Estimating the Seroprevalence of Cytomegalovirus (CMV), Epstein-Barr Virus (EBV) and the Risk of Transfusion-Transmitted and Community-Acquired CMV Infection in Solid Organ Transplant Donors and Recipients

by

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Abstract

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are ubiquitous herpesviruses that establish lifelong, often asymptomatic, infections in healthy people, but are responsible for significant morbidity and mortality in certain hosts. CMV is the most common congenital infection worldwide, causing sensorineural hearing loss, neurodevelopmental delays, and other significant sequelae. CMV seronegative women of childbearing age who experience primary CMV infection during pregnancy are at greatest risk of maternal-fetal CMV transmission. Immunocompromised individuals, such as solid organ transplant (SOT) recipients, are also at serious risk of harm from these viruses. In SOT recipients, CMV infection is known to have “direct” effects including CMV syndrome and tissue-invasive disease as well as “indirect” effects including organ rejection, organ dysfunction and increased risk of opportunistic infections. EBV infection is a major risk factor for virus-associated cancers in SOT recipients, especially post-transplant lymphoproliferative disorders (PTLD) such as Hodgkin and Burkitt lymphoma. There is a paucity of data regarding the prevalence of these viruses in Canada; such data would be valuable to assess disease burden as well as to inform public health interventions including vaccine development and vaccine deployment strategies.

Community-acquired CMV (CA-CMV) is generally transmitted from person to person via contact with infected secretions (including saliva, urine, respiratory and genital secretions); in SOT recipients, CMV can also be acquired from infected donor organs and cellular blood products. CMV seronegative recipients who receive CMV seronegative donor organs are not considered at risk of donor-transmitted (DT)-CMV

infections, thus infections occurring in the post-transplant period are either due to CA-CMV or transfusion-transmitted (TT)-CMV. Two main strategies are used to reduce risk of TT-CMV: leukoreduction of blood products and screening for CMV-seronegative blood. Presently, Canadian Blood Services (CBS) uses leukoreduction as the main strategy for preventing TT-CMV, but there is no consensus as to whether additional screening for seronegative blood is merited. The risk of TT-CMV infection in SOT has not yet been evaluated in the current era of universal leukoreduction, and this risk must be assessed while accounting for the risk of CA-CMV.

Our first objective of the research program was to estimate the age and sex-specific seroprevalence of CMV and EBV in Canada using available data from first time Canadian blood donors who donated blood between 2005 - 2014 and SOT donors and recipients who were transplanted at the University of Alberta/Stollery Children's Hospitals between 1984 – 2013. Our results show that the age and sex-specific prevalence trends for CMV and EBV in our study populations are similar to those of other western developed countries.

Our second objective was to estimate the risk of TT-CMV and CA-CMV infection in D-/R- SOT recipients transplanted at our center during the current era of universal leukoreduction. Patients transplanted between 2000 – 2011 were evaluated for receipt of blood products and incidence of CMV infection during follow-up. Our results show that after the implementation of universal leukoreduction, we did not observe any confirmed cases of TT-CMV in our cohort and that the risk of CA-CMV exceeds the risk of TT-CMV.

Together, our studies give insight into the seroprevalence of CMV and EBV in the Canadian population, and the incidence of CMV infections in our SOT population. Notably, the prevalence of CMV was much lower in blood donors (42%) than in SOT recipients (62%) and the prevalence of the general Canadian population is likely in between the two values. Our results also show that EBV prevalence rises rapidly early in life among our SOT recipients which supports the targeting of infants in potential future EBV immunization programs. Lastly, the negligible risk of TT-CMV observed in our D-/R- SOT population supports current CBS policy of using universal leukoreduction as the primary strategy to prevent TT-CMV. The risk of CA-CMV is thus a more important consideration than TT-CMV in the follow-up of D-/R- SOT recipients.

Preface

This thesis is an original work by Curtis Mabilangan. The research project, of which this thesis is a part of, received research ethics approval from the University of Alberta Research Ethics Board: “Cytomegalovirus transfusion transmission in solid organ transplant recipients in the era of universal leukoreduction” (Pro00035419, Nov. 22, 2013).

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Chapter 1: Introduction

1.1 Statement of the Problem

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are ubiquitous viruses with adult prevalence rates ranging from 40 to over 90% around the world(1). Both viruses are members of the human herpesvirus family and share the unique characteristic of establishing lifelong latent infections(2,3). They can be transmitted by most bodily secretions including urine, saliva, blood, breast milk (CMV) and genital secretions, and typically cause asymptomatic infections in healthy immunocompetent individuals. These viruses can also present symptomatically as a febrile or mononucleosis-like illness, with EBV being the primary cause of infectious mononucleosis(2,4). In women of childbearing age, CMV is a significant concern as it is a major cause of congenital infection, and among immunocompromised individuals such as solid organ transplant (SOT) recipients, both CMV and EBV infections can have severe health consequences.

Although the epidemiology of CMV and EBV has been widely studied in other nations, in Canada there is little data regarding the prevalence of these viruses. Such data would be immensely valuable not only for assessing disease burden and associated costs in the general population, but also to inform vaccine development and potential deployment strategies for preventing CMV and EBV infection. In particular, age and sex-specific prevalence are useful because vaccine modeling uses prevalence data to determine the impact of vaccine deployment at different ages and whether both males and females should be vaccinated.

CMV infections are known to be acquired either by exposure in the community, receipt of an infected donor organ, or receipt of infected cellular blood products. The

latter case, also known as transfusion-transmitted (TT)-CMV infection, has not been well studied in the SOT setting where some patients may be heavily transfused. Prevention of TT-CMV infection is primarily achieved using two strategies: universal leukoreduction of blood products and screening for CMV seronegative blood products. The current approach employed by Canadian Blood Services (CBS) is universal leukoreduction, but it remains unknown whether screening for CMV seronegative blood would grant any additional benefit.

1.2 Clinical Manifestations and Consequences of CMV and EBV Infection

While the illnesses caused by CMV and EBV are generally benign and self-limited in immunocompetent hosts, congenital CMV infection in the fetus/newborn and CMV and EBV infection in SOT recipients are responsible for significant morbidity and mortality. CMV infection acquired during pregnancy is the leading cause of congenital infection with long term sequelae including sensorineural hearing loss, neurodevelopmental delays and other serious abnormalities(5). Approximately 30-40% of primary CMV infections and 1% of non-primary infections in pregnant women are transmitted to the fetus(1). In the SOT population, CMV is known to have “direct effects” causing CMV syndrome, characterized by fever, fatigue, leukopenia, thrombocytopenia and elevation of hepatic aminotransferases, and tissue-invasive disease, which can affect almost any organ or tissue but has a predilection for the transplanted organ. CMV also has a wide variety of “indirect effects” in SOT recipients contributing to organ rejection and dysfunction, and increased risk of opportunistic infections(6). Antiviral prophylaxis and post-transplant surveillance are important strategies employed to

prevent CMV infection and disease post-transplant especially in CMV-mismatched recipients, i.e. CMV seronegative recipients receiving CMV seropositive organs (D+/R-), as they have the highest risk of donor-transmitted (DT)-CMV infection.

EBV is an oncogenic virus and EBV infection, especially primary EBV infection acquired post-transplant, is a major risk factor for post-transplant lymphoproliferative disorders (PTLD) such as Hodgkin, Burkitt, and diffuse large B-cell lymphoma, as well as epithelial malignancies such as nasopharyngeal and gastric carcinoma(7–9). EBV prevalence is highly dependent on age. Pediatric patients are disproportionately affected by PTLD as they are often seronegative and commonly receive organs from older seropositive donors (EBV mismatch: D+/R-), and thus are at very high risk of primary EBV infection post-transplant(10).

1.3 Prevalence of CMV and EBV

The prevalence of CMV and EBV in Canada remains largely unknown. CMV data are typically limited to studies of women of childbearing age which estimate CMV prevalence to be between 60-70%(11), while no large scale seroprevalence data exists for EBV. Unlike the USA, no national seroprevalence survey akin to their National Health and Nutrition Examination Survey (NHANES) has been conducted. NHANES data from 1988-1994 indicates overall CMV prevalence among Americans at least 6 years of age to be an estimated 58.9%, while NHANES data from 1999-2004 estimates prevalence to be 50.4% among Americans between the age of 6-49 years(12,13). As prevalence depends on numerous factors such as age, sex, race/ethnicity, socioeconomic status, cultural practices (hygiene, childcare, sexual activity) and

population density, CMV and EBV prevalence may differ significantly between Canada and the US. Prevalence tends to vary highly across regions especially in nations like Canada and the US who have heterogenous demographics(11). Seroprevalence data obtained from NHANES has been used to mathematically model both clinical and cost effectiveness of CMV vaccines(12–16). We sought to estimate the Canadian prevalence of CMV and EBV using available data from routine SOT donor and recipient screening from our center in Alberta, Canada, and Canadian blood donor screening data.

Despite the pressing need for both CMV and EBV vaccines, no vaccine currently exists for these viruses. In the year 2000, the Institute of Medicine earmarked the development of a CMV vaccine to be a top research priority, but nearly 20 years later no effective vaccine has been developed(17). Similarly, there is urgent need for an EBV vaccine, and several candidates are in development(18). Canadian seroprevalence data would be immensely useful in the calibration of vaccine models and lead to a substantial public health impact for Canadians once successful vaccines are developed and deployed.

1.4 Transmission of CMV Infection

CMV infection can be classified into three categories: community-acquired (CA)-CMV infection, donor-transmitted (DT)-CMV infection, and transfusion-transmitted (TT)-CMV infection. CA-CMV is largely related to behaviors where bodily fluids are exchanged. The two most important risk factors for CA-CMV are thus sexual activity and exposure to young children. Young children are especially contagious due to their high rate of viral shedding(19–21). In addition to CA-CMV, SOT recipients who are CMV

seronegative (R-) pre-transplant are at risk of incident TT-CMV infections, and if they receive an organ from a CMV seropositive donor (CMV mismatch: D+/R-), DT-CMV infections. If receiving a CMV seronegative donor organ (D-/R-) then infection is most likely CA-CMV or TT-CMV.

The pathogenic mechanism of TT-CMV infection is not completely understood. There are several possible ways for CMV to be transmitted: the blood donor may have primary infection, in which case their blood will have high levels of infectious virions, the donor may have an acute reactivation of latent infection, or the donor may have a latent infection that reactivates post-transfusion. In all cases, it is believed that TT-CMV is only possible when a patient receives cellular blood products, i.e. red blood cells or platelets, but not plasma. Indeed, no case of TT-CMV has ever been etiologically linked to transfusion with plasma, and it has been shown that the vast majority of DNA in plasma is in fact free DNA and not infectious virions(22,23).

Two main strategies are used to reduce the risk of TT-CMV: universal leukoreduction of blood products and screening for CMV seronegative blood products. In universal leukoreduction, white blood cells (WBC) are filtered from all blood products to reach a “CMV safe” level of $< 5 \times 10^6$ WBCs per unit(22). In contrast, screening for seronegative blood is done to maintain a separate inventory of blood products to be specifically administered to patients considered at high-risk of TT-CMV infection. No consensus exists regarding whether screening provides additional risk-reduction benefit to leukoreduction as opposed to leukoreduction alone due to lack of evidence and/or poor quality of existing studies(24,25). Screening adds logistical complexity and costs related to additional testing and management of dual inventories of blood products.

These costs may in fact be unjustified in the current era of universal leukoreduction, and we endeavored to resolve this controversy using data from CMV seronegative recipients of organs from CMV negative donors (D-/R-) at our center. We have the unique opportunity to study this phenomenon in our SOT population, controlling for DT-CMV infection by studying D-/R- transplants and accounting for CA-CMV infection by comparing the incidence of CMV infection in the first year post-transplant between the transfused and non-transfused cohorts.

We previously reported the risk of TT-CMV in 127 adult D-/R- transplants performed in the era prior to universal leukoreduction (1984-1998) to be 2.4% (n=3)(26). Thus, we sought to estimate the residual risk of TT-CMV in D-/R- transplants performed in the current era of universal leukoreduction while accounting for confounding sources of infection. We hypothesize that in the current era of universal leukoreduction, the residual risk of TT-CMV is negligible and that additional screening for seronegative blood is accordingly unwarranted.

1.5 Summary

CMV is the leading cause of congenital infection worldwide, and along with EBV is a major risk factor for morbidity and mortality in the immunocompromised such as our SOT population. The current prevalence of these viruses in Canada is largely unknown. Knowledge of seroprevalence would be useful for assessing disease burden, developing vaccines and designing effective public health intervention strategies.

The incidence of TT-CMV in the current era of universal leukoreduction of blood products is unknown, thus no consensus exists as to whether additional screening for

seronegative blood is needed in the current era. Given the additional complexity and costs related to additional testing and management of dual inventories of unscreened and CMV-seronegative blood products, resolving this question will help to determine if this additional resource use is justified.

1.6 Objectives

1) To estimate the prevalence of CMV and EBV in Canada using available SOT donor, recipient and blood donor serology data.
2) To estimate the risk of TT-CMV and CA-CMV infection in CMV D-/R- SOT patients transplanted in the era of universal leukoreduction

The first objective was attained through retrospective analysis of CMV and EBV serology for all SOT performed at the University of Alberta/Stollery Hospitals (UAH/SCH) between January 1984 – December 2013, as well as first time blood donors who donated between January 2005 and May 2014 (Chapter 2).

