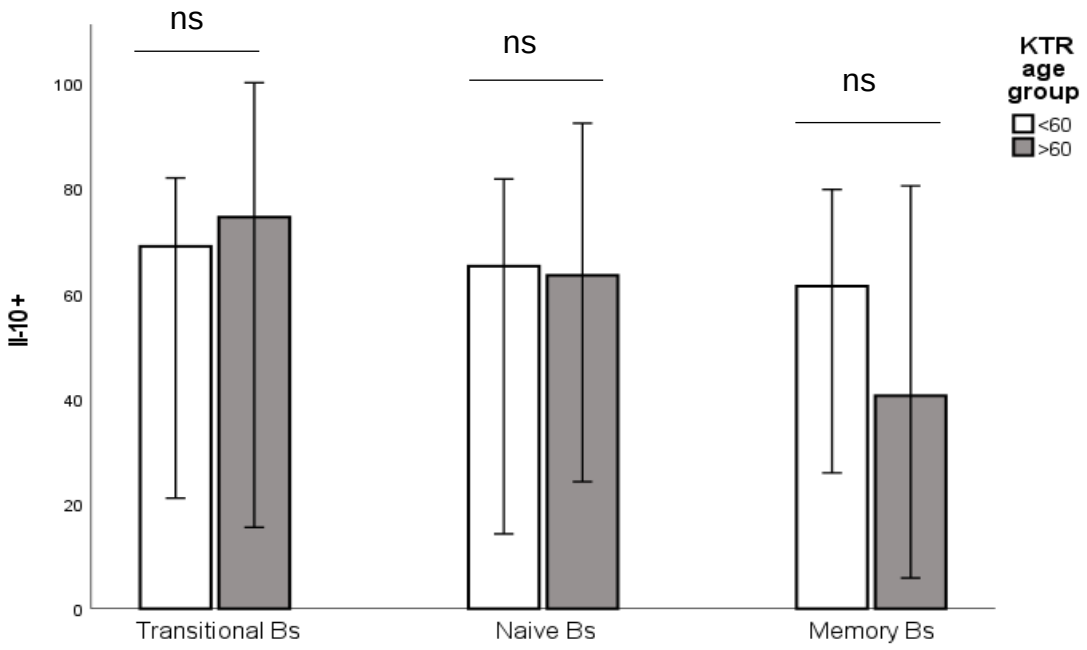


Figure 5.6: Proportions of IL-10 expressed on transitional, naive and memory B cells in KTR patients aged younger (<60; n =13) or older (>60; n =10) than 60. Data are medians with interquartile ranges. Medians were compared between the two-age group using Mann-Whitney U test.

Table 5.6: Proportions of IL-10 expression on B cell subsets in KTR patients according to age at transplantation, frailty prior transplantation and development of CMV viremia post transplantation.

Parameters (n)	IL-10 expression (median (IQR))		
	Transitional Bs	Naïve Bs	Memory Bs
KTR age:			
Young (<60) (n=13)	69(34-81)	65 (18-79)	61(28-79)
Old (>60) (n=10)	74 (33-96)	63 (24-88)	40 (21-76)
p value	0.85	0.51	0.13
Frailty score:			
Non frail (1 & 2) (n=11)	68.9(50-81.9)	64(21.7-76)	59.7(25.8-79)
Moderately frail (3&4) (n=9)	64.5(18-97.5)	62.7(14.9-89.6)	38.6(15.8-72.2)
p value	0.84	0.84	0.30
CMV post transplantation:			
CMV viremia (11)	75(54-84.4)	62.7(21.7-86)	44.4(25.9-75)
No CMV viremia (12)	56.3(18.3-93)	65.8(25.6-80.4)	65.4(33-80.2)
P value	0.41	0.44	0.34

The median percentage of memory B cells expressing IL-10 was 59.7(25.8-79) in the non-frail group which was higher than in the moderately frail 38.6(15.8-72.2), but it was not statically significant p=0.3 (Figure 5.7).

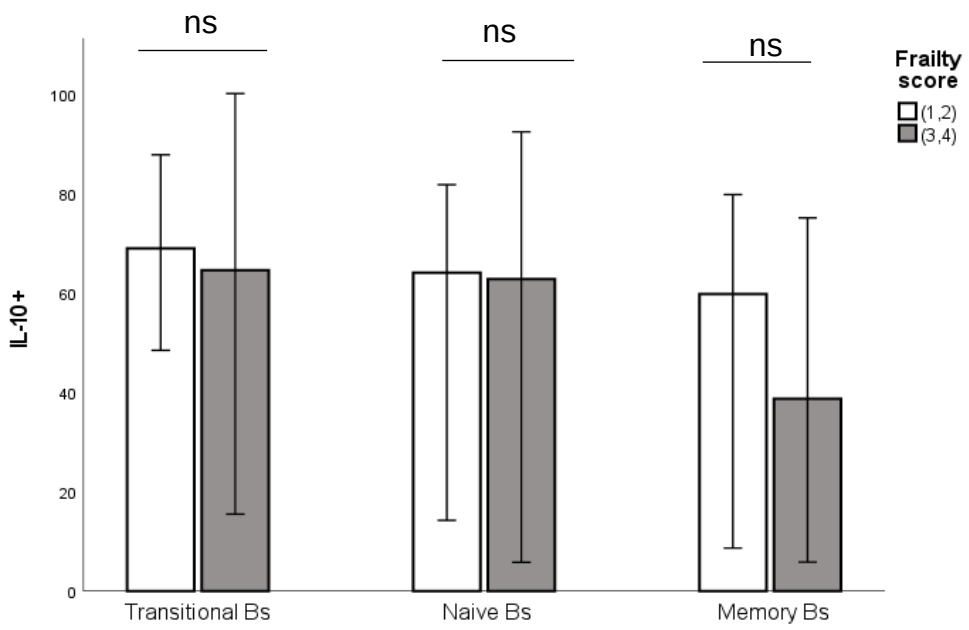


Figure 5.7: Proportions of IL-10 expressed on transitional, naive and memory B cells in KTR patients with frailty score 1&2(n =11) or 3&4(n=9). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

The median percentage of different B cell subsets expressing IL-10 according to CMV viremia is shown in table 5.6. Although transitional, naïve, and memory B cells expressed more IL-10 in KTR who did not develop CMV, these differences were not statistically different (Figure 5.8).

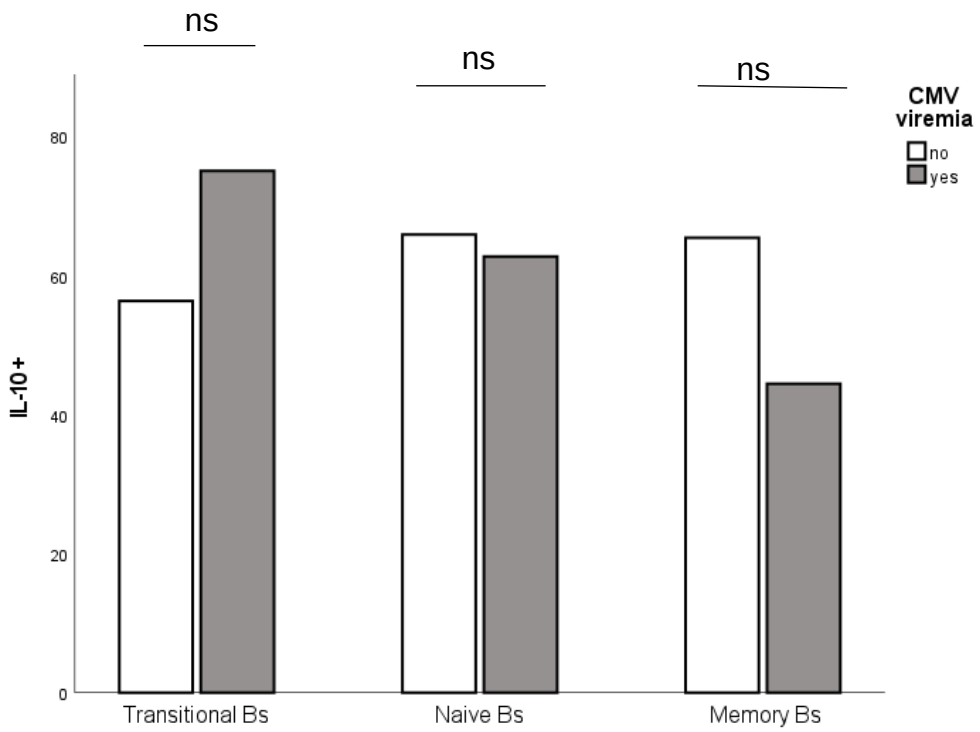


Figure 5.8: Proportions of IL-10 expressed on transitional, naive and memory B cells in KTR patients with CMV viraemia (n =11) or with no CMV viremia(n=12). Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

5.4.B.C. TNF expression of different B cell subsets relation to KTR age at transplantation, frailty and CMV viremia

The percentage of transitional B cells expressing TNF-α + B was 31(16-57) in the younger group compared with the old one 18(9-67), p0.51. Similarly, naïve Bs expressing TNF-α were non-statistically significant in the two age groups. However, the memory B cells expressing TNF-α were higher in the older KTR compared with the younger ones but not statistically significant (Table 5.7&Figure 5.9).

