The Risk and Timing of Hematologic Malignancy Following Solid Organ Transplant

By Scott C. Quinlan

Bachelor of Science, June 2000, The University of Delaware
Master of Science, January 2004, The George Washington University

A Dissertation submitted to

The Faculty of the

Columbian College of Arts and Sciences and the
School of Public Health and Health Sciences of The George Washington University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

August 31, 2010

Dissertation directed by

Eric A. Engels
Senior Investigator, Infections and Immunoepidemiology Branch,
National Cancer Institute

and

Paul H. Levine
Research Professor of Epidemiology and Biostatistics

The Columbian College of Arts and Sciences of The George Washington University certifies that Scott C. Quinlan has passed the Final Examination for the degree of Doctor of Philosophy as of March 24, 2010. This is the final and approved form of the dissertation.

The Risk and Timing of Hematologic Malignancy Following Solid Organ Transplant

Scott C. Quinlan

Dissertation Research Committee:

Paul H. Levine, Research Professor of Epidemiology and Biostatistics, Co-Director

Eric A. Engels, Senior Investigator, National Cancer Institute, Co-Director

Dante A. Verme, Professor of Epidemiology and Biostatistics, Committee Member

Madeline Rice, Associate Professor of Epidemiology and Biostatistics, Committee Member

Dedication

The author wishes to thank his family and friends for their love and support. In particular, the author wishes to thank his wife Leila. Your love, support, and encouragement were the fuel that kept me going. Words cannot express my gratitude and I will be forever in your debt.

Abstract of Dissertation

The Risk and Timing of Hematologic Malignancy Following Solid Organ Transplantation

Purpose: To develop a better understanding of the risk and timing of hematologic malignancy (HM) following solid organ transplantation.

Methods: To examine the association between specific HM subtypes and solid organ transplantation we conducted a population-based case-control study using the Surveillance, Epidemiology, and End Results-Medicare linked database. The prevalence of solid organ transplant history was compared between HM subtypes and frequency-matched controls using polytomous logistic regression.

To compare the characteristics and risk factors between early-onset and late-onset post-transplant lymphoproliferative disorder (PTLD) we conducted a retrospective cohort study of U.S. kidney recipients using data from the Scientific Registry of Transplant Recipients. Hazard ratios (HRs) for risk factors for early-onset and late-onset PTLD were estimated using proportional hazards models.

Results: In the U.S. elderly population, transplantation was associated with increased risk for non-Hodgkin lymphoma (NHL, OR=2.13, 95%CI 1.67-2.72), especially diffuse large B-cell lymphoma (OR=3.29, 95%CI 2.28-4.76), the most common NHL subtype; marginal zone (OR=2.48, 95%CI 1.17-5.22), lymphoplasmacytic (OR=3.32, 95%CI 1.41-7.81), and T-cell lymphoma (OR=3.07, 95%CI 1.56-6.06). Transplantation was

also associated with Hodgkin lymphoma (OR=2.53, 95%CI 1.01-6.35), plasma-cell (OR=1.91, 95%CI 1.24-2.93) and myeloid neoplasms (OR=1.99, 95%CI 1.41-2.81).

For early-onset PTLD, significantly increased risk was associated with young age at transplantation (HR 3.97 for <20 vs. 20-50 years), non-Hispanic white race/ethnicity (HR 1.82, vs. other races/ethnicities), and glomerular disease, tubular/interstitial disease, or malignancy leading to kidney transplant (HRs 1.57, 1.71, and 4.38, respectively, vs. hypertensive nephrosclerosis), while EBV and CMV seropositivity at transplantation decreased risk (HR 0.32 and 0.67, respectively). Late-onset PTLD risk was associated with younger and older age (HR 2.68 and 1.28 for <20 and >50 respectively, vs. 20-50 years) and non-Hispanic white race/ethnicity (HR 1.77, vs. other races/ethnicities).