The second objective was achieved by retrospective review of all D-/R- SOT performed at UAH/SCH between January 2000 and December 2011 (Chapter 3). All available CMV serology, antigenemia and DNAemia results were reviewed. In patients lacking adequate serology follow-up post-transplant, supplemental testing of archived specimens was performed when available. All cases of suspected incident CMV infection underwent clinical chart review and were evaluated in conjunction with available laboratory testing results to verify CMV infection.

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Chapter 2: Using Blood Donors and Solid Organ Transplant Donors and Recipients to Estimate the Seroprevalence of Cytomegalovirus and Epstein-Barr Virus in Canada

2.1 Introduction

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections are almost universal during one's lifetime in the general population and once acquired, infection persists for life(3,27). Both CMV and EBV can be transmitted by exposure to infected saliva, leukocytes present in blood transfusions, organ transplants, and hematopoietic stem cells from seropositive donors. Other infected secretions such as urine, genital secretions, and breast milk are known to transmit CMV.

Most CMV infections are asymptomatic, but symptomatic CMV infection can lead to significant morbidity and even mortality primarily in two settings. First, CMV infection of an infant in utero is the most common congenital infection in Canada, occurring in an estimated 0.5% of live births with up to 15% of these children having neurodevelopmental disabilities including sensorineural hearing loss most commonly(1,28–31). It is estimated that 32% of primary CMV infections and 1.4% of recurrent infections are vertically transmitted(32). Second, despite significant advances in CMV prevention including the use of anti-viral drugs, a significant proportion of immunosuppressed patients who receive hematopoietic or solid organ transplants (SOT) continue to experience CMV infection in the post-transplant period with direct CMV effects of fever, gastrointestinal disease, hepatitis, pneumonitis, and retinitis as well as indirect effects such as graft loss, increased risk of co-infection with other pathogens, and the development of post-transplant lymphoproliferative disorder

(PTLD)(2,33,34). As a consequence of the burden of CMV in these two populations, the Institute of Medicine in the United States has identified the development of a prophylactic and/or therapeutic vaccine for the prevention of CMV disease as a public health priority(17,33). Vaccine modeling using American seroprevalence data has suggested that a CMV vaccine would be cost-effective(15).

Like CMV, most EBV infections are also asymptomatic, but infection can cause infectious mononucleosis characterized by pharyngitis, fever, and lymphadenopathy in adolescents and young adults. However, the greatest disease burden related to EBV is its association and presumed role in the pathogenesis of a large number of hematopoietic and epithelial malignancies in both immunocompetent (Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, diffuse large B cell lymphoma, gastric carcinoma) and immunocompromised patients (PTLD, smooth muscle tumors)(3,4,7,8,35). As with CMV, significant efforts are underway to develop an effective vaccine for EBV(36,37).

In Canada, there is a paucity of data regarding age-specific CMV and EBV prevalence. Such data is critical to allow modeling of disease burden and health resource expenditures associated with these viral infections and for designing future vaccine strategies. Over 90% of Canadians will be infected with EBV by age 40, but data pertaining to seroprevalence in early childhood is particularly scarce. At our transplant center in the Canadian province of Alberta, we routinely test organ donors and recipients for CMV and EBV serostatus pre-transplant to risk stratify patients and inform post-transplant management strategies aimed at preventing CMV and EBV-associated morbidity(38). Acquisition of primary CMV or EBV infection post-SOT, often

donor-transmitted, has been identified as a major risk factor for both CMV disease and EBV-associated PTLD. In addition, a subset of blood donors in Canada have been screened for CMV antibody to identify CMV seronegative blood products that could be provided to patients at high risk of morbidity from transfusion-transmitted CMV infection.

The objective of this study was to analyze the age and sex specific prevalence of CMV and EBV in available data from routine organ donor, recipient and blood donor screening as an approach to obtaining seroprevalence information regarding infection with these viruses in Canada.

2.2 Methods

Organ Transplant Population: We retrospectively analyzed all SOT recipients and donors (kidney, kidney-pancreas, pancreas, liver, small bowel, multivisceral, heart, lung and heart-lung) transplanted at the University of Alberta Hospital/Stollery Children's Health Center, Edmonton, Alberta, Canada between Jan. 1, 1984 and Dec. 31, 2013. Recipients were analyzed using the first transplant event within the study period, including re-transplant events for those patients who had been initially transplanted prior to Jan. 1, 1984 and were re-transplanted within the study period. Transplant data from 1984 – 1992 was collected manually from a database of index cards. From 1993 – 2013 data was collected from the Provincial Laboratory for Public Health (ProvLab) information systems, University of Alberta Organ Transplant Tracking Registry (OTTR) and chart review at the Edmonton Human Organ Procurement and Exchange (HOPE) program.

Blood Donor Population: We analyzed first time blood donors (age >17 years) who donated between Jan. 1, 2005 and May 3, 2014. Every collection center randomly tested a proportion of blood donors for CMV in order to maintain a national inventory of CMV negative blood products. Blood donor data including CMV serostatus, age, sex and region were provided by Canadian Blood Services (CBS). Blood donor data is maintained in a National Epidemiology Donor Database that contains all Canadian blood donors except those from Quebec. Region data was grouped as follows: BC & Yukon, Alberta, Prairies (Saskatchewan and Manitoba), Ontario (Central Ontario, Southern Ontario, North & Eastern Ontario), and Atlantic (New Brunswick, Newfoundland & Labrador, Prince Edward Island and Nova Scotia).

Serology Testing: In local transplant donors and recipients, the presence of CMV IgG was determined using an enzyme immunoassay (EIA) (Siemens Enzygnost Anti-CMV/IgG, Siemens Healthcare Diagnostics Products, Marburg, Germany) during the entire study period. Presence of EBVCA IgG or EBNA-1 IgG was assessed using the following EIAs: From 1994 – 2001, Gull Laboratories, Salt Lake City, Utah, USA was used, and from 2002 – 2013, Captia^{TM} Trinity Biotech, Bray, Ireland was used. EBV serology prior to 1994 was tested retrospectively on available samples. The CMV and EBV serostatus of non-local donors were obtained from HOPE records (assay details unknown).

CMV screening in blood donors was performed using an Olympus particle agglutination assay (PK 100, PK 200, or PK 300) which detects CMV IgG and IgM.

Variables: We investigated age, sex, organ, time period, region and year (blood donors only) and living versus cadaveric organ donor as predictors of CMV and EBV seroprevalence. For organ transplant data, analyses were stratified by age at transplant or donation (adult ≥ 17 years. and pediatric <17 years) and donor or recipient status. Age was modeled as a continuous variable in regression analysis but presented as age groups in tables. Because of the issue of passive maternal antibody, infants <12 months of age at transplant or donation were included in tables but excluded from regression analyses. For risk category analysis, infants with positive or indeterminate serology were reclassified according to the highest risk scenario: recipients were considered seronegative, while donors were considered seropositive. Indeterminate serostatus donors and recipients were also reclassified as D+ or R- in risk category analysis. In all other analyses, indeterminate serology results were treated as missing values. We grouped multiple organ transplants as follows: kidney-pancreas with kidneys, small bowel and multivisceral with liver, and heart-lung with lung. Time trends were analyzed using a continuous year variable in all groups and a binary period variable representing the first 15 years (1984-1998) and the last 15 years (1999-2013) in organ donors and recipients. Women of childbearing age were defined as women age 17 – 45 years.

Statistical Analysis: All tested donors and recipients were compared using Chi-square test for independence or Fisher's exact test across categorical variables. Pairwise comparisons were adjusted using Benjamini-Hochberg procedure. Confidence intervals for proportions were calculated using the binomial exact method. Trends over time were analyzed using linear regression or Cochran-Armitage test for trend. Prevalence odds

ratios (POR) were obtained via logistic regression with a purposeful model building strategy. Briefly, each predictor was fit to a univariate logistic regression model and entered into multivariate regression if $p < 0.20$. Age, sex and recipient organ group were considered clinically important predictors and were included in the multivariate model regardless of p-value in univariate analysis. Predictors in the multivariate model were considered statistically significant if $p < 0.05$. Before dropping predictors from the multivariate model, confounding was assessed by comparing changes in regression coefficients with and without the predictor in the model using a threshold of 15% change as evidence of confounding. Presence of interaction was tested between age, sex, and organ group. All analyses were performed using R 3.5.2(39).

2.3 Results

A flow chart outlining the solid organ transplant study groups analyzed is given in Figure 2.1. Complete cases not missing age, sex, or serology were analyzed. Donors were excluded because of missing age (n=80), and further exclusions from analysis due to missing serology are indicated by table footnotes. Over 90% and 65% of missing donor and recipient EBV serology respectively was from the period prior to implementation of routine EBV screening in 1994.

In linear regression of blood donor age versus donation year, average age at donation decreased from 40 years in 2005 to 31.1 years in 2014 ($p<0.001$). Average adult organ donor age decreased from 44.4 years in 1984 to 40.9 years in 2013 ($p=0.003$), while adult recipient age increased from 42.8 years in 1984 to 52.7 years in 2013 ($p<0.001$). Excluding infants less than 12 months, the average age of pediatric

donors decreased from 12.8 years in 1984 to 8.3 years in 2003 (p=0.002) while in pediatric recipients, it decreased from 9.6 years in 1984 to 7 years in 2013 (p=0.027). Age and sex distributions of blood donors were not significantly different between provinces.

CMV Seroprevalence

CMV seroprevalence in blood donors, organ donors, and recipients by age and sex is illustrated in Table 2.1 and Figure 2.2.

Adult CMV Seroprevalence

Multivariate regression showed increasing age and female sex were significantly associated with CMV seropositivity in all three study groups (Table 2.2). Among recipients, organ type and period were also significant predictors of seropositivity: liver recipients were more likely to be seropositive compared to kidney recipients [adjusted odds ratio (aOR): 1.38, 95% CI: (1.17, 1.62)], and recipients transplanted between 1999 and 2013 were more likely to be seronegative [aOR: 0.70, 95% CI: (0.60, 0.81)] compared with those transplanted before 1999. In blood donors, an interaction model showed that the effect of age on CMV seroprevalence was stronger in females than in males, the prevalence decreased over time and was region-dependent (Table 2.2). Among blood donors, CMV seroprevalence was highest in BC and Yukon (48.3%) and lowest in the Atlantic provinces (30.8%) (Figure 2.3). Age and sex-specific CMV seroprevalence was similar between Alberta blood and organ donors (data not shown). Living donor status was not significantly associated with CMV seroprevalence.

Pediatric CMV Seroprevalence

Among pediatric recipients, multivariate regression (Table 2.2) indicated that females were more likely to be seropositive than males [aOR: 1.66, 95% CI: (1.04, 2.66)], and that heart recipients were more likely to be seropositive compared to kidney recipients [aOR: 2.64, 95% CI: (1.44, 4.91)].

Donor/Recipient CMV Risk Stratification Categories

The proportion of transplants in each of the four D/R CMV risk categories is shown in Table 2.3. Nearly 40% of adult recipients were still recipient seronegative (R-) at transplant with 18% being CMV mismatched (D+/R-). The proportion of adult mismatches did not change between periods but the D-/R- group increased significantly from 15.7% to 21.1% ($p<0.001$). Among pediatric transplants, two thirds of transplant recipients are CMV negative pre-transplant with one third being mismatched. The proportion of pediatric mismatches significantly increased from 25.5% in 1984-1998 period to 35.5% in the 1999-2013 period ($p=0.04$).

Women of Childbearing Age

In our study, 689 organ donors, 605 recipients and 475,869 blood donors were women of childbearing age. The seroprevalence of CMV in organ donors of childbearing age was 56.2% [95% CI: (52.4%, 60.0%)], and was 56.8% in recipients [95% CI: (52.7%, 60.8%)]. The seroprevalence in blood donors was much lower at 39.0% [95% CI: (38.8%, 39.1%)], and in blood donors from Alberta, the seroprevalence was 43.4% [95% CI: (43.1%, 43.8%)]. Canadian blood donors and Alberta only donors had

significantly lower prevalence compared to organ donors and recipients ($p<0.001$ for all comparisons).

EBV Seroprevalence

EBV seroprevalence in adult and pediatric organ donors and recipients is described in Table 2.4. EBV seroprevalence reached over 90% by age 40, and the rapid acquisition of EBV in childhood by age 10-16y can be seen in Figure 2.4.

Adult EBV Seroprevalence

Multivariate regression results in Table 2.5 show that in adult donors, females were more likely to be seropositive compared to males [aOR: 1.59, 95% CI: (1.10, 2.31)], and this effect was similar in adult recipients [aOR: 1.67, 95% CI: (1.17, 2.43)]. A significant interaction was found between age and lung recipients as the effect of age on EBV seroprevalence was stronger in lungs compared to other organs. EBV seroprevalence was significantly higher in the 1999 – 2013 period compared to the 1984 – 1998 period for adult organ donors [aOR: 1.49, 95% CI: (1.02, 2.16)], but not for adult recipients.

