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CYTOMEGALOVIRUS (CMV) DISEASE IN RENAL ALLOGRAFT RECIPIENTS IN THE NEW IMMUNOSUPPRESSION ERA

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ABSTRACT

Despite aggressive prophylaxis with antivirals, CMV infection remains a major complication of immunosuppression in renal transplantation with remarkable health and economic impacts. The incidence of new and recurrent CMV disease in adult renal transplant recipients at a single tertiary care hospital were studied, and multivariable analyses conducted to identify major predictors for CMV disease in the current immunosuppression era. Patients transplanted between January 1, 1999 and December 31, 2002 were included and followed prospectively until December 31, 2006. The primary end point was development of CMV disease and the incidence was 14.6% (95% CI, 11.7–18%). None had recurrent CMV disease. Using multivariable analysis, factors associated with increased risk of developing CMV disease were CMV sero-status and positive B-cell cross match at time of transplantation. Patients with a positive B-cell cross-match had a 3 times greater associated risk for developing CMV disease than those with a negative cross-match (OR = 3.23, 95% Confidence Interval, 1.16 – 9.0, p = 0.025). This association has not been previously reported and should be considered when identifying risks and complications with patients.

Keywords:

Cytomegalovirus, CMV, CMV disease, Kidney Transplant, Multivariable analysis, Regression analysis, Immunosuppression, predictors.

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ALG	Anti-Lymphocyte Globulin
APD	Automated Peritoneal Dialysis
AR	Acute Rejection
ATG	Anti-Thymocyte Globulin
BPAR	Biopsy Proven Acute Rejection
CAPD	Chronic Ambulatory Peritoneal Dialysis
CIHI	Canadian Institute of Health Information
CKD	Chronic Kidney Disease
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitors
CORR	Canadian Organ Replacement Register
CRF	Chronic Renal Failure
CS	Corticosteroids
CsA	Cyclosporine A
DGF	Delayed Graft Function
EBV	Epstein Barr Virus
ESRD	End Stage Renal Disease
FK506	Tacrolimus
GFR	Glomerular Filtration Rate
HD	Hemodialysis
HLA	Human Leukocyte Antigen

Ig	Immunoglobulin
IS	Immunosuppression
IVIG	Intravenous Immunoglobulin
KDIGO	Kidney Disease: Improving Global Outcomes
LHSC-UH	London Health Sciences Center-University Hospital
MMF	Mycophenolate Mofitil
NKF	National Kidney Foundation
OKT3	Orthoclone
OPTN	Organ Procurement and Transplantation Network
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PD	Peritoneal Dialysis
PRA	Panel Reactive Antibody
QOL	Quality of Life
RRT	Renal Replacement Therapy
SRTR	Scientific Registry of Transplant Recipients
USRDS	United States Renal Database System

INTRODUCTION

The objective of this thesis is to evaluate the incidence and predictors of Cytomegalovirus (CMV) disease among renal transplant recipients, in the era of immunosuppression between 1999 - 2006.

The aim of the first four chapters is to present a summary of published literature related to the thesis' topic. Each of these chapters deals with a specific sub-topic. These subtopics include review of the Epidemiology and Burden of Illness in Chronic Kidney Disease and Renal Transplantation for End Stage Renal Disease (Chapter 1), Immunosuppression for Kidney Transplantation (Chapter 2), and Cytomegalovirus Infection and Kidney Transplantation (chapter 3). These are followed by a review on Multivariable analysis (Chapter 4), the main analytical method utilized in the study.

Literature review chapters are followed by Chapter 5, which presents the study rationale and objectives. Chapter 6 presents the study methods. Results are presented in Chapter 7. Chapter 8 is the final chapter and it presents the discussion on the study results, limitations, and future directions.

CHAPTER 1:

CHAPTER 2: Epidemiology and Burden of illness in Chronic Kidney Disease

Chronic Kidney Disease (CKD) is a major global health problem. As of the 2006, according to Coresh et al., it is estimated that 20 million people have CKD in the United States[1]. The consequences of developing CKD are now well recognized. In addition to complications that include anemia, hypertension, and abnormalities of mineral metabolism, patients with CKD have been shown to have an increased risk for cardiovascular disease and associated mortality[2, 3].

1.1 Definition and Classification of CKD

In February 2002 the National Kidney Foundation (NKF), one of the major international voluntary health organizations promoting renal care, published its first guidelines, which included the definition and classification of kidney diseases[4]. Since then the definition and classifications have been widely discussed and largely adopted in research and practice communities[5]. The NKF classification system of CKD was recently endorsed in a position statement by the Kidney Disease: Improving Global Outcomes (KDIGO) group, a newly formed and independent organization dedicated to the improvement of care of kidney disease patients worldwide[6].

CKD is defined as either kidney damage or a decrease in the estimated Glomerular Filtration Rate (GFR) of <60 ml/min/1.73m^2 for 3 or more months. Kidney damage is defined as pathologic abnormalities of markers of damage, including abnormalities in blood or urine tests or imaging studies. APPENDIX I lists criteria used to define CKD. Regardless of the underlying etiology, any loss or damage of renal mass will potentially result in progressive decline in GFR.

1.2 Prevalence of CKD

In the US the prevalence of CKD has shown a remarkable growth. A recent analysis of a representative sample of the US population revealed an ongoing increase in the prevalence rate of CKD. These rates rose from 10% (95% CI, 9.1-10.9), for 1988-1994 estimates, to 13.1% (95% CI, 12.0-14.1), for 2000-2004 estimates. The authors concluded that the increasing prevalence of CKD is likely related to the increase in prevalence of diabetes mellitus and hypertension in the general population [1, 7].

1.3 Complications of CKD

CKD is a major cause of cause of morbidity and mortality. Complications associated with it include cardiovascular diseases and the associated increase in mortality, and increased hospitalization; among many others.

1.1.1 Cardiovascular diseases

Cardiovascular diseases are the most important of these. When compared to non-CKD patients, the overall incidence of cardiovascular disease in patients with CKD is more than twice as high[2, 8-11]. In fact, cardiovascular diseases are established as the most common cause of mortality in CKD patients in the developed world[9, 10]. The clear and strong association between CKD and cardiovascular events has lead to listing CKD as an independent risk factor for cardiovascular disease in reports from major organizations, such as the Joint National Committee on Prevention, Detection, and Treatment of High Blood Pressure (JNC VII) and the American Heart Association (AHA)[12, 13].

1.1.2 Hospitalization

In both the CKD and non-CKD populations, hospitalization rates are highest in patients with both diabetes and congestive heart failure (CHF). In the US the general dialysis population has 2 hospital admissions per patient per year.

Patients who have a kidney transplant have an average of 1 hospital admission per year[8, 14, 15].

1.1.3 Mortality

Due to the high rate of complications related to CKD it is now well established that the survival rate of CKD population is lower than it in the general population. The 5-year survival rate for a patient undergoing chronic dialysis in the US is approximately 35%; it is approximately 25% for patients with diabetes mellitus[9-11].

Go et al. conducted a longitudinal follow up of over 1 million CKD patients not requiring dialysis. Multivariable analyses of predictors of different outcomes revealed an independent, graded association between lower levels of the estimated GFR and the risks of death, cardiovascular events, and hospitalization. The adjusted hazard ratio for death was 1.2 with an estimated GFR of 45 to 59 ml per minute per 1.73 m^2 (95% CI, 1.1 to 1.2), 1.8 with an estimated GFR of 30 to 44 ml per minute per 1.73 m^2 (95 % CI, 1.7 to 1.9), 3.2 with an estimated GFR of 15 to 29 ml per minute per 1.73 m^2 (95% CI, 3.1 to 3.4), and 5.9 with an estimated GFR of less than 15 ml per minute per 1.73 m^2 (95% CI, 5.4 to 6.5)[8] .

1.4 End-Stage Renal Disease (stage 5 CKD)

End stage renal disease (ESRD) is defined as the stage of progressive renal failure when renal replacement therapy (RRT), such as dialysis or transplantation becomes necessary. "End stage" refers to the end of the kidney function. For most patients with progressive CKD, the decision to start dialysis is based on a combination of uremic symptoms and laboratory parameters. These symptoms include nausea, vomiting, anorexia, unexplained weight loss, development of malnutrition, decrease in mentation, changes in sleeping patterns, peripheral neuropathy, restless leg syndrome, and pruritus [16]. The presence of these signs and symptoms significantly affect the patient's quality of

life (QOL). There is usually 5-10% of the kidney function remaining when patients start RRT [17].

1.4.1 Epidemiology of End Stage Renal Disease

Much of our epidemiological information comes from the United States of America due the completeness of their ESRD registry data. The United States Renal Data System (USRDS) is a national data system that collects, analyzes, and distributes information about ESRD in the United States. The USRDS contains data on over 93% of all patients treated for ESRD in the United States[18]. Submission of this information is mandatory and linked to reimbursement for patients who are covered by Medicare, who comprise the majority of ESRD patients. The Canadian Organ Replacement Register (CORR) data is submitted on a voluntary basis and it includes 93.3% of all the patients treated for ESRD in Canada[19].

1.4.2 The USRDS Data Base

The USRDS is funded directly by the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) in conjunction with the Health Care Financing Administration (HCFA). HCFA provides most of the existing data in the USRDS database. This national data system collects, analyzes, and distributes information about ESRD in the United States. It includes comprehensive data needed to describe the incidence and prevalence of treated ESRD, modality of treatment, cause of death, patient survival, hospitalization, cost and cost effectiveness, and institution providers of ESRD treatment. The University of Michigan, Ann Arbor was the coordinating center for the USRDS at the time of this study.

1.4.3 The CORR Data Base

The Canadian Organ Replacement Register (CORR) at the Canadian Institute of Health Information (CIHI) is a national information system on organ failure and transplantation, with a mandate to record and analyze the level of activity and outcome of vital organ transplantation and renal dialysis activities, information is collected from a number of sources including 26 transplant hospitals, 86 dialysis facilities and 8 organ procurement organizations. The most recent data available is from 1994 to 2004, reported in the 2006 CORR annual report[19].

1.5 Incidence and Prevalence of ESRD

1.5.1 Definition of Incidence and Prevalence

Incidence refers to new cases of ESRD during a given time period and is a key population measure of kidney disease and access to renal replacement therapy. Prevalence refers to all patients receiving ESRD treatment at a particular time (point prevalence) or during a given time period (period prevalence) and is a population measure of disease burden and resource requirements. Prevalence is determined by incidence and patient life expectancy.

1.5.2 Measuring Incidence and Prevalence of ESRD

ESRD is defined by treatment with any form at chronic dialysis or renal transplantation. Patients who die of renal failure without first receiving dialysis or a transplant are not considered ESRD patients. Dialysis for acute renal failure is not considered ESRD unless renal function fails to recover. As a practical matter, the degree of renal failure or the reason for initiation of dialysis does not impact the ESRD classification. Most prevalence statistics reported by the USRDS and CORR refer to point prevalence. Prevalence is a direct function, of incidence and survival. Prevalence rates are on average four to five times higher than incidence rates because the average survival time is four to five

years for ESRD patients. Changes in prevalence are attributable to changes in incidence and survival. Patients who return to dialysis after a failed transplant are not counted as incident ESRD patients. This situation is classified as a modality change. Similarly, patients who stop chronic dialysis and then restart are counted as prevalent, not incident patients. In the USRDS, patients are maintained in the ESRD database until death. Incidence and prevalence will be referred to as rates; incidence is expressed as rate (number per million population per year), while prevalence is expressed as a proportion (number per million population).

Both the USRDS and the CORR databases adjust incidence and prevalence rates to a reference population (for age, gender, and race) using a direct method, use of an adjusted rate accounts for growth and aging of the general population and permits meaningful comparisons across years. In other words, the adjusted rate assumes a constant reference population. This classic approach is used because it is often difficult to define the true at-risk population for a specific disease.

1.5.3 Incidence and Prevalence of ESRD

The ESRD program in the United States has grown from approximately 10,000 beneficiaries in 1973, when the Medicare entitlement became effective, to 86,354 in 1983 to 484,693 patients as of Dec 31, 2005. The incidence rate was 287 per million per year. The annual percent increase was near 10% at the start of the decade and has fallen to 2% increase in 2005. The prevalence rate was 1105 per million population, or 1 in every 1000 persons is receiving RRT as of December 31, 1997. Prevalence growth rates provide important information for determining future ESRD resource needs and it has risen every year, it has more than tripled since 1988. Adjusted prevalence of ESRD reached 1,569 in 2005, 1.4 times greater than in 1995. Annual growth in the rate, however, has slowed, and has been 3.0 percent or lower since 2001. Most of the change in prevalence rates is due to change in incidence rates because death rates have been

comparatively stable. By 2020, the incident population is projected to grow to 150,000. It is also been estimated that a prevalent population nearing 800,000, including a dialysis population of 534,000[18].

In Canada, the rate of incident renal replacement therapy rose 41%, from 112 per million population in 1995 to 158 per million population in 2004. At the end of 2004, there were 18,827 patients on dialysis and 12,099 living with a functioning kidney transplant, for a total of 30,924 Canadians with end-stage renal disease registered in CORR[19].

1.5.4 Burden of Illness in ESRD

1.5.4.1 Morbidity in the ESRD Population

Patients with ESRD experience significantly greater morbidity, including a substantial decline in QOL compared to aged-matched controls[20]. The frequency and duration of hospitalization has been used as a measure of QOL because of the Impact that it can have on the lifestyle of patients[21].

According to the USRDS data, all-cause hospitalizations in patients age 20–44 and those 75 and older have been nearly equal since the beginning of the decade, at 2.2 admissions per patient year. Admissions for cardiovascular disease, in contrast, rise with age, while the youngest patients have the highest rates of admission for infection due to internal devices. The average number of hospital days per year was 12 for ESRD patients[18].

1.5.4.2 Mortality in the End-Stage Renal Disease population

The availability of renal replacement therapy (RRT) has allowed the survival of patient with ESRD, previously a fatal illness. Despite improvement in the overall quality of dialysis therapy, the mortality among dialysis patients remains high. The expected lifetime of dialysis patients is 16% to 37% that of the age-, gender-, and race-matched US population. As an example, the mean expected remaining life span is only 9.3 years for a person beginning dialysis at

40 and 4.3 years for a person beginning dialysis at 59[18] . These values in older patients are only slightly better than those in patients with lung cancer, but much worse than the general population (37.4 and 20.4 years at 40 and 59 years respectively).

1.5.4.3 Cause of Death in the End-Stage Renal Disease Population

There are three major causes of death in dialysis patients: cardiovascular disease, accounting for approximately 50% of cases, infection, accounting for 15-20%, and withdrawal from dialysis, accounting for 5-10%. While a decline in cardiovascular death has recently occurred in the general population, a similar trend has not been seen in the dialysis patient. This may be due to the high prevalence of co-morbid conditions the ability of dialysis to fully replace the functions of the native kidney, and adverse consequences or side effects of RRT. The average age, of ESRD patients is over 60 years and approximately 16% are over 70 years; and many have underlying cardiac disease [22]. It is estimated that only 27% of patients about to enter the dialysis regimen have a normal echocardiogram, while 19% already have severe left ventricular hypertrophy[23, 24].

Coronary artery disease is very common among patients on dialysis and factors that promote the develop of coronary disease and cardiovascular mortality include hypertension, which is present in approximately 80% of patients at the onset of dialysis, left ventricular hypertrophy, due both to hypertension and chronic anemia, and possibly hyperlipidemia, as the most predominant abnormality in maintenance dialysis is hypertriglyceridernia. Several factors have been associated with increased mortality. These include dialysis time, dialysis clearance or dose, RRF, type of diaiyzer, fluid balance, malnutrition, mode of dialysis and calcium-phosphate product[25].