Conclusion: Solid organ transplantation is associated with the increased risk of a wide spectrum of HMs in the elderly. The increased risk is not confined to the period immediately following transplantation, but extends for many years thereafter. The characteristics and risk factors for malignancies that occur shortly after transplantation are different from those that occur later. Our results support monitoring for a wide spectrum of HM for many years following solid organ transplantation.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Definition
AIDS	Acquired Immunodeficiency Syndrome
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
DLBCL	Diffuse Large B-cell Lymphoma
EBV	Epstein-Barr Virus
ESRD	End-stage Renal Disease
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
HLA	Human Leukocyte Antigen
HM	Hematologic Malignancy
HMO	Health Maintenance Organization
HR	Hazard Ratio
ICD-9	International Classification of Diseases - 9
MM	Multiple myeloma
mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
NHL	Non-Hodgkin Lymphoma
NK	Natural Killer
OPTN	Organ Procurement and Transplantation Network
OR	Odds Ratio
PAK	Pancreas After Kidney
PTLD	Post-transplant Lymphoproliferative Disorder
SEER	Surveillance, Epidemiology, and End Results
SIR	Standardized Incidence Ratio
SMAHRT	SEER-Medicare Assessment of Hematopoietic Risk Traits
SRTR	Scientific Registry of Transplant Recipients
TGF	Tumor Growth Factor
WHO	World Health Organization

Chapter 1: Introduction

Hematologic malignancies (HMs) consist of leukemias, lymphomas, and related proliferative disorders. Although there is a large amount of heterogeneity in etiology and other factors, all HMs share derivation from related precursor cells in the bone marrow, blood, and lymphatic system. The incidence of many types of HM has been steadily increasing over the last several decades. Better diagnostic and classification tools, as well as the epidemic of human immunodeficiency virus (HIV) infection can explain some of this increase, but these factors do not explain all of the increase in incidence. It is estimated that in 2009 in the U.S., 139,860 new cases of HM will be diagnosed and 53,240 people will die from the disease.[1] The largest number of cases and deaths is expected to be attributable to non-Hodgkin lymphoma (NHL), which is expected to account for 65,980 new cases and 19,500 deaths in 2009.[1] For the majority of sub-types of HM, more than half of new cases are diagnosed in persons older than 65 years of age, in particular NHL and chronic leukemias.[2]

The etiology of many sub-types of HM is largely unknown. Previous studies have demonstrated an increased risk of lymphoma development in individuals infected with HIV or who have recently undergone an organ transplant.[3] This indicates that immune dysfunction may play a key role in HM development. Studies have shown an increase in the incidence of NHL following solid organ transplant, but did not delineate which particular subtypes of NHL are increased most in incidence.[3-9] Recent research has demonstrated etiologic heterogeneity among NHL subtypes, and immune dysfunction impacts some subtypes more than others.[10;11] In addition, the role of immunodeficiency

in the development of other HM subtypes, such as myeloid neoplasms, is less well understood.

Solid organ transplantation is a life-saving treatment for people with end-stage organ disease. The number of solid organ transplants has been increasing steadily with time due to increasing need and better surgical procedures. In 2007, a total of 27,578 organ transplantations were performed in the United States. This represents a 40% increase over the number of transplants performed in 1998.[12] In addition to the increasing number of solid organ transplants, short-term patient and allograft survival have improved due to better medical care and immunosuppressive strategies that prevent rejection of the transplanted organ. One- and five-year survival post kidney transplant is now 98% and 91%, respectively.[12] Similar improvements in survival have been witnessed for other types of transplant. This has led to a growing population of transplant survivors within the United States. At the end of 2006, there were a total of 173,339 people living in the United States with a functioning transplanted organ. This represents a 1.6-fold increase since 1998.[12]

Due to the increasing number of transplants performed each year, as well as improved short-term patient and allograft survival, it is imperative that the long-term health risks in solid organ transplant recipients be fully understood. One of the major risks following solid organ transplantation is malignancy development, particularly HM.[13] It was the aim of this project to study the association between transplantation and the risk of HM development in more detail.