Pediatric EBV Seroprevalence

In pediatric donors, age was the only important predictor for EBV seroprevalence. In pediatric recipients, multivariate analysis revealed organ group to be an important predictor along with age and period. Liver recipients were significantly more likely to be positive compared to kidney recipients [aOR: 2.21, 95% CI: 1.12,

4.50)]. Recipients transplanted in the 1999 - 2013 period were more likely to be seronegative compared to the 1984 – 1998 period [aOR: 0.49, 95% CI: (0.26, 0.88)].

D/R EBV Risk Stratification Categories

The proportion of patients in each D/R EBV risk category is shown in Table 2.6. In adults, 5% of adult recipients were EBV seronegative at time of transplant, and if seronegative, 92% were in the highest risk EBV mismatched (D+/R-) category. In contrast, 54% of pediatric recipients were EBV seronegative at the time of transplant, and 83% were mismatched. Overall, half of all pediatric liver and heart transplants and a third of kidney transplants were mismatched. In adult transplants, EBV mismatches decreased from 5.7% in the 1984 – 1999 period to 4.0% in the 1999 – 2013 period (p=0.03). Conversely, among pediatric transplants, EBV mismatches increased from 32.6% to 48.1% in the 1999 – 2013 period (p=0.01).

High Risk D/R Serostatus for both CMV and EBV

When analyzing adult transplants, 2.8% (n=123) were seronegative pre-transplant for both CMV and EBV, and 1.4% (n=63) were mismatched for both viruses. Among pediatric transplants including those under 12 months of age, 24.1% (n=112) were co-negative and 20.1% (n=93) were co-mismatched. Excluding recipients under 12 months of age, 22.6% (n=76) were co-negative and 9.3% (n=31) were co-mismatched.

2.4 Discussion

The age–specific CMV seroprevalence reported in our study of Canadian blood donors and adult and pediatric solid organ transplant donors and recipients, is similar to that reported in comparable populations in the United States and many areas in Western Europe(11,13,40–44), as is the association of increasing prevalence with age and female sex. This trend is in contrast to that found in developing countries of Africa, Central and South America and Asia where infection is almost universal in early childhood, a pattern that has also been observed in the indigenous population in Northern Canada(11,45). Even some industrialized countries such as the Scandinavian countries, Australia, Italy, and Spain have significantly higher age-specific seroprevalence than we observed, likely reflecting fertility rates, child care and breast-feeding practices, immigration history, and socioeconomic status of these populations(11,46). The proportion of the Canadian population in specific regions such as British Columbia that are immigrants from countries of high CMV prevalence or are indigenous will impact CMV seroprevalence in that region, possibly explaining some of the geographic differences in blood donor CMV prevalence we observed. British Columbia and the Prairies receive a significant proportion of Canada's immigrants each year, and almost half of Canada's foreign-born population is from Asia(47). The lower prevalence seen in blood donors from the Atlantic provinces is supported by older reports of CMV prevalence from this region(48,49).

Despite the usefulness of our data for modeling CMV prevalence, none of the three subgroups likely reflect true CMV seroprevalence in the general Canadian population, particularly with respect to foreign-born and indigenous representation. A

2006 study of first time Canadian blood donors suggest that donors born in Canada and the US were over-represented (90.2%) relative to the general population (84.3%)(50). Alberta blood donor and organ donor age–specific CMV seroprevalence are similar. We know that in Western Canada, the site of our study, Caucasian organ donors are under 90% of the donor population compared to Eastern Canada where they make up over 90%, and Caucasian donors tend to have much lower prevalence compared to non-Caucasians donors(51,52). During the period of our study, 39% of our adult liver transplant recipients had hepatitis B (HBV) or C (HCV) liver disease with or without hepatocellular carcinoma as the indication for transplant. Areas where HCV and HBV have high prevalence (Asia, Africa, the Mediterranean basin and the Middle East) also have very high CMV seroprevalence. Overrepresentation of immigrants from these countries may explain the higher CMV seroprevalence observed in adult liver transplant recipients relative to other organ types. In addition, Canadian indigenous populations with higher CMV seroprevalence have a disproportionate burden of diabetes, immune-mediated kidney disease and associated complications of chronic kidney disease and ischemic heart disease that might result in kidney or heart transplantation(53). This may be an additional factor explaining the higher CMV seroprevalence rates observed in organ transplant recipients compared to Canadian blood donors.

A decrease in overall and age-specific CMV prevalence has been observed by some international investigators serially studying the same population(54–56) but not all(12). We have limited historical data on CMV seroprevalence in blood donors. Two smaller studies document seroprevalences of 38% in 1983-85 and 40.5% in 1989-94 in Alberta blood donors(26,57). The 45.9% CMV seroprevalence documented in Alberta

blood donors in our current study is a marked increase by comparison, but we also observed that the CMV seroprevalence appeared to be decreasing in our blood donor pool from 2005-2014. Disproportionate (relative to the general population) and increasing donations from young blood donors aged 17-25y may account for this change(58). The best evidence for decreasing age-specific CMV seroprevalence rates over time is in the adult organ transplant recipient population where CMV seroprevalence decreased over time despite increasing age at transplant, and this trend is also echoed by the multivariate regression model in our study that demonstrated a marked decrease in prevalence in the 1999-2013 period after accounting for age, sex, and organ group.

In our study, we estimated CMV prevalence in women of childbearing age to be 56% in organ donors and recipients, and this was nearly identical to the 55% reported previously in Edmonton(52) and two other studies in Canadian pregnant women reporting 55%(31) and 54%(59) respectively. However, the largest known Canadian study of pregnant women, which was conducted in Quebec, reported a prevalence of 42%(60) which is closer to the 39% we observed in Canadian blood donors. These prevalence estimates indicate a significant opportunity to protect Canadian women and their children from the risk of congenital CMV infection with an effective CMV vaccine. Furthermore, the prevalence estimates we report are comparable to those from American NHANES data used for calibration in recent vaccine modeling studies(14,16). These models demonstrate tremendous potential reductions in congenital CMV with universal immunization of infants, but even vaccinating adolescent females can realize

not only significant resource and cost savings but also an immense gain in quality-adjusted life years for infants born to vaccinated mothers(15).

The potential value of a CMV vaccine cannot be understated, and extensive benefits could also be gained in the domain of transplantation as 18% of our adult transplants and 33% of our pediatric transplants fall into the highest risk D+/R- (CMV mismatched) serostatus subgroup for serious CMV disease after transplant. This is similar to the 20% reported in a similar group of transplant recipients in the United States(61). Relative to other CMV D/R subgroups, CMV mismatched patients use an inordinate amount of health care resources in the form of prophylactic anti-viral therapy and laboratory monitoring for evidence of CMV infection as strategies to prevent CMV disease. Relieving these patients from direct morbidity associated with CMV disease and the possible indirect effects of CMV related to graft loss, as well as antiviral side effects is therefore critical both clinically and economically.

EBV prevalence is strongly dependent on age, and infection tends to be acquired at older ages in developed countries with low population density and high hygiene standards(9). From our study, it is clear that Canadians acquire EBV much later in comparison to countries such as Thailand(62), Taiwan(63), and China(64), who attain 90% seroprevalence between the ages of 5-8y, as we do not approach 90% seroprevalence until age 30. However, in our transplant population, 39% of recipients are already EBV seropositive by the age of 2 years. While the donor prevalence was 70% by 2 years of age, this is likely an overestimation as donors may have been transfused and serology could be falsely positive from passive antibody. Few sources of similar data exist in young children. A Minnesota study reported 31% EBV prevalence

among children age 1-5y(65), and a birth cohort study reported 7% prevalence by age 1 and 18% prevalence by age 2(66). Taken together with our study, these data suggest that a vaccine, if available and able to block transmission, would have most impact if it targeted children under the age of 2 years.

In the transplant setting, EBV-mismatched patients (D+/R-) are at highest risk of developing PTLD after transplant. Our study illustrates why this is such a significant problem in pediatric recipients who have estimated PTLD incidences of up to 10% and a 45-fold greater risk of cancer compared to the general population(10,61). This problem may also escalate if the trend of transplanting younger recipients we observed continues and with increased rates of living donor transplantation and use of older donor organs for liver and kidney transplant recipients. In contrast, a report using American registry data indicated a lower pediatric EBV mismatch rate of 31% in heart recipients compared to our data, although if we exclude children <12 months of age then our EBV mismatch rate in hearts is more comparable at 28%. It was unclear whether they accounted for potential passive maternal antibody in children <12 months of age(61). The incidence and predictability of EBV transmission and the high rates of PTLD observed in EBV mismatched organ transplant recipients make it an apt setting to test future EBV vaccines with respect to blocking infection and preventing malignancy.

A trend of waning age-specific EBV prevalence over time has been observed by others in both healthy pediatric and adult(67) populations(67,68). In our study, age-specific EBV seroprevalence did not significantly change in the adult recipient population over time, although we observed that age-specific EBV prevalence increased in adult organ donors and decreased in pediatric recipients. This may be attributable to

the older average age of our adult recipient population in comparison to adult organ donors and pediatric recipients, precluding observation of changing prevalence over time as prevalence is already universal in older adults.

A limitation of our study is that additional demographic variables such as race, ethnicity and socioeconomic status were unavailable for analysis. Also, data was missing in early years due to lack of testing especially for EBV serology. Furthermore, fewer transplants were performed in the early period so data in that era may be underpowered compared to the later period. Transfusion data in organ donors and recipients was unavailable and the presence of passive antibody may inflate seroprevalence. Lastly, our data is from a single centre in Western Canada and not necessarily representative of all Canadians. Overall, our study's strength is the large sample of transplant patients among all age groups over a 30-year period as well as the vast blood donor data derived from the majority of Canada.

In summary, we illustrate how CMV and EBV serology data obtained by routine screening in the setting of blood donor and organ transplant screening might be used to inform modeling with respect to determining the burden of disease and associated health-care resource expenditures as well as future vaccine deployment strategies in Canada. Given the prevalence rates observed in our study, the development of effective vaccines would be expected to have a substantial impact on the public health of Canadians.

Table 2.1. Adult and Pediatric CMV Seroprevalence in Blood Donors, Organ Donors, and Recipients by Age and Sex. Pediatric patients age <12M are included in the table for descriptive purposes only and were not analyzed.
*Exclusions due to missing or indeterminate serology: 35 adult donors (32 indeterminate), 75 adult recipients (49 indeterminate), 8 pediatric donors (8 indeterminate) and 6 pediatric recipients (4 indeterminate).
[abc]Superscript letters indicate statistically significant pairwise comparisons with adjusted p<0.05

Characteristics	Blood Donors			Organ Donor			Recipients		
	N (median age, IQR)	Prevalence% 95% CI	POR 95% CI	N (median age, IQR)	Prevalence% 95% CI	POR 95% CI	N (median age, IQR)	Prevalence% 95% CI	POR 95% CI
Overall Adult Prevalence	1253350 (25.0, 15.0)	42.2[bc] (42.1 – 42.3)	-	2588* (42.6, 23.0)	53.3[ab] (51.4 – 55.3)	-	4113* (50.9, 19.2)	62.3[ac] (60.8 – 63.8)	-
Adult Age Group									
17y-29y	499529	33.8[bc] (33.7 – 33.9)	1 (ref)	658	43.0[ab] (39.2 – 46.9)	1 (ref)	445	48.3[ac] (43.6 – 53.1)	1 (ref)
30y-39y	218959	42.4[ab] (42.2 – 42.6)	**1.44 (1.43 – 1.46)**	475	50.9[a] (46.4 – 55.5)	**1.38 (1.08 – 1.75)**	599	49.6[b] (45.5 – 53.7)	1.05 (0.82 – 1.34)
40y-49y	264977	46.4[bc] (46.2 – 46.6)	**1.70 (1.68 – 1.71)**	626	55.8[ab] (51.8 – 59.7)	**1.67 (1.34 – 2.08)**	916	59.4[ac] (56.1 – 62.6)	**1.56 (1.25 – 1.97)**
50y-59y	205037	51.4[bc] (51.2 – 51.7)	**2.08 (2.05 – 2.10)**	535	57.0[ab] (52.7 – 61.2)	**1.76 (1.40 – 2.21)**	1249	68.0[ac] (65.3 – 70.6)	**2.27 (1.82 – 2.83)**
60y-69y	63520	59.1[bc] (58.9 – 59.5)	**2.84 (2.79 – 2.89)**	193	65.8[ab] (58.6 – 72.4)	**2.55 (1.83 – 3.58)**	819	72.9[ac] (69.7 – 75.9)	**2.88 (2.26 – 3.67)**
>70y	1328	66.7 (64.1 – 69.2)	**3.93 (3.51 – 4.41)**	101	73.3 (63.5 – 81.5)	**3.63 (2.30 – 5.88)**	85	69.4 (58.5 – 79.0)	**2.43 (1.49 – 4.05)**
Adult Sex									
Male	590675	40.8 (40.7 – 40.9)	1 (ref)	1308	47.7 (45.0 – 50.5)	1 (ref)	2712	59.4 (57.5 – 61.2)	1 (ref)
Female	662675	43.4[bc] (43.3 – 43.5)	**1.11 (1.10 – 1.12)**	1280	59.1[ab] (56.3 – 61.8)	**1.58 (1.35 – 1.85)**	1401	67.9[ac] (65.4 – 70.3)	**1.45 (1.26 – 1.66)**
Overall Pediatric Prevalence	-	-	-	250* (10.7, 10.5)	44.4 (38.1 – 50.8)	-	302* (8.1, 10.1)	44.4 (38.7 – 50.2)	-

Pediatric Age Group									
<12M	-	-	-	52	44.2 (30.5 – 58.7)	-	112	50.0 (40.4 – 59.6)	-
12M – 2y	-	-	-	24	29.2 (12.6 – 51.1)	1 (ref)	50	34.0 (21.2 – 48.8)	1 (ref)
2y – 4y	-	-	-	42	47.6 (32.0 – 63.6)	2.21 (0.78 – 6.74)	53	45.3 (31.6 – 59.6)	1.61 (0.73 – 3.60)
5y – 9y	-	-	-	52	38.5 (25.3 – 53.0)	1.52 (0.55 – 4.52)	65	43.1 (30.8 – 56.0)	1.47 (0.69 – 3.19)
10y – 16y	-	-	-	132	48.5 (39.7 – 57.3)	2.29 (0.92 – 6.25)	134	48.5 (39.8 – 57.3)	1.83 (0.94 – 3.66)
Pediatric Sex									
Male	-	-	-	147	40.8 (32.8 – 49.2)	1 (ref)	148	37.8 (30.0 – 46.2)	1 (ref)
Female	-	-	-	103	49.5 (39.5 – 59.5)	1.42 (0.86 – 2.37)	154	50.6 (42.5 – 58.8)	**1.69 (1.07 – 2.67)**

Table 2.2. CMV Multivariate Regression Models for Blood Donors, Organ Donors and Recipients. An interaction model was used for Blood Donors. Pediatric regression excluded patients <12M. No variable was significant in Pediatric Organ Donor Regression. *Asterisks indicate statistical significance as follows: <0.001***, <0.01**, <0.05*.