1.5.4.4 Economic Costs of End-Stage Renal Disease

Total expenditure for ESRD patients in the United States has increased dramatically, as a result of the growing patient population and the increasing cost of treating older and sicker patients[18]. Information on ESRD cost is available through Medicare payments on per patient year at risk by treatment modality costs. The estimated total US ESRD costs in 1997 was 15.64 billion dollars, reflecting Medicare and non-Medicare payments. In 2005, total Medicare spending per year at risk for dialysis patients averaged $65,406 per year. ESRD costs rose to $21 billion, 6.4 percent of the entire Medicare budget[18]. Total Medicare costs related to ESRD are projected to approach $54 billion by 2020. In the 2000 ESRD budget was estimated to be $28 billion by 2010, and actual numbers have been ahead of these projections[26].

The rate of incident renal replacement therapy rose 41% in Canada, from 112 per million population in 1995 to 158 per million population in 2004. At the end of 2004, there were 18,827 patients on dialysis and 12,099 living with a functioning kidney transplant, for a total of 30,924 Canadians with end-stage renal disease registered in CORR[19]. Zelmer recently conducted a study to measures the economic burden of ESRD in 2000. The total expenditure on ESRD in Canada in 2000 was $1.9 billion. Direct and indirect (morbidity and mortality) costs were 69% and 31%, respectively[27].

1.5.5 Modalities of renal replacement therapy (RRT)

The modalities of RRT available for treatment of ESRD include hemodialysis (HD) and peritoneal dialysis (PD) and renal transplantation. Hemodialysis is subdivided into in-centre provided HD, the most commonly used modality, self-care and home hemodialysis. The majority of PD comprises continuous ambulatory PD (CAPD), and automated PD (APD).

1.5.5.1 Hemodialysis

Hemodialysis removes toxins and excess fluid via extracorporeal circulation of blood through a dialyzer, or so-called "artificial kidney". Treatments are usually scheduled for three times weekly and last three to five hours. A vascular access is required, using an arterio-venous (AV) fistula, an A-v graft, or in-dwelling vascular catheter. The treatment is performed predominantly as In-centre HD in a hospital based dialysis unit.

1.5.5.2 Peritoneal Dialysis

Peritoneal dialysis uses the patient's own peritoneal membrane as a "dialyzer". It requires placement of a catheter into the abdominal cavity, and repeated instillation and drainage of sterile dialysate. PD involves the movement of small salutes and water across the semi-permeable membrane. Toxins move from the plasma to the dialysate due to concentration gradients during the dwell time while other solutes (e.g. calcium and lactate) move in the opposite direction, fluid is removed by osmotic ultrafiltration using hypertonic glucose containing dialysate solutions. The rate of movement of small solutes, such as creatinine, between blood and dialysate differs from one patient to another and this peritoneal function characteristic is quantified in the peritoneal equilibrium test (PET). Using this test, each patient's peritoneal membrane can be categorized as having a high, high average, low average, or low peritoneal transport characteristics. Patients with high peritoneal transport have rapid clearance of small molecules, but poor ultrafiltration due to dissipation of the osmotic gradient between the dialysate and the blood by glucose absorption. Patients with low transport ultrafiltrate well but have slow equilibration requiring the continuous presence of larger dwell volumes in the peritoneal cavity[28].

Several PD options are available. The most common is continuous ambulatory PD (CAPD). The patient usually performs four or five exchanges with a dialysate volume of two to three liters on a daily basis. Automated PD (APD) includes exchanges with the use of a programmed machine cycler and includes

continuous cycling PD (CCPD), a home treatment utilizing several exchanges through a programmed machine cycler, typically every night with one long dwell time throughout the day.

Hemodialysis and peritoneal dialysis have similar effects on patient outcomes. Survival of ESRD patients has improved dramatically since the discovery of dialysis.

1.5.5.3 Kidney Transplantation for ESRD

Kidney transplantation, both from living and deceased donors, provides better survival outcomes when compared to other modalities of renal replacement therapies. This has been demonstrated in a large landmark study done by Wolfe et al. in 1999 in this study, data from the USRDS. Analysis compared patients on dialysis to recipients of kidney transplants with regards to long term survival. Results from this analysis showed an unequivocal evidence of superior survival among transplant recipients[29]. Furthermore, Loubeau et al. showed a superior economic advantage for kidney transplantation over dialysis. This advantage is first seen (break-even point) 2 years and 10 months post-transplant[30]. Despite these advantages a remarkable proportion of ESRD patients remains on dialysis due to the lack of suitable kidney donors. This donor shortage is a global issue that ultimately resulted in a dramatic prolongation of waiting time for kidney transplantation[31].

1.5.5.4 Historical Overview

Transplantation was one of the major achievements in the field of medicine in the 20th century. The first successful kidney transplant was performed in 1954 by Dr Joseph Murray and his colleagues at Peter Bent Brigham Hospital in Boston; from an adult male to his twin brother. Since the twins were identical, no immunosuppression was needed[32-35]. This success was preceded with extensive bench and clinical work by scientists that lasted over 50 years before it became reality.

During the 1960s, there have been remarkable advances in the field of kidney transplantation. In 1962 the first successful kidney transplantation from a deceased donor was performed, nearly 3 decades after the first (failed) attempt at kidney transplantation in the 1930s[36]. Early attempts to control the immune response to foreign tissue involved total body irradiation[37, 38]. These attempts together with use of immunosuppressive drugs resulted in the advancement of transplantation one step further. In 1959, two transplant recipients of kidneys from non-identical twins received total body irradiation with successful outcomes[39, 40]. Another major step along the success path was made in 1960, when 6-mercaptopurine was used successfully to reverse acute rejection[41, 42].

In the following three decades efforts were geared toward developing new techniques, new immunosuppressive medications[43, 44]. These efforts resulted in tremendous progress in the field of transplantation.

Table 1: Important landmarks in the history of kidney transplantation.

Year	Name of Surgeon & Place	Type of Surgery
1906	Jaboulay, France	First kidney transplant from an animal to a human.
1908	Carrel, USA	First kidney transplant in the canine model with long-term graft survival.
1936	Voronoy. Russia	First unsuccessful kidney transplant between humans.
1954	Murray JE, USA	First successful kidney transplant in humans between identical twins.
1958	Murray JE. USA	First kidney transplant in humans using immunosuppression.
1959	Murray JE. USA	First successful kidney transplant between dizygotic twins.
1960	Kuss. France	First successful kidney transplant between nun-twin siblings.
1961	Kuss. France	First kidney transplant between non-siblings.
1962	Murray JE, USA	First successful cadaver kidney transplant using immunosuppression.

1.5.5.5 Current Data on Kidney Transplantation

The US Organ Procurement and Transplantation Network (OPTN) is an organization developed by the government to ensure the success and efficiency of the US organ transplant system. One of OPTN's responsibilities is to provide data to the Scientific Registry of Transplant Recipients (SRTR), which supports the ongoing evaluation of scientific and clinical status of solid organ transplantation in the US. According to SRTR data, a total of 16,477 kidney transplants have been performed during the year 2005. These included 9,914 (60%) transplants from deceased donors and 6,563 (40%) transplants from living donors[45].

In Canada, a total of 10,109 kidney transplants have been performed between 1995 and 2004; 65% of which were from deceased donors. The total number of kidney transplants in the year 2004 was 1,112. In London Ontario, the first kidney transplant was performed at Victoria hospital in the late 1950s. Between 1973 and 2005, a total of 1,720 kidney transplants have been performed in London Ontario[19].

1.5.6 Summary

CKD is a major global health problem. In addition to complications that include anemia, hypertension, and abnormalities of mineral metabolism, patients with CKD have been shown to have an increased risk for cardiovascular disease and associated mortality.

The ESRD population continues to grow in size, resulting in an enormous need for adequate renal replacement therapies in the form of dialysis and transplantation. Kidney transplantation confers a better survival and quality of life benefit over dialysis, which makes it the renal replacement therapy of choice for this population.

Kidney transplantation, both from living and deceased donors, provides better survival outcomes when compared to other modalities of renal replacement therapies. It provides a superior economic advantage for kidney transplantation over dialysis. Despite these advantages a remarkable proportion of ESRD patients remains on dialysis due to the lack of suitable kidney donors.

CHAPTER 2: Review of Immunosuppression Therapy for Kidney Transplantation

2.1 The immune response

Immune system reactions were the main obstacle to organ transplantation, resulting in the failure of earlier trials of transplantation from non-identical-twin donors. To adequately understand the immune response to transplanted tissue, it is helpful to also review the general immune response; with an emphasis upon those elements involved in the response to donor antigens. The immune response is divided into two closely interrelated components, natural and adaptive immune responses.

2.2 Natural and Adaptive Immunity

Natural or innate immunity is part of the immune system which involves both cellular and non-cellular components (macrophages, neutrophils, natural killer cells, cytokines, and complement), which are recruited to the site of infection to initiate an inflammatory response. This process does not involve recognition of specific antigens; and therefore, this type of immune response is often referred to "non-specific immune response".

The adaptive immunity, on the other hand, involves recognition of specific foreign antigens, and therefore referred to as "specific". Antigen-specific T cell activation leads to the production and secretion of cytokines and chemokines which recruit components of the "natural immune" system as well as the specific mechanisms of alloantibody production and T cell-mediated cytotoxicity.

2.2.1 Importance of T and B Cell Cross-match

In situation of organ transplantation, in the absence of immunosuppression, the final outcome of immune system activation is the

destruction of the foreign body, the transplanted organ or tissue; a process known as rejection. Recipients with preformed antibodies directed against donors' cells could result in immediate destruction of those cells, a process called "hyperacute rejection". To avoid such dire situations, patients should be tested for the presence of anti-donor antibodies in their serum, prior to transplantation. T Cell Cross match is the most commonly used test to predict the likelihood of developing hyperacute rejection. This test involves mixing of donor's cells (T and B, separately) with recipient's serum followed by the addition of complement the mix. A positive T Cell cross match is a contraindication to transplantation as it predicts the high likelihood for developing hyperacute rejection.

Positive B Cell cross match, however, is not associated with hyperacute rejection and therefore not a contraindication to kidney transplantation. The utility of positive B Cell cross match in the field of kidney transplantation is yet to be determined. Despite of this lack of understanding of its significance most transplant centers continue to perform this test in their patients prior to transplantation.

2.3 Human Leukocyte Antigen (HLA) gene complex

HLA gene complex are a group of genes in the short arm of chromosome 6 (p6) which encode for the strong transplantation proteins, class I and II antigens (HLA I and HLA II). This complex is composed of three pairs of genes designated as HLA-A, HLA-B, and HLA-DR). These proteins are antigen presenting structures that bind molecules inside the cells and display them on the cell surface for T cells to recognize, as a sign for intracellular invasion by foreign organisms. In kidney transplantation HLA molecules on the surface of donor cells play a major role in initiating an immunologic response by the recipient's immune system. The aggressiveness of the recipient immune response depends on the degree of dissimilarity (mismatch) in HLA molecules between the donor and the recipient; with 0-mismatch (or six-antigen match) being the least immunogenic.

2.4 Immunosuppression and Immunosuppression Eras

2.4.2 Importance of Immunosuppression

Acute rejection, the process by which the immune system fights foreign bodies and organisms, is the main challenge to organ transplantation. Since the advent of transplantation over 50 years ago, acute rejection has been a major challenge for short and long-term graft survival, especially in the early decades[42]. This has led to the proliferation of studies resulting in a large body of research in this field[43, 46-54]. The main focus of these studies was to identify a treatment or intervention that would reduce the rate of acute rejection and therefore prolong kidney and patient survival. Unfortunately, to-date, acute rejection remains an important post-transplant complication that seems far from being eradicated. This research, however, resulted in a remarkable improvement in acute rejection rates, which reflected positively on the long-term survival of transplanted kidneys (allografts). With current immunosuppressive regimens acute rejection rates have been decreased from over 50% in the 1970s, to approximately 10% to 15% in the late 1990s and early 2000s[55]. In most centers, 1-year kidney (graft) survival rates are 90% to 95% with most of this success being attributed the remarkable developments in the field of immunosuppression over the decades.

2.4.3 Immunosuppression eras

The work in the field of transplantation by scientists in many fields (immunology, surgery, pharmacology and others) has resulted in a constant advancement of knowledge, leading to progressive change in immunosuppressive regimens over time[55-62].

The classification of immunosuppressive eras is somewhat arbitrary, providing only an approximate outline of main drug regimens used at different time periods since the first successful kidney transplant was performed. Nonetheless, it reflects the general practices carried out by transplant professionals around the world in different time periods. Based on this and available literature there are five main eras that can be described[63]. These main

eras are (I) the early immunosuppression (pre-cyclosporine) era, extending between the early 1960s and February 1986; (II) the cyclosporine era, between February 1986 and 1992; (III) the post-Minnesota, antilymphocyte globulin era, 1992 - 1995, during which Minnesota Antilymphocyte Globulin was withdrawn; (IV) between 1995 and 1998, the new anti-proliferative agent mycophenolate mofetil (Cellcept) replaced azathioprine as a maintenance immunosuppressive agent; and (V) the current era of immunosuppression which started in 1999 and extends to 2007. Chronologically, each of the immunosuppression eras has set a new standard for the graft survival in kidney transplantation. Each of these eras was characterized by specific immunosuppressive protocols used to prevent acute rejection and sustain graft survival. During the early years of the history of kidney transplantation only a few immunosuppressive drugs were available, and therefore few options were available to clinicians to use to prevent and treat rejection. As the field was developing over time more drugs were made available to use for immunosuppression. This development elicited further research aiming at identifying which of the available drugs and combinations (regimens) provided the best outcomes. Drug regimens used in each of the five eras will be described with special emphases on the most recent, or current, era.

Corticosteroids and azathioprine were the two drugs used in the first era[56]. It is also during this era when biologic agents targeted toward T lymphocytes were introduced as part of the regimens use to treat and prevent acute rejection[64].

At the end of the 1st era (late 1970s) a new pharmacologic agent, cyclosporine, had become available and soon became a popular immunosuppressant in kidney transplantation. Numerous studies were then conducted to assess the efficacy of this new agent in improving renal allograft outcomes[57-59, 65]. The second era (II) was launched by the results of these trials which has set new standards in immunosuppression and even paved the way for non-renal solid organ transplantation (the heart and the liver)[58]. This era extended one full decade, 1982 through 1992; during this phase induction with a

short T-cell depleting (Anti Lymphocyte Globulin, ALG) course followed by maintenance therapy with cyclosporine, azathioprine and prednisone formed the standard immunosuppressive protocol.

The third era (III) began in July of 1992 when the anti T-cell monoclonal antibody Orthoclone (OKT3) replaced the polyclonal ALG as the induction agent of choice. The maintenance immunosuppression regimen consisted of a combination similar to that in era (II), i.e. cyclosporine, azathioprine and prednisone.