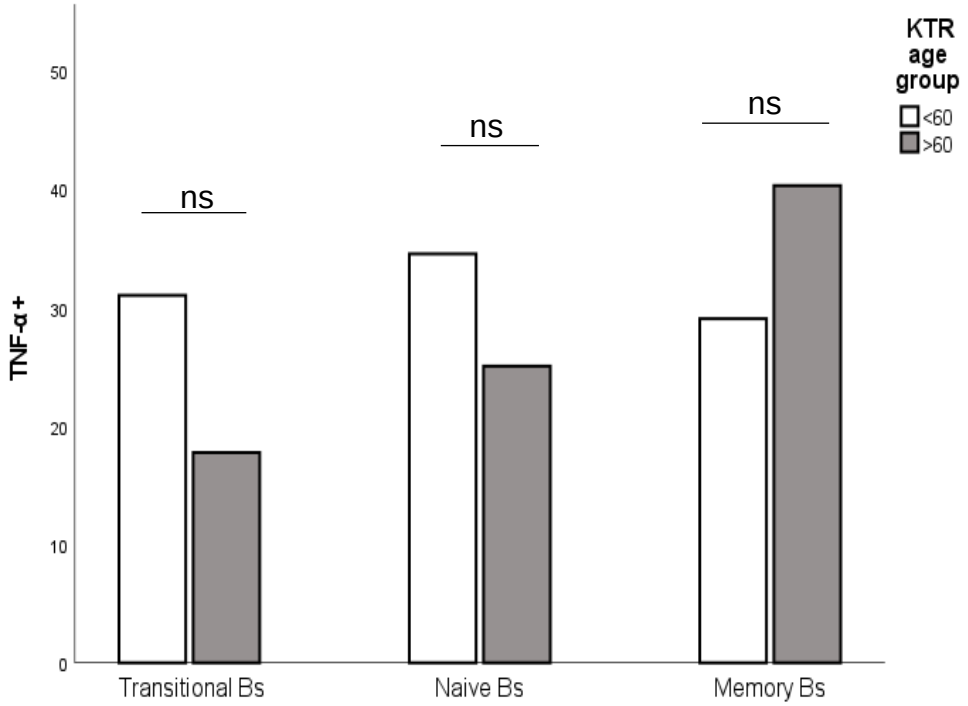


Figure 5.9: Proportions of TNF-α expressed on transitional, naive and memory B cells in younger KTR aged younger (<60; n =13) or older (>60; n =10) than 60. Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

Table 5.7: Proportions of TNFα expression on B cell subsets in KTR patients according to age at transplantation, frailty prior transplantation and development of CMV viremia post transplantation.

Parameters	TNFα expression median (IQR)		
	Transitional Bs	**Naïve Bs**	**Memory Bs**
KTR age:			
Young (<60) (n=13)	31(16-57)	34(15-71)	29 (18-51)
Old (>60) (n=10)	18(9-67)	25(6-51)	40(9-58)
p value	0.51	0.51	0.30
Frailty score:			
Non frail (1 & 2) (n=11)	41.2(15.2-65.5)	34.5 (12.9-72)	31.6(21.3-55.8)
Moderately frail (3&4) (n=9)	27(14.3-51.9)	23.9(6.4-45.7)	17.7(8.3-42.6)
p value	0.6	0.29	0.23
CMV post transplantation:			
CMV viremia (11)	41.2 (16-59)	37.6(12.9-72.8)	40 (25.8-55.8)
No CMV viremia (12)	17.7(10.5-56.8)	26.4(6.5-42)	24.4(8.7-46.2)
P value	0.34	0.21	0.23

The median percentage of TNF- α expression in different B subsets was not statistically different in non-frail groups compared to moderately frail (Figure 5.10 and Table 5.7).

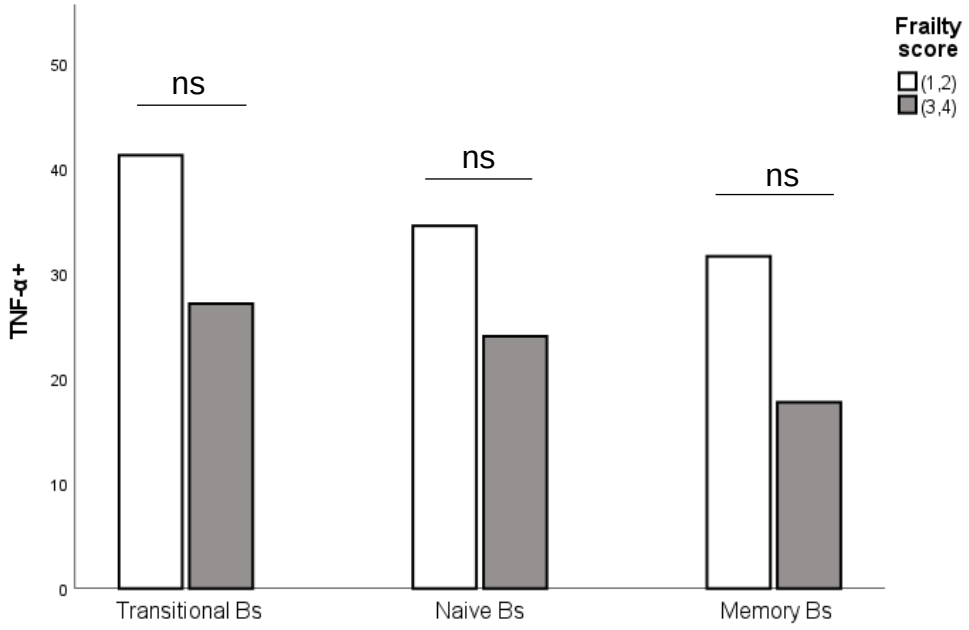


Figure 5.10: Proportions of TNF-α expressed on transitional, naive and memory B cells in KTR patients with frailty score 1&2(n =11) or 3&4(n=9). Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

The percentage of TNF-α expression in transitional, naïve, and memory B subsets was higher in the KTR who developed CMV viremia compared to those who did not, however, this difference was not significant (Figure 5.11 and Table 5.7).

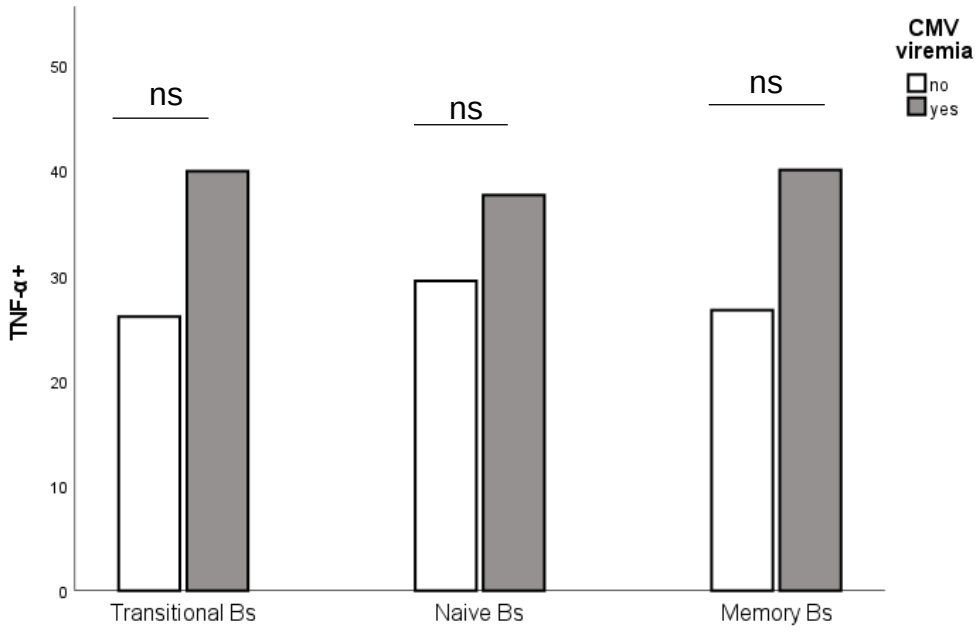


Figure 5.11:Proportions of TNF-α expressed on transitional, naive and memory B cells in KTR patients with CMV viremia (n =11) or with no CMV viremia (n=12). Data are medians with interquartile ranges. Medians were compared between the two groups using Mann-Whitney U test.