Chapter 2: Specific Aims

General Purpose

The purpose of this project was to develop a better understanding of the role played by solid organ transplantation in the development of HM. Studies have shown that solid organ transplantation increases the risk of HM development, in particular the risk of NHLs. However, these studies were not able to confirm which particular subtypes of NHL are most impacted by solid organ transplant, or whether other subtypes of HM such as leukemia, multiple myeloma, or HL are impacted. HM comprises a heterogeneous group of malignancies that show stark differences in etiology, tumor characteristics such as morphology, and outcome. It was hoped that this project would better elucidate the association between the development of HM and history of solid organ transplant, in particular to assess which subtypes of HM are most strongly associated. **It was hypothesized that high-grade NHL subtypes, such as diffuse large B-cell lymphoma (DLBCL), would be most strongly associated with a history of solid organ transplant compared to low-grade NHL subtypes and other subtypes of HM.** This hypothesis was tested by conducting a population-based case-control study among the elderly population in the United States.

While the risk of lymphoma development following solid organ transplant has been confirmed previously, the exact risk factors that impact the timing of this development are not well understood. Prior studies often lacked sufficient statistical power or adequate post-transplant follow-up time to address this issue. In this project we also assessed which particular risk factors impacted the risk and timing of post transplant lymphoproliferative disorder (PTLD) development following solid organ transplant.

PTLD includes a wide spectrum of lymphoid proliferations ranging from non-malignant lymphocyte hyperplasia to frank lymphoma. **It was hypothesized that that there are distinct differences in risk factors between early and late-onset PTLD.** This hypothesis was examined by conducting a retrospective cohort study among kidney transplant recipients in the United States.

Specific Aims

There were two major aims of this project. **The first was to assess the association between particular sub-types of HM and history of solid organ transplant in the elderly**. To accomplish this aim we conducted a population-based case-control study using data from the linkage of Surveillance, Epidemiology, and End Results (SEER) and Medicare databases. The cases included incident cases of HM reported to SEER between 1987 and 2002 for persons aged 66 years and older. Cancer free individuals were selected as controls from a 5% random sample of Medicare recipients (age 66 years and older) living in the SEER areas and were matched to cases on calendar year of selection, age in five strata, and sex. Exposure to solid organ transplant was assessed through Medicare claims files. As related secondary aims, we assessed the association between solid organ transplant and extranodal and central nervous system lymphomas, as well as the association between particular types of solid organ transplant and sub-types of HM.

The second major aim was to compare the characteristics and identify whether risk factors differ between early-onset and late-onset post transplant lymphoproliferative disorder (PTLD). To accomplish this aim we conducted a retrospective cohort study using data from the Scientific Registry of Transplant

Recipients (SRTR). The SRTR contains baseline and follow-up data on all solid organ transplants performed within the United States, including data on any malignancies that develop post-transplant.

Developing a better understanding of the specific subtypes of HM most strongly associated with solid organ transplant may provide further clues to the mechanisms of carcinogenesis. In addition, by examining the risk factors that impact the timing of lymphoma development following solid organ transplantation, we will gain a better understanding of the etiology of early-onset and late-onset PTLD.