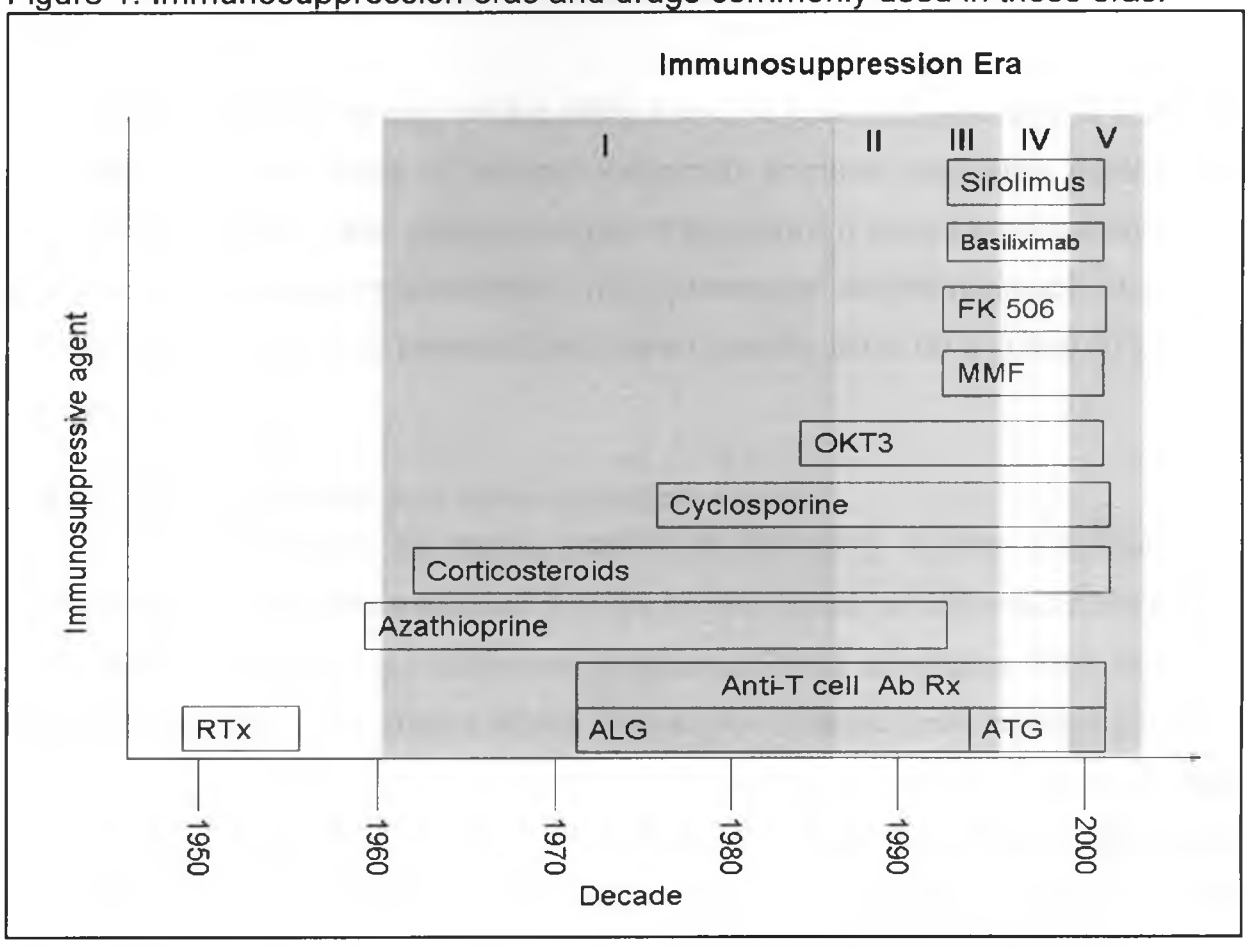
In era four (IV), 1995 to 1998, the new anti-proliferative agent mycophenolate mofetil (Cellcept) replaced azathioprine as a maintenance immunosuppressive agent. Results of large-scale trials comparing this drug with azathioprine (in regimens including cyclosporine and prednisone) showed that it was superior to azathioprine in preventing rejection of kidney transplants[66-71]. These regimens (which included mycophenolate mofetil and calcineurin inhibitors) improved patient survival and graft survival and reduced early and late allograft rejection[72, 73].

The year 1999 marks the beginning of era five (V), which represents the current era of immunosuppression. In this era, a wider variety of immunosuppressive drugs for both induction and maintenance is utilized. For induction, ALG, OKT3, or basiliximab (a monoclonal antibody directed toward the Interleukin 2 -IL2- receptors) were used for induction of immunosuppression in the early post transplant days. The choice of which agent to use for induction relied on the recipients' immunologic risk and the risk for developing delayed graft function. Patients at very low immunologic risk received no induction immuno-therapy, whereas those with high immunologic risk received OKT3 or ALG, while those with intermediate risk received the IL2 receptor monoclonal antibody.

Tacrolimus (FK 506), a calcineurin inhibitor that became popular in the late 1990s, was shown to be as effective as cyclosporine in lowering acute rejection rates and improving graft survival. Therefore, it became a part of the maintenance immunosuppression during era V. The maintenance

immunosuppression protocol in this era consists of prednisone, cellcept, and a calcineurin inhibitor (cyclosporine or tacrolimus). Other drugs were made available during this era, for example, sirolimus and everilomus. However, no regimen that utilizes these newer agents has been shown to be as effective as (nor superior to) the standard immunotherapy in the current era. A recently published study by Ekberg et al. evaluated the effect of different immunosuppressive regimens on the outcome (graft function, survival and acute rejection rates) of transplanted kidneys in the first year. Of these regimens, daclizumab, MMF, corticosteroids in combination with low-dose tacrolimus was shown to be superior to regimens containing daclizumab induction plus either low-dose cyclosporine or low-dose sirolimus or with standard-dose cyclosporine without induction[74]. Figure 1 depicts all five eras and the common immunosuppressive drugs used in these eras.

Figure 1: Immunosuppression eras and drugs commonly used in those eras.



RTx, total-body radiation therapy; OKT3, orthoclone; Ab Rx, antibody therapy; ALG, anti-lymphocyte globulin; ATG, anti-thymocyte globulin; FK 506, tacrolimus.

2.4.4 Immunosuppressive Regimens

2.4.4.1 Standard protocols

As of the year 2007, the standard immunosuppressive protocols used include induction and maintenance immunosuppression.

Induction therapy is an intensive form of immunosuppression usually given in the preoperative period (prior to transplantation, lasting a few days after the procedure) and involves a non-depleting antibody thymoglobulin (ALG), or Anti CD 25 (IL 2 receptor) antibody, daclizumab or basiliximab, which are considered non-depleting antibody agents. OKT3 has become a much less popular antibody for induction in North America, mainly because of its first dose phenomena, which

results from a profound cytokine release resulting from the antibody attack on CD3 positive cells.

Maintenance therapy, on the other hand, is less intensive and is aimed to maintain a chronic state of altered (reduced) immune response against the transplanted kidney, and therefore prevent rejection. It consists of a calcineurin inhibitor (cyclosporine or tacrolimus), mycophenolate mofetil (cellcept) at a dose 1 gram twice daily, and prednisone tapered rapidly from 60 mg/day to 5 - 7.5 mg/day.

2.4.4.2 Thymoglobulin and other depleting agents

The advantages of these lymphocyte-depleting agents are that they deplete the lymphocytes and allow a delay in introduction of calcineurin inhibitors. The delay in introducing calcineurin inhibitors (CNIs) eliminates their potential nephrotoxic effect in situations where the risk for delayed graft function (DGF) is high. In such situations depleting antibodies provide the needed immunosuppression until the kidney function starts to recover. Patients at risk for developing DGF include those whose donor cold ischemia time is greater than 24 hours, and whose donors are considered to be "expanded criteria donors".

2.4.4.3 Anti-IL2 monoclonal antibodies

Non depleting monoclonal antibodies are excellent alternative agents to depleting antibodies in reducing acute rejection. These antibodies do not deplete lymphocytes and therefore have no side effects. Unlike depleting agents and other immunosuppressants, they are not associated with malignancy. Their use allows the minimization of maintenance immunonosuppressive drugs post-transplant.

2.4.4.4 Calcineurin Inhibitors (CNIs)

2.4.4.4.1 Cyclosporine (CsA)

CsA is a prodrug that engages cyclophilin, an intracellular protein of the immunophilin family, forming a complex that then engages calcineurin. Its main adverse effects include nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, and tremor. Less common side effects of cyclosporine include hemolytic–uremic syndrome and post-transplantation diabetes mellitus.

2.4.4.4.2 Tacrolimus (FK 506)

Tacrolimus exerts its action by engaging an intracellular protein (immunophilin), FK506-binding protein 12 (FKBP12), to create a complex that inhibits calcineurin with greater molar potency than does cyclosporine. Tacrolimus resembles cyclosporine in that it can result in nephrotoxicity and the hemolytic–uremic syndrome, but it is less likely to cause hyperlipidemia, hypertension, and cosmetic problems and more likely to induce post-transplantation diabetes.

2.4.4.5 Mycophenolate Mofitil (MMF)

MMF is a prodrug that releases mycophenolic acid which inhibits inosine monophosphate dehydrogenase, a key enzyme in purine synthesis. It acts on a lymphocyte selective enzyme, IMPDH, to inhibit de novo purine biosynthesis. Because the lymphocytes have a unique requirement for high levels of de novo purine synthesis, this effect selectively suppresses lymphocyte clonal expansion.

Side effects include gastrointestinal (mainly diarrhea) and hematologic (anemia, leukopenia). Mycophenolate mofetil may increase cytomegalovirus disease but in vitro manifests antipneumocystis activity. Mycophenolate mofetil lacks the marrow toxicity of azathioprine.

2.4.5 Complications related to immunosuppression

Complications related to immunosuppression can be grouped in two main categories, general and specific. General complications result from the overall state of decreased immune response, regardless of the nature of the

immunosuppressive agent used. Data from many studies have suggested that the incidence of cancer after kidney transplantation is higher than that found in the general population. However, the incidence varies substantially by cancer type and site. Particularly common are malignancies that have a putative viral cause. Skin cancers, for example, are most common. Lymphomas also occur frequently after kidney transplantation[75].

Specific complications (discussed in the previous section) are unique to individual agents and are not related to the drug effect on the immune system. The higher incidence of diabetes mellitus associated with FK 506, diarrhea associated with MMF, and gum hypertrophy associated with CSA, are examples for the drug-specific complications related to immunosuppressive agents.

CHAPTER 3: Cytomegalovirus (CMV) Infection and Kidney Transplantation

3.1 Introduction

Cytomegalovirus, a member of the human herpesviridae family of viruses, is the most common opportunistic infection after kidney transplantation; causing about two-thirds of all febrile episodes in the first 6 months post-transplant[76-82] Some authors consider infections with CMV the most important infection in renal transplant recipients[83].

In addition to its short-term morbidity and mortality risks, there is a growing body of literature suggesting that this infection may affect graft function and survival. Aiello et al. conducted a retrospective analysis of 30 subjects utilizing a case-control design (15 cases and 15 controls). In this study, the estimated 1, 5, and 8 year graft survivals were significantly lower in patients who had had viral infections (CMV and or EBV); 80%, 66%, and 57%, respectively ($p < 0.05$). The authors concluded the following: "renal transplanted patients experiencing viral diseases undergo chronic allograft nephropathy and reduced graft survival more frequently than patients without viral infections"[84].

Since CMV disease is a cause for increased morbidity (direct and indirect) it may result in higher hospitalization rates in the post-transplant population. Consequently, CMV infection constitutes an economic burden on transplant programs. In the United States, for example, the treatment of a single case of CMV disease costs between $25,000 and $50,000[85].

The significant associated morbidity and mortality together with the tremendous cost implications make CMV infection the single most important infectious complication in kidney transplantation.

3.2 Epidemiology of CMV Infection and Disease

3.2.1 Incidence of CMV Disease

Without prophylaxis, CMV reactivation occurs in almost 100% of transplant recipients[86] and in over 50% of patients viral reactivation occurs despite prophylaxis with antiviral therapy. The incidence of CMV disease among renal transplant recipients ranges between 20% and 60%[87]. The incidence varies according to several factors including CMV donor/recipient CMV sero-status (CMV-positive donor into a negative recipient), whether patients were given prophylaxis with antiviral or CMV hyper-immunoglobulin, and the type of immunosuppressive regimen. In the 1990s and earlier, prior to the use of effective antiviral therapy, the mortality from CMV disease was very high; reaching 90% in some reports.

In a retrospective analysis done by Schintzler and Brennan et al. the incidence of CMV disease was 9.1%, and varied by recipient/donor CMV sero-status. In this study the time to 1st episode of CMV disease ranged between 19 and 518 days post-transplant[87].

3.2.2 Modes of virus transmission in the transplant population

Sources of infection can be exogenous or endogenous. The majority of infections from exogenous sources are from infected allografts, other exogenous sources include leukocyte-containing blood products. Endogenous infections occur when CMV is present latently in the kidney recipient.

3.2.3 Predictors of CMV Disease

Over the past two decades many studies have been conducted to identify risk factors for developing CMV disease in different organ transplant populations. In a study by Peterson et al. done in the early 80s, CMV serology, the relationship of the donor to the recipient, and HLA matching were the most important factors associated with the development of CMV disease[88]. Almost a

decade later, an analysis by Boland et al. showed that the main predictors for CMV disease, in 106 solid organ transplant recipients reviewed, were CMV sero-status, treatment with OKT3, in addition to HLA type and number of mismatches[89] . More recent studies confirmed findings from previous ones and showed other new predictors for CMV disease including the type of immunosuppressive regimen, recipient age, female gender, rejection episodes, and blood type A[90-94].

Most studies showed that HLA has no effect on overall incidence of CMV. However, for recipients of kidneys from sero-positive donors, HLA-DR matching was strongly associated with incidence of CMV disease; the associated risk was 2.1 for those with zero HLA-DR matching (incidence: 16.7%), compared to 1 or 2 matches (8%). Effect of double CMV sero-positive status (donor and recipient) was stronger for patients with 0 HLA-DR matching (incidence: 10.8%), compared to 1 or 2 match (3%), RR = 3.6; p = 0.04 for positive donor/negative recipient (D+/R-), negative donor/positive recipient (D-/R+), and double CMV sero-negative (D-/R-). In this paper, no other recipient or donor characteristics were associated with the incidence of CMV disease[87].

3.3 Definitions of CMV infection and disease

The importance of CMV as the most common infection in post-transplant population has caught the interest of many scientists as indicated by the ever expanding research that has advanced the understanding on this virus and its interactions in such population. However, the continuing research resulted in constantly changing knowledge and inconsistency in the definitions related to CMV infection and associated diseases among studies. In a study by Rytel and Baily, in the late 1970s, CMV was defined by CMV Immunoglobulin G (IgG) titer and the presence of clinical features consistent with the disease[95]. In a study by Boland et al. CMV disease was defined by the presence of CMV antigenemia and/or evidence of viral excretion in addition to any two of the following symptoms: fever (38 5°C for at least 2 days and not due to other causes),

leucopenia or thrombocytopenia, elevated hepatic enzyme levels, kidney dysfunction, retinitis, pneumonia[89].

To improve the understanding of clinical trials and their utility in this field, uniform definitions related to CMV and its effects were needed. This need was fulfilled through the collaborative effort of scientists in the field who recommended such definitions[96, 97]. CMV definitions were classified according to the mechanism by which the virus causes tissue injury. Direct CMV infections are those from which the virus can be isolated. Indirect infections (or effects) are those caused by the virus through an indirect mechanism and the virus can not be isolated from the affected tissues and organs. Detailed description of definitions, of direct effects of CMV infections, is presented in Appendix II. It is important to distinguish between the first two definitions, CMV infection and CMV disease, since each points out a particular state of the virus activity in the host and has a different morbidity impact; with CMV disease being more aggressive.

3.4 Clinical Features, Pathogenesis and Diagnosis of CMV disease

3.4.1 Clinical Features of CMV Disease

CMV disease is characterized by the detection of CMV in a clinical specimen accompanied either by CMV syndrome with fever, muscle pain, or leukopenia and/or thrombocytopenia (other causes excluded), or by organ involvement such as hepatitis, gastrointestinal ulceration, pneumonitis, or retinitis.

Clinical features of indirect CMV effects vary according to the transplanted organ. In renal transplant recipients acute rejection is the main indirect effect of CMV infection. Other patients may suffer from chronic renal allograft dysfunction attributed to preceding CMV infections. Some studies have suggested other indirect effects such as coronary artery disease. This link however is not well established and further studies are needed to such relationship[98].