Variable	Blood Donor OR coefficient (OR 95% CI)	Adult Organ Donor OR coefficient (OR 95% CI)	Adult Recipient OR coefficient (OR 95% CI)	Pediatric Recipient OR coefficient (OR 95% CI)
Age	1.021 (1.021 – 1.022)***	1.02 (1.01 – 1.03)***	1.03 (1.03 – 1.04)***	1.05 (1.00 – 1.10)
Sex				
Male	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Female	0.96 (0.94 – 0.98)***	1.47 (1.26 – 1.73)***	1.53 (1.33 – 1.76)***	1.66 (1.04 – 2.66)*
Age X Female (Interaction)	1.0053 (1.0047 – 1.0058)***	-	-	-
Organ				
Kidney	-	-	1 (ref)	1 (ref)
Liver	-	-	1.38 (1.17 – 1.62)***	1.31 (0.71 – 2.43)
Heart	-	-	0.95 (0.78 – 1.16)	2.64 (1.44 – 4.91)**
Lung	-	-	0.92 (0.75 – 1.12)	0.54 (0.08 – 2.56)
Period				
1984-1998	-	-	1 (ref)	-
1999-2013	-	-	0.70 (0.60 – 0.81)***	-
Region				
BC & Yukon	1 (ref)	-	-	-
Alberta	0.92 (0.91 – 0.94)***	-	-	-
Prairies	0.90 (0.88 – 0.91)***	-	-	-
Ontario	0.74 (0.74 – 0.75)***	-	-	-
Atlantic	0.46 (0.45 – 0.47)***	-	-	-
Year	0.98 (0.98 – 0.98) ***	-	-	-

Table 2.3. Adult and Pediatric CMV Risk Categories by Organ Type. Seropositive and indeterminate donors and recipients age <12M were risk adjusted to be D+ or R- respectively. Indeterminate donors and recipients age >12M were risk adjusted to be D+ or R- respectively. *Exclusions due to missing serology in adults (n=34), and pediatrics (n=4)

Adult	**D-/R-** % of transplants (n)	**D+/R-** % of transplants (n)	**D-/R+** % of transplants (n)	**D+/R+** % of transplants (n)	**Total N**
Kidney	21.3% (451)	17.4% (369)	28.3% (600)	33.1% (701)	2121
Liver	15.5% (179)	17.2% (199)	32.6% (377)	34.7% (402)	1157
Heart	20.1% (120)	18.9% (113)	30.7% (183)	30.3% (181)	597
Lung	20.5% (117)	20.3% (116)	23.6% (135)	35.6% (203)	571
Total	19.5% (867)	17.9% (797)	29.1% (1295)	33.4% (1487)	4446*
Pediatric					
Kidney	31.6% (30)	30.5% (29)	11.6% (11)	26.3% (25)	95
Liver	39.2% (85)	34.1% (74)	14.3% (31)	12.4% (27)	217
Heart	27.8% (42)	32.5% (49)	23.8% (36)	15.9% (24)	151
Lung	37.5% (3)	37.5% (3)	12.5% (1)	12.5% (1)	8
Total	34.0% (160)	32.9% (155)	16.8% (79)	16.3% (77)	471*

Table 2.4. Adult and Pediatric EBV Seroprevalence in Organ Donors and Recipients by Age and Sex. Pediatric patients age <12M are included in the table for descriptive purposes only and are not analyzed. *Exclusions due to missing or indeterminate serology: 226 adult donors (22 indeterminate), 224 adult recipients (34 indeterminate), 19 pediatric donors (4 indeterminate) and 16 pediatric recipients (4 indeterminate).
[a]Superscript letters indicate statistically significant comparisons with p<0.05

Characteristics	**Organ Donor**			**Recipients**		
	N (median age, IQR)	**Prevalence% 95% CI**	**OR 95% CI**	**N (median age, IQR)**	**Prevalence% 95% CI**	**OR 95% CI**
Overall Adult Prevalence	2397* (42.1, 22.4)	94.3 (93.3 – 95.2)	-	3964* (51.2, 19.0)	95.8 (95.1 – 96.4)	-
Adult Age Group						
17y-29y	622	89.5 (86.9 – 91.8)	1 (ref)	420	86.7 (83.0 – 89.8)	1 (ref)
30y-39y	450	95.8[a] (93.5 – 97.4)	**2.65 (1.59 – 4.60)**	566	92.6[a] (90.1 – 94.6)	**1.91 (1.26 – 2.94)**
40y-49y	59	94.9 (92.8 – 96.5)	**2.17 (1.40 – 3.45)**	874	97.0 (95.7 – 98.0)	**5.02 (3.13 – 8.24)**
50y-59y	497	96.6 (94.6 – 98.0)	**3.29 (1.95 – 5.87)**	1228	98.0 (97.0 – 98.7)	**7.40 (4.61 – 1.22)**
60y-69y	175	98.3 (95.1 – 99.6)	**6.69 (2.45 – 27.60)**	792	97.9 (96.6 – 98.7)	**7.01 (4.11 – 1.26)**
>70y	64	96.9 (89.2 – 99.6)	**3.62 (1.10 – 22.37)**	84	100 (95.7 – 100)	**-**
Adult Sex						
Male	1189	92.7 (91.1 – 94.1)	1 (ref)	2608	95.3 (94.4 – 96.1)	1 (ref)
Female	1208	95.9[a] (94.7 – 97.0)	**1.87 (1.31 – 2.69)**	1356	96.8[a] (95.7 – 97.6)	**1.46 (1.04 – 2.10)**
Overall Pediatric Prevalence	239* (10.1, 11.0)	74.5 (68.5 – 79.9)	-	292* (8.1, 10.0)	65.4 (59.6 – 70.9)	-
Pediatric Age Group						
<12M	54	72.2 (58.4 – 83.5)	-	117	58.1 (48.6 – 67.2)	-
12M – 2y	23	69.6[a] (47.1 – 86.8)	1 (ref)	46	39.1[a] (25.9 – 54.6)	1 (ref)

2y – 4y	43	62.8 (46.7 – 77.0)	0.74 (0.24 – 2.14)	54	61.1 (46.9 – 74.1)	**2.44 (1.10 – 5.56)**
5y – 9y	51	68.6 (54.1 – 80.9)	0.96 (0.32 – 2.73)	64	59.4 (46.4 – 71.5)	**2.27 (1.06 – 5.00)**
10y – 16y	122	82.0 (74.0 – 88.3)	1.99 (0.70 – 5.28)	128	79.7 (71.7 – 86.3)	**6.10 (2.97 – 12.91)**
Pediatric Sex						
Male	140	73.6 (65.5 – 80.6)	1 (ref)	145	63.4 (55.1 – 71.3)	1 (ref)
Female	99	75.8 (66.1 – 83.8)	1.12 (0.62 – 2.05)	147	64.6 (56.3 – 72.3)	1.19 (0.73 – 1.93)

Table 2.5. EBV Multivariate Regression Models for Organ Donors and Recipients. Pediatric regression excludes patients <12M. An interaction model was used for Adult Recipients. Pediatric Donor regression revealed only age as a significant predictor. *Asterisks indicate statistical significance as follows: <0.001***, <0.01**, <0.05*.

Variable	Adult Organ Donor OR coefficient (OR 95% CI)	Adult Recipient OR coefficient (OR 95% CI)	Pediatric Donor OR coefficient (OR 95% CI)	Pediatric Recipient OR coefficient (OR 95% CI)
Age	1.04 (1.02 – 1.05)***	1.05 (1.04 – 1.07)***	1.08 (1.02 – 1.14)**	1.16 (1.10 – 1.23)***
Sex				
Male	1 (ref)	1 (ref)	-	-
Female	1.59 (1.10 – 2.31)*	1.67 (1.17 – 2.43)**	-	-
Organ				
Kidney	-	1 (ref)	-	1 (ref)
Liver	-	0.88 (0.19 – 4.51)	-	2.21 (1.12 – 4.50)*
Heart	-	2.41 (0.54 – 12.59)	-	1.60 (0.80 – 3.26)
Lung	-	0.09 (0.02 – 0.35)***	-	1.06 (0.22 – 5.80)
Age X Lung	-	1.06 (1.02 – 1.10)***	-	-
Age X Liver	-	1.02 (0.98 – 1.06)	-	-
Age X Heart	-	0.98 (0.94 – 1.00)	-	-
Period				
1984-1998	1 (ref)	-	-	1 (ref)
1999-2013	1.49 (1.02 – 2.16)*	-	-	0.49 (0.26 – 0.88)*

Table 2.6. Adult and Pediatric EBV Risk Categories by Organ Type. Seropositive and indeterminate donors and recipients age <12M were risk adjusted to be D+ or R- respectively. Indeterminate donors and recipients age >12M were risk adjusted to be D+ or R- respectively. *Exclusions due to missing serology in adults (n=515), and pediatrics (n=38)

Adult	**D-/R-** % of transplants (n)	**D+/R-** % of transplants (n)	**D-/R+** % of transplants (n)	**D+/R+** % of transplants (n)	**Total N**
Kidney	0.7% (12)	5.1% (91)	8.0% (142)	86.3% (1539)	1784
Liver	0.3% (3)	1.8% (20)	6.4% (71)	91.5% (1018)	1112
Heart	0.0% (0)	6.0% (31)	8.3% (43)	85.7% (442)	516
Lung	0.2% (1)	6.3% (35)	6.7% (37)	86.8% (480)	553
Total	0.4% (16)	4.5% (177)	7.4% (293)	87.7% (3479)	3965*
Pediatric					
Kidney	2.5% (2)	34.2% (27)	3.8% (3)	59.5% (47)	79
Liver	8.2% (17)	51.0% (106)	10.1% (21)	30.8% (64)	208
Heart	12.7% (18)	45.1% (64)	8.5% (12)	33.8% (48)	142
Lung	37.5% (3)	0.0% (0)	12.5% (1)	50.0% (4)	8
Total	9.2% (40)	45.1% (197)	8.5% (37)	37.3% (163)	437*

Figure 2.1. Study Population Flow Chart. *4548 first event transplants, 362 second event transplants, 42 third event transplants, 4 fourth event transplants. 407 total re-transplant events. **4548 recipients first transplanted during study period plus 66 recipients first transplanted prior to study period. [1-6]Numeric footnote indicates table in which this data was analyzed. †80 donors were missing age and thus not split into adult or pediatric datasets.