5.4.B.D. Ratios of IL-10+ and TNF-a+ cells within different B cell subsets in relation to KTR age at transplantation, frailty and CMV viremia

The median IL-10/TNF-α ratio of the memory B cells in the younger age group was 1.45 (0.92-3.16) higher than the old age group 0.98 (0.62-2.94) but again not statistically different. Figure 5.12 and table 5.8 show the IL-10/TNF-α ratio in different B cell subsets in the younger and older age groups.

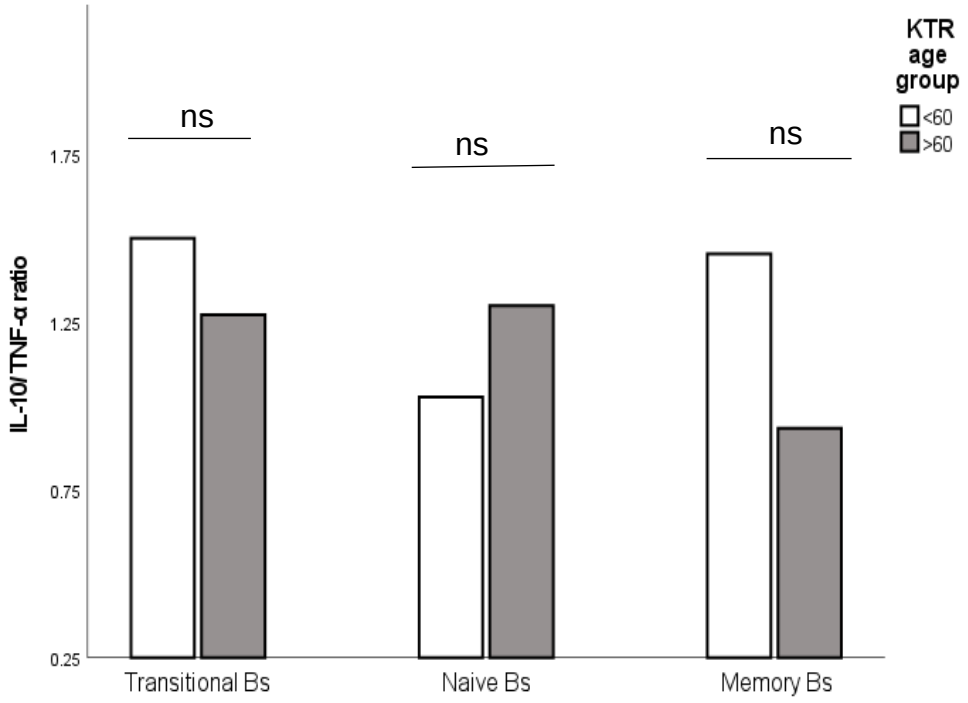


Figure 5.12: Ratios of IL-10+ and TNF-a+ cells within transitional, naïve and memory B cell subsets in KTR patients aged younger (<60; n =13) or older (>60; n =10) than 60. Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

Table 5.8**:** Ratios of IL-10+ and TNF-a+ cells within B cell subsets in KTR patients according to age at transplantation, frailty prior transplantation and development of CMV viremia post transplantation.

parameters	IL-10/ TNF-α ratio median (IQR)		
	Transitional Bs	**Naïve Bs**	**Memory Bs**
KTR age:			
Young (<60) (n=13)	1.49 (1.02-3)	1.45 (0.90-1.80)	1.45 (0.92-3.16)
Old (>60) (n=10)	1.31 (0.90-3.96)	1.38 (1.03-8.34)	0.98 (0.62-2.94)
p value	0.66	0.3	0.13
Frailty scores:			
Non frail (1 & 2) (n=11)	1.58(1.05-3.01)	0.98(0.92-1.42)	1.05(0.67-1.88)
Moderately frail (3&4) (n=9)	1.22(0.77-1.8)	1.38(0.86-3.67)	1.02(0.65-3.97)
p value	0.23	0.51	0.84
CMV post transplantation:			
CMV viremia (11)	1.49(1.22-1.71)	1.03(0.92-1.42)	1(0.67-2.6)
No CMV viraemia (12)	1.22(0.73-1.71)	1.31(0.97-7.3)	1.73(0.88-4.2)
P value	0.75	0.17	0.13

The moderately frail KTR had a higher IL-10/TNF-α ratio than the non-frail group in the naïve B cells, 1.38(0.86-3.67) in the moderately frail compared with 0.98(0.92-1.42) in the non-frail group but it was not statistically significant (Table 5.8 and Figure 5.13). The median IL-10/ TNF-α ratio in different B cell subsets in the KTR who developed viremia or who did not are shown in Figure 5.14 and Table 5.8.

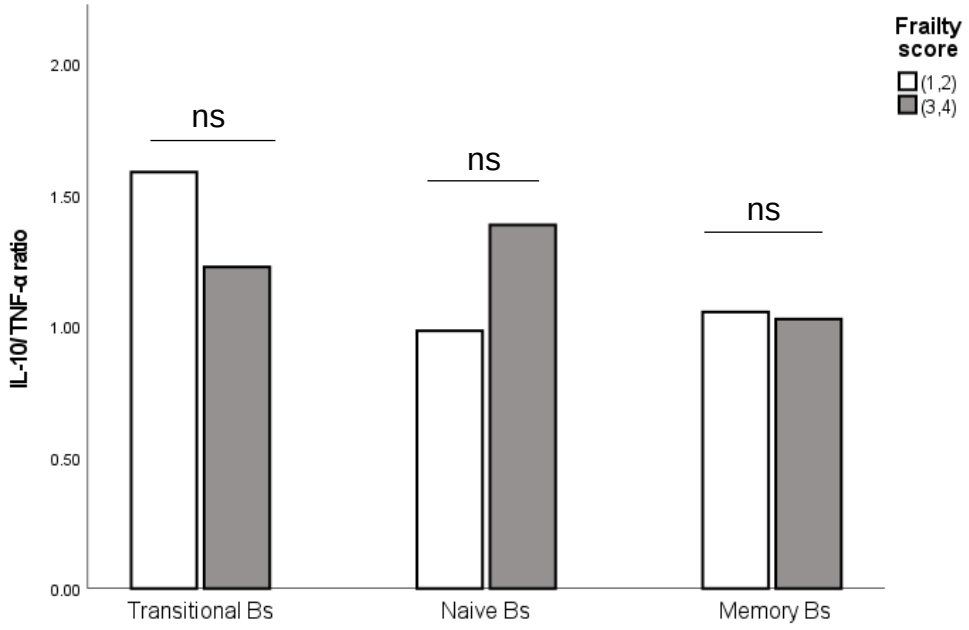


Figure 5.13**:** Ratios of IL-10+ and TNF-a+ cells within transitional, naive and memory B cells in KTR patients with frailty score 1&2(n =11) or 3&4(n=9). Data are medians with interquartile ranges. Medians were compared between the 2 groups using Mann-Whitney U test.

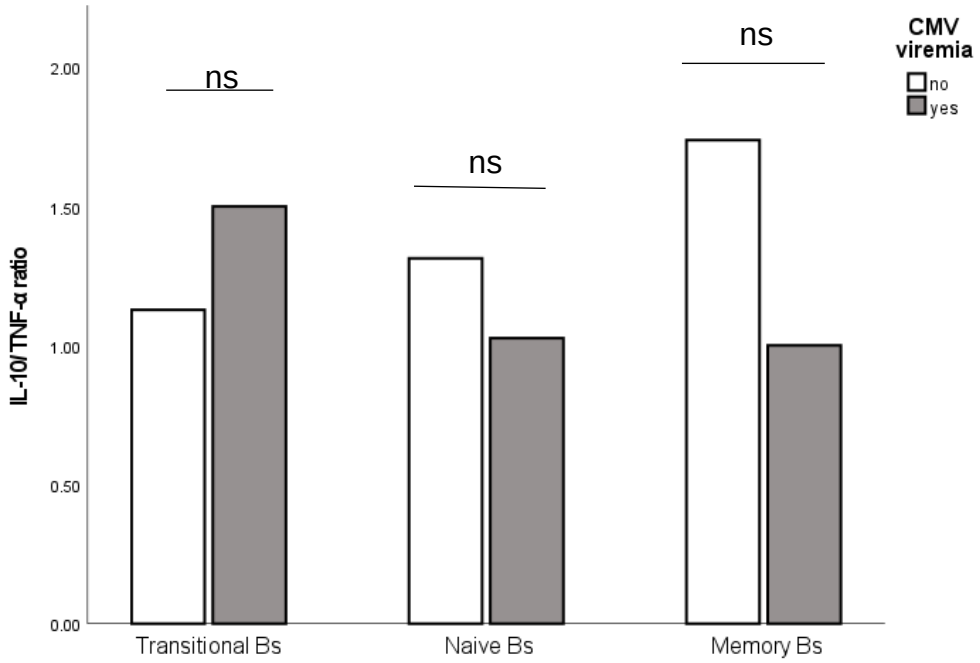


Figure 5.14**:** Ratios of IL-10+ and TNF-a+ cells within transitional, naive and memory B cells in KTR patients with CMV viraemia (n =11) or with no CMV viremia(n=12). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

On further analysis and dividing each age group according to developing CMV, half of the older KTR developed CMV (5/10) while 6 out of 13 developed CMV in the young KTR. The median transitional B cells IL-10/ TNF-α ratio in the older KTR who developed CMV was 1.22(0.78-1.46) compared to 1.99 (0.55-6.6) who did not develop CMV, p=0.42. Interestingly, older KTR who developed CMV had lower transitional IL-10/ TNF-α ratio than younger KTR who developed CMV, 1.22(0.78-1.46) compared to1.65(1.47-3), p=0.02(Figure 5.15). Naïve B cell IL-10/ TNF-α ratio

was significantly lower in the older KTR who developed CMV viremia 0.93(0.86-1.26) compared to 4.33(2.11-50.9) in the older KTR who did not, p=0.01 figure 5.16.