Chapter 3: Background/Significance

Solid Organ Transplant

The first successful kidney transplantation was performed in 1954. Following this success, numerous technical achievements allowed for transplantation to be expanded to heart, liver, pancreas, lung, and small intestine.[14] The introduction of cyclosporine in the 1980's followed by other immunosuppressive agents in later years reduced the risk of organ rejection, thus overcoming the major obstacle that had prevented organ transplant from becoming a more viable medical option. The use of these agents reduced the risk of organ rejection, but also led to transplant recipients being severely and chronically immunosuppressed due to the medications they receive.[14]

Table 1 compares the number of transplants performed in the United States in 1998 and 2007. As can be seen, the number of transplants performed annually has increased by more than 40% during this time. Large increases have been observed for almost all transplant types over this time period. One of the factors that has led to the increase is the rising prevalence of conditions that lead to transplant, such as end stage renal disease (ESRD) leading to kidney transplant.[12]

Table 1 – A summary of transplants performed in the United States in 2007 and 1998 by transplant type.[12]

Transplant Type **Year**	**1998**	**2007**
Kidney	12,318	16,119
Liver	4,369	5,890
Heart	2,310	2,141
Lung	866	1,461
Heart and Lung	46	29
Pancreas alone	73	110
Pancreas after kidney (PAK)	156	259
Simultaneous Kidney and Pancreas	969	848
Intestine	28	57
Multiple organ	184	664
Total	21,319	27,578

In 2006, the majority of transplant recipients in the United States were male and white.[15] There has been a trend over the last ten years towards increasing age of transplant recipients, in particular a large increase in the number of transplants performed in persons 65 years of age or older. In 1998, 7% of the 21,319 transplants performed in the United States were in persons older than 65 years. This percentage had doubled by 2006.[15]

The primary short-term risk following transplantation is organ rejection. Organ rejection is primarily mediated by T-lymphocytes (T-cells) and requires organ transplant recipients to take immunosuppressive medications chronically. Different strategies for immunosuppression have evolved over time to include a number of different classes of medications that exhibit different properties and actions. These classes include corticosteroids, calcineurin inhibitors, anti-metabolites, monoclonal and polyclonal antibodies, and mammalian target of rapamycin (mTOR) inhibitors. Corticosteroids, such as prednisone, inhibit glucocorticoid-receptor mediated gene transcription of important cytokines and other proteins involved in the immune response. Calcineurin

inhibitors, which include cyclosporine and tacrolimus, block signaling pathways within T-cells that are responsible for the production of pro-inflammatory cytokines. Anti-metabolites include azathioprine and mycophenolate mofetil and derive their immunosuppressive activity by preventing the intracellular synthesis of nucleotides required for T-cell proliferation. Monoclonal and polyclonal antibodies bind to B-cells or T-cells and either destroy them, or block cytokine binding sites thus inhibiting their activation. Lastly, mTOR inhibitors such as sirolimus, bind to mTOR in T-cells and prevent transition through the cell cycle, effectively inhibiting T-cell proliferation.[16;17]

There are three phases of the transplant process in which these medications are utilized; induction, maintenance, and anti-rejection.[16] Induction involves the use of medications for a short, finite amount of time before, during, and immediately following surgery to prevent acute rejection. These medications are typically monoclonal or polyclonal antibodies.[16] Maintenance therapy involves medications given for intermediate or long lengths of time, usually for periods of months or years following transplant. Anti-rejection therapy is used to treat instances of acute rejection.

The last ten years have been highlighted by an increase in the use of new immunosuppressive agents and regimens. The use of induction therapy with antibodies has become far more common and is now used in the majority of kidney and pancreas transplants, as well as more than half of intestine, heart, and lung transplants.[16] Over the last several years, tacrolimus with mycophenolate mofetil has been the most commonly used initial maintenance regimen for solid organ transplant recipients.[16] There has also been a trend in recent years to avoid corticosteroid-based maintenance regimens, or to

limit their use as much as possible. This is due to the potentially harmful side effects that have been associated with long-term use of these medications such as diabetes, osteoporosis, and liver damage.[18] These improvements in immunosuppressive therapy have resulted in the incidence of acute rejection declining steadily over the last decade.[16]

Long-term complications of transplantation have taken on greater importance as short-term patient and graft survival has improved. While transplantation offers a survival advantage over other treatments for end-stage organ failure, the life-expectancy of transplant recipients lags behind that of the general population.[19] This difference in life-expectancy is the result of a variety of long-term health risks following solid organ transplantation, including malignancy.[19] There are several reasons that recipients of organ transplants are at increased risk of malignancy. The first is that recipients of organ transplants are severely and chronically immunosuppressed as a result of the medications used to prevent and treat rejection of the transplanted organ. The immune system may play a vital role in detecting and destroying cancer cells at an early stage (the "immune surveillance" hypothesis).[13;20] When the immune system is impaired, these cells have a greater chance of escaping detection and developing into malignancies.