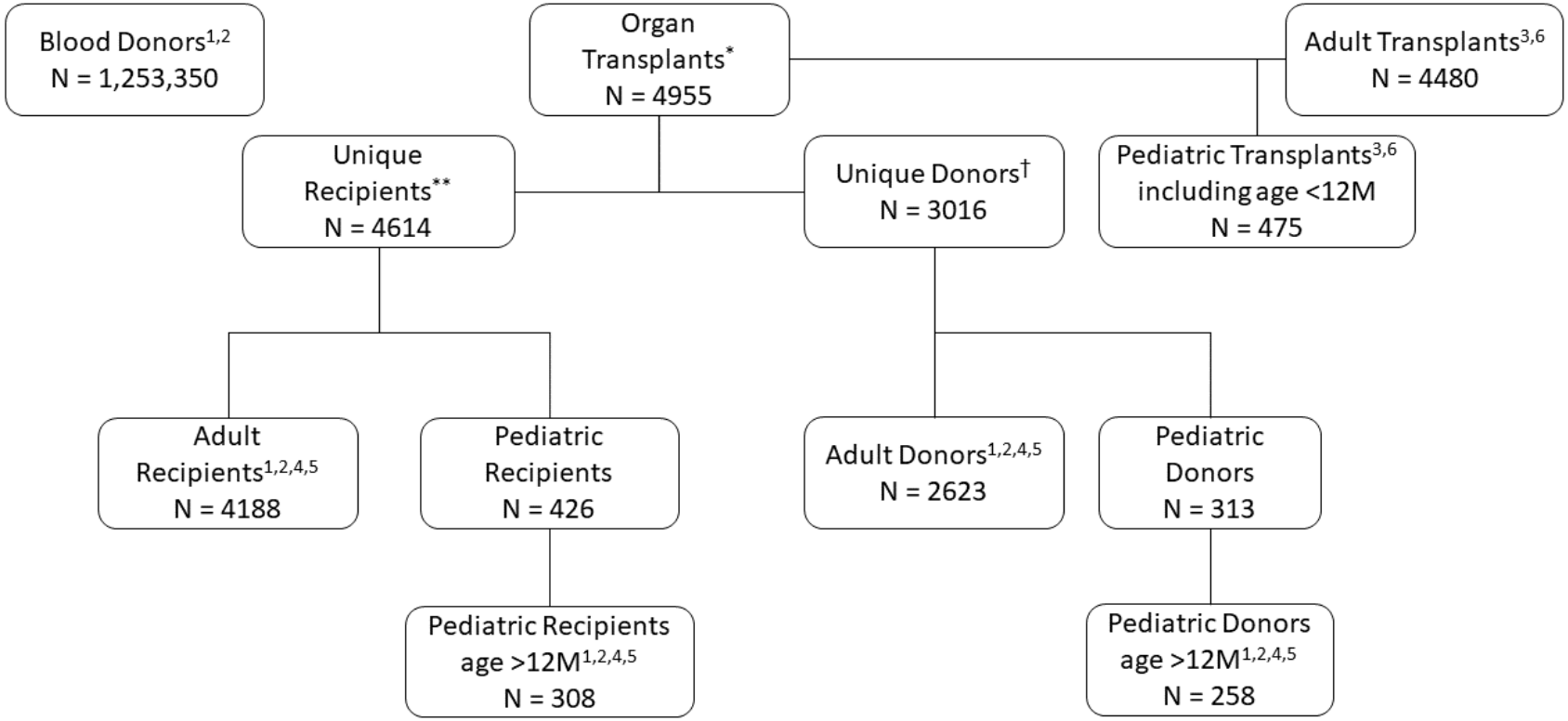


Figure 2.2. CMV Seroprevalence vs Age among Blood Donors, Organ Donors and Recipients. Seroprevalence in <12M individuals is inflated by presence of maternal antibodies. Female seroprevalence was higher than males in all age groups except donors <12M.

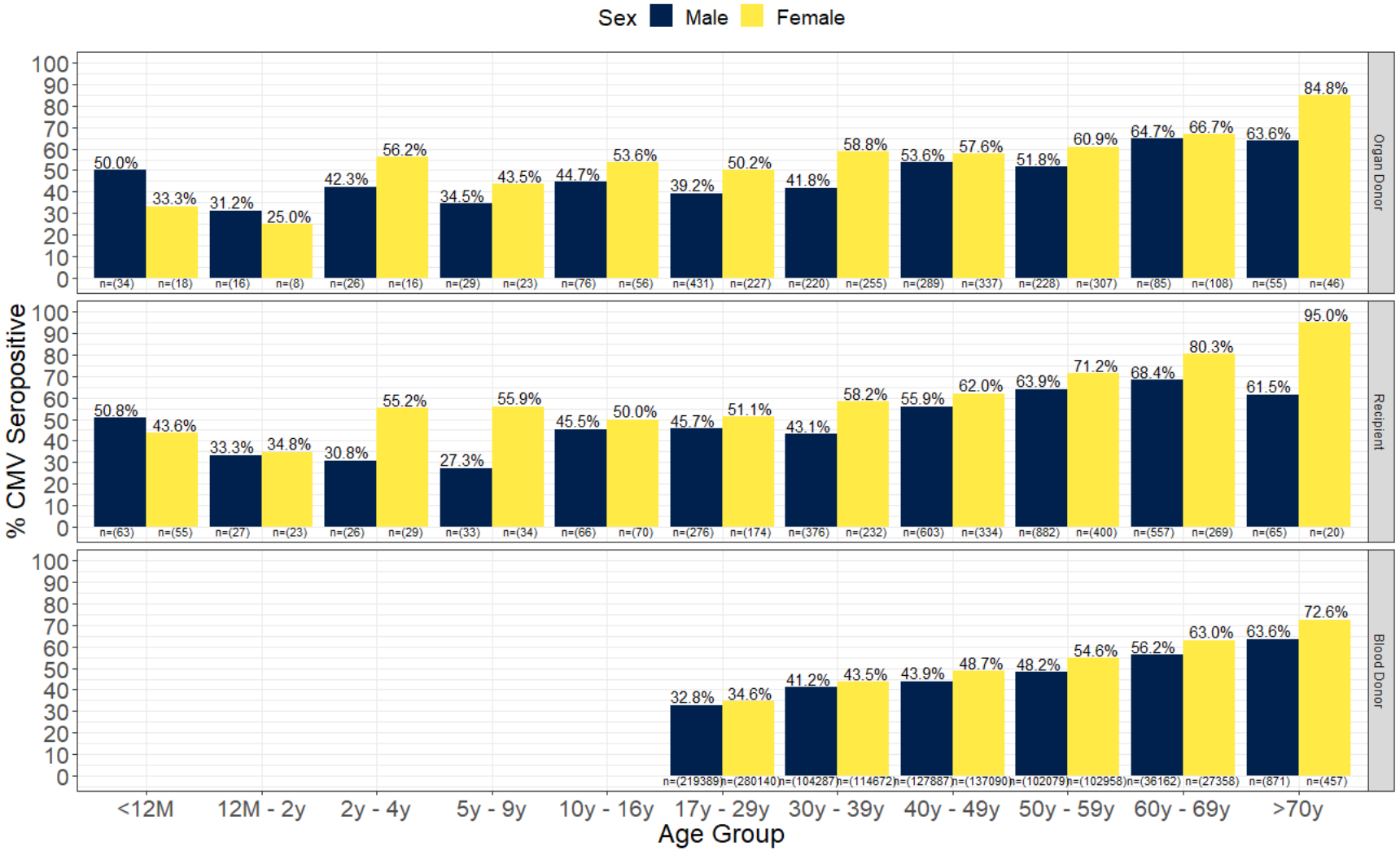


Figure 2.3. CMV Prevalence in Blood Donors across Canada. A trend of decreasing prevalence across Canada is visible. Female seroprevalence is higher than males in all regions.

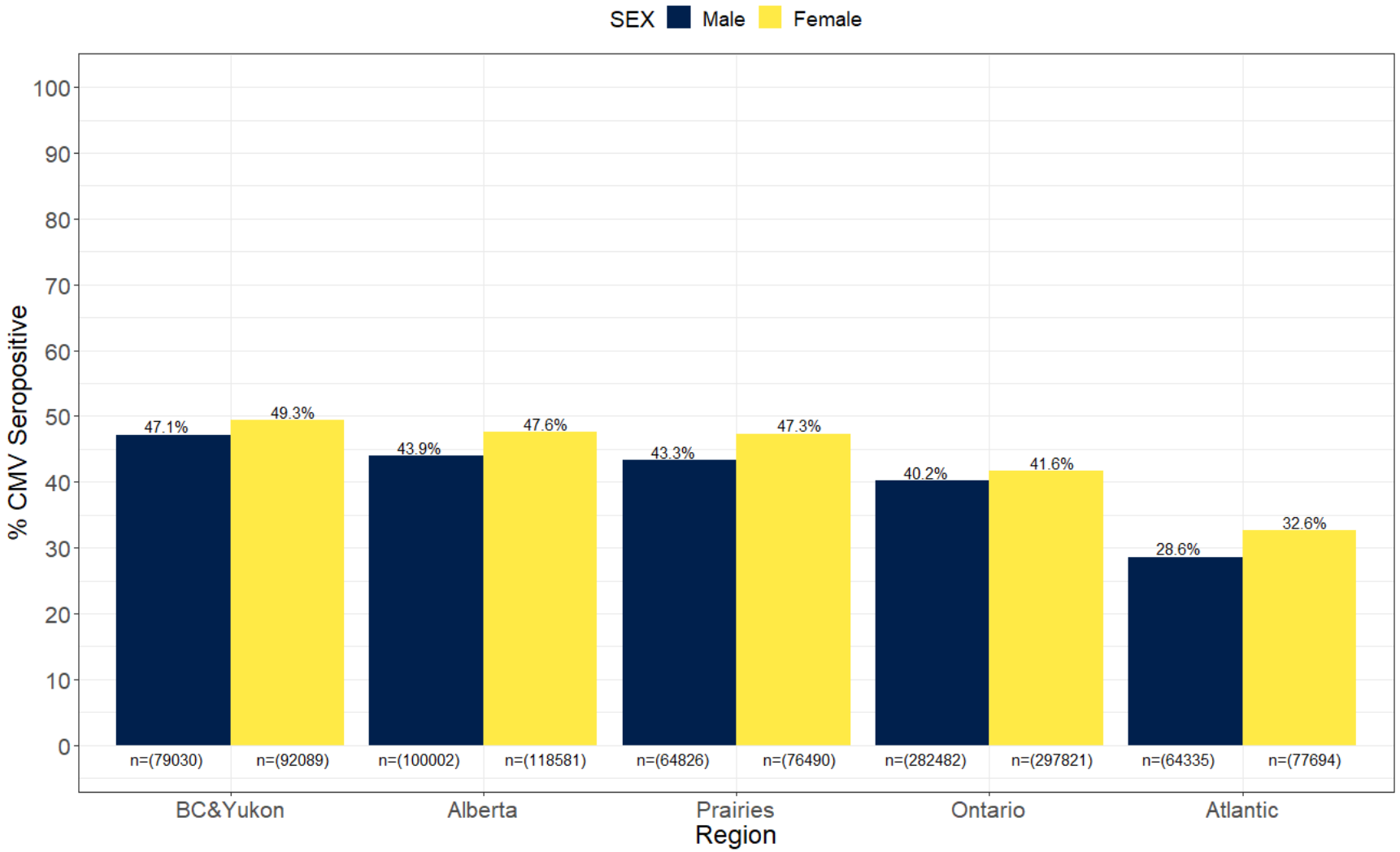
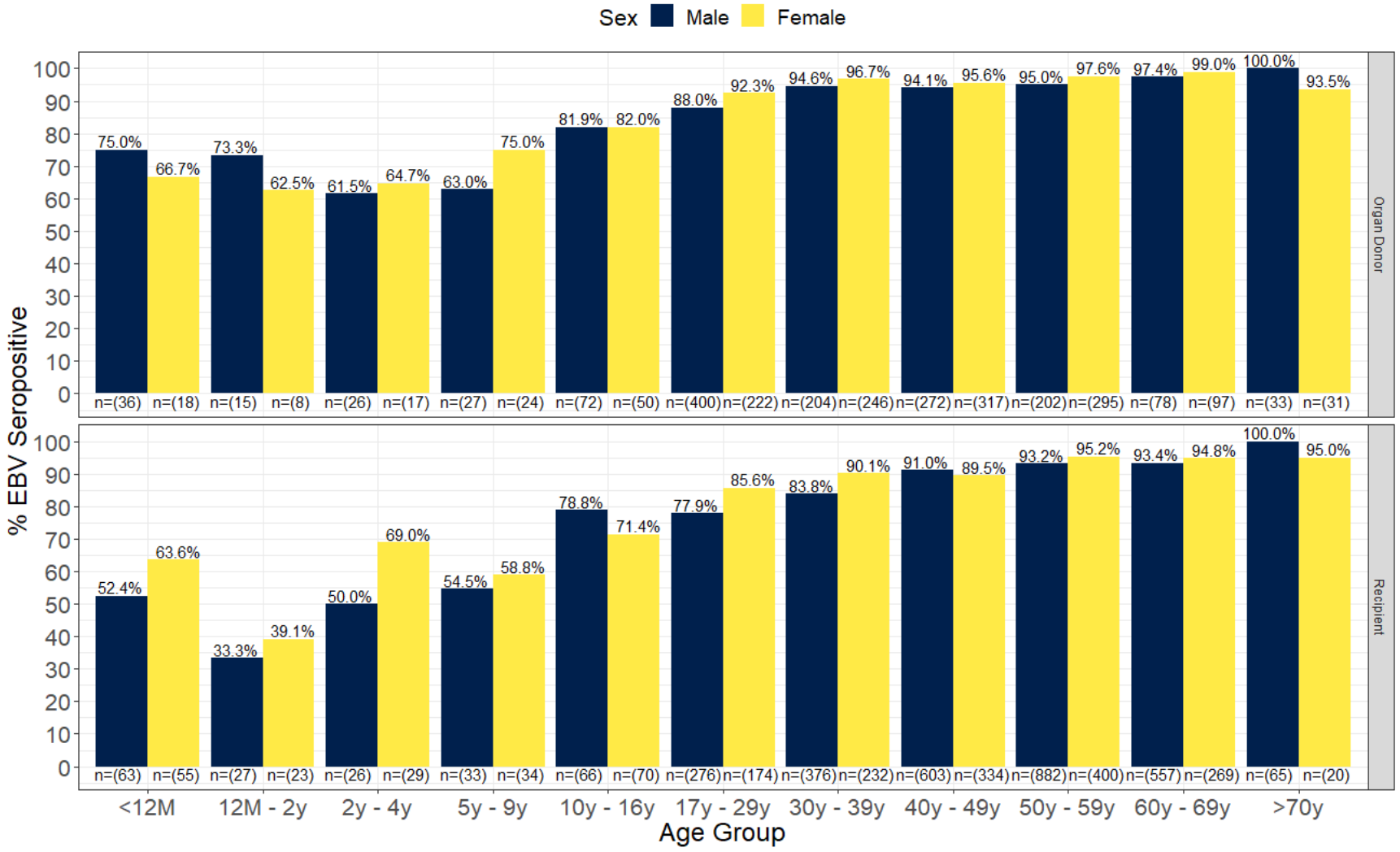


Figure 2.4. EBV Seroprevalence vs Age. The rapid rise of EBV seroprevalence is clear in recipients. This pattern is less clear in donors.