The memory B cell IL-10/ TNF-α ratio was lower in the older KTR who developed CMV 0.73 (0.62-0.95) compared to 1.4 (0.49-4.47) in the older KTR who did not develop CMV, however, this difference was not statistically significant, p=0.17.

There was no significant difference in IL-10/ TNF-α ratio in the different B cell subsets in the younger KTR who developed CMV and in those who did not.

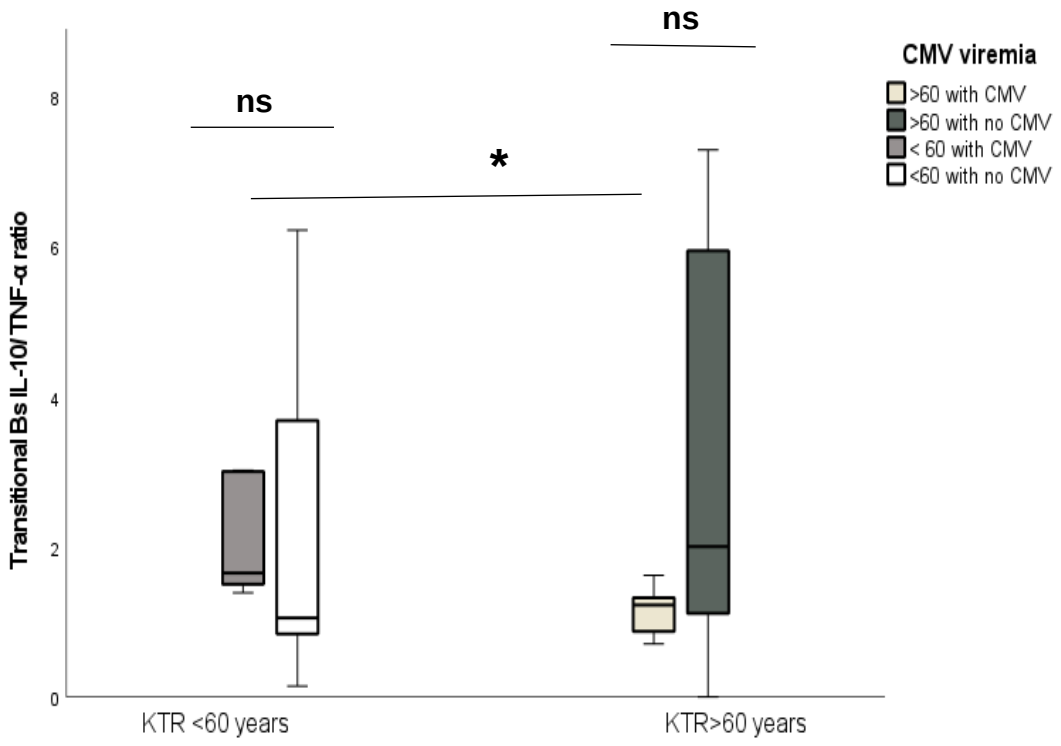


Figure 5.15**:** Ratios of IL-10+ and TNF-a+ cells within on transitional B cells in KTR patients with CMV viremia and aged <60 (n =6), CMV and aged >60(n =5), no CMV and aged <60 (n =7), or with no CMV viremia and aged >60 (n=). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

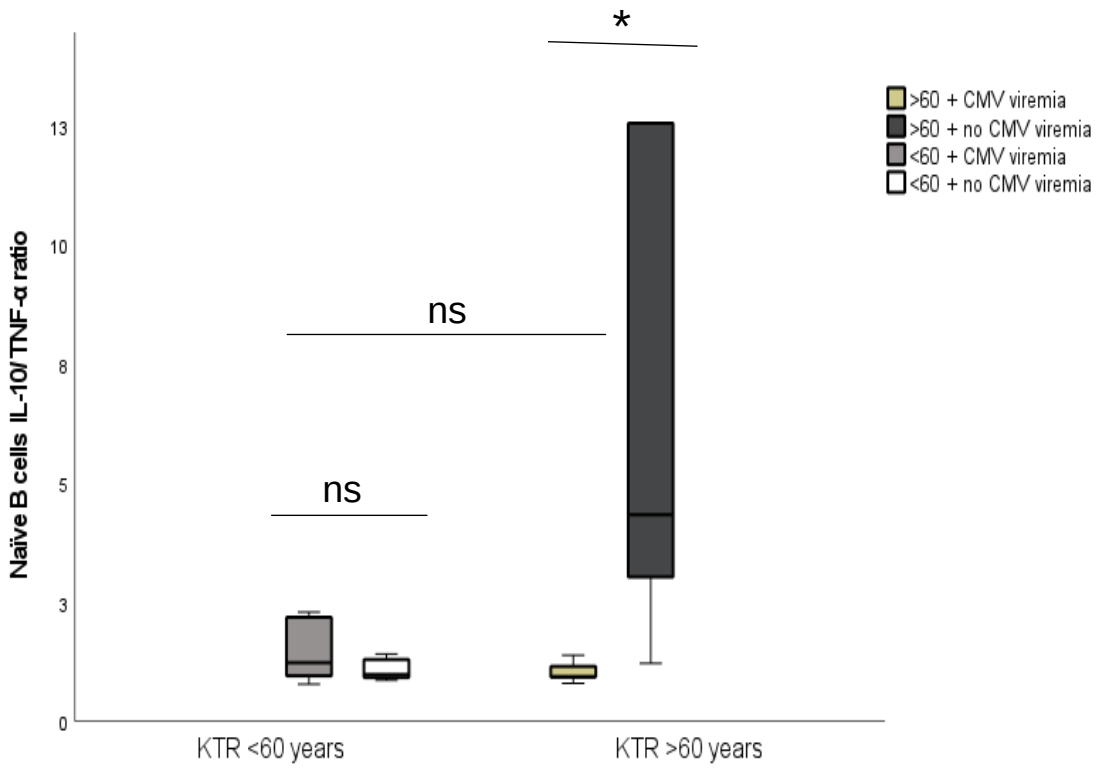


Figure 5.16**:** Ratios of IL-10+ and TNF-a+ cells within B cells in KTR patients with CMV viremia and aged <60 (n =6), CMV and aged >60(n =5), no CMV and aged <60 (n =7), or with no CMV viremia and aged >60 (n=5). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

5.4.B.E. Dual IL-10 and TNFα expression in different B cell subsets in relation to KTR age at transplantation, frailty and CMV viremia

The percentage of transitional B cells expressing dual IL-10 and TNFα in the older age group was 22.9(9.86-66) compared to 16 (4.55- 47.7) in the younger age group, however, this difference was not statistically significant (Figure 5.17). Figure 5.18 and Table5.9 show the percentage of B cell subsets expressing both IL-10 and TNF-α in the non-frail and moderately frail KTR.

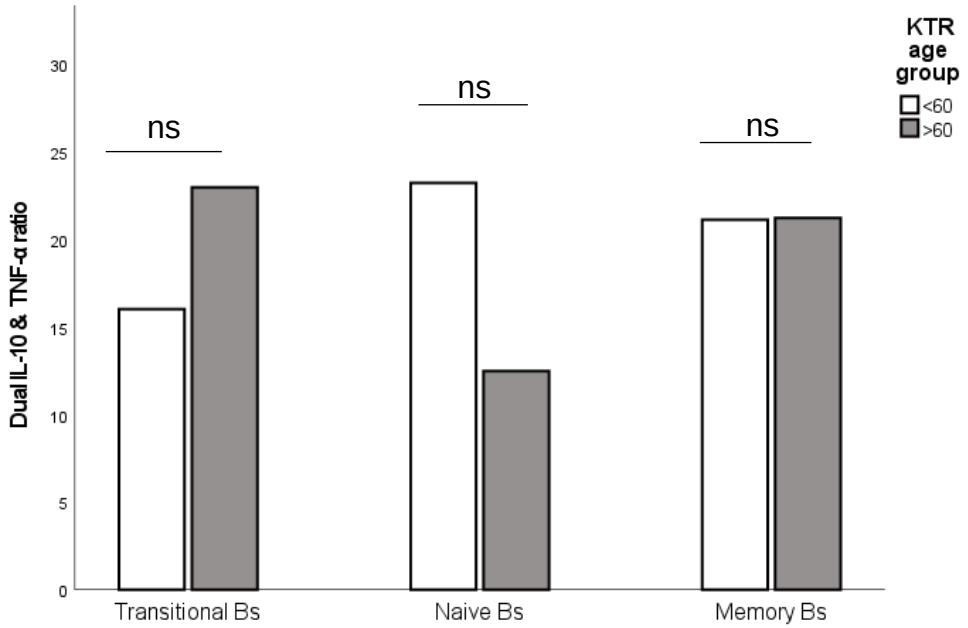


Figure 5.17: Proportions of dual IL-10 and TNF-α expressed on transitional, naive and memory B cells in younger KTR aged younger (<60; n =13) or older (>60; n =10) than 60. Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

Table 5.9: Proportions of dual L-10/ TNF-α ratio expression on B cell subsets in KTR patients according to age at transplantation, frailty prior transplantation and development of CMV viremia post transplantation.