The second reason for the increased incidence of malignancy in organ transplant recipients is the increased susceptibility to infection with, or reactivation of, oncogenic viruses. These include hepatitis B and C viruses, which are associated with liver cancer, and human papillomavirus, which is the primary cause of cervical cancer. Epstein-Barr virus (EBV) plays an important role in the etiology of several subtypes of HM, including PTLD.[13;20] These important malignancies will be described in more detail later.

Prior research has also demonstrated that specific immunosuppressive medications may increase the risk of malignancy in transplant recipients.[21] For example, prolonged exposure to azathioprine has been observed to increase the risk of a variety of malignancies, including skin cancer and leukemia.[22;23] DNA damage resulting from prolonged exposure to azathioprine has been postulated as a mechanism for this increased risk.[22;23]Lastly, chronic antigen stimulation due to the allograft has been postulated as another factor increasing post-transplant malignancy risk.[22]This chronic stimulation may over stimulate a partially depressed immune system, leading to the development of lymphoma.[22]

The excess of malignancies in solid organ transplant recipients is observed for many different tumor sites, but is most pronounced for non-melanoma skin cancer and HMs.[3-9] These excesses are typically profound and persist for many years after transplantation.[9] Although the incidence of non-melanoma skin cancer is highest, HM accounts for a larger proportion of mortality in this population.[24] Previous studies have highlighted the increased risk of HM following organ transplantation, but have often lacked sufficient numbers of cases to carefully examine which particular subtypes of HM are elevated most in incidence.[3] This project offers a unique opportunity to further assess the association between solid organ transplantation and HM, with special emphasis on identifying which particular subtypes of HM are most strongly associated.

Hematologic Malignancy (HM)

HM consists of leukemias, lymphomas, and related proliferative disorders. The two primary categories of HM are myeloid and lymphoid. Lymphoid malignancies include

lymphomas, multiple myeloma, and certain subtypes of leukemia. Myeloid malignancies include various leukemia subtypes.[25] Lymphomas are malignancies of the lymphatic system and are typically divided into two broad categories, NHLs and HL. Nearly 85% of lymphomas are of the NHL type. Leukemias comprise a group of malignancies that begin in the blood-forming cells of the bone marrow. Multiple myeloma is a malignancy of plasma cells.[25]

Lymphomas comprise the majority of HMs diagnosed in the United States in a given year. It is estimated that out of 139,860 expected new HM diagnoses in 2009, 74,490 will be lymphomas and 44,790 will be leukemias, with the remainder being multiple myeloma and other proliferative disorders.[1]

Lymphoma

These malignancies occur at progressive stages of lymphoid development. The two primary cells involved are known as B- and T-cells, which mature in the bone marrow and the thymus respectively. The mature cells then migrate to the secondary or peripheral lymphoid organs.[26] The World Health Organization's (WHO) classification scheme for lymphoid neoplasms combines morphologic, immunophenotypic, genetic and clinical features to group lymphomas into three major categories, B-cell neoplasms, T- and natural killer (NK)-cell neoplasms, and HL. Within the B- and T-cell categories, neoplasms are subdivided into those derived from precursor or mature cells and then further into even finer divisions based on cell appearance, location, and other factors. Mature B-cell lymphomas account for more than 90% of all NHLs.[26]