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Chapter 3: Transfusion-Transmitted and Community-Acquired Cytomegalovirus (CMV) Infection in CMV Seronegative Solid Organ Transplant Recipients Receiving CMV Seronegative Organs and Leukoreduced Cellular Blood Products

3.1 Introduction

Cytomegalovirus (CMV) is highly prevalent in the general population and acute primary infection is characterized by viremia, predominantly in polymorphonuclear cells, and plasma CMV DNAemia. Acute infection is followed by CMV persistence and latency in CD34+ myeloid progenitor cells and their derivative CD14+ monocytes with CMV DNA found in 0.004% to 0.01% of mononuclear cells from healthy seropositive donors after granulocyte-colony-stimulated mobilization(69). This tropism results in CMV being transmitted by blood transfusion, which is well-documented(70). Although CMV infection is usually asymptomatic, significant morbidity is observed in low birthweight neonates, in pregnant women with resulting congenital infection and in immunosuppressed patients including hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients.

Historical reports of transfusion-transmitted (TT)-CMV infection rates have reached as high as 60% when giving fresh whole blood to CMV seronegative recipients(71). The use of CMV “safe” blood products in the form of CMV seronegative or leukoreduced cellular blood components (<5 x 10^6 WBC/unit) has had a major impact on this risk, reducing transfusion-transmitted (TT)-CMV infection by an estimated 93.1% and 92.3%, respectively, in high risk populations(72). However, residual “breakthrough” CMV infection is believed to occur in 1.2-3.0% of high-risk populations receiving these

CMV “safe” products. There is ongoing controversy regarding whether these two strategies for TT-CMV prevention are equally efficacious and whether CMV serological testing adds additional benefit to leukoreduction for reducing TT-CMV. A recent systematic review and meta-analysis comparing the two strategies was unable to resolve this controversy because of the low or very low quality of the studies and the absence of studies performed within the last decade(24). These factors prevented the American Association of Blood Banks from making recommended clinical practice guidelines regarding this issue(25). The issue is further complicated by the lack of clear understanding related to the pathogenesis of TT-CMV specifically the relative contributions of viremic donors experiencing primary infection versus latently infected donors who reactivate CMV or even transfused latently infected cells reactivating virus after transfusion in the recipient. We recently demonstrated that CMV DNA in plasma during acute infection is entirely cell-free DNA, highly fragmented and unlikely to be infectious(23) confirming the results of a previous study(73).

Studies of TT-CMV must also account for confounding by community-acquired (CA)-CMV infection, which is particularly common in infants and toddler-aged children as CMV is also frequently transmitted by breastmilk from seropositive mothers and by mucosal exposure to infected saliva, urine and genital secretions(27). To account for this confounding, we will compare the incidence of CMV infection between transfused and non-transfused CMV D-/R- SOT recipients in the first post-transplant year.

We previously reported the risk of TT-CMV in 127 adult CMV seronegative SOT recipients of CMV seronegative organs (kidney, liver, heart, and lung) transplanted between 1984 and 1996 at the University of Alberta Hospitals (UAH) transfused with

random non-leukoreduced blood not screened for CMV seronegativity(26). Three cases (2.4%) of presumed TT-CMV infection were observed. In Canada, pre-storage WBC reduction of platelets was implemented in January 1998 and was extended to red blood cell (RBC) units in July 1999. We therefore repeated the study to examine both TT-CMV and CA-CMV in adult and pediatric seronegative SOT recipients receiving CMV seronegative organs in the era of universal blood product leukoreduction.

3.2 Methods

Patient population/data elements collected: This retrospective cohort study included all pediatric and adult (≥ 17-year-old) patients who were CMV seronegative (R-) and received a solid organ transplant (lung, heart, liver, kidney, whole pancreas, or multivisceral) from a CMV seronegative donor (D-) at the University of Alberta/Stollery Children's Hospitals (UAH/SCH), Edmonton, Alberta, Canada between January 2000 and December 2011. CMV seropositive children < 12 months of age were excluded from our study, although we recognized that many may have had false positive CMV serology due to passive maternal antibody. Patients who died within one month of transplant were also excluded but were reviewed to confirm that the death was unrelated to CMV. Data including organ type (grouped as kidney including kidney-pancreas and pancreas, liver, heart, and lung including heart-lung), date of transplant, pre-transplant donor and recipient CMV serology, EBV serology, age, sex, history of previous transplants and results of all routine post-transplant CMV testing (serology and antigenemia/CMV DNAemia) were obtained from a prospectively maintained transplant

database. Additional CMV serology follow-up testing was performed to supplement available data.

Immunosuppression: In all allograft types, triple therapy using tacrolimus, mycophenolate mofetil (MMF) and steroids were used as routine maintenance immunosuppression. Steroids were tapered to steady state levels by the end of first post-transplant year in non-liver recipients and discontinued in rejection-free liver recipients by 3-6 months post-transplant. Subsets of adult liver recipients with hepatocellular carcinoma or renal dysfunction were converted to sirolimus monotherapy by one year post-transplant if the patient remained rejection free. Induction therapy included either an IL2-receptor antagonist (dacluzimab or basiliximab) or anti-thymocyte globulins (thymoglobulin, ATGAM) with use depending on recipient immunologic risk and varying across allograft programs. Further details regarding immunosuppression are available in supplementary table S3.1.

Antiviral prophylaxis: D-/R- patients received no CMV-directed antiviral prophylaxis. All patients mismatched with respect to pre-transplant EBV serostatus (D+/R- SOT) recipients were expected to receive 14 weeks of prophylaxis using either oral ganciclovir (prior to Jan 2005) or valganciclovir (after Jan 2005) using standard CMV prophylaxis doses adjusted for renal impairment. Anti-HSV patients not receiving ganciclovir/valganciclovir who received HSV-directed acyclovir prophylaxis for one month post-transplant (except liver transplant recipients not receiving anti-lymphocyte globulin induction).

Patient and Laboratory Monitoring: All CMV serology and viral load testing is performed at a single laboratory, the Provincial Laboratory for Public Health (ProvLab) in Alberta. During the study period, all residual plasma from samples submitted for antigenemia and CMV PCR assays were archived and stored at -70°C.

Throughout the study period all recipients and local donors were tested for CMV IgG using the Enzygnost^{R} Anti-CMV IgG assay (Behring/Siemens). The CMV serostatus of distant donors was provided by the Human Organ Procurement and Exchange program (details of assays unknown). Standardized management protocols recommend rescreening all CMV seronegative recipients for CMV IgG at 6 and 12 months post-transplant, and further serology testing was sometimes performed at the discretion of the physician. Additionally, archived CMV viral load and HLA lab plasma samples were retrieved (when available) and tested for CMV IgG using the Enzygnost^{R} Anti-CMV IgG assay to supplement missing data and extend follow-up as required in subsets of patients who continue to be transfused such that last follow-up occurs 6 months after the last cellular blood transfusion and > 2 months after receipt of blood products that might result in false positive serology results.

Patients are followed closely clinically and asked to report all illnesses; “for cause” CMV viral load (VL) testing is performed in settings where CMV disease is suspected. From January 2000 – September 2005, CMV VL was measured in whole blood using a CMV pp65 antigenemia assay. From October 2005 – February 2012, plasma CMV DNAemia testing was performed using an in-house developed real-time PCR assay [Limit of Detection (LOD) 500 copies/mL, 5.65 genome copies/mL = 1

IU/mL) as previously described(74). From March 2012 onwards, a commercial assay calibrated to the WHO international standard RealStar CMV PCR (Altona Diagnostics, Hamburg, Germany) was used [LOD 41 IU/mL].

Transfusion records: All patients received random leukoreduced blood products processed at Canadian Blood Services (CBS) not pre-selected for CMV seronegativity. Transfusion records of each recipient were obtained from the University of Alberta Hospitals laboratory information system. Date of transfusion and type of product (red blood cell [RBC], platelet [PLT], plasma [PLA] and IVIG) were recorded. Patients were considered at risk for TT-CMV only if they received cellular blood products (RBC, PLT). CMV IgG positive results in recipients without positive antigenemia or CMV DNAemia and recent transfusion were considered possible false positive results if they occurred within 8 weeks of plasma, red blood cell, platelet transfusion or within 16 weeks of administration IVIG. Later post-transplant samples were tested to confirm seroconversion.

Definitions: CMV infection was defined based on the recommendation for clinical trials; recipients were considered to have had CMV transmitted if they had evidence of CMV antigenemia or CMV DNAemia and/or seroconverted to CMV IgG positive(75). Possible TT-CMV infection was defined as CMV infection occurring in the first year post-transplant for transfused patients. CMV infection after the first year post-transplant and at any time in non-transfused patients was considered to be community acquired. One year was chosen as a cut-off for possible TT-CMV as in our historical pre-

leukoreduction study of TT-CMV, all cases of presumptive TT-CMV occurred within the first 100 days post-transplant, and other studies have shown that even when three months of antiviral prophylaxis is used (as was the case for EBV mismatched recipients in our study), almost all CMV infection events occur in the first post-transplant year(76,77). Patients were considered transfused at transplant if they received cellular blood products within 30 days pre-transplant or 90 days post-transplant. Patients with suspected CA-CMV infection were reviewed to ensure that they had not received cellular blood products within 3 months prior to CMV infection. Non-transfused recipients were additional controls for CA-CMV.

Adequate follow-up was defined as a serology testing result by 12 months post-transplant for transfused patients (or > 6 months post-transplant after last cellular blood product if CMV infected and transfused post-transplant). In non-transfused patients, adequate follow-up was defined as a serology testing result at 6 months post-transplant if not EBV mismatched and 12 months otherwise. The date of first positive result (serology or VL) was considered the date of infection and confirmed by chart review. Date of first positive result or last negative serology result was used as the date of last follow-up.

Retrospective chart review was performed on all patients with CMV infection and/or seroconversion to assess CMV-associated morbidity (CMV syndrome and tissue invasive disease) and mortality.

Ethics review: This study was approved by the University of Alberta Health Research Ethics Board (HREB_Pro 00035419).

Statistical Analysis: We calculated incidence rates in adult and pediatric transfused and non-transfused patient groups. Each transfused patient contributes their first year of follow-up to the TT-CMV risk period. Transfused patients followed for more than one year had their remaining follow-up time added to the CA-CMV risk period. Exact Poisson 95% confidence intervals were calculated and incidence rates were compared using an exact rate ratio test. Blood products in adult D-/R- patients from our current study were compared to those from our historical study of patients transplanted between January 1984 – October 1996 in the era prior to universal leukoreduction using chi-square test(26). All analyses were performed using R 3.5.2(39).

3.3 Results

A total of 536 patients were initially identified for inclusion. Upon review, we excluded 14 patients who died within 1 month of transplantation, 26 patients with incorrect donor or recipient serology, and 10 recipients with missing transfusion data. Table 3.1 describes the characteristics of the 486 patients analyzed. A total of 23 patients were re-transplanted during follow-up, and 12 of these recipients were re-transplanted with CMV seropositive donors resulting in follow-up termination at time of re-transplant. In 10 recipients who were re-transplanted with CMV seronegative donors, 6 were re-transplanted between 2000-2011 and included as separate events (2 heart recipients received kidney transplants and 1 kidney recipient received a pancreas transplant), and 4 were re-transplanted after 2011 so these events were treated as follow-up serology only. Adequate follow-up was observed in 81.8% (n=398) of the

study population. Reasons for inadequate follow-up included: 4 deaths within one year of transplant, 23 transferred out-of-province and 61 lost to follow-up.

Incidence of Post-Transplant CMV Infection

Table 3.2 describes the incidence of CMV infection in transfused and non-transfused adult and pediatric patients. A total of 231 patients (58%) were transfused at transplant and had adequate follow-up, receiving a total of 1626 units of RBC, 470 units of PLT and 690 units of PLA. We identified 17 cases of CMV infection: 13 confirmed CA-CMV, 2 potential TT-CMV infections and 2 infections that were unclassifiable.