Parameters	Dual IL-10 & TNF-α expression		
	Transitional Bs	**Naïve Bs**	**Memory Bs**
KTR age:			
Young (<60) (n=13)	16 (4.55-47.7)	23.2 (5.17-63.6)	21 (6.25-42.55)
Old (>60) (n=10)	22.9(9.86-66)	12.46(1.62-40.27)	21.2 (5.15-37.95)
p value	0.85	0.51	0.85
Frailty scores:			
Non frail (1 & 2) (n=11)	32.4(5.4-84.7)	23.2(5.66-65)	21.1(6.1-45.2)
Moderately frail (3&4) (n=9)	16(9.8-74.5)	7.8(1.21-35.7)	12(3.65-23.6)
p value	0.7	0.21	0.27
CMV post transplantation:			
CMV viremia (11)	46.7(9.6-56)	33.6(4.3-62.2)	35.3(4.7-62)
No CMV viremia (12)	12.3(4.1-37.6)	6.8(2.8-33.6)	18.6(6.18-42.8)
P value	0.15	0.29	0.58

Figure 5.18 and Table 5.9 show the percentage of B cells subsets expressing both IL-10 and TNF-*α* in the non-frail and moderately frail KTR.

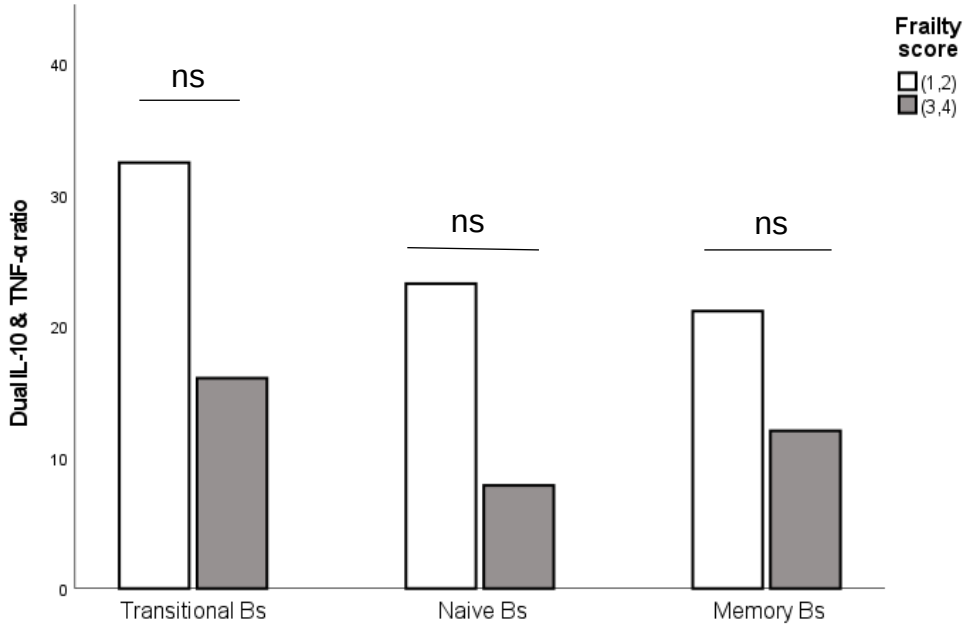


Figure 5.18: Proportions of dual IL-10 and TNF-α expressed on transitional, naive and memory B cells in KTR patients with frailty score 1&2(n =11) or 3&4(n=9). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test

Figure 5.19 and Table 5.9 show the percentage of B cells subsets expressing both IL-10 and TNF-*α* according CMV viremia post transplantation.

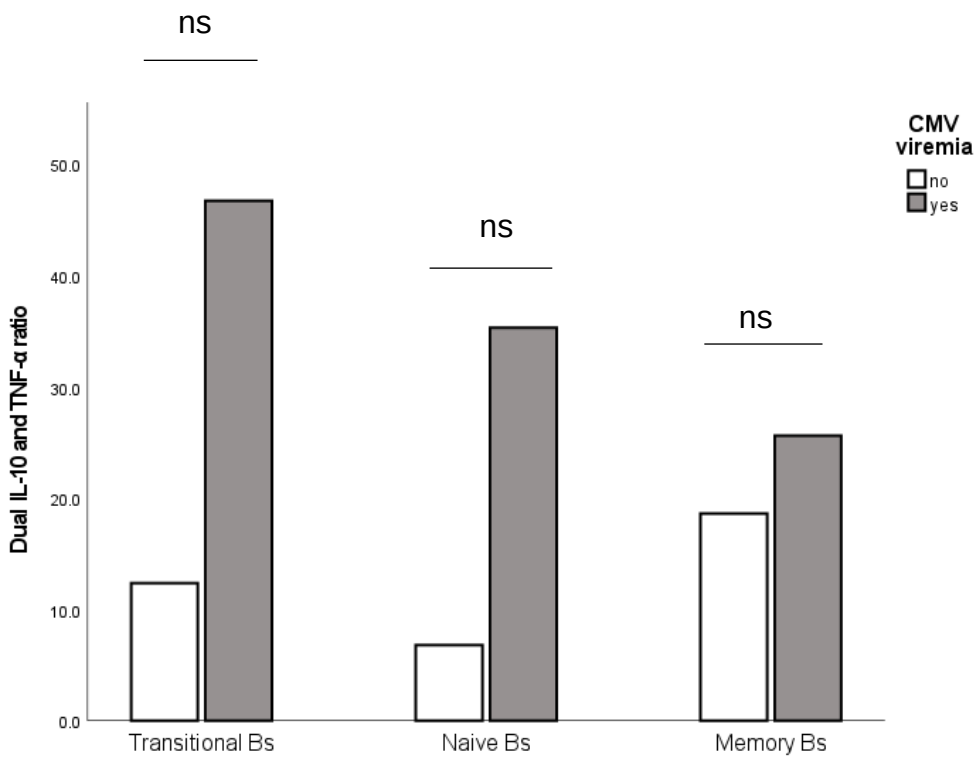


Figure 5.19: Proportions of dual IL-10 and TNF-α expressed on transitional, naive and memory B cells in KTR patients with CMV viremia (n =11) or with no CMV viremia (n=12). Data are medians with interquartile ranges. Medians were compared between the 2 age groups using Mann-Whitney U test.

5.5. Discussion

In this work, there was no difference in IL-10/TNF expression in transitional, naïve, and memory B cell subpopulations between the two age groups, < 60 and >60. As mentioned earlier, B cell subsets and their IL-10/TNF ratio can predict transplant outcomes [181] [255] [180]. Recent data demonstrated low Transitional B cell IL-10/TNF ratio in KTR at three months was associated with a higher risk of progressing to acute rejection and worse graft survival at 7 years post-transplant [32]. However, borderline rejection in KTR with a higher transitional B cell IL-10/TNF ratio, had similar outcomes to KTR with no rejection in the biopsy [32].

In this work, the median age of KTR was 57 with no KTR <30-year-old which limited accurate comparison with younger and older KTR. However, this work demonstrated that KTR age at transplantation does not influence transitional IL-10/TNF ratio at three months post-transplant. As mentioned above, transitional B cell IL-10/TNF ratio in KTR at three months was associated with a higher risk of progressing to acute rejection therefore, it could also be used as a biomarker to risk stratify older KTR without worrying about age influence on B cells cytokines profile. This is consistent with a previous report where KTR age did not affect transitional IL-10/TNF α ratio which was predictive of developing rejection equally in >50 and <50 years old (the age cut was the median of the cohort in this report) [172].