3.4.2 Pathogenesis

The development of CMV disease involves reactivation of the latent virus in various body organs, followed by amplification and dissemination of the replicating virus. This process results in endothelial and tissue injury and the release of inflammatory mediators which fuels the process leading to more tissue damage.

3.4.3 Effects of Different Immunosuppressive Agents on Latent CMV Infection

Renal transplant recipients have a significant exposure to immunosuppressive drugs, especially in the early post-transplant period. These drugs affect different components of the immune system, including T-cell-lymphocyte function, an important factor in defense against viruses such as CMV. Studies have shown that different immunosuppressive drugs vary in their effect on anti-viral mechanisms and therefore have different associated risks to developing CMV disease in the post-transplant population.

Biologic agents such as ATG/OKT3 have been shown to carry the highest associated risk for activating a latent CMV, followed by anti-proliferative drugs such as mycophenolate mofetil acid. Calcineurin inhibitors and steroid were not associated with heightened risk for latent CMV.

3.4.4 Effects of Immunosuppression on Replicating Virus

In situations where CMV is actively replicating, calcineurin inhibitors and other immunosuppressive drugs lead to increased viral replication and in many instances the development of CMV disease, i.e. clinical syndrome.

3.4.5 Diagnosis of CMV disease

Diagnosis of CMV disease requires the detection of the virus or its components in tissues or body fluids. Many techniques are currently available for clinical use[99]. The most common diagnostic methods will be described:

1. Shell vials, a method by which immunoflurescence is used to detect the viral nuclear antigen pp 72 in mink lung epithelial cells infected with patients' urine or sputum samples.
2. CMV antigenemia, again, immunofurescence is used to detect the viral nuclear antigen p 65 in patients' peripheral blood polymorphonuclear cells.
3. CMV Polymerase Chain Reaction (PCR) of buffy-coat specimens.
4. The CMV hybrid-capture RNA-DNA hybridization assay.
5. Immunohistochemistry, which is a histologic study that utilizes specific monoclonal antibody-based staining. Biopsy specimens initially undergo a process of microwave antigen retrieval. Sections are then stained using an envasion technique (Dako, Glostrop, Denmark). Multiple monoclonal antibodies are available for use, these include:
    a. anti-CD79a (Immunotech, Marseille, France).
    b. anti-CMV clone CCH2 (Dako, Glostrup, Denmark), which reacts with a nuclear early antigen and a nuclear and cytoplasmic late antigen.
    c. anti-CMV MAB 810 (Chemicon, Temecula, CA, USA), which reacts with a nuclear immediate early antigen that is detected throughout the complete infection cycle. Tissue specimens were examined for the presence of CMV effect (inclusion bodies).
6. CMV culture.

3.5 Complications Associated with Cytomegalovirus (CMV) Infection

In the 1970s, two studies have described multiple clinical patterns for CMV infection in immunosuppressed populations[100, 101]. Simmons et al. presented two

patterns of the disease, one being benign and a more severe (lethal) form. Both patterns were characterized by leukopenia, fever, occurring in the first six weeks post transplant. In addition, the severe form was associated with invasive multi-systems involvement and high mortality[100]. In more recent publications, three patterns have been reported, these include: primary infection, reactivation infection, and superinfection. Primary infection carries the greatest clinical risk; this is the result of exposing a weak (suppressed) immune system to a virulent virus for the first time, i.e. in the absence of memory and preformed antibodies, two major determinants of efficiency of secondary immune response[81].

Coexisting infections, (be they viral, bacterial or fungal) are not uncommon in patients with CMV disease. It is imperative that these co-pathogens are reported together with CMV, to allow timely intervention with specific antimicrobials.

3.5.1 Effect of CMV on Graft Loss Rates

In the retrospective analysis of 333 patients done by McLaughlin et al., CMV donor/recipient sero-status was not associated with decreased three-year graft function. Similarily, Schintzler and Brennan et al. found no relationship between donor/recipient CMV sero-status and 5-years graft survival. For patients with functioning grafts 6 month post-transplant, there was a significant graft survival difference between those who developed CMV disease (56.8%) compared to those who did not (79.1%), $p < 0.001$[87].

3.5.2 Association between CMV and graft rejection

CMV has been implicated in the pathogenesis of rejection in different types of organ transplants; such as what is seen in kidney transplant patients, vanishing bile ducts, which is a chronic type of rejection seen in liver transplants, and cardiac rejection[102, 103, 103]. The increased incidence of acute rejections seen in CMV infected patients was pointed out by Pouteil-Noble et al. in the early 1990s[104]. More recently, an analysis 477 kidney transplant recipients, done by

Sageda et al. showed an increased risk of acute rejection in patients who have had CMV disease or CMV infection[105].

3.5.3 CMV infection and chronic renal rejection

Solez et al. conducted an analysis of patients involved in a randomized controlled trial comparing the effect of tacrolimus and cyclosporine on the prevention of rejection. While biopsies at 2 years post transplant were not different between the two groups, patients with prior CMV disease had a 15% higher incidence of chronic rejection (OR = 2.15; p = 0.038). The authors' conclusion was that CMV early post transplant was a predictor for chronic rejection at 2 years[62].

3.5.4 CMV Disease and Graft survival

In a retrospective analysis of two hundred and fifty six kidney transplant recipients, Giral et al. demonstrated a significantly lower 5-year kidney allograft survival among patients with CMV disease compared to those who did not have CMV disease. The graft survival was lower in patients with CMV disease[106].

3.5.5 Post-transplant cardiac complications

The risk for developing cardiac complications is 1.5 times higher (OR=1.5; p=0.01) among patients with history of CMV disease compared to those without[107]. Kalil et al. found that CMV sero-positivity, among others, to be an independent risk factor for cardiovascular death after renal transplant[108].

3.5.1 CMV increases risk of post-transplant diabetes

The incidence of diabetes mellitus is significantly increased post-transplant; ranging between 10% to 20%. This is mostly the result of hyperglycemic and diabetogenic effect of immunosuppressive medications used in this population, tacrolimus and steroids having the strongest effect.

Interestingly, CMV replication has been found to act as an independent risk factor for the development of post-transplant diabetes (OR=4.0; p=0.025)[109].

3.5.6 CMV and oncogenesis

CMV has been shown to be associated with an increased risk of B-cell lymphoma; Epstein Barr Virus (EBV) associated lymphoma[110-114]. It has also been detected in many solid tumors. However, the associated risk with these remains to be confirmed with further studies.

3.6 Anti-CMV Prophylaxis in kidney transplantation

Due to the relatively high prevalence of CMV infection, the seriousness of CMV disease, and the related complications, the presence of an effective prophylactic regimen against this disease is of great importance. Optimal prophylaxis against cytomegalovirus (CMV) disease for organ transplant patients at risk for disease is a widely debated subject. To date, multiple prophylactic regimens have been widely used in different transplant programs, with variable degrees of success. The most common prophylactic strategies are the use of antiviral prophylaxis for individuals at risk, immunoglobulin prophylaxis (with CMV hyperimmune globulin, CMV immunoglobulin), and initiating preemptive therapy on obtaining a positive antigen assay. However, there is no consensus regarding the most appropriate prevention method[115, 116].

Data from prospective randomized trials of antiviral prophylaxis in solid-organ transplant recipients have established a clinically significant beneficial effect of antiviral agents in reducing the incidence of both CMV infection and disease.

In 2000, Couchoud et al. conducted a meta-analysis of studies evaluating the use CMV prophylaxis with antiviral agents for solid organ transplantation. CMV prophylaxis treatment was associated with a significant decrease in cytomegalovirus disease compared with placebo or no treatment (RR= 0.51, 95%

CI 0. 41-0.64). Prophylaxis also decreased the rate of CMV infection (RR=0.62; 95%CI 0.53-0.73, $p < 0.001$)[117].

To evaluate the effect of gangiclovir prophylaxis, Rondeau et al. conducted an open-label prospective randomized study of ganciclovir administration in CMV sero-negative recipients of a renal allograft from CMV sero-positive donors. Ganciclovir (5 mg/kg bid for 14 days) was started on day 14 after transplantation. Thirty-two patients were included in this study (15 in the control group, 17 in the ganciclovir group). Renal and patient outcomes were similar in both groups. The rate of CMV infection and CMV disease were similar in both groups (80% and 73.3% in the control group versus 70.6% and 47.1% in the ganciclovir group; P = NS) [118].

In 2005, Kalil et al. conducted a meta-analysis to assess the efficacy of universal prophylaxis and preemptive approaches in preventing CMV organ disease and other complications in solid organ transplant recipients. Randomized, controlled trials that evaluated antiviral strategies for preventing CMV and associated complications in solid organ transplant recipients were included. Compared with placebo or no therapy, both universal prophylaxis (odds ratio [OR], 0.20 [95% CI, 0.13 to 0.31]) and preemptive strategies (OR, 0.28 [CI, 0.11 to 0.69]) reduced CMV organ disease. However, only universal prophylaxis seemingly reduced CMV organ disease in subgroups of patients at highest risk (donors with positive CMV serostatus and recipients with negative CMV serostatus and induction with antibodies)[119].

More recently, Small et al. conducted another meta-analysis to compare the efficacy of universal prophylaxis and preemptive therapy using ganciclovir. The relative risk of CMV disease for study subjects in all preemption trials was 0.30 (95% confidence interval, 0.15-0.60), compared with that for control subjects. There was no statistically significant difference in CMV disease between prevention strategies[120].

Balfour et al. conducted a randomized, placebo-controlled trial using high-dose oral acyclovir has shown acyclovir to have a moderately beneficial effect in

preventing CMV disease following kidney transplantation[121]. Recently published data shows valacyclovir, the prodrug of acyclovir, to be effective in preventing CMV disease in kidney transplant recipients.

In a similar fashion, a 12-week course of oral ganciclovir (1 g three times a day) given to recipients of kidneys from CMV-seropositive donors, begun at the time of transplantation, prevented CMV infection and disease during the period of prophylaxis[122] . Although achievable ganciclovir levels in serum following oral administration are significantly lower than those achieved following parenteral administration, they may be sufficient to inhibit viral replication following transplantation.

In their meta-analysis, published in 2005, Kalil et al. found that both acyclovir and ganciclovir significantly prevented CMV organ disease in the universal prophylaxis trials; when compared to placebo. They concluded that both acyclovir and ganciclovir are effective for universal prophylaxis[119].

In another meta-analysis, Hodson et al. evaluated randomized controlled trials of prophylaxis with antiviral medications for cytomegalovirus disease in solid organ transplant recipients[123]. They compared prophylaxis with acyclovir, ganciclovir or valacyclovir with placebo or no treatment. The authors found that prophylaxis significantly reduced the risk for CMV disease (RR 0.42, 95% CI 0.34 to 0.52), CMV infection (RR 0.61, 95% CI 0.48 to 0.77), and all-cause mortality (RR 0.63, 95% CI 0.43 to 0.92) primarily due to reduced mortality from CMV disease (RR 0.26, 95% CI 0.08 to 0.78).

Consequently, the provision of antiviral prophylaxis has become the standard of care in many renal transplant programs. However, there is some variability with regards to whether populations will or won't receive prophylaxis. The majority of renal transplant centers now provide anti-CMV prophylaxis to most of their new renal transplant recipients (except those who are CMV sero-negative receiving kidneys from sero-negative donors). Prophylactic therapy with ganciclovir or valganciclovir for at risk patients (either donor or recipient or both

being CMV positive) is currently the standard of care for transplant recipients in Canada and the US.

3.6.2 Acyclovir vs. Ganciclovir

Earlier studies assessing the efficacy of acyclovir prophylaxis in high-risk renal transplant patients showed a high incidence of breakthrough CMV disease and CMV-related mortality in patients who received oral acyclovir prophylaxis. These studies, however, were criticized of being retrospective and lacking control for important variables that may affect the risk of CMV disease[124, 125]. Flechner et al. randomized 101 renal transplant recipients to either oral acyclovir or oral ganciclovir to assess the efficacy and safety of the two agents in preventing CMV infection in this population. Both agents were well tolerated, and no drug interruptions for toxicity occurred. CMV infection rates were significantly lower in the ganciclovir group; and when they stratified by CMV serology the rates were (for acyclovir vs. ganciclovir, respectively): D+R-, 54 vs. 0%, P=0.0008; D+R+, 43 vs. 6.6%, P=0.01; D-R+, 8.3 vs. 0%, P=NS. They concluded that: "Oral acyclovir provides effective CMV prophylaxis only for recipients of sero-negative donor kidneys. Oral ganciclovir is a superior agent providing effective CMV prophylaxis for recipients of sero-positive donor kidneys. Recipients who are treated for acute rejection are at risk for delayed CMV infection during the first post-transplantation year"[126].

In a randomized trial evaluating the efficacy of different antiviral prophylaxis regimens in reducing the incidence of CMV infection in sero-negative recipients organs from sero-positive donors (D+/R-), Rubin et al. included a total of 155 organ transplant recipients from 13 transplant centers. Patients received intravenous ganciclovir (5 mg/kg/day) for 5-10 days and then either oral acyclovir (400 mg tid) or oral ganciclovir (1 g tid) for an additional 12 weeks. The primary endpoint was the incidence of CMV disease in the first six months post-transplant. Treatment with oral ganciclovir was associated with a significant decrease in the incidence of symptomatic disease or viremia when compared

with the oral acyclovir group (32% vs. 50%, P<0.05). Furthermore, there was a significant difference in the time to CMV disease or viremia in the two groups; with a mean time *212±17* days post-transplant for the acyclovir group vs. 291±13 days for the ganciclovir group (P<0.001) [127].

Most trials comparing the two commonly used antiviral agents for CMV prophylaxis showed that ganciclovir to be more effective than acyclovir in preventing CMV disease.

3.6.3 Valgancilovir vs. Ganciclovir

In a randomized, double blind, prospective trial, Paya et al. examined the safety and efficacy of oral valganciclovir compared to oral ganciclovir prophylaxis in solid organ transplant recipients. In this study, the incidence of CMV disease was comparable in the two groups (valganciclovir 30.5%, ganciclovir 28%; p>0.05)[128-130]. More recently, Said et al. reported their results of a randomized controlled trial comparing the efficacy of oral valganciclovir to iv ganciclovir in preventing CMV disease among kidney transplant recipients. Oral valganciclovir was more effective in reducing the incidence of CMV disease than two-week therapy with iv ganciclovir (incidence 8.7% and 14.5%, respectively)[131].

3.6.4 Immunoglobulin Prophylaxis

Passive immune prophylaxis (against CMV infection) with human immunoglobulin preparations have been widely studied in different settings, including post-kidney transplant[132-136]. Prophylaxis with hyperimmune CMV immunoglobulin preparations have shown significant reductions in CMV disease in studies including kidney transplant recipients[132, 136]. One major limitation to wide use of such intervention (despite its low toxicity) is the remarkably high cost. Furthermore, its protection against CMV disease is partial[133].

3.6.5 Other advantages of CMV prophylaxis

Ricart et al. showed that ganciclovir prophylaxis was associated with significantly lower risk for acute rejection than no-prophylaxis or prophylaxis with acyclovir[137]. Furthermore, Opelz et al. found that the long-term graft survival was significantly improved by CMV prophylaxis (OR=0.8; 95% CI, 0.57 – 0.75; $p < 0.0001$)[103]. This improvement in graft survival has been attributed to the reduction in acute rejection rate.

In a meta-analysis by Hodson et al. CMV prophylaxis was shown to reduce the incidence of herpes virus infections by 73%, of bacterial infections by 35% and of protozoal infections by 70%[138].

3.7 Treatment of CMV Disease

Intravenous (i.v.) ganciclovir and oral valganciclovir are considered the standard therapy for CMV disease. For patients with invasive CMV disease or non-invasive disease with high viral load the treatment consists of i.v. ganciclovir in a dose of 5 mg/kg q 12 hours given for 2–3 weeks. The dose should be adjusted for patients with impaired graft function. Oral ganciclovir can be used for the treatment of less severe cases of CMV disease, where patients' symptoms are mild and there is no evidence of invasive disease.