TT-CMV Infection

Although we observed 2 infections occurring within the TT-CMV timeframe (one possible and one confirmed), we concluded based on laboratory and clinical data that the first likely represented a false positive result, and the second was likely CA-infection not TT-CMV. Clinical details of each case are presented in Table 3.3 (Cases 1 and 2).

The first equivocal case of TT-CMV infection (Case 1) was an adult liver recipient who received 4 PLT units at transplant. Six weeks post-transplant he presented with a diffuse erythematous rash, confusion, pancytopenia, and hyponatremia, which was rapidly corrected from 119 mEq/L to 125 mEq/L overnight. Rash and pancytopenia were attributed to possible cotrimoxazole toxicity. The patient had negative plasma CMV DNA at 3 weeks post-transplant and at 7 weeks post-transplant (one week after onset of illness). Because of ongoing confusion, three lumbar punctures were performed one to three weeks from illness onset with cell counts (RBC/WBC) of 63/11, 768/12, and 566/27 x 10^6; WBC's were 100% lymphocytes. Protein was also elevated at 1.68, 1.15

and 1.34 g/L. A brain MRI 10 days after illness onset was normal. Bacterial and mycobacterial testing of the CSF were negative as were PCR viral studies for VZV, HSV, enterovirus, West Nile virus and HHV-6. The patient suffered a cardiac arrest two weeks after onset of illness from which he did not have functional CNS recovery. Bone marrow biopsy confirmed severe marrow hypoplasia, and the patient had a persistent desquamative exfoliative dermatitis. Endoscopy and colonoscopy performed one day prior to death found HSV esophagitis and diffuse colonic ulceration; biopsies were CMV negative on immunohistochemistry. A plasma CMV viral load performed three weeks after illness onset had an equivocal result of <500 copies/mL (at LOD of assay). No follow-up viral testing was available as the patient died one month after presentation with a diagnosis remaining unclear. This result is likely a false positive as clinical features and the very low viral load only documented several weeks after onset of symptoms are not compatible with expected clinical and laboratory manifestations of primary CMV infection occurring early post-transplant. Two other CMV seronegative recipients of organs from the same donor did not have evidence of infection after >3 years of follow-up, supporting that this was not donor-transmitted CMV.

The second possible case of TT-CMV infection (Case 2) was a pediatric heart recipient transfused at transplant with 2 RBC and 1 PLT who had routine follow-up CMV serology performed at 0.75 years post-transplant, which was negative, and then at 1.36 years, which was positive. The patient did not have any symptoms of CMV infection. As the interval between the 2 serologic results encompasses both the TT-CMV and CA-CMV risk periods, we cannot determine if seroconversion occurred before or after 1 year and thus cannot rule out TT-CMV. As the patient did not receive any anti-viral

prophylaxis, which can delay CMV seroconversion post-transplant, the negative CMV serology result 9 months after transfusion makes TT-CMV less likely.

CA-CMV Infection

Thirteen CA-CMV infections were identified. The median (IQR) time to diagnosis of CA-CMV infection was 5.7 (4.4) years in adult patients and 7.7 (4.9) years in pediatric patients.

The 231 transfused patients contributed 1164 years to the CA-CMV risk period (1016 adult and 148 pediatric), and the 166 non-transfused patients contributed 1010 years to the CA-CMV risk period (926 adult and 84 pediatric) for a total of 2174 patient years of follow-up (PYFU) in the CA-CMV risk period (1942 adult and 232 pediatric). The incidence rate for CA-CMV in adults was thus 0.36 per 100 PYFU in adults (95% CI: [0.14, 0.74]) and 2.59 per 100 PYFU in pediatrics (95% CI: [0.94, 5.62]) giving an incidence rate ratio of 7.18 (95% CI: [1.99, 24.94], p=0.0026). When including Case 2 as a case of CA-CMV, 9 of 14 cases (64%) had symptomatic CMV infection.

Unclassifiable Cases

We identified 2 patients in whom there was insufficient follow-up to classify their CMV infection as either TT-CMV or CA-CMV. Case 3 was transfused at transplant but had no CMV testing until 11 years post-transplant at which time CMV serology was positive. Case 4 was not transfused at transplant but received 1 unit of PLT at 1.29 years post-and seroconverted between 0.53-2.82 years post-transplant.

Comparison with Historical Cohort

Blood product use in our previous study of adult D-/R- transplants is compared with those transplants in our current study in Table 3.4. The proportion of patients transfused has decreased across all organs except lungs (kidney $p<0.001$, liver $p=0.005$, heart $p=0.02$, lung $p=0.4$). The average number of RBC and PLT units transfused has decreased across all organ groups.

3.4 Discussion

In our current study of SOT recipients transplanted in the era of universal leukoreduction, no definitively confirmed cases of TT-CMV infection were observed among patients transplanted over a period of 11 years at our center, providing additional evidence that leukoreduction alone is an effective strategy to prevent TT-CMV infections, and that a dual strategy including screening for seronegative blood products is unnecessary. Our results also show the risk of TT-CMV is inconsequential in comparison to CA-CMV and provide an impetus to remember the ongoing risk of CA-CMV when monitoring D-/R- patients long-term as all the confirmed cases of CMV infection were CA-CMV.

In our previous 2002 study of SOT recipients receiving non-leukoreduced, unscreened blood products during the era prior to universal leukoreduction and effective antiviral prophylaxis, we identified 3 cases of presumed TT-CMV infection among 127 adult D-/R- transplants, all occurring within the first year post-transplant and within 100 days of last transfusion(26). All cases were symptomatic with fever or gastrointestinal symptoms. Although the average overall recipient exposure to cellular blood products

has decreased over time, it is more likely that the risk reduction in TT-CMV was due to adoption of a universal leukoreduction policy.

Our study highlights the effectiveness of leukoreduction in reducing the risk of TT-CMV infection as we saw no confirmed cases of TT-CMV associated with any symptoms of CMV infection. Leukoreduction is the dominant strategy for reducing risk of TT-CMV infection although some inventory of seronegative blood may be maintained for “high-risk” patients. Screening for seronegative blood presents significant logistical considerations and becomes especially difficult in regions where CMV seroprevalence is high and the donor pool is small. Furthermore, CMV screening and management of dual inventories is expensive and resource intensive, making use of CMV seronegative blood an unattractive strategy for reducing TT-CMV risk. In Canada, additional screening for seronegative blood is no longer required by CBS except to maintain inventory for intrauterine transfusion. This recent change in practice was based on a 2017 position paper from the National Advisory Committee on Blood and Blood Products recommending leukoreduced and seronegative products be considered equivalent in safety except for intrauterine transfusion(78). In America, a 2017 survey reported 90% of responding institutions use leukoreduction as the primary strategy for preventing TT-CMV infection, and 40% of respondents indicated that use of seronegative blood was by physician discretion as a result of lacking clinical guidelines(79). Expert committees have not been able to reach a consensus, and the most recent international guidelines for managing CMV in SOT recommend the use of either leukoreduced or CMV seronegative blood products, but not combined due to lack of evidence(25,80). Our observations support this recommendation, and our study will

be important in informing future clinical guidelines regarding prevention of TT-CMV infection.

The negligible risk of TT-CMV infection observed in our study is concordant with other studies conducted in HSCT recipients during the last decade, although our study is unique as it occurs in a SOT setting in which data is lacking. Three studies of D-/R- HSCT recipients have failed to identify a single documented case of TT-CMV infection among a total of 141 recipients receiving 6978 leukoreduced and unscreened blood products(81–83). A study by Kekre et al purportedly detected one case of TT-CMV infection when using leukoreduced blood alone and three cases when using CMV leukoreduced and seronegative blood, casting doubt on the effectiveness of additional seronegative screening adjunct to leukoreduction(84). The authors further concluded that screening in the current era of universal leukoreduction is unwarranted. The risk of TT-CMV infection appears to be nearly infinitesimal, and one estimate of the residual risk of TT-CMV using a mathematical model suggests an estimate of approximately 1 in 13 million in Australia(22). However, this model is predicated on the assumption that the probability of TT-CMV viremia can be modeled from aggregated rates of CMV DNA detection from 4 published studies(85–88) as a surrogate measure for detecting donors able to transmit CMV. Furthermore, they equate DNA detection with CMV infectivity which may not be the case, and do not consider the possibility that transfused cells from seropositive latently infected donors who are CMV DNA negative could reactivate after transfusion into a recipient. Despite these caveats, the model provides a useful estimate when cautiously interpreted. Although the pathogenesis of TT-CMV infection is still unclear, especially in regards to the aforementioned mechanisms of transmission, and

some may still suspect that free CMV contribute to infectious risk of TT-CMV, we believe that free CMV in plasma does not meaningfully contribute to transmission(89). Our 2017 study showed that the biologic form of CMV in plasma is almost exclusively free DNA unassociated with infectious virions(23). While leukoreduction does not reduce levels of free CMV in plasma, there is an absence of documented TT-CMV infection from plasma in the literature(90,91).

In our study, the risk of CA-CMV exceeded that of TT-CMV and was higher in children than in adults. Sexual activity and exposure to young children are two important risk factors for CA-CMV, and one review estimates the annual CMV seroconversion rate in pregnant women to be 2% worldwide(92), while annual seroconversion rates among German blood donors are estimated to be 0.55%(43) and 0.8%(85). In our adult cohort, we observed a CA-CMV incidence rate of 0.36 per 100 PYFU which is mathematically equivalent to an annual seroconversion rate of 0.36%. Incidence rate estimates from birth cohorts range from 5.75(93), 8.33(94) and 13.27 per 100 PYFU(66), all of which exceed the CA-CMV incidence rate of 2.59 per 100 PYFU observed in our pediatric cohort. The lower incidence rates observed in our study may be explained by our chronically ill population being less likely to be exposed to sexual activity or young children compared to healthy individuals.

Our study confirms that CMV IgG is a good marker for previous CMV infection and virus latency, although CMV IgG assay results are not always concordant with discordance rates for CMV IgG detection as high as 4% among assays reported(95). While recent literature suggests as many as 25% of CMV seronegative renal transplant recipients have evidence of previous CMV exposure in the form of detectable CMV-

specific T-cells and therefore may have latent CMV that can be reactivated, we did not see evidence of transmission from CMV seronegative donors or any early reactivation in CMV seronegative recipients(96,97), calling into question the specificity associated with cell-mediated immunity assays when used to identify seronegative donors and recipients who may be latently infected with CMV.

Our study is not without limitations as it is observational and does not directly compare leukoreduction alone to leukoreduction combined with seronegative screening. A large scale randomized controlled trial would be valuable to evaluate this comparison directly, although such a trial is unlikely. We were able to obtain sufficient follow-up for over 80% of patients in the cohort. We examined the impact of transfusion at transplant and disregarded transfusion occurring post-transplant except in the cases of documented CMV infection, which underreports the total transfusion exposure per patient, but inclusion of these products would diminish the risk of TT-CMV even further. Due to the low overall risk of CMV infection among D-/R- transplants, post-transplant monitoring is not routinely performed over long durations, thus CA-CMV may be underreported in this study, although we sought to ameliorate this fact through retrospective testing of available samples. In those patients who were transfused and later presented with CMV infection, we were unable to investigate the CMV serostatus or DNA levels of implicated blood donors. Despite these limitations, we believe our study has several notable strengths. Our sample size is larger than any recent comparable study with a significant number of products transfused in our unique population of SOT recipients. The duration of follow-up in our study also grants insight into the risk of CA-CMV in SOT recipients, a phenomenon in which data is lacking.

Lastly, repeating the study at our center permits comparison with our previous study while controlling for some potential confounders such as using the same assays and transplant programs over time.

In summary, the residual risk of TT-CMV is negligible, and we find it difficult to justify use of incremental safety measures such as use of seronegative blood products or NAT screening of blood donors. The risk of CA-CMV is much higher than the risk of TT-CMV, and this diagnosis should remain in the differential during the long-term follow-up of D-/R- patients when presenting with signs and symptoms of CMV disease.

Table 3.1. Study Population Characteristics. EBV mismatched patients should have received 3 months of antiviral prophylaxis.