Our data demonstrated no difference in the percentage of different B cell subsets between the younger and older age groups. This is in keeping with the work where the total B cells were reduced in the elderly (>90 years) compared with younger individuals (age 19-29), however, the subpopulation percentages of B cells were not

different in the two age groups [204]. In this work, there was no KTR <30 years, therefore comparison between the younger ages group (19-29) and >60 was not possible. In this work, IL-10 production by B cell subsets was not different in the older age group which is like data reported in animal models. In mice, B cell numbers and function (IL-10 production) were not affected by age. However, aged mice with skin transplants were found to have a higher proportion of aged-associated B cells (ABCs) and lower production of donor-specific antibodies [170].

In this work, IL-10 production by B cell subsets was not different between the KTR who developed CMV viremia and those who did not. Previous work has shown that viral infections stimulate B cell IL-10 production via TLR-induced mechanisms. Type-I (Interferons) IFN production during the inflammatory process caused by viral infections also triggers IL-10 production by B cells [171] [256]. However, five KTRs had CMV viremia and were on antiviral agents before recruiting or at the time of this study which might have confounded the results.

Increased levels of IL-6 and TNF α, the pro-inflammatory cytokines, are associated with frailty in older individuals (>85 years) [257]. The late memory B (CD19+ CD27-IgD-) B cell subset percentage was reported higher in frail men (>90 years old) with an interestingly higher association with IL-6 production. This confirms the association between IL-6 and frailty [258]. Memory (CD19+CD24hiCD27+) B cells express TNF α alone more than other B cell subsets in addition to IL-10 or dual IL-10 and TNF α [145]. In this work, there was no significant correlation between the TNF-α expression in memory cells and frailty score. However, the elderly cohort in this work is younger than the previously published studies on frailty or immunosenescence

which might also explain my findings. Furthermore, there was mostly non-frail and moderately frail KTR in this work which again confounds these findings.

Interestingly, the data showed a significantly lower ratio of IL-10/TNF-α of naïve B cells in the older KTR who developed CMV compared to those who did not. This is consistent with the previous report of the association between CMV viremia and chronic rejection especially when associated with acute rejection [259] [260]. The explanation for this association is not clear, however, treating the rejection was suggested as a possible causative factor [260]. In this work, there was no difference in IL-10/TNF-α of transitional B cells concerning developing CMV viremia, however, older KTR who developed CMV viremia had lower ratios than younger KTR who developed CMV viremia. A previous report has shown CMV infection was not associated with the change in IL-10/TNF-α ratio in transitional B cells at three months post-transplant [172]. However, there is a complex effect on IL-10 and TNF-α production following CMV infection, with one study showing a rise in serum IL-10/ TNF-α ratio in KTR with asymptomatic CMV compared with symptomatic CMV. In these data, there was a profound TNF-α activation with moderate IL-10 expression in KTR who developed symptomatic CMV infection compared to KTR with asymptomatic CMV[261]. Other data show that CMV infection increases the expression of interferon-gamma which subsequently increases the expression of MHC (major histocompatibility complex) class II antigens on the graft parenchymal cells leading to acute rejection [262].

Five of the seven KTR who developed CMV viremia had contracted the infection before the blood collection for this experiment (appendix 5). Thus, changes in the transitional B cell cytokine expression may have been influenced by the CMV

infection rather than representing a risk factor for causing the CMV infection. With the evidence mentioned above that low Transitional-1 B cell IL-10/TNF-α ratio was associated with increased risk of rejection, it is possible that low IL-10/ TNF-α ratio in transitional B and naïve b cells at 3 months post-transplant, may have been caused by CMV infection, and increased the risk for developing subsequent rejection as immunosuppression was reduced.

5.6. Conclusions

The transitional B cell IL-10/TNF ratio at three months post-transplantation in older subjects compared to younger ones was like <60 years compared to>60 years in this small cohort of 23 KTR. On the other hand, older KTR with CMV infections had lower naive IL-10/ TNF-α ratios than older ones who did not develop CMV. In the KTR who developed CMV viremia, the transitional B cell IL-10/TNF ratio was lower in older KTR compared to young ones, raising the question of whether CMV infection altering the B cell cytokine profile puts the patients at risk of subsequent rejection. Larger numbers of KTR especially of two extreme ages and frail ones before transplantation may provide a better understanding of the impact of age and frailty on Immune subsets and their subsequent role in mediating graft rejection.

CHAPTER 6. DISCUSSION

Summary of results and interpretation

This research project, investigating factors predicting infectious outcomes, specifically CMV infections, in older kidney transplant recipients, consists of three main areas. The initial work focused on confirming that elderly transplant recipients have a higher risk of infection and a lower risk of rejection. Then, I investigated whether frailty before transplantation predicted CMV infection risk in elderly kidney transplant recipients. Finally, I looked at B cell subsets in older and younger kidney transplant recipients at three months post-transplant and their IL-10/TNF-α expression after stimulation ex-vivo to understand how the immune phenotype was related to age, frailty, and infection

In Chapter 3, I demonstrated that the risk of rejection was significantly lower in the older >60-year-old KTR compared to younger matched recipients, especially those < 35. This supports previous work showing that older KTR (>40 years old) are less likely to develop antibody-mediated rejection compared to younger recipients [219]. Conversely, the rate of CMV viremia, the most common opportunistic infection in KTRs, was higher in older KTRs than younger ones. In our unit, we do not use prophylaxis for CMV infection which permits studying the rate of CMV in the acute period post-transplantation. This finding is also consistent with published data that the risk of infections in general is higher in the elderly KTR [43]. However, it shows that we may be able to utilize CMV viraemia as a readout of the degree of

immunosuppression needed in older KTR and could pave the way for a clinical trial of reduced immunosuppression in elderly recipients.

This project focused on service improvement, and it suggests modifying the immunosuppressive protocol for older kidney transplant recipients. Additionally, implementing CMV prophylaxis is recommended, particularly for frail older patients. In Chapter 4, I demonstrated that in the frail, older KTRs, the rate of CMV infection was significantly higher compared with the non-frail older KTR. After adjusting for confounders in two models, the CMV rate remained higher in the frail elderly group compared with non-frail elderly KTR. This clear association between CMV infection and prevalent frailty is in keeping with other work showing increases in inflammatory states, measured by increased IL-6 production, may contribute to frailty [240]. I also demonstrated that frail elderly KTRs have a higher risk of recurrent admissions due to infectious causes compared with non-frail ones. Re-hospitalization post-kidney transplant is higher in frail kidney transplant recipients [83]. Although survival was not different in the frail and non-frail groups in this work, the frail KTR had a three-fold increase in graft loss compared to the non-frail.

This project was also a service initiative. The frailty assessments conducted on non-white older transplant recipients raised concerns about potential stereotypes associated with certain ethnic groups. Therefore, it is recommended that frailty assessments be performed for all recipients in the clinic and on the day of transplantation. Importantly, these assessments should not be used as a basis for declining transplant eligibility, but rather as a tool for optimizing care and ensuring close monitoring.

In Chapter 5, I demonstrated that there was no difference in transitional B cell IL-10/TNF production at three months post-transplantation between younger and older KTR in this small cohort. Previous work has demonstrated that B cell subsets and their IL-10/TNF ratio can predict transplant outcome [172] [247] [171] and this was the rationale for investigating the IL-10/TNF ratio in older KTR which may have explained some of the changes found in graft outcomes. One interesting finding was a reduction in this ratio in older KTR with CMV viremia compared to young KTR with CMV viremia which could be due to the actual infection rather than increasing the susceptibility to CMV infection. Finally, in this work, IL-10/TNF ratios were not different in the moderately frail KTR compared with non-frail KTR. There was no significant correlation between the TNF expression on memory cells and frailty scores. This contrasts with published data on increased levels of the pro-inflammatory cytokines, IL-6, and TNF, in frail older individuals (>85 years of age) [250].

6.1. Limitations and further work

The work in Chapter 3 is a retrospective study with all the problems related to data acquisition and loss of follow-up of some patients. I tried to eliminate this by only including those patients for whom we had complete follow-up data. The matching strategy was limited to a few key factors, although I adjusted for other factors in the analysis, this may not be complete. The rates of rejection were low in our cohort, therefore comparing the rates of rejection and alloimmune reactions across four age groups was affected by group size. Investigating the malignancy risk or rate was not straightforward due to the complexities of assessing malignancy risk factors. Data

shows the risk of malignancy after 10 years of immunosuppression is 20 %, however, the risk of malignancy is multifactorial, and calculating the rate related to transplant antirejection medication alone is not possible [263].

I would propose conducting similar work with a more robust matching strategy.

Again, the work in Chapter 4, was retrospective with known challenges related to collecting retrospective information, including loss of follow-up and changes in clinical hospital databases and IT systems. In this work, a clinical frailty scale was used to assess frailty, and it was done within one year before transplantation which could affect the outcome due to the possibility of changes in the frailty status in the more immediate run-up to transplantation. Most published data used either the frailty index or the Fried frailty tool, thus comparison with other publications was not straightforward. Furthermore, the clinical frailty scale is a more subjective tool and relies on clinician judgment in one of the clinic visits. Despite this, the clinical frailty scale was predictive of survival in pre-dialysis cohorts with a median age of 70 years [230]. The small study population, especially the frail group, and the short follow-up time limited seeing the effect on long-term adverse effects like malignancy.