Overall, there has been a worldwide increase in the incidence of NHL over the last 50 years. The age-adjusted incidence increased by 50% in the United States from 1970 to 1990.[27] While some of this increase can be explained by the emergence of the HIV epidemic and changes in diagnostic practices, the reason for the remainder of the increase has not been discovered. The impact of HIV on the increased incidence is due to the creation of a population that is chronically and severely immunosuppressed. Incidence trends over time varied substantially by lymphoma subtype. For many subtypes of lymphoma, incidence rates increased most significantly in the elderly populations such as for DLBCL, follicular, mantel cell, and Burkitt lymphomas.[27]

The incidence rates of lymphoid neoplasms tend to be higher in males than in females and in white populations compared to other races.[27] In general, the incidence of total lymphoid neoplasms increases monotonically with age in all racial and gender groups.[27] There are a few notable exceptions to this rule, including Burkitt lymphoma and HL which increase with age more gradually than other subtypes, and lymphoblastic lymphoma/leukemia which is more commonly diagnosed in children.[27]

The etiology of many lymphoid malignancies is largely unknown. The one factor that has consistently been demonstrated to be associated with lymphoma development is immunosuppression.[28] Approximately one-quarter of subjects with congenital immunodeficiency will eventually develop a tumor during their lifetime and nearly half of these observed tumors are NHLs.[28] Patients with acquired immunodeficiency syndrome (AIDS) due to infection with HIV have been shown to have a 60- to 100-fold higher risk of NHL development when compared to the general population.[28] The risk of

NHL development tends to increase as immune function decreases and AIDS-related lymphomas tend to be extranodal, aggressive, and have a poorer prognosis than those observed in the general population.[10;28] The risk of HL has also been shown to be increased in the setting of HIV infection, with the excess risk more than 9 times what is observed in the general population.[29]

Several infectious agents have been implicated in the etiology of lymphoma, particularly in NHLs.[30;31] One of the most prominent is EBV, particularly in subjects who are immunocompromised. The risk of lymphoma development is highest if primary infection with EBV occurs while a person is immunosuppressed.[31] EBV has been linked to a number of different lymphoma types, including HL and high-grade NHLs arising in the setting of immunosuppression.[30;31]

Several studies have evaluated the association between solid organ transplantation and lymphoma and generally found an increased incidence of NHL and HL following solid organ transplant.[3-9;32] These studies were either registry-based or cohort studies and did not have a sufficient number of cases, or the necessary information on the cases, to examine which particular subtypes of NHL are elevated most in incidence. The majority of reported NHLs in the transplant setting are of B-cell origin and are more likely to be extranodal than NHLs in the general population.[33] EBV has been shown to play an important role in the etiology of post-transplant NHL, as the vast majority of malignancies are found to be EBV positive.[31;34] Due to the rarity of HL, previous research in the transplant setting has typically been limited to small case series.[35-37] Similar to HL in the setting of HIV infection, post-transplant HL has been observed to be

of mixed cellularity pathology and almost uniformly EBV positive.[35-37] It has also been shown to be a later developing complication of transplantation than NHL.[35-37]

The general increased risks of lymphoma development observed in both patients with HIV/AIDS and those that have undergone a solid organ transplant highlight the importance of immune disturbance in the etiology of these malignancies. The incidence of lymphoma in HIV/AIDS patient tends to be higher than in transplant patients, perhaps due to more severe immunosuppression.[3] In addition, the lymphomas that develop under conditions of immune disturbance tend to be more aggressive than those that develop in persons with a normally functioning immune system.[38] The proposed mechanism for this observation is that in the setting of severe immunosuppression the development of more aggressive (high-grade) lymphomas is the result of uncontrolled proliferation of EBV-infected lymphocytes. Nonetheless, recent research has demonstrated etiologic heterogeneity among lymphoma subtypes, and immune dysfunction appears to impact some subtypes more than others.[10;11]

Leukemia

There are four major subtypes of leukemia that are included in most cancer registries. These include acute lymphocytic leukemia (ALL), which is typically classified as either a pre-cursor B-cell lymphoma or a T-cell NHL, acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Additional subtypes reported to cancer registries include myelodysplastic syndrome and chronic myeloproliferative disorder.