In addition to antiviral therapy, the treatment includes reducing the doses and/or the number of immunosuppressive drugs as CMV disease is traditionally perceived to occur as a result of over-immunosuppression. The sequence and extent of reduction vary from one transplant physician to the other, but in general it involves reducing MMF dose to the lowest possible or holding it (in cases of severe episodes of CMV disease). This adjustment is done under careful monitoring for acute rejection that may occur with lower exposure to immunosuppressive drugs.

3.8 Summary

The ESRD population continues to grow in size, resulting in an enormous need for adequate renal replacement therapies in the form of dialysis and transplantation. Kidney transplantation confers a better survival and quality of life benefit over dialysis, which makes it the renal replacement therapy of choice for this population. Transplantation, both from living and deceased donors, provides better survival outcomes when compared to other modalities of renal replacement therapies. Except for transplantation between identical twins, success of kidney transplantation would never be possible without the use of immunosuppressive drugs. However, these agents have major side effects which include increasing the risk of infections in recipients of kidney transplants. CMV infection is the most common infection in recipients of renal allografts. The high prevalence of CMV and the associated significant morbidity and mortality post kidney transplant, together with the tremendous cost implications, make it the single most important infectious complication in kidney transplantation. Many techniques are currently available for clinical use, allowing for easy diagnosis of the infection and follow up of patients affected by it. To minimize the complications related to CMV disease, multiple prophylactic regimens have been widely used in different transplant programs, with variable degrees of success. Patients suffering from invasive CMV disease or non-invasive disease with high viral load require specific antiviral therapy, in addition to reducing immune suppression.

CHAPTER 4: Multiple Logistic Regression Analysis

Multiple logistic regression is a form of regression used when the dependent variable is dichotomous and the independent variables are continuous, ordinal, or dichotomous[139-142].

Multiple logistic regression analysis can be used for several purposes[139]:

- **To verify the association between a single explanatory variable and the response variable when controlling for one or more other explanatory variables.** If the explanatory variable continues to be highly associated with the response variable when included in the model with other explanatory variables, it is likely to be an important independent predictor of the response variable. If its association is strengthened or weakened as a result of its relationship with another variable or variables, these relationships can be investigated.
- **To reduce a large number of variables to a "best" subset of variables of manageable size.** Large clinical registries or administrative databases may contain data for hundreds of explanatory variables. Instead of testing the association between each explanatory variable and the response variable separately, variable-selection techniques can be used to reduce the number of variables included in the final regression model by identifying those that meet specified statistical thresholds. Clinicians, however, must still identify that the clinically important variables are included in the model.
- **To quantify the risk associated with individual explanatory variables.** In the study of risk factors, it is sometimes useful to determine the change in risk associated with an incremental change in an explanatory variable, such as the change in risk of stroke for every 20-mmHg decrease in systolic blood pressure. In this application, the regression coefficients are converted to odds ratios. Furthermore, quantifying risks associated with

different independent variables allows ranking the relative importance of these variables.

- **To assess for different sources of confounding (interaction, effect modification) and to understand the impact of covariate control variables.**

Logistic regression estimates the probability of a certain event occurring by applying maximum likelihood estimation (MLE) after transforming the dependent variable into the natural log of the odds of the dependent variable occurring or not. The transformed variable is sometimes referred to as "logit" variable. The logistic regression does not calculate changes in the dependent variable itself; but rather, it calculates changes in the log odds of the dependent variable.

The success of the logistic regression can be assessed by goodness-of-fit tests, such as model chi-square (used as indicators of model appropriateness), and the Wald statistic (used to test the significance of individual independent variables).

The "model building" of regression analysis is a process of selecting the best combination of explanatory variables to predict the response variable. One of the first steps in building a regression model is to identify the explanatory variables that are significantly related to the response variable. Those values identified as significant by the univariate analysis are considered for inclusion in the model[139].

CHAPTER 5: Study Objectives

CMV disease is an important health problem for immunosuppressed populations including kidney transplant recipients. The ongoing advances in the field of transplantation especially the development of new immunosuppressive regimens could potentially alter the pattern of CMV disease incidence and severity in kidney transplant recipients. Monitoring of CMV incidence and associated factors (predictors) in kidney transplant recipients is necessary for early detection of altered patterns of disease occurrence, and subsequently the design and implementation of measures addressing these changes. The constant change in immunosuppressive regimens could potentially result in changing the way traditional risk factors predispose to the development of CMV disease.

5.1 Research Hypotheses

- **Hypothesis 1:** the incidence of CMV disease in LHSC kidney transplant recipients is not known for the current era of immunosuppression.

- **Hypothesis 2** the incidence of recurrent CMV disease in LHSC kidney transplant recipients is not known for the current era of immunosuppression.

- **Hypothesis 3:** the factors associated with CMV disease in the current immunosuppression era will have different risk associations than previous eras.

5.2 Study Aims

1. determine the incidence of CMV disease in renal transplant recipients in the current era of immunosuppression (IS).

2. determine the incidence of recurrent CMV disease in renal transplant recipients.

3. identify potentially modifiable predictors (risks) of CMV disease in this population.

5.3 Specific Aims, and How to Achieve Them?

- **Aim 1:** Estimate the incidence of CMV disease in kidney transplant recipients. This aim was addressed by measuring the incidence of CMV disease in kidney transplant recipients at a single centre during the period January 1999 until December 2006.

- **Aim 2:** Estimate the incidence of recurrent CMV disease in kidney transplant recipients. This aim was addressed by measuring the incidence of CMV disease in kidney transplant recipients at a single centre during the same period.

- **Aim 3:** Test (using logistic regression modeling) whether traditional CMV risk factors (as reported in the literature) are still associated with this disease and assess for other potential risks not reported previously.

CHAPTER 6: Methodology

6.1 Study Design

This is an observational study involving retrospective identification of the patient population with prospective follow-up. The retrospective component was performed during the time of initial screening at the beginning of the study (January 2004); charts of eligible patients were reviewed and data were collected regarding their base-line characteristics and outcomes. Patients were then followed prospectively until the occurrence of a censoring event, as will be described in details later.

6.2 Data Sources and Collection

Baseline and follow up data were collected from patients' hospital charts. Other sources of data included electronic records, the renal program database at LHSC and the regional renal transplant database. Additional data were obtained from patients' local programs if they were jointly followed with LHSC post-transplant. All patients were followed prospectively from time of transplant until death, or until the last follow-up date of December 31, 2006.

Potential risk factors for CMV disease and other study variables were obtained from data collected for demographics, laboratory investigations, clinical characteristics and donor source (appendix III). Data on patients' demographics (age, sex, and race) were collected from patients' hospital records. For donor source and clinical characteristics (cause of ESRD, retransplantation, immunologic risk, immunosuppressive therapy, and CMV prophylaxis) data were obtained from hospital charts, pharmacy records, as well as the LHSC renal transplant database. Laboratory results (serum creatinine, and recipients' and donors' CMV serologic tests) were obtained from the hospital's electronic records and the LHSC renal transplant database. Data on development of CMV disease and other complications, for example acute rejection episodes and delayed graft

function, were obtained from patients charts and diagnostic tests results were obtained from the LHSC electronic patients' records.

Records for each patient were obtained and reviewed. Data needed for the study were initially abstracted in specific paper forms and subsequently entered (by A.H. and C.N.) in an electronic database using Microsoft Excel 2003, which contained all study variables. At the end of the study, follow-up data were entered in the same database and identifiers were removed after confirming the accuracy of the data and completing missing values (by A.H.).

Accuracy of data collection from patients' hospital charts was assessed by a different investigator, who collected the same data for a random sample of subjects. Furthermore, to ensure agreement between different data sources, randomly selected values (entries) were checked for discrepancies from primary data sources used in the study. Apart from missing values observed variably in hospital records or electronic databases, no major discrepancies in data were noted.

The protocol was approved by the Research Ethics Board of the University of Western Ontario and was conducted in conformity with the declaration of Helsinki.

6.5 Sample Size Calculation

There is wide range in the reported prevalence of CMV disease in the literatures due to variability in definitions used for CMV disease, demographic characteristics and other differences in participants in various studies. However, based on recent data from other centers in Canada and the US, the reported incidence of CMV disease was between 15 to 20%.

Sample size calculation was performed a priori to estimate the number of patients needed to be included in the study. The following formula was used to determine the sample size requirements to produce an estimate for the incidence of CMV disease within a standard error (SE) of 5%:

$n = p*(1-p)*[Z_{1-\alpha/2}/E]^2$; where n is the sample size, p = the estimated proportion of subjects with CMV, $Z_{1-\alpha/2}$ reflects the desired level of confidence, and E = margin of error. This formula is expected to produce the minimum number of subjects required to ensure a 5% SE (i.e. the estimate of proportions of subjects with CMV disease within 5%), with 95% confidence (α=0.05). According to these calculations, the total number of subjects estimated for inclusion in the study was 230 (range, 196 – 246). Approximately, 70-100 kidney transplants are performed at LHSC. Therefore, it was estimated that a four-year period would include the minimum number of consecutive kidney transplant recipients needed to be included in the study.

6.3 Patient Population

The study population consisted of all patients who received kidney transplants between January 1, 1999 and December 31, 2002, at the London Health Sciences Center-University Hospital (LHSC-UH), London Ontario, Canada. A total of 243 (consecutive) kidney transplants were performed at LHSC-UH, during the study's enrollment period.

6.3.1 Inclusion and Exclusion Criteria

Table 2 lists the inclusion and exclusion criteria. Based on available literature it is apparent that recipients of multiple different organs are different from recipients of a single organ in many aspects, including factors that would likely alter their CMV disease development. For example, the type and intensity of immunosuppression is remarkably different in recipients of a non-renal transplant who undergo a subsequent renal transplant. Furthermore, the clinical course and natural history of CMV disease are different among recipients of only-renal transplants compared to recipients of non-renal organs and to recipients of multiple different organs. Therefore, patients were excluded from the study if they were recipients of non-renal organ transplants, or if they were recipients of

multiple organs (i.e. recipients of other organ transplants, in addition to their kidney transplant).

Since the majority of CMV disease cases occur after 3 months post-transplant, patients who had their transplanted kidneys removed in the first three month post-transplant were excluded from the study, as they wouldn't be receiving immunosuppressive therapy, and their CMV disease risk is significantly altered.

Also excluded from the study were patients followed in programs other than LHSC-UH (or supervised by LHSC-UH) after organ transplantation, as no follow-up data could be available; however, only 3 patients were followed in programs outside LHSC-UH's supervision.

Table 2: Study's Inclusion and Exclusion Criteria

Inclusion Criteria

- Recipients of kidney transplants during the period January 1, 1999 – December 31, 2002.
- Followed at LHSC-UH or affiliated center.

Exclusion Criteria

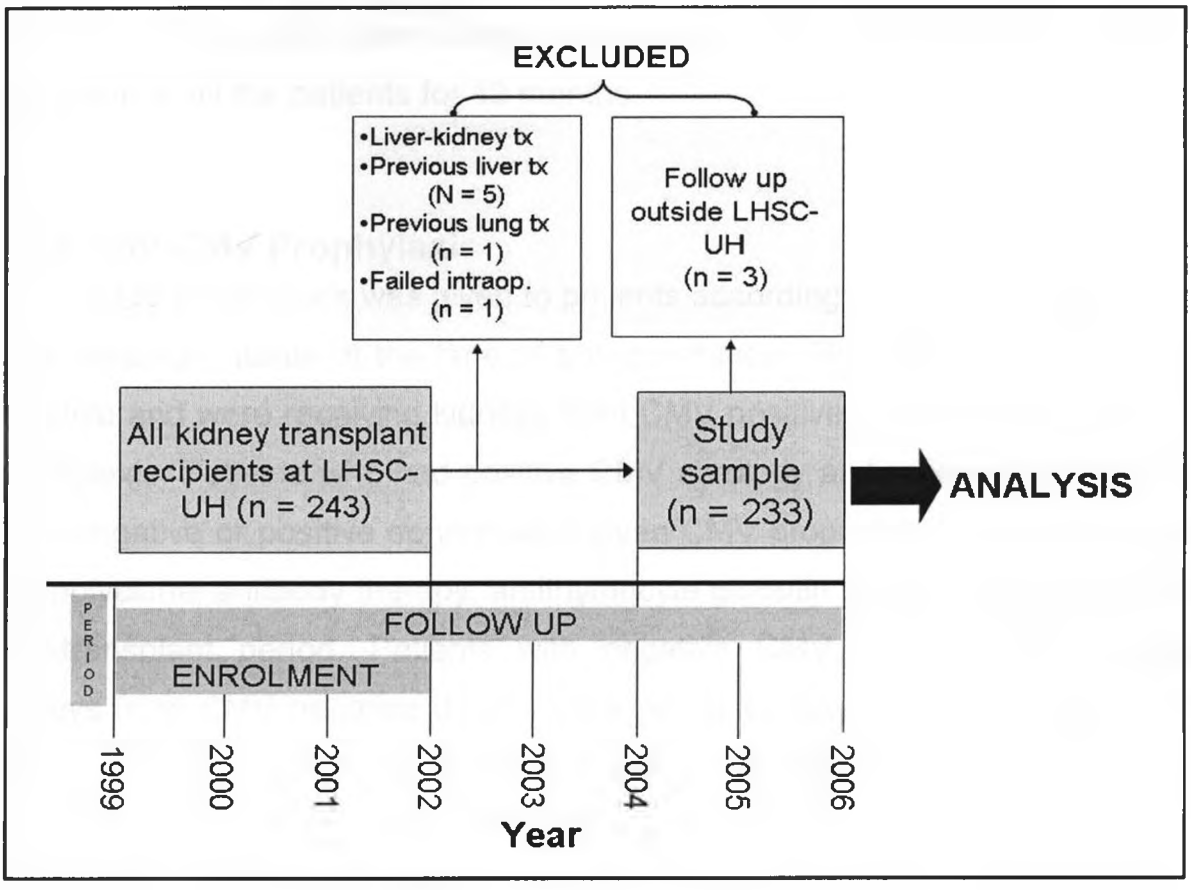
- Recipients of kidneys from identical twins
- Recipients of multiple different organs.
- Recipients of non-renal organ transplants.
- Patients who had their transplanted kidneys removed in the first three month post-transplant.
- Patients followed in programs other than LHSC-UH (or supervised by LHSC-UH) after organ transplantation.

6.3.2 Patients' Follow Up

All patients were followed for the occurrence of study outcome (CMV disease), until December 31, 2006, or until death if the latter occurred at an

earlier date. Figure 2 depicts the study design, patients' enrolment and follow-up periods.

Figure 2: Study design, population, and follow-up



tx = transplant, intraop = intraoperatively.

6.4 Study Outcomes

The primary study end point was the incidence of CMV disease during the post transplant follow up period specified above. Secondary end points were the occurrence of biopsy proven acute rejection (BPAR), and renal allograft failure (defined as loss of renal function requiring return to dialysis or re-transplantation). BPAR was defined as the occurrence of an acute rejection episode, confirmed by biopsy, according to Banff criteria[143].

6.5 Antimicrobial Prophylaxis

6.5.1 Anti-Pneumocystis carinii pneumonia (PCP) Prophylaxis

All patients received primary prophylaxis against with trimethoprim-sulfamethoxazole, one double strength tablet, three times a week (or oral dapsone, 100mg daily when allergy against sulfa was suspected). This antibiotic was given to all the patients for 12 months.