There were more non-white kidney transplant recipients who underwent frailty assessments prior to transplantation, which may raise concerns about potential selection bias. Additionally, the frailty assessments conducted at the time of evaluation for transplantation were not updated while patients were on the waiting list, raising concerns about inconsistent measurements and their potential impact on the results. Lastly, frailty was not assessed at or following transplantation, which may explain some of our findings, as deterioration or improvement in frailty post-

transplantation for example could explain the lack of difference in mortality between the two groups.

I would propose investigating frailty at the time of transplantation when the potential KTR is deemed at their best health and thereafter to test if frailty truly predicts outcomes. I will also propose investigating if transplantation improves frailty compared to remaining on dialysis in the older KTR. Finding factors determining the above might be a potential area of change to improve the outcomes or highlight the patients who would need more support.

In Chapter 5, specific phenotypic markers of regulatory B cells are lacking and contradictory in the literature. IL-10 expression was used as a signature cytokine to identify B reg. IL-10 expression is very low in vivo therefore in vitro stimulation is needed to promote measurable levels of IL-10 and TNF expression. Prolonged in vitro stimulation can affect the phenotype of B cells [247]. That was addressed in a previous publication by purifying B cells before culturing them and analyzing cytokine expression by ELISA [145]. The overall immune regulatory function was not fully assessed, as only B cell subsets and cytokine secretion were assessed, without investigating T cell function. Additionally, IL-10 and TNF production was not confirmed in culture supernatants. The average age of the young age group in this work was 43 compared to the average age of 65 in the older group with no KTR < 30 years old included. In my earlier data, most of the differences were between the very young and older cohorts which might affect some of the findings. There were only 3 KTR who were frail according to the frailty classification made earlier in this work, therefore, comparing frail and non-frail was underpowered. Thus, I re-classified the cohorts into non-frail and moderately frail to permit making a better comparison.

Finally, this work was not prospective, thus B cell phenotypes and regulatory profiles might not truly predict the outcome as cytokine profiles could potentially be affected by induction and maintenance of immunosuppressive agents.

I would propose investigating B cell phenotype and expression of IL-10 and TNF after a short period of stimulation to minimize changes in B cell phenotype and improve cell viability. I also propose to do the investigation in elderly transplant recipients from high immunological risk groups in a longitudinal study to see if testing B cell subsets before transplantation truly predicts outcomes.

6.2. Summary of planned and potential future work

-Prospective and longitudinal trial to validate the clinical frailty scale used in our unit, in our kidney transplant recipient's cohort

- Investigate whether B cell subsets and their cytokines differ in extreme age and frailty to get true predictive value and help personalize the immunosuppressive therapy

- Investigate using anti-TNF therapy in vitro can reset B cells' regulatory function in high-risk groups (highly sensitized)

6.3. Concluding remarks

Transplantation is an effective treatment for end-stage kidney disease, the incidence of which increases with age. It improves both survival and quality of life for older individuals, and is economically beneficial, but is also associated with morbidity consequent to the necessary pharmacological manipulation of the immune system, including infection and malignancy. The work in this thesis added to the existing evidence that older transplant recipients (> 60 years old) are at significantly increased risk of viral infections, particularly cytomegalovirus (CMV), post-transplantation. This can lead to increased frequency of hospitalization, frailty, and increased mortality. We believe that reduced responsiveness of the older immune system may be responsible, and that tailored reduction in immunosuppression (IS) would mitigate these complications, improving outcomes for older transplant recipients. However, reducing immunosuppression or personalizing immunosuppression in older adults should be guided by evidence of clinical or laboratory parameters. These parameters or biomarkers could create the potential development of novel measures of immune status that could be more valuable or informative in older adults. Thus, in this thesis, I demonstrated that frailty predicts infectious outcomes, particularly CMV and hospitalization. Frailty before transplantation can then be used as an important clinical parameter to guide reduced immunosuppression. These biomarkers and parameters may provide the beginning of personalized immunosuppression in older KTR but need to be validated in larger cohorts.

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Appendix

Appendix 1: Occurrence of the Individual Deficits and Their Odds Ratios for 10-Year Death[91]

	%, Odds Ratio (95% Confidence Interval) *P*-Value	
Variable Description	**Men (1,073)**	**Women (1,667)**
Has long-term disability or handicaps	32.1, 2.37 (1.86–3.01) <.001	30.6, 1.34 (1.12–1.61).002
Restriction of activities	40.4, 2.29 (1.85–2.83) <.001	41.5, 1.21 (1.03–1.41).02
Needs help for preparing meals	6.3, 8.57 (3.92–18.75) <.001	7.8, 4.22 (2.70–6.58) <.001
Needs help for shopping for necessities	9.1, 5.79 (3.28–10.20) <.001	16.7, 3.16 (2.38–4.20) <.001
Needs help for house work	10.3, 6.79 (3.87–11.89) <.001	17.3, 1.93 (1.51–2.49) <.001
Needs help for heavy household chores	21.9, 4.11 (2.96–5.72) <.001	35.7, 1.63 (1.37–1.94) <.001
Needs help for personal care	4.6, 15.00 (4.66–48.27) <.001	5.2, 4.61 (2.64–8.06) <.001
Needs help moving about inside house	2.5, 11.50 (2.71–48.78) .001	2.6, 6.18 (2.61–14.66) <.001
Has arthritis or rheumatism	35.5, 1.55 (1.25–1.92) .01	48.8, 0.95 (0.79–1.06) .23
Has high blood pressure	23.7, 1.48 (1.14–1.92) .003	33.6, 0.88 (0.74–1.05) .15
Has chronic bronchitis or emphysema	8.3, 2.25 (1.76–4.64) <.001	6.1, 1.25 (0.82–1.89) .30
Has diabetes mellitus	11.7, 1.79 (1.24–2.59) .07	9.5, 1.92 (1.37–2.69) .001
Has heart disease	18.7, 2.38 (1.75–3.24) <.001	16.5, 1.46 (1.13–1.89) .004
Has cancer	5.1, 3.07 (1.64–5.73) <.001	5.4, 1.50 (1.07–2.10) .007
Has stomach or intestinal ulcers	5.6, 1.90 (1.10–3.26) .02	5.2, 1.19 (0.77–1.84) .44
Suffers from the effect of stroke	3.9, 5.65 (2.37–13.43) <.001	3.5, 1.54 (0.90–2.64) .11
Suffers from urinary incontinence	3.1, 2.49 (1.10–5.65) .29	4.5, 1.53 (0.95–2.47) .08
Has migraine headache	2.9, 1.89 (0.88–4.07) .10	5.1, 0.95 (0.60–1.49) .81
Has cataracts	9.6, 2.09 (1.37–3.18) .001	18.3, 1.23 (0.97–1.56) .09
Has glaucoma	3.4, 1.40 (0.72–2.71) .32	5.2, 1.39 (0.89–2.18) .15
Has other medical conditions	8.2, 1.42 (0.91–2.22) .12	8.5, 0.94 (0.66–1.33) .72
Have no regular physical exercise	48.8, 1.77 (1.46–2.14) <.001	55.7, 1.13 (0.98–1.30) .08
Has vision problem	5.6, 1.87 (1.08–3.23) .03	9.4, 2.01 (1.42–2.85) <.001
Has hearing problem	8.3, 2.75 (1.70–4.46) <.001	7.0, 1.43 (0.97–2.11) .07
Feeling hopeless	3.8, 2.19 (1.06–4.56) .04	6.2, 1.65 (1.08–2.54) .02
Has dexterity problem	2.9, 5.52 (1.86–16.35) .02	2.8, 2.01 (1.07–4.00) .03
Has emotional problem	4.3, 2.05 (1.08–3.91) .03	4.0, 1.56 (0.89–2.56) .12
Has memory problem	34.2, 1.80 (1.44–2.24) <.001	33.8, 0.85 (0.71–1.01) .06
Has bodily pain	26.4, 1.78 (1.38–2.31) .01	29.5, 1.04 (0.87–1.26) .66
Has speech problem	2.1, 3.29 (1.20–9.04) .02	1.5, 0.71 (0.32–1.60) .41
Taking 5 or more medications	10.6, 4.61 (2.74–7.76) <.001	13.0, 1.53 (1.13–1.53) .006
Has difficulty carrying or lifting light loads	30.3, 2.41 (1.89–3.07) <.001	31.7, 1.52 (1.26–1.82) <.001
Mobility problem	13.0, 4.98 (3.16–7.84) <.001	17.1, 2.34 (1.80–3.05) <.001
Has limited kind or amount of activity	30.2, 2.58 (2.01–3.30) <.001	33.7, 1.27 (1.07–1.51) .007
Feels tired all the time	8.4, 1.63 (1.09–3.31) .03	7.4, 1.58 (1.23–2.80) <.001
Weight loss	4.3, 2.15 (0.59–3.80) .48	3.9, 1.42 (1.15–2.46) <.001

Appendix2: Fried Frailty

Criteria used to define Fried Frailty [92]

Weight loss: “In the last year, have you lost more than 10 pounds unintentionally (i.e., not due to dieting or exercise)?