Leukemia is the most common malignancy diagnosed among children and adolescents, but the majority of leukemia cases occur in older adults.[39] The incidence of leukemia by age is multimodal, with an early peak in childhood, a second peak at around 25 years of age and a subsequent gradual rise.[39] In almost all age groups blacks have a lower risk of leukemia than whites and males tend to have a slightly higher incidence compared to females.[39]

The etiology of most cases of leukemia is largely unknown.[40] There is little consistent evidence to implicate any particular infectious agents or immunosuppression as risk factors for leukemia development. Several potential etiologic associations have been postulated, but none have been consistently demonstrated. A meta-analysis demonstrated a small increase in the incidence of leukemia in persons with HIV/AIDS or following organ transplant, with standardized incidence ratios of 3.20 and 2.38 respectively.[3] Two studies demonstrated an increased risk of myelodysplastic syndrome and AML following solid organ transplantation, thought to be related to the prolonged use of azathioprine, but these risk estimates were based on small case numbers.[23;41]

Multiple Myeloma (MM)

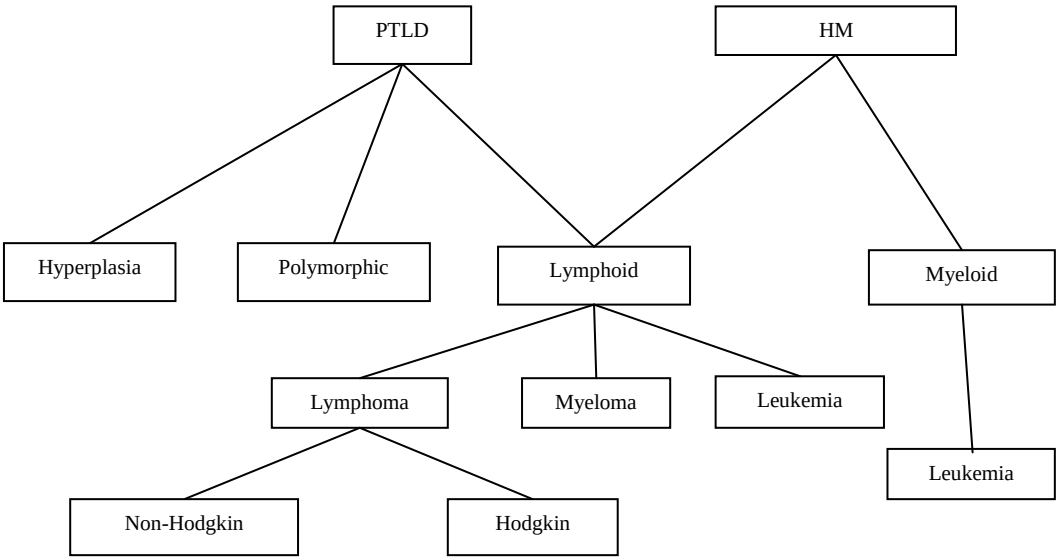
Multiple myeloma is a neoplasm of the plasma cells and accounts for 1% of all malignancies and 14% of all malignant hematologic disorders diagnosed annually in the United States.[42] Plasma cells are B-cells that are committed to the production of antibodies. The incidence of MM is nearly twice as high in blacks compared to whites and is slightly higher in males than in females. As with other HMs, multiple myeloma is more common at older ages, with 65 years being the median age of onset.[42]