6.5.2 Anti-CMV Prophylaxis

CMV prophylaxis was given to patients according to their (and the donors') CMV serologic status at the time of transplantation. All patients who were CMV negative and were receiving kidneys from CMV positive donors were given CMV prophylaxis. Patients who had positive CMV serology and received kidneys from CMV-negative or positive donors were given CMV prophylaxis if they were given any polyclonal antibody therapy, antithymocyte globulin or OKT3, during the early post-transplant period. Patients with negative CMV serology who received kidneys from CMV negative donors were not given any CMV prophylaxis. CMV prophylaxis was given to all CMV positive recipients any time polyclonal antibodies were given, to prevent CMV activation and development of CMV disease as a result of intensified immunosuppression. However, CMV prophylaxis varied among patients who had positive CMV serology and received kidneys from CMV-positive or negative donors and were not given any polyclonal antibodies (antithymocyte globulin or OKT3), reflecting the controversy in the literature (at that time) on whether CMV prophylaxis was indicated in this patient population[115]. CMV prophylaxis was initiated immediately post transplant and consisted of IV ganciclovir, followed by oral valganciclovir for a total of three months post transplant. Both medications were adjusted for renal function.

6.6 Immunosuppressive treatment

6.6.1 Standard immunosuppressive therapy

Standard immunosuppressive therapy consisted of a combination of calceineurin inhibitor: Cyclosporine (CsA) or Tacrolimus, Steroids, and Mycophenolate mofitil (MMF).

6.6.1.1 Corticosteroids (CS)

All recipients were given 250 mg of methylprednisolone i.v. intra-operatively, and then a tapering oral steroid regimen is initiated postoperatively starting at 1 mg/kg of prednisone. When a dose of 20 mg/day of prednisone is reached, usually 7 – 10 days post-transplant, tapering is withheld until the end of the first month of the post-transplant period. From the beginning of the 2nd month, oral prednisone dose was further tapered gradually (over 4 weeks period) to 10mg/day. After 6-12 months, prednisone dose was further tapered to 7.5 mg daily or 15 mg every 48 hours.

6.6.1.2 Calcineurin Inhibitors (CNI)

Calcineurin inhibitors were started post-operatively, CsA was given 10 mg/kg orally in 2 divided doses, and tacrolimus was given 0.2 mg/kg orally in two divided doses. Later the doses were adjusted according to drug levels.

Patients were treated with cyclosporine microemulsion or tacrolimus from the time of transplantation unless they received induction with polyclonal antibodies.

6.6.1.3 Mycophenolate Mofitil (MMF)

All patients were treated with mycophenolate mofitil immediately post-operatively (2 g orally per day). At the end of the first year, the dose is reduced gradually to a total of 1-1.5 grams per day.

6.6.1.4 Biologic Agents

Patients at high immunological risk were also given induction therapy with OKT3 (Orthoclone) or rabbit antithymocyte globulin (ATG Fresenius). A minority of patients received induction with Interleukin-2 monoclonal antibodies.

6.6.1.5 Sirolimus

A total of 13 patients were on immunosuppressive regimens which contained sirolimus. All of these patients were initially receiving either cyclosporine or tacrolimus at the time of transplant and were "switched" to sirolimus to minimize CNI related toxicities.

6.6.1.6 Azathioprine

None of the patients included in this study were on azathiprine as an initial immunosuppressive therapy. Only one patient was switched from MMF to azathiprine shortly after transplantation for severe gastrointestinal complication (colonic perforation) felt by treating team to be related to MMF.

6.7 Acute Rejection

6.7.1 Diagnostic Criteria

Acute rejection episodes were suspected clinically by a >20% rise in serum creatinine in the absence of dehydration, infection, and drug nephrotoxicity (including CsA). Urinary tract obstruction and renal graft artery stenosis were excluded (by an ultrasonography and duplex Doppler examination of the renal graft), prior to attributing acute allograft dysfunction to acute rejection.

In all patients with suspected acute rejection, ultrasound guided allograft core biopsy was performed to confirm the diagnosis. All cases of AR were confirmed by biopsy prior to anti-rejection therapy and classified using the 1997 Banff criteria, developed and updated by investigators using the Banff Schema and the Collaborative Clinical Trials in Transplantation (CCTT) modification for diagnosis of renal allograft pathology [143]. According to these criteria, diagnosis of acute

rejection (AR) requires renal biopsy specimens from patients with suspected rejection; it was defined and classified as follows:

- "borderline/suspicious for rejection." Are biopsies with only mild inflammation.
- Type I AR is tubulointerstitial rejection without arteritis. Type II is vascular rejection with intimal arteritis.
- Type III AR is severe rejection with transmural arterial changes.
- Chronic/sclerosing allograft changes are graded based on severity of tubular atrophy and interstitial fibrosis.
- Antibody-mediated rejection, hyperacute or accelerated acute in presentation, is also categorized, as are other significant allograft findings.

Acute rejection episodes were treated with a total of 250-500 mg of methylprednisolone i.v. for 3-5 days. The dose of oral prednisone was increased to 1 mg/kg/day and tapered gradually back to patients' previous maintenance dose of prednisone. Steroid resistant rejections were diagnosed if no fall in maximum serum creatinine was observed on the 4th day after the start of standard methylprednisolone treatment. Such cases were treated with antithymocyte globulin or OKT3. If patients were on cyclosporine emulsion at the time of the rejection, the cyclosporine was stopped and the patient was switched to tacrolimus in addition to receiving pulse methylprednisolone therapy.

6.8 CMV Screening

Techniques for CMV screening and diagnosis were described in detail in chapter 2. There was no routine screening for CMV, post-transplant, for asymptomatic patients.

6.8.1 CMV Serology Testing

At LHSC, CMV screening prior to transplantation (for living and deceased donors as well as for recipients) is done using CMV serology for IgG and IgM. The ABBOTT Axsym system was used for detection of CMV IgG antibodies (ABBOTT, Chicago IL).

6.9 CMV Diagnosis

As described in chapter 2, active CMV infection can be confirmed using several techniques. At LHSC, for patients with suspected CMV disease, both pp65 antigenemia assay CMV PCR have been utilized. Additionally, for patients with suspected invasive CMV disease specimens obtained from affected tissues (gastro-esophageal, colonic, bronchial, and renal biopsies) are examined for viral effect (viral inclusions) and viral cultures were also obtained from these specimens.

6.9.1 CMV antigenemia (pp65) assay

pp65 lower matrix protein antigenemia was determined on polymorphonuclear cells isolated from buffy coat from EDTA blood samples (CINA kit, Argene BIOSOFT; France). The result was presented as the number of CMV pp65 antigen-positive cells per 100,000 polymorphonuclear leukocytes.

6.9.2 CMV PCR

All patients with CMV DNA levels of >500 copies/μg of total DNA in peripheral blood had clinical evidence of disease, although some with lower viral burdens were also symptomatic.

6.10 CMV Disease

In this study, recent definitions of CMV infection, CMV disease, and CMV tissue invasive disease were used. CMV disease was defined as the presence of CMV DNAemia (by PCR), or positive pp65 antigenemia (≥5 positive

cells/100,000), in the presence of clinical symptoms suggestive of CMV disease (either CMV syndrome or tissue invasive CMV disease)[97, 144]. Since screening of asymptomatic transplant recipients was not utilized in our centre, patients with asymptomatic CMV infection were not included in this study. In this study the outcome involves two components: the presence of the virus in patients' tissues or blood and the presence of clinical symptoms and signs. Since the study design was observational, additional testing to screen asymptomatic patients was not possible as it was not part of the current standard of care.

6.11 CMV Treatment

Treatment of CMV disease consisted of reducing immunosuppressive therapy and specific antiviral therapy with i.v. ganciclovir. Patients with confirmed CMV disease were treated with i.v. ganciclovir, 5 mg/kg q 12 hours, given for a minimum of 3 weeks. The ganciclovir dose was adjusted for renal function. At the end of antiviral therapy, CMV antigenemia and/or PCR were repeated to confirm the eradication of the antigenemia/viremia. If these tests remained positive or patients remained symptomatic after three weeks, duration of antiviral therapy was prolonged until the infection was controlled with no antigenemia or viremia and with the patient being asymptomatic.

6.12 Patient Follow-up and Clinical Monitoring

After being discharged from the hospital, patients were followed in the transplant nephrology clinic. Frequency of outpatient clinic visits were as follows: twice a week during the 1st 2 weeks, then once a week for two weeks, then every two weeks for one month and then monthly until the end of the 6th month. Frequency of visits was gradually reduced and by the end of 1st year post-transplant patients were then followed at four to six month intervals. During these visits, patients were evaluated clinically for their general condition, symptoms of renal dysfunction, infections and for other immunosuppression-related

complications, such as malignancy, hypertension and diabetes. Laboratory investigations were also done during these visits, including drug levels (cyclosporin, tacrolimus, and sirolimus) and routine hematologic and renal function evaluation. Additional laboratory and radiologic investigations were done as indicated by clinicians' evaluation.

In our centre, routine CMV screening with antigenemia and DNAemia tests were not performed. These tests were only performed if CMV disease was suspected.

6.13 Data Analysis

6.13.1 Selection of Variables

All previously identified variables in the literature were included in the study. Other variables were also included if available literature or clinical experience suggested the presence of relationship with CMV disease or with its known predictors.

Table 3 shows the variables selected to be included in the model to evaluate for potential independent predictors of CMV in our study population. These variables included demographic information, relevant pre-transplant clinical and laboratory data, and post-transplant clinical and outcome data.

Table 3: Variables selected to be included in the multivariable model to evaluate for potential independent predictors of CMV disease.

Variable[a]	**Reference**[b]
Demographics	
Age at the time of transplant	-
Gender	Female
Ethnic group	Caucasian
Weight at the time of transplant	-
Pre-transplant Clinical Data	
Number of previous kidney transplants	-
Underlying cause of ESRD ✓ Glomerulonephritis (GN) ✓ Hypertension ✓ Diabetic nephropathy ✓ Others	GN
Pre-transplant Test Results	
Number of Human Leukocyte Antigen (HLA) matches ✓ 0-2 ✓ 3-4 ✓ 5-6	5-6
B cell cross-match	Negative
Recipient's CMV serologic status ✓ D-/R- ✓ D-/R+ ✓ D+/R+ ✓ D+/R-	D-/R-
Estimated Pre-transplant Immunologic Risk ✓ Low	Low

✓ High	
History of prior immunosupprression (IS) or Transplantation	No
Post-transplant Data	
History of CMV prophylaxis	Yes
Drugs used for initial IS ✓ Cyclosporine-based ✓ Tacrolimus-based ✓ Other	Cyclosporine-based
Induction with biologic agents (thymoglobulin, OKT3)	No
Delayed Graft Function (DGF)	No
Biopsy proven acute rejection (BPAR)	No
Donor Information Organ source ✓ Living ✓ Deceased donor	Living

[a]variables shown to be associated with CMV disease in previous literature[81, 86, 87, 88, 89, 92, 93, 94]. [b]Reference group for logistic regression analyses.

6.13.2 Data Screening

After the end of last follow up and data entry, study database was screened for missing and ambiguous data. Each variable was assessed individually and further data collection was decided based on this initial screening.

6.13.3 Missing and Ambiguous Values

Initial review of study database revealed < 5% missing values. Efforts were made to minimize the number of missing entries include reviewing other data sources available at LHSC (transplant program electronic database, transplant clinic data, and data from nephrology program database in addition to data available from patients' hospital health records). Therefore, after obtaining missing data from these different databases, the proportion of missing values for all variables in final dataset was very small (0.6%). B-cell cross-match and D/R CMV sero-status were the variables with highest missing values, 3.9% and 2.6%, respectively. To maximize the strength of the results ambiguous data were also clarified prior to embarking on analysis.

6.13.4 Data Analysis

SPSS version 13.1 (Chicago, IL) was used for all data analyses, unless otherwise specified.

Period prevalence of CMV disease was defined as the number of patients who developed CMV disease at any time during the study period divided by the total number of patients included in the study.

6.13.5.1 Demographic Differences

Differences between groups in categorical demographic variables were assessed with the chi-square (χ^2) test or Fisher's Exact Test for categorical variables. Continuous (interval) variables were compared using the Student t-test

or the Mann-Whitney test, according to their distribution; normal and non-normal, respectively.

6.13.5.2 Descriptive (Univariate analysis)

Univariate analysis was performed to describe frequencies and proportions for discrete variables. For continuous variables, central tendencies (means and medians) and spread/dispersion measures (SD and SE) were described, in addition to variables distribution and skewness.

6.13.5.3 Outcomes Analyses

A) CMV Disease Rate

Overall CMV disease rate as well as the incidence of proven CMV disease in different sub-groups was calculated from different aspects including, demographic, management, and transplant characteristics (number of transplant, whether a deceased donor kidney had been used, immunosuppressant regimens and dosages, and whether the donor or recipient had serologic evidence of CMV infection). Rate ratios (RR) were calculated as estimates of risk, and 95% confidence intervals (CIs) and the Fisher Exact Test two-tailed p-value were used to assess statistical significance.

For the primary outcome analyses, data were censored at the time of occurrence of any of the following events: the time of CMV diagnosis, patient's death, or the end of the follow up period. For the secondary outcome analyses, censoring events were patient's death before December 31, 2006, or the end of the follow up period.

B) Bivariate Analysis

Association of CMV disease with continuous and categorical variables was assessed using Wilcoxon rank-sum test and chi-square (χ^2) or Fisher Exact Test, respectively.

C) Multivariable Modeling (Multiple Logistic Regression Analysis)

Multiple logistic regression analysis was used to model the relationship between CMV sero-status and CMV disease while adjusting for the presence of potential confounders. Backward stepwise logistic regression analysis was performed separately to assess factors that were associated independently with the development of CMV disease. Variables that were associated at $P < 0.15$ in bivariate analysis were included in the logistic regression model for assessment in the multivariable analysis. Significant predictors in the bivariate analysis were included in a backward, stepwise multiple logistic regression model to determine the most important risk factor for developing CMV disease. Variables included in the model regardless of the statistical significance of their bivariate analysis are: Receipt of ATG, DGF, B Cell Cross-match (positive vs. negative), Calceinurin Inhibitor type (FK 506 vs. CSA), and BPAR.

CMV sero-status has been recognized as the strongest risk factor for developing CMV disease. Therefore, CMV sero-status was chosen as the risk factor in this study. Other variables were examined for their effect on the development of CMV disease and on the relationship between CMV sero-status and CMV disease. The first step was to determine which variables were "classical confounders".

The second step determined confounding variables as defined by Kleinbaum et al.[140]. In this method, models are created without the potential confounder and compared to the 'full' model. If the regression coefficient of the study variable changes by a specified percentage or more, the variable removed is considered a confounder, and is replaced in the model. A change of 10% has previously been suggested, by Mickey and Greenland, to be an appropriate selection criterion for confounders in logistic regression. Since Kleinbaum et al. do not specify at which percentage the study variable must change in multiple linear regression, 10% was chosen as a reasonable change by the investigator. All covariates were analyzed in this manner for confounding between CMV disease and CMV sero-status.

The final step was a backward selection while forcing the study variable, effect modifiers, and confounders to stay in the model, eliminating any non-significant covariates.

Cox proportional-hazards analysis was used to estimate the relative contribution (RR) of various factors to the risk of developing CMV disease[145]. A p-value of <0.05 was considered to indicate statistical significance; all tests were two-tailed.

6.13.5.4 Sensitivity analysis (to compare different regression models)

We assessed goodness of fit and predictive value of logistic regression models by using the Hosmer-Lemeshow test and area under the receiver operating characteristic curve.

CHAPTER 7: Results

Between January 1, 1999 and December 31, 2002, a total of 243 adult patients received kidney transplants at the London Health Sciences Center (LHSC), London Ontario, Canada. Of these, ten patients were excluded from the study; six had received either a previous (1 lung, 2 liver) or concomitant (3 liver-kidney) non-renal organ transplants, three patients were followed in programs other than LHSC, and one patient underwent graft nephrectomy shortly after transplantation for primary non-function.