Group	Organ Group	Median Age (IQR)	%Male	Re-Transplants	%EBV Mismatched	%Adequately Followed	Median Follow-up Time, Years (IQR)
Adult Transfused	Kidney	49.2 (21.8)	66.2% (51)	1.3% (1)	7.8% (6)	89.6% (69)	6.2 (6.26)
	Liver	49.7 (16.3)	70.0% (42)	3.3% (2)	1.7% (1)	66.7% (41)	6.58 (6.43)
	Heart	53.6 (17)	82.5% (33)	2.5% (1)	7.5% (3)	92.5% (37)	6.11 (7.21)
	Lung	49.2 (24.6)	62.3% (43)	1.4% (1)	8.7% (6)	78.3% (54)	4.33 (4.57)
	Total	49.8 (20.1)	68.7% (169)	2.0% (5)	6.5% (16)	80.9% (200)	5.74 (5.6)
Adult Non-Transfused	Kidney	43.4 (23.2)	73.0% (111)	7.9% (12)	9.9% (15)	82.2% (125)	5.53 (5.77)
	Liver	46.1 (16.2)	80.0% (16)	5.0% (1)	10.0% (2)	80.0% (16)	8.3 (7.75)
	Heart	34.4 (29.9)	85.7% (6)	0.0% (0)	28.6% (2)	100.0% (7)	1.34 (1.11)
	Lung	38.4 (10.2)	0.0% (0)	0.0% (0)	0.0% (0)	100.0% (4)	1.97 (0.51)
	Total	43.5 (21.8)	72.7% (133)	7.1% (13)	10.4% (19)	83.1% (152)	5.45 (6.25)
Pediatric Transfused	Kidney	2.55 (3.62)	80.0% (4)	0.0% (0)	80.0% (4)	100.0% (5)	5.55 (2.07)
	Liver	1.31 (6.54)	52.2% (12)	13.0% (3)	56.5% (13)	69.6% (16)	4.44 (7.7)
	Heart	1.54 (4.31)	41.7% (5)	8.3% (1)	41.7% (5)	75.0% (9)	5.4 (4.85)
	Lung	16.4 (0.60)	50.0% (1)	0.0% (0)	0.0% (0)	50.0% (1)	3.47 (0)
	Total	1.94 (6.44)	52.4% (22)	9.5% (4)	52.4% (22)	73.8% (31)	4.89 (5.57)
Pediatric Non-Transfused	Kidney	12.6 (2.19)	42.9% (3)	0.0% (0)	28.6% (2)	100.0% (7)	6.16 (3.25)
	Liver	6.56 (3.73)	66.7% (4)	16.7% (1)	50.0% (3)	100.0% (6)	5.15 (7.8)
	Heart	0.84 (0.11)	50.0% (1)	0.0% (0)	100.0% (2)	100.0% (2)	7.43 (1.95)
	Lung	-	-	-	-	-	-
	Total	7.49 (8.36)	53.3% (8)	6.7% (1)	46.7% (7)	100.0% (15)	6.16 (6.25)

Table 3.2. Adult and Pediatric Transfusion History and Infection Classification. RBC = red blood cells, PLT = platelets, PLA = plasma units transfused. Infected <12M refers to CMV infections occurring within 1 year, and >12M after 1 year. Unclassifiable refers to unclassifiable CMV infections.

Organ	Transfused % (N)	RBC median (range) [total]	PLT median (range) [total]	PLA median (range) [total]	Infected <12M	Infected >12M	Unclassifiable	Non-transfused%	Infected <12M	Infected >12M	Unclassifiable
Adult											
Kidney	34.1% (78)	2 (1-10) [244]	3 (1-5) [6]	3 (1-20) [96]	0	0	0	65.9% (151)	0	4	0
Liver	75.0% (60)	2 (1-21) [365]	1 (1-15) [135]	2 (1-17) [216]	1	0	0	25.0% (20)	0	0	1
Heart	85.1% (40)	2 (1-15) [329]	2 (1-11) [132]	2 (1-11) [158]	0	1	1	14.9% (7)	0	0	0
Lung	94.5% (69)	1 (1-10) [452]	1 (1-6) [116]	2 (1-8) [134]	0	2	0	5.5% (4)	0	0	0
Total	**57.6% (247)**	**2 (1-21) [1390]**	**1 (1-15) [389]**	**2 (1-20) [604]**	**1**	**3**	**1**	**42.4% (182)**	**0**	**4**	**1**
Pediatric											
Kidney	41.7% (5)	1 (1-1) [6]	0 (0-0) [0]	1 (1-1) [1]	0	0	0	58.3% (7)	0	0	0
Liver	79.3% (23)	1 (1-5) [119]	1 (1-6) [34]	1 (1-3) [45]	0	1	0	20.7% (6)	0	2	0
Heart	85.7% (12)	1 (1-9) [107]	1 (1-8) [46]	1 (1-8) [38]	1	2	0	14.3% (2)	0	1	0
Lung	100.0% (2)	4 (4-4) [4]	1 (1-1) [1]	2 (2-2) [2]	0	0	0	0.0% (0)	0	0	0
Total	**73.7% (42)**	**1 (1-9) [236]**	**1 (1-8) [81]**	**1 (1-8) [86]**	**1**	**3**	**0**	**26.3% (15)**	**0**	**3**	**0**

Table 3.3. Clinical History of CMV Infected Patients. RBC = red blood cells, PLT = platelets, PLA = plasma units transfused. *EBV mismatched patients should have received 3 months of antiviral prophylaxis.

Case	Age Group	Organ	Infection	Time of Infection	Transfused at Transplant	Transfused Units	Signs and Symptoms	Treatment	EBV Mismatch*
1	Adult	Liver	TT-CMV: indeterminate infection	Viral load at limit of detection (<500 copies/ml) detected at 0.18 yr	Yes	4 PLT at transplant 10 RBC and 8 PLT between 6-10 weeks post-transplant	Hyponatemia, desquamative rash, severe marrow aplasia six weeks after transplant, encephalopathy/encephalitis, died one month later, two weeks after cardiac arrest. Diagnosis uncertain; unlikely CMV related	None	No
2	Pediatric	Heart	TT-CMV: Asymptomatic seroconversion	Seroconversion between 0.75-1.36 yr	Yes	2 RBC, 1 PLT	No evidence of CMV disease. Asymptomatic seroconversion	None	No
3	Adult	Heart	Unclassifiable asymptomatic seroconversion	Seroconversion between 0-11.09 yr	Yes	2 RBC, 8 PLT	No evidence of CMV disease. Asymptomatic seroconversion	None	No
4	Adult	Liver	Unclassifiable asymptomatic seroconversion	Seroconversion between 0.53-2.82 yr	No	None, but received 1 PLT at 1.29 yr	No evidence of CMV disease. Asymptomatic seroconversion	None	No
5	Adult	Lung	CA-CMV: Acute infection	Acute infection with viral load at 7.19 yr	Yes	5 RBC, 5 PLT, 1 PLA	CMV syndrome, fever, chills, malaise	IV ganciclovir and valganciclovir	Yes

6	Adult	Lung	CA-CMV: Acute infection	Acute infection with viral load at 10.55 yr	Yes	4 RBC, 5 PLT	CMV syndrome, prominent GI symptoms, nausea, vomiting	IV ganciclovir, valganciclovir and cytogam	No
7	Adult	Kidney	CA-CMV: Acute infection	Acute infection with seroconversion at 3.04 yr	No	None	CMV syndrome, anorexia, malaise, severe diarrhea	Valganciclovir	No
8	Adult	Kidney	CA-CMV: Acute infection	Acute infection with viral load at 3.49 yr	No	None	Tissue-invasive gastrointestinal disease	IV ganciclovir and valganciclovir	Yes
9	Adult	Kidney	CA-CMV: Acute infection	Acute infection with viral load at 5.47 yr	No	None	CMV syndrome, fever, malaise	IV ganciclovir and valganciclovir	No
10	Adult	Kidney	CA-CMV: Acute infection	Acute infection with viral load and seroconversion at 12.93 yr	No	None	CMV syndrome, fever, leukopenia	None	No
11	Pediatric	Heart	CA-CMV: Acute infection	Acute infection with viral load and seroconversion at 7.46 yr	No	2 RBC, 2 PLT	CMV proctitis	Valganciclovir	No
12	Pediatric	Liver	CA-CMV: Acute infection	Acute infection with viral load at 2.35 yr	No	17 RBC, 3 PLT, 1 PLA	CMV hepatitis	Valganciclovir	Yes

13	Pediatric	Liver	CA-CMV: Acute infection	Acute infection with viral load at 1.04 yr	No	None	CMV hepatitis	Ganciclovir	No
14	Adult	Heart	CA-CMV: Asymptomatic seroconversion	Seroconversion between 3.88-5.74 yr	Yes	9 RBC, 14 PLT	No evidence of CMV disease. Asymptomatic seroconversion	None	No
15	Pediatric	Heart	CA-CMV: Asymptomatic seroconversion	Seroconversion between 3.27-7.84 yr	Yes	4 RBC, 5 PLT	No evidence of CMV disease. Asymptomatic seroconversion	None	Yes
16	Pediatric	Heart	CA-CMV: Asymptomatic seroconversion	Seroconversion between 8.25-9.38 yr	No	None	No evidence of CMV disease. Asymptomatic seroconversion	None	Yes
17	Pediatric	Liver	CA-CMV: Asymptomatic seroconversion	Seroconversion between 6.06-8.81 yr	No	None	No evidence of CMV disease. Asymptomatic seroconversion	None	Yes

Table 3.4. Historical vs Current Use of Blood Products in Adult D-/R- Transplants. RBC = red blood cells, PLT = platelets, PLA = plasma units transfused.

Study	Organ	Transfused % (N)	RBC	RBC per patient	PLT	PLT per patient	PLA	PLA per patient
Historical	Kidney	80.3% (57)	286	5.0	109	1.9	36	0.6
Historical	Liver	100% (20)	248	12.4	228	11.4	168	8.4
Historical	Heart	100% (29)	332	11.4	214	7.4	164	5.7
Historical	Lung	85.7% (6)	93	15.5	64	10.7	57	9.5
Current	Kidney	34.1% (78)	244	3.1	6	0.1	96	1.2
Current	Liver	75% (60)	365	6.1	135	2.3	216	3.6
Current	Heart	85% (40)	329	8.2	132	3.3	158	4.0
Current	Lung	94.5% (69)	452	6.6	116	1.7	134	1.9

Table S3.1. Details of Immunosuppression Administered at our Center. CNI = calcineurin inhibitor, HCC = hepatocellular carcinoma, MMF = mycophenolate mofetil, pts = patients. *In all programs, steroid-resistant rejection was treated with anti-lymphocyte globulin preparations being used within the program during that time.

Organ	Induction*	Maintenance	Calcineurin inhibitor (CNI)	Antiproliferative Agent
Kidney and kidney/pancreas	Thymoglobulin (2000-2013) for highly sensitized (high-panel reactive antibodies) or delayed graft rejection. Low immunologic risk pts given IL2-receptor antagonists daclizumab (2000-2004) or basiliximab (2005-2013).	Triple therapy throughout study period, using CNIs, anti-proliferative agent and steroids, tapered to steady state levels by the end of the first post-transplant year. Sirolimus used only in CNI toxicity.	Tacrolimus (2000-2013)	MMF (2000-2013)
Liver	Daclizumab (2000-2009) Basiliximab (2009-2013)	Steroid-free maintenance with low-dose tacrolimus plus sirolimus with steroids tapered and discontinued in rejection-free patients by 3-6 months (2000-2002) Tacrolimus and MMF with steroid withdrawal (2002-2013); pts with HCC (25%) or renal dysfunction converted within 4-12 weeks to sirolimus and MMF (HCC) or sirolimus and low dose tacrolimus, with the goal of sirolimus monotherapy by one year if rejection-free.	Cyclosporine (2000-2002) Tacrolimus (2002-2013)	Azathioprine (2000-2002) MMF (2002-2013)
Heart and/or Lung	RCT ATGAM vs. daclizumab (2001-2005 heart, 2001-2003 lung) Heart recipients received ATGAM (2005-2011) or thymoglobulin (2011-2013)	Triple maintenance immunosuppression including a CNI, antiproliferative agent, and steroids throughout study period. Sirolimus used only in CNI toxicity.	Cyclosporine (2000-2001) Tacrolimus (2001-2013)	MMF (2000-2013)

	Lung recipients received daclizumab (2003-2011)/ basiliximab (2011-2013) or ATGAM (2003-2013) at the physician's discretion			
Intestinal/multi-visceral	Thymoglobulin (2003-2013)	Triple maintenance immunosuppression with low-dose tacrolimus, sirolimus and steroids tapered to steady state levels over one year	Tacrolimus (2003-2013)	

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Chapter 4: Summary

4.1 Summary

This research program set out to address the data gap regarding the seroprevalence of CMV and EBV in Canada and estimate the risk of TT-CMV and CA-CMV in D-/R- SOT recipients. The lack of nationally representative seroprevalence data for CMV and EBV is an obstacle to developing public health interventions and strategies, including effective CMV and EBV vaccines for Canadians. Information on prevalence of these infections is also important to estimate disease burden and the associated costs and resource use. The prevalence of CMV in the general Canadian population likely lies between the prevalence of 42% found in our national blood donor population and 53-62% found in our local SOT donor and recipient population. Furthermore, the seroprevalence of CMV in women of childbearing age in our populations indicates that 44-61% are seronegative and at risk of primary CMV infection. This proportion represents a significant opportunity to prevent congenital infection. Our results also show the EBV seroprevalence in our SOT population rapidly increases very early in life suggesting that potential vaccines should likely target infants.

Importantly, we have also shown that there is no significant risk of TT-CMV in the current era of universal leukoreduction, and that additional screening for CMV seronegative blood products is unjustified. Our observations support the current policy adopted by Canadian Blood Services, which no longer recommends CMV screening except for intrauterine transfusion. While leukoreduction appears to essentially eliminate the risk of TT-CMV, CMV D-/R- recipients remain at risk of CA-CMV which can result in symptomatic CMV disease even when acquired late post-transplant.

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