” If yes, then frail for weight loss criterion. At follow-up, weight loss was calculated as:(Weight in previous year – current measured weight)/ (weight in previous year) =K.

If K ≥ 0.05 and the subject does not report that he/she was trying to lose weight (i.e., unintentional weight loss of at least 5% of previous year’s body weight), then frail for weight loss =Yes.

• Exhaustion: Using the CES–D Depression Scale, the following two statements are read. (a) I felt that everything I did was an effort; (b) I could not get going. The question is asked “How often in the last week did you feel this way?” 0 =rarely or none of the time (<1 day), 1 +some or a little of the time (1–2 days), 2 = a moderate amount of the time (3–4 days), or 3 =most of the time. Subjects answering “2” or “3” to either of these questions are categorized as frail by the exhaustion criterion.

• Physical Activity: Based on the short version of the Minnesota Leisure Time Activity questionnaire, asking about walking, chores (moderately strenuous), mowing the lawn, raking, gardening, hiking, jogging, biking, exercise cycling, dancing, aerobics, bowling, golf, singles tennis, doubles tennis, racquetball, calisthenics, swimming. Kcals per week expended are calculated using standardized algorithm. This variable is stratified by gender.

Men: Those with Kcals of physical activity per week <383 are frail.

Women: Those with Kcals per week <270 are frail.

• Walk Time, stratified by gender and height (gender-specific cutoff a medium height).

Men	Cutoff for Time to Walk 15 feet criterion for frailty
Height ≤173 cm	≥7 seconds
Height > 173 cm	≥6 seconds
Women	
Height ≤159 cm	≥7 seconds
Height > 159 cm	≥6 seconds
Grip Strength, stratified by gender and body mass index (BMI) quartiles:	Cutoff for grip strength (Kg) criterion for frailty
Men	
BMI ≤ 24	≤29
BMI 24.1–26	≤30
BMI 26.1–28	≤30
BMI > 28	≤32
Women	
BMI ≤ 23	≤17
BMI 23.1–26	≤17.3
BMI 26.1–29	<18
BMI > 29	≤21

Appendix 3: Example of Frailty index[94].

Part A Determinants of frailty		
1. Which sex are you?	0 male	0 female
2. What is your age?	.....years	
3. What is your marital status?	0 married/living with partner	
	0 unmarried	
	0 separated/divorced	
	0 widow/widower	
4. In which country were you born?	0 The Netherlands	
	0 Former Dutch East Indies	
	0 Suriname	
	0 Netherlands Antilles	
	0 Turkey	
	0 Morocco	
	0 Other, namely.	
5. What is the highest level of education you have completed?	0 none or primary education	
	0 secondary education	
	0 higher professional or university education	
6. Which category indicates your net monthly household income?	0 €600 ($742) or less	
	0 €601– €900 ($744 – $1114)	
	0 €901– €1200 ($1115 – $1485)	
	0 €1201– €1500 ($1486 – $1856)	
	0 €1501– €1800 ($1857 – $2227)	
	0 €1801– €2100 ($2228 – $2598)	
	0 €2101 ($2600) or more	
7. Overall, how healthy would you say your lifestyle is?	0 healthy	
	0 not healthy, not unhealthy	
	0 unhealthy	
8. Do you have two or more diseases and/or chronic disorders?	0 yes	0 no
9. Have you experienced one or more of the following events during the past year?		
- the death of a loved one	0 yes	0 no
- a serious illness yourself	0 yes	0 no
- a serious illness in a loved one	0 yes	0 no
- a divorce or ending of an important intimate relationship	0 yes	0 no
- a traffic accident	0 yes	0 no
- a crime	0 yes	0 no
10. Are you satisfied with your home living environment?	0 yes	0 no

Part B Components of frailty			
B1 Physical components			
11. Do you feel physically healthy?	0 yes		0 no
12. Have you lost a lot of weight recently without wishing to do so? *('a lot' is: 6 kg or more during the last six months, or 3 kg or more during the last month)*	0 yes		0 no
Do you experience problems in your daily life due to:			
13. .....difficulty in walking?	0 yes		0 no
14. .....difficulty maintaining your balance?	0 yes		0 no
15. .....poor hearing?	0 yes		0 no
16. .....poor vision?	0 yes		0 no
17. .....lack of strength in your hands?	0 yes		0 no
18. .....physical tiredness?	0 yes		0 no
B2 Psychological components			
19. Do you have problems with your memory?	0 yes	0 sometimes	0 no
20. Have you felt down during the last month?	0 yes	0 sometimes	0 no
21. Have you felt nervous or anxious during the last month?	0 yes	0 sometimes	0 no
22. Are you able to cope with problems well?	0 yes		0 no
B3 Social components			
23. Do you live alone?	0 yes		0 no
24. Do you sometimes miss having people around you?	0 yes	0 sometimes	0 no
25. Do you receive enough support from other people?	0 yes		0 no

The TFI was translated into English using the method of back-translation.

Scoring Part B Components of frailty (range: 0–15)

Question 11:	yes = 0, no = 1
Question 12–18:	no = 0, yes = 1
Question 19:	no and sometimes = 0, yes = 1
Question 20 and 21:	no = 0, yes and sometimes = 1
Question 22:	yes = 0, no = 1
Question 23:	no = 0, yes = 1
Question 24:	no = 0, yes and sometimes = 1
Question 25:	yes = 0, no = 1

Appendix 4: Edmonton Frail Scale[96].

The Edmonton Frail Scale:				Score: ___/17
Frailty domain	Item	0 point	1 point	2 points
Cognition	Please imagine that this pre-drawn circle is a clock. I would like you to place the numbers in the correct positions then place the hands to indicate a time of 'ten after eleven'	No errors	Minor spacing errors	Other errors
General health status	In the past year, how many times have you been admitted to a hospital?	0	1–2	≥2
	In general, how would you describe your health?	'Excellent', 'Very good', 'Good'	'Fair'	'Poor'
Functional independence	With how many of the following activities do you require help? (meal preparation, shopping, transportation, telephone, housekeeping, laundry, managing money, taking medications)	0–1	2–4	5–8
Social support	When you need help, can you count on someone who is willing and able to meet your needs?	Always	Sometimes	Never
Medication use	Do you use five or more different prescription medications on a regular basis?	No	Yes	
	At times, do you forget to take your prescription medications?	No	Yes	
Nutrition	Have you recently lost weight such that your clothing has become looser?	No	Yes	
Mood	Do you often feel sad or depressed?	No	Yes	
Continence	Do you have a problem with losing control of urine when you don't want to?	No	Yes	
Functional performance	I would like you to sit in this chair with your back and arms resting. Then, when I say 'GO', please stand up and walk at a safe and comfortable pace to the mark on the floor (approximately 3 m away), return to the chair and sit down'	0–10 s	11–20 s	One of >20 s patient unwilling, or requires assistance
Totals	Final score is the sum of column totals			

Appendix 5: list of patient B cell subsets and their associated cytokine profiles

Age	Frailty score	CMV	Gender	IL-10/TNF-a ratio of CD38hiCD24hi	IL-10/TNF-a ratio of CD38interCD24inter	IL-10/TNF-a ratio of CD24hiCD27+	Gap between CMV infection & biomarker analysis
73	NA	0	Male	7.280	12.539	1.473	
70	1	1	Female	1.319	1.383	1.025	
70	1	0	Male	1.999	3.023	4.414	35
67	1	1	Male	1.617	.795	.734	-7
64	0	1	Male	.706	.928	.678	-56
63	1	1	Male	.870	.928	.879	
63	1	1	Male	1.223	1.147	.572	
63	1	0	Female	1.111	4.327	.000	190
63	NA	0	Male	.000	1.214	.987	-14
61	1	0	Female	5.938	89.231	4.542	
59	NA	1	Male	1.499	2.287	2.776	-21
57	0	0	Female	1.052	1.171	1.272	
56	0	0	Male	1.144	.977	.313	
55	0	0	Male	1.000	.980	.846	
53	0	0	Male	1.714	1.429	1.000	-42
53	0	0	Male	1.385	1.026	1.052	
52	0	0	Male	6.212	8.259	11.809	
45	1	0	Male	.144	.236	2.003	
43	0	1	Male	3.014	2.179	1.889	
43	0	0	Male	3.000	.950	1.453	-38
39	1	0	Female	.677	1.412	3.545	
35	0	0	Male	1.584	.778	.399	
31	0	0	Male	7.987	.855	3.709	