7.1 Results

7.1.1 Patients Characteristics

Table 4 describes baseline patient characteristics including demographics, cause of ESRD, immunologic profile, immunologic risk, B-cell cross match, immunosuppressive therapy used, and CMV serologic status. Donors' type (living vs. deceased) and CMV serologic status are also described.

At time of kidney transplantation, patients' ages ranged from 16 to 78 years, with an average age of 46.7 years (S.D., 13.8; 95% C.I., 44.81 – 48.52); median age was 48 years. The age distribution of patients was roughly bell shaped between 20-70, reflecting the distribution of the overall transplant population during the study period. When age was divided into groups by decades, the largest group was those between age 41 and 50 years (24%). After age 70, the distribution tails off rapidly, reflecting the lower number of older transplant recipients. Eighty-four of the 233 (36%) renal transplant recipients were female. Eighty seven percent of patients were Caucasians, and 13% were from other ethnic groups.

Diabetic nephropathy (DN) was the underlying cause of ESRD in over a third of all patients, with glomerulonephritis second (19%), and hypertension a distant third (7%). The remaining 40% were equally distributed between reflux

nephropathy, obstructive nephropathy, tubulointerstitial diseases and hereditary renal diseases (table 4).

Of the total number of transplanted kidneys, 170 (73%) were from deceased donors and 63 (27%) from living donors.

Table 4: Baseline characteristics of study population (reported as percent or mean ± SD)

Variable	Total population (N = 233)
Age (years)	46.7 ± 13.8
Gender	
% Female	84 (36)
Race	
% White	199 (86.9)
Weight (Kg)	74.6 ± 15.7
Cause of ESRD	
Glomerulonephritis	43 (18.5)
Hypertension	17 (7.3)
Diabetic nephropathy	79 (34.1)
Others	93 (40.1)
First-time Kidney Recipients	195 (83.6)
Matched HLA[a]	
0-2	223 (96.5)
3-4	5 (2.2)
5-6	3 (1.3)
Immunologic Risk[b]	
Low	190 (81.5)
High	43 (18.5)
B Cell Cross-match	
% Positive	49 (21.9)

CMV Serologic Status[c]	
D-/R-	54 (23.8)
D-/R+	60 (26.4)
D+/R+	71 (31.3)
D+/R-	42 (18.5)
Donor Source	
Living related	51 (21.9)
Living non-related	12 (5.2)
Deceased	170 (73)
Pre transplant IT exposure	35 (15)

[a]Human Leukocyte Antigen, [b]Immunologic Risk is arbitrarily determined by transplant team prior to transplantation based on patient's history of presensitization (blood transfusion, pregnancy, previous transplantation), and PRA (Panel Reactive Antibody), [c]Cytomegalovirus serostatus is determined by recepient's and donor's serum CMV IgG at the time of transplantation.

7.1.2 Immunologic Profile

As shown in table 4 approximately 84% of patients were recipients of first kidney transplant, the remaining were those with second (15%) or third kidney transplant (1%). The vast majority of patients (95%) had two or less HLA matches with their kidney donors. Over 80% of the patients were deemed as having low immunologic risk at the time of transplantation. B-cell cross-match at the time of kidney transplant was positive in 22% of patients.

7.1.3 Immunosuppression:

Almost all patients were on Mycophenolate Mofetil (MMF) as part of their triple immunosuppressive therapy (table 5). Only 3 patients were not on MMF and were receiving a triple therapy consisting of Prednisone, Tacrolimus, and Sirolimus. 226 patients (97%) were receiving a Calcineurin (CNI) based immunosuppression. Of these, 125 (55%), and 101 (45%) were receiving

Tacrolimus and Cyclosporine, respectively. The remaining seven (3%) patients were receiving Sirolimus based triple immunosuppressive therapy.

7.1.4 Antibody Therapy

Less than half (45%) of the patients received antibody therapy (Anti-thymoglobulin, 39%; OKT3, 4%; Basiliximab, 2%) during the study period. For those who have received ATG, the mean dose was 7.8 mg per kilogram body weight (SD, ± 3 mg; range, 1-16), and the mean number of ATG doses was 6 (SD, ± 3; range, 1-12).

7.1.5 CMV Sero-status

At the time of kidney transplant, 41% of patients had CMV negative serology; 56% of these received kidneys from CMV sero-positive donors.

7.1.6 CMV Disease

7.1.6.1 Incidence of CMV disease

The overall incidence of CMV disease in the study population was 14.6% (95% CI, 11.7–18%).

7.1.6.2 Characteristics of patients with CMV disease

For patients who have had CMV disease, the mean age was 47 years (range, 22-78; SD, 15.2 years); this was not significantly different from those who did not develop CMV disease.

7.1.6.3 Factors Associated with CMV Disease

Results of bivariate analysis are shown in table 6. The donor and recipient pair status was evaluated. For this part of the analyses (as well as the multivariable analyses), the risk factor, CMV sero-status was grouped into four

categories, determined by donor and recipient CMV serology at the time of transplantation (tables 4 and 6). The patient group with negative CMV serology in both the donor and recipient (D-/R-) was chosen as the reference group. In bi-variate analysis, the combination of CMV-positive donor and CMV-negative recipient sero-status was associated with CMV disease (OR = 11.66; 95% CI, 2.46 – 55.27; p= 0.002). The associated risk for D+/R+ sero-status was lower (OR = 6.39; 95% CI, 1.39 – 29.45; p = 0.017). The lowest associated risk was for D-/R+ sero-status (OR = 1.86; 95% CI, 0.326 – 10.57), however this was not significant (p = 0.45). Lack of CMV prophylaxis was significantly associated with risk for development of CMV disease (OR = 4.05; 95% CI, 1.79 – 9.17; p = 0.001). Risk of CMV disease did not differ significantly by donor source, receipt of anti-lymphocyte globulin, or positive B cell cross-match.

Table 5: Patient's follow up data on exposures and outcomes.

Variable	n (%)
Antibody induction	
ATG[a]	90 (38.6)
OKT3[b]	9 (3.9)
Basiliximab	5 (2.1)
Total ATG dose/Kg body weight	7.83 ± 3
Total no. of ATG doses	6 ± 3
Maintenance IT[c]	
Cyclosporine-based	103 (44.2)
Tacrolimus-based	117 (50.2)
Other	13 (5.6)
CMV Prophylaxis	
Delayed graft function	66 (28.3)

[a]Antithymocyte Globulin, [b]Orthoclone, [c]Immunosuppressive Therapy.

7.1.7 Multivariable Modeling of CMV Disease

As discussed above, the first step in multivariable modeling was to perform bivariate analyses assessing the relationship between the outcome (CMV disease) and the chosen covariates. This is followed by performing bivariate analyses to assess the relationship between the strongest predictors for the outcome, recipient/donor (R/D) sero-status and other covariates. From these analyses the following is concluded (at alpha = 0.15):

- CMV disease was associated with B Cell Cross-match, positive vs. negative, ($p = 0.053$); and CMV prophylaxis ($p = 0.001$).
- D/R sero-status was associated with Race ($p = 0.005$), CMV prophylaxis ($p < 0.001$), Age ($p = 0.018$), IVIG ($p < 0.001$), and Donor source ($p = 0.14$).
- Therefore, CMV prophylaxis is a CLASSICAL CONFOUNDER, and other covariates are not.

7.1.8 Effect of CMV Sero-status on CMV Disease

7.1.8.1 Identification of Effect Modifiers

Interaction terms for each covariate and the study variable, CMV sero-status, were analyzed using multiple logistic regression with a backward selection algorithm at a significance level of 0.15 indicated that none of the interaction terms was significant, we then concluded that there are no effect modifiers among the model covariates.

7.1.8.2 Identification of Operational Confounders

All covariates were analyzed for effect as operational confounders. A multiple logistic regression model was constructed with the risk factor (D/R sero-status), the classical confounder (CMV prophylaxis) and other possible covariates. By removing only one covariate at a time, the regression coefficient of the study variable, CMV sero-status, was analyzed for a difference of 10%[140] .

Covariates which caused a change of 10%, when absent from the full model, were considered operational confounders, and forced into all subsequent models. Race, number of HLA cross-matches, B Cell cross-match, history of prior transplantation or immunosuppression, maintenance immunosuppression (tacrolimus vs. cyclosporine), delayed graft function, BPAR, and donor source (deceased vs. living) were shown to cause over 10% increase in the regression coefficient of R/D CMV sero-status, and were concluded to be operational confounders. All other covariates caused changes of less than 10%.

7.1.8.3 Backward Elimination for Significant Covariates

Backward elimination algorithms were used to select significant covariates in the multiple logistic regression model. The risk factor D/R CMV sero-status, the classical confounder CMV prophylaxis, together with all operational confounders were forced into the model. After adjusting for the risk factor and confounding variables, no additional covariates remained in the backward elimination model (alpha < 0.05). Therefore, the final model for the association of CMV Sero-status and CMV disease included the primary explanatory variable D/R CMV sero-status, the classical confounder CMV prophylaxis, the operational confounders race, number of HLA cross-matches, B cell cross-match, history of prior transplantation or immunosuppression, maintenance immunosuppression (tacrolimus vs. cyclosporine), delayed graft function, BPAR, and donor source (deceased vs. living).

D/R CMV sero-status D+/R- was strongly associated with increased risk for developing CMV disease in renal transplant recipients (OR = 9.63; 95% CI, 1.29 – 71.84). Similarly, D+/R+ sero-status was associated with development of CMV disease (OR = 10.49; 95% CI, 1.53 – 71.66). D-/R+ sero-status was not associated with increased risk for developing CMV disease in this population (OR = 1.21; 95% CI, 0.13 – 11.02).

In addition to D/R CMV sero-status, B-cell cross match was the only variable that remained significant in the final model with an OR = 3.23 (95% CI, 1.16 – 9.0).

7.1.8.4 Summary of multivariable modeling

Results of a series multivariable models show that, in addition to CMV sero-status, B-cell cross-match was associated with the risk for the development of CMV disease. When all important variables were adjusted for, the risk of CMV disease associated with D-/R+ was not different from the D-/R- sero-status (p = 0.86). The associated risk for D+/R+ (OR = 10.49, p = 0.017) was 60% higher, and that associated with D+/R- (OR = 9.63, p = 0.027) was 17% lower than from what is seen in bivariate analyses (OR = 6.39, 11.66, respectively). The associated risk for B-cell cross-match (OR = 3.23, p = 0.025) was 40% higher than estimated risk in bivariate analysis (OR = 2.24, p = 0.05).

Table 6: Summary of bivariate and multivariable analyses.

Step	**Significant Variables[a]**
Identifying factors associated with CMV disease	B Cell cross match (p = 0.053), and CMV prophylaxis (p = 0.001)
Identifying factors associated with CMV sero-status	Race (p < 0.001), IVIG (p < 0.001) CMV prophylaxis (p < 0.001), and Donor source (p = 0.14).
Classical confounder (s)	CMV prophylaxis.
Testing for effect modification	No effect modifiers detected.
Testing for operational confounders	Race, No. of HLA[b] cross matches B Cell cross match, History of prior transplantation or IS[c], Maintenance IS[c], DGF[d], BPAR[e], and Donor source.
Testing for significant covariates	[f]D+/R- (OR = 9.63; 95% CI, 1.29 – 71.84), [f]D+/R+ (OR = 10.49; 95% CI, 1.53 – 71.66), B-cell cross match (OR = 3.23; 95% CI, 1.16 – 9.0)

[a]alpha level selected: 0.15 for bivariate analyses and 0.05 for multivariable analyses. [b]HLA: Human Leukocyte Antigen, [c]IS: Immunesuppression, [d]DGF: Delayed Graft Function, [e]BPAR: Biopsy Proven Acute Rejection, [f]R-: recipient with negative CMV IgG, R+: recipient with positive CMV IgG, D+: donor with positive CMV IgG.

7.1.8.5 Regression Diagnostics

A) Assessing assumptions for logistic regression

To ensure that all assumptions for logistic regression were fulfilled, a series of exploratory analyses were carried out. These analyses included testing for linearity assumption and distribution of the dependant variable (CMV disease).

Age was the only continuous variable in the dataset, therefore it was tested for linearity with the occurrence of CMV disease. To do this age was categorized into multiple dichotomous variables of equal units on the variable scale. Box-Tidwell Transformation was then performed. This test involves adding to the logistic model the interaction terms which are the cross-product of each age category times its natural logarithm [(age category) ln (age category)]. Interaction terms were not significant. Additionally, a quadratic form of the age group variable was created (by subtracting out the mean of untransformed age variable and then the result was squared). The quadratic variable was then entered in the model (with the untransformed variable). Neither terms were statistically significant in the model. From results of both procedures, it was concluded that linearity assumption was not violated. However, when the age variable was grouped into three categories and cross-tabulated with the outcome (CMV disease) the test was not statistically significant (p=0.35), suggesting a non-linear relationship between the variables. However, when age was categorized into 6 one-decade groups, and mid points of these categories were plotted against the coefficient for CMV disease in the regression model, the appearance was of a "U" shape, i.e. non-linear. Similar findings were obtained when age was divided into three (two-decade) categories; these age groups were Young (15-35), Middle-age (36-55), and Old (56 and older). To resolve the linearity concern, age was then entered in the logistic regression analyses as three distinct dichotomous variables.

CMV disease is dichotomous, it is assumed to have a binomial distribution. The occurrence of first episode of CMV disease in individual is independent of

the outcome from another subject, i.e. the outcomes are not clustered owing to being from same individuals or families.

B) Assessment of how well the model accounts for the outcome

To assess whether the full model accounts for the outcome better than would be expected by chance, the final model's Chi-square test resulted in Chi-square=31.9 (df = 9, $p < 0.001$); indicating that the model accounts for the outcome better than chance.

To quantitatively assess how well the model accounts for the outcome, Hosmer-Lemeshow goodness-of-fit test was used. In this test the estimated probability (according to the model) of the outcome, CMV disease, is compared to the observed probability of CMV disease (in the original data). The test resulted in a small Chi-square (5.45, df = 8) and non-significant p-value (0.71). From this it was concluded the final model is a well-fitting model, and the estimated likelihood is close to the observed likelihood of CMV disease.

7.1.9 Secondary Outcomes

7.1.9.1 Renal allograft outcomes

Delayed graft function occurred in 66 (28%) patients, and 49 (21%) developed biopsy proven acute rejection during the follow up period. In patients who developed CMV disease, acute rejection data was obtained from the period prior to developing CMV disease.

Table 7: Bivariate and Multivariable-Adjusted Associations of Recipient and Donor CMV sero-status With the Development of CMV Disease in White Patients.

Variable (reference)	**Bivariate Odd Ratio (95% CI)**	**P-value**	**Multivariable Odd Ratio (95% CI)**	**P-value**
Demographics				
Age				
> 55 (reference)				
36 – 55	0.77 (0.29 – 2.05)	0.61	-	-
15 – 35	0.97 (0.36 – 2.64)	0.95	-	-
Female (vs. male)	1.29 (0.6 – 2.7)	0.50	-	-
Nonwhite race (vs. white)	2.68 (0.61 – 11.83)	0.19	2.03 (0.39 – 10.55)	0.40
Cause of ESRD				
Glomerulonephritis (reference)			-	-
Diabetic nephropathy	0.32 (0.04 – 2.83)	0.31	-	-
Hypertension	0.99 (0.37 – 2.64)	0.98	-	-
Other causes	0.83 (0.30 – 2.33)	0.73	-	-
Retransplantation (vs. 1st transplant)	0.94 (0.36 – 2.43)	0.90	0.8 (0.10 – 6.23)	0.83
High immunologic risk (vs. low)	1.07 (0.41 – 2.76)	0.90	0.91 (0.12 -7.13)	0.93
Positive B cell crossmatch (vs. negative)	**2.24 (0.99 – 5.08)**	**0.05**	**3.23 (1.16 – 9.0)**	**0.025**
Recipient &Donor				

CMV Status[1]				
D-/R- (ref)				
D-/R+	1.86 (0.326-10.57)	0.49	1.21 (0.13 – 11.02)	0.864
D+/+	**6.39 (1.39 – 29.45)**	**0.017**	**10.49 (1.53 – 71.66)**	**0.017**
D+/R-	**11.66 (2.46 – 55.27)**	**0.002**	**9.63 (1.29 – 71.84)**	**0.027**
Antibody Induction (vs. no induction)	0.82 (0.39 – 1.72)	0.59	1.25 (0.48 – 3.22)	0.65
CMV Prophylaxis (no. vs. yes)	**4.05 (1.79 – 9.17)**	**0.001**	1.04 (0.3 – 3.6)	0.95
DGF[3]	1.71 (0.80 – 3.65)	0.169	1.95 (0.66 – 5.8)	0.23
Calcineurin Inhibitor type[4]: FK 506 (vs. CSA)	0.65 (0.31 – 1.36)	0.25	0.82 (0.3 – 2.27)	0.7
CMVIG (no vs. yes)	1.28 (0.35 – 4.71)	0.71	1.07 (0.27 – 4.35)	0.92
BPAR (vs. no BPAR)	2.026 (0.91 - 4.51)	0.08	2.32 (0.8 – 6.67)	0.12

(-): not included in the multivariable analysis, [1]R-: recipient with negative CMV IgG, R+: recipient with positive CMV IgG, D-: donor with negative CMV IgG, D+: donor with positive CMV IgG, [2]Antithymoglobulin, [3]Delayed Graft Function, [4]FK 506: Tacrolimus, CSA: Cyclosporine.

CHAPTER 8: Discussion, Conclusion, and Future Directions

8.1 Discussion

CMV is the most common opportunistic infection in renal transplant recipients. It is a well recognized cause of significant morbidity (CMV disease, acute rejection, hospitalization and graft loss) and mortality. Many risk factors for CMV disease have been identified over the past two decades. Among these factors are certain immunosuppressive agents such as antibody therapy for immunosuppression induction or rejection treatment. Immunosuppressive strategies have evolved over the decades to provide the best outcomes with regards to rejection prevention and prolonging graft survival. This progress is likely to be associated with change in the pattern of risk factors for developing CMV disease in renal transplant recipients.

The main focus of this study is to determine the incidence of CMV disease (and recurrent CMV disease) in adult renal transplant recipients at a tertiary care transplant centre, in the current era of immunosuppression (IS) and to identify the major predictors for CMV disease in this population.

In this study, the incidence of CMV disease in renal transplant recipients was approximately 15%; which is lower than incidences reported in older literature addressing similar populations[86, 146]. This finding is consistent with results from recent studies on CMV disease in renal transplant populations[81, 147]. The lower incidence of CMV disease has multiple potential explanations. First, CMV prophylaxis was given to most kidney transplant recipients in the early post-transplant period; many of these patients receive prolonged courses of oral ganciclovir[148]. Second, the use of IV ganciclovir/oral valganciclovir at the time of antibody treatment of rejection episodes. Third, the lower target levels of cyclosporine and tacrolimus used for renal transplant recipients. Finally, the less aggressive use and dosing of ATG for prevention and treatment of rejection.

Recurrent CMV disease has been reported to occur in 6-59% of solid organ transplant recipients[149]. In this study, none of the patients with CMV disease developed recurrence during the follow up period.

In our multivariable analyses, only D/R CMV sero-status and B-cell cross match were found to be associated with increased risk of developing CMV disease.

Traditionally, ATG use has been associated with the development of CMV disease in recipients of solid organ transplants[150]. However, this association has been questioned in recent studies. For instance a study by Hassan-Walker et al. showed that CMV viral load was the only risk factor for development of CMV disease and treatment with ATG was not an independent risk factor in their multiple logistic regression model[151]. Similarly, Abbott et al. in their multivariable logistic regression analysis of USRDS data, found no association between induction antibody therapy and hospitalization for CMV infections[152]. Similarly, in our study, induction antibody therapy was not associated with higher risk of developing CMV disease in renal transplant recipients. One potential reason for the absence of association is small sample size relative to the variables studied. However other alternative explanations should be also considered. These include: (1) the increased risk seen earlier studies was likely due to confounding, and association is eliminated when these factors were controlled for. (2) the absence of association in recent studies (including our study) is due to the use of smaller total doses of antibody (ATG) for induction therapy in the current immunosuppression era.

This study shows that, interestingly, patients who had a positive B-cell cross-match had a higher risk for developing CMV disease than those with negative cross-match. Such association has not been previously reported. One possible explanation for this association is that patients with positive B-cell cross-match might have received more intensive immunosuppression post-transplant, to minimize the risk for developing acute rejection episodes. The present study did not look at immunosppressive drug levels, so this hypothesis can not be

tested. Further studies are needed to evaluate this interesting relationship between B-cell cross match and CMV disease. For these studies, immunosuppressive drug levels as a measure of drug exposure should be included and adjusted for in the analysis.

An association between HLA mismatch and CMV disease has been proposed previously. Schintzler et al. and Carstens et al. have demonstrated a tendency toward a higher risk for CMV disease in the presence of donor and recipient HLA mismatch. In this study, such an association was not seen. This is most likely because in these studies important confounders (intensity and type of immunosuppression etc.) were not adjusted for, the observed associated risk may be explained by other differences in study populations[81, 87].

The limitations of our study are related to the relatively small sample size and that our data come from a single center, when compared to some previous studies that were based on large clinical and administrative databases. However, large databases may suffer from under-reporting of certain clinical variables, incomplete follow-up, misclassification, centre effect and heterogeneity of laboratory testing, which can lead to less reliable estimates of risk or association than well conducted single center studies involving exhaustive review of clinical records[153, 154]. Secondly, this study lacks comparative analyses between estimates from current and earlier studies with regards CMV disease incidence and predictors. Since the baseline rate (in LHSC kidney transplant patients) for CMV disease is not known, such comparison was not feasible.

The strength of our analysis rests on the use of multivariable model building that controlled for important confounders allowing the identification of those factors that significantly affect the outcome.

8.2 Conclusions

- This study confirms the lower incidence of CMV disease, in the current era of immunosppression, among renal transplant recipients in the first two to five years post-transplant.
- Positive B-cell cross match at the time of transplantation are strongly associated with increased risk for CMV disease; after adjusting for all known potential confounders for the relationship between CMV sero-status (donor and recipients) and CMV disease.

8.3 Future Directions

The association of B-cell cross-match at the time of kidney transplant with CMV disease has not been described in previous literature. Further study of such association in a larger population of kidney transplant recipients is warranted; after adjusting for potential confounders such as immunosuppressive drugs levels, the occurrence of antibody-mediated rejection, and the use of IVIG in the post transplant period.

The evaluation for dose-response relationship between IV antibody therapy and CMV disease warrants attention. A study involving larger number of patients is needed to test this hypothesis, to identify whether there is a dose of ATG that is safe with regards to the risk for developing CMV disease. Alternatively, the optimum duration of anti-viral therapy in patients receiving ATG requires further investigation. This group may benefit from more intensive surveillance and consideration of pre-emptive strategies[148].

APPENDIX I

Classification of chronic kidney disease (CKD)

Stage	Description	Classification by severity: GFR mL/min/1.73 m^2	Classification by severity: Related terms	Classification by treatment
1	Kidney damage with normal or ↑ GFR	≥90	Albuminuria, proteinuria, hematuria	
2	Kidney damage with mild ↓ GFR	60–89	Albuminuria, proteinuria, hematuria	
3	Moderate ↓ GFR	30–59	Chronic renal insufficiency, early renal insufficiency	T if kidney transplant recipient
4	Severe ↓ GFR	15–29	Chronic renal insufficiency, late renal insufficiency, pre-ESRD	
5	Kidney failure	<15 (or dialysis)	Renal failure, uremia, end-stage renal disease	
				D if dialysis (hemodialysis, peritoneal dialysis)

Abbreviations are: GFR, glomerular filtration rate; ESRD, end-stage renal disease.
Related terms for CKD stages 3 to 5 do not have specific definitions, except ESRD.

APPENDIX II: DIFINITIONS OF CMV RELATED INFECTIONS[a]

CMV Infection
"CMV infection" is defined as isolation of the CMV virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen.

CMV Detection in Blood
Several specific definitions for CMV detection in blood are recommended.
Viremia. "Viremia" is defined as the isolation of CMV by culture that involves the use of either standard or shell vial techniques.
Antigenemia. "Antigenemia" is defined as the detection of CMV pp65 in leukocytes.
DNAemia. "DNAemia" is defined as the detection of DNA in samples of plasma, whole blood, and isolated peripheral blood leukocytes or in buffy-coat specimens.
RNAemia. "RNAemia" is defined as the detection of RNA (e.g., by nucleic acid sequence-based amplification or noncommercial reverse transcriptase PCR) in samples of plasma, whole blood, or isolated peripheral blood leukocytes or in buffy-coat specimens.

Primary CMV Infection
"Primary CMV infection" is defined as the detection of CMV infection in an individual previously found to be CMV sero-negative. The appearance of de novo specific antibodies in a sero-negative patient may also be acceptable for the diagnosis of CMV, provided that passive transfer of antibodies via immunoglobulin or blood products can be excluded.

Recurrent Infection
"Recurrent infection" is defined as new detection of CMV infection in a patient who has had previously documented infection and who has not had virus detected for an interval of at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous).
Reinfection. "Reinfection" is defined as detection of a CMV strain that is distinct from the strain that was the cause of the patient's original infection. For cases in which infection can be demonstrated on 2 different occasions, reinfection may be documented by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.
Reinfection is diagnosed if the 2 strains are distinct. Reinfection may also be inferred if the patient develops new immune responses to epitopes known to be polymorphic; however, interference from passive antibody must be excluded.
Reactivation. Reactivation is assumed if the 2 strains are found to be indistinguishable either by sequencing specific regions of the viral genome or by using a variety of molecular techniques that examine genes known to be polymorphic.

CMV End-Organ Disease
Pneumonia. "CMV pneumonia" is defined by the presence of signs and/or symptoms of pulmonary disease combined with the detection of CMV in bronchoalveolar lavage fluid or lung tissue samples. Detection of CMV should be performed by virus isolation, histopathologic testing, immunohistochemical analysis, or in situ hybridization. Detection of CMV by PCR alone may be too sensitive for the diagnosis of CMV pneumonia and is therefore insufficient for this purpose. The presence of fungal copathogens, such as *Aspergillus* species, together with radiologic signs typical of *Aspergillus* pneumonia (e.g., a halo sign or a crescent sign) indicates fungal pneumonia rather than CMV pneumonia.
Gastrointestinal disease. "CMV gastrointestinal disease" is defined by identification of a combination of clinical symptoms from the upper or lower gastrointestinal tract, findings of macroscopic mucosal lesions on endoscopy, and demonstration of CMV infection (by culture, histopathologic testing, immunohistochemical analysis, or in situ hybridization) in a gastrointestinal tract biopsy specimen. Detection of CMV by PCR alone is insufficient for the

diagnosis of CMV gastrointestinal disease. Patients with CMV disease that involves the intestinal tract usually have mucosal abnormalities that can be seen by the endoscopist, but the appearance of some of these lesions is subtle. The spectrum of endoscopic lesions is variable and ranges from patchy erythema, exudates, and microerosions to diffusely edematous mucosa, to multiple mucosal erosions, to deep ulcers and pseudotumors. The diagnostic yield for CMV is higher when mucosal abnormalities are targeted for study. If CMV is detected in normal mucosa near a lesion consistent with those typical of CMV infection, this can be accepted as CMV gastrointestinal disease.

Hepatitis. "CMV hepatitis" is defined by findings of elevated bilirubin and/or enzyme levels during liver function testing, absence of any other documented cause of hepatitis, and detection of CMV infection (by culture, psychopathologic testing, immunohistochemical analysis, or in situ hybridization) in a liver biopsy specimen. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV hepatitis because it can imply the presence of transient viremia. Documentation of CMV (i.e., by immunohistochemical analysis) within the liver tissue is needed. Other pathogens, such as hepatitis C virus, may be present without excluding the diagnosis of CMV hepatitis.

CNS disease. "CNS disease" is defined by the identification of CNS symptoms together with the detection of CMV in CSF samples, by culture or PCR, or in brain biopsy specimens, by culture, histopathologic testing, immunohistochemical analysis, or in situ hybridization.

Retinitis. Lesions typical of CMV retinitis must be confirmed by an ophthalmologist.

Nephritis. "CMV nephritis" can be defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of histologic features of CMV infection in a kidney biopsy specimen obtained from a patient with renal dysfunction. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV nephritis. Furthermore, detection of CMV in the urine of a patient with kidney dysfunction does not fulfill the definition of CMV nephritis.

Cystitis. "CMV cystitis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a bladder biopsy specimen obtained from a patient with cystitis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV cystitis. Furthermore, detection of CMV in urine combined with identification of symptoms does not fulfill the definition of CMV cystitis.

Myocarditis. "CMV myocarditis" is defined by the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a heart biopsy specimen obtained from a patient with myocarditis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV myocarditis.

Pancreatitis. The definition of CMV pancreatitis requires the detection of CMV infection (by culture, immunohistochemical analysis, or in situ hybridization) together with the identification of conventional histologic features of CMV infection in a pancreatic biopsy specimen obtained from a patient with pancreatitis. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV pancreatitis.

Other disease categories. CMV can also cause disease in other organs, and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of CMV by biopsy (detection of CMV by PCR alone is insufficient), with other relevant causes excluded.

CMV syndrome. The term "CMV syndrome" should be avoided. Although it is recognized that CMV can cause the combination of fever and bone marrow suppression that is usually used to define the disease entity, the same symptoms can have several other different causes in stem cell transplant recipients, including such viral infections as human herpesvirus 6 (HHV-6), possibly human herpesvirus 7, and adenovirus. Antiviral drugs might have some effect against these viruses, making interpretation of causality difficult. Thus, if the term "CMV syndrome" is to be used, it must be used only after testing has been done for HHV-6, at the very least.

In solid-organ transplant recipients, CMV syndrome is better defined. At present, the minimum requirements for its definition are the documented presence of fever (temperature, >38°C) for at least 2 days within a 4-day period, the presence of neutropenia or thrombocytopenia, and the detection of CMV in blood.

[a]Definition of CMV infection, Syndrome, and Disease[97].

APPENDIX III: Collected data by source.

Variable	**Data Source**	**Data collected by:**
Demographic (Age, Gender, Race)	LHSC-UH health records	AH[1], CN[2], AAH[4]
Cause of ESRD	Health records files	CN[2]
History of prior immunosuppression	Health records files, & pharmacy records	CN[2], KD[3]
Number of previous transplants	Health records files	CN[3]
Number of HLA matches	Health records files & transplant database	CN[3], AH[1]
History of blood transfusion	Health records files	CN[2]
Donor Source	Health records files	AH[1], CN[2]
Antibody induction and type	Health records files, & pharmacy records	AH[1], CN[2], KD[3]
Maintenance immunosuppression	Health records files, & pharmacy records	AH[1], CN[2], KD[3]
CMV Prophylaxis and type	Health records files, & pharmacy records	CN[2], KD[3]
CMV culture results, CMV p65 antigenemia results, CMV serology, CMV PCR, CMV pathology (biopsy) results	Health records files	AH[1], CN[2]
Delayed graft function	Health records files	CN[2]
Donor source (Living vs. deceased)	Health records files	CN[2]
Donor CMV serology	Health records files	AH[1], CN[2]

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