The etiology of multiple myeloma is unknown, but the best established risk factors include exposure to radiation and certain chemical agents such as pesticides and certain petroleum products.[43] The role of particular infectious agents or immunosuppression in the etiology of multiple myeloma has been postulated but has not been consistently demonstrated.[43] Previous research has found mildly increased MM risk following solid organ transplantation, but these risk estimates were based on very few observed cases.[3;44]

Post transplant Lymphoproliferative Disorder (PTLD)

PTLDs include a wide spectrum of lymphoid proliferations ranging from non-malignant hyperplasia to frank lymphoma that develop after solid-organ or bone marrow transplantation. Except for non-melanoma skin cancer, PTLDs are the most common malignancy following solid organ transplant in adults and are the most common malignancy among younger transplant recipients.[45] The WHO divides PTLDs into four main categories with each category having smaller subtypes. The first category is termed early lesions and includes either plasmacytic hyperplasia or infectious mononucleosis-like disorders. The second category, polymorphic PTLDs, show the full range of B-cell maturation and are more likely to have monoclonal derivation. The third category, monomorphic PTLDs, includes overtly monoclonal malignant lymphomas of B-cell and rarely T-cell derivation. The B-cell neoplasms include NHL subtypes, multiple myeloma, and plasmacytoma-like lesions.[45;46] More than 80% of monomorphic PTLDs arise from B-cells and the most common subtype is DLBCL.[46] The last broad category is Hodgkin and Hodgkin lymphoma-like PTLD.[46] **Figure 1** below provides a summary of how these categories of PTLD are related to HM.

Figure 1 – An overview of post-transplant lymphoproliferative disorder (PTLD) and hematologic malignancy (HM).



Most cases of PTLD are associated with EBV and occur in the setting of diminished T-cell immune surveillance due to the use of immunosuppressive drugs to prevent rejection of the transplanted organ. There is great variability in incidence based on the type of organ transplanted. The cumulative incidence is 1-3% for liver and kidney transplants, but can be as high as 20% in small intestine transplants.[46] The difference in incidence by transplanted organ is strongly related to the duration and intensity of immunosuppression.[46] Additionally, the differences in incidence may also be related to the amount of lymphoid tissue that is present in the grafts since this tissue may serve as a reservoir for viruses such as EBV and cytomegalovirus (CMV) which will then be transmitted to the organ recipient.[46]

PTLD can develop at any time after transplantation and is typically categorized as either early or late-onset. PTLDs occurring within the first 1-2 years after transplantation are typically referred to as early-onset, while those developing later are considered late-

onset.[47-51] While the incidence of PTLD is highest in the period immediately following transplant, the early incidence is typically only a fraction of the total cumulative incidence after long-term follow-up. In kidney transplant recipients, for example, the median time from transplant to development of PTLD was found to be nearly 5 years.[32]

In comparison to lymphomas in the general population, PTLDs are more likely to involve locations outside of the lymph nodes, be disseminated to multiple organ sites, and have a poorer prognosis.[33;45] The most common locations of extranodal involvement are the liver, lungs and central nervous system. In addition, particularly for early-onset PTLDs, the transplanted organ is often involved.[50;52]

The main risk factors for PTLD include the EBV serostatus of the donor and recipient, the type of transplanted organ, the intensity and duration of immunosuppression, and the age of the transplant recipient.[46] The most well established risk factor is EBV infection, particularly if primary infection occurs while the transplant recipient is immunosuppressed.[34;45;46] Other risk factors associated with PTLD development have also been reported, including the type of disease that led to the need for transplant[46;53], the degree of human leukocyte antigen (HLA) mismatch between the donor and the recipient [54], the use of antibody induction therapy[51;55-57], and other viral agents such as CMV.[34;48;58] Lastly, the use of antiviral medications has been demonstrated to decrease the risk of PTLD development, particularly in EBV naïve recipients.[59]

Studies have highlighted important morphologic and pathologic differences between early-onset PTLD and late-onset PTLD. The vast majority of early-onset PTLDs are EBV-positive.[45;50] By contrast, a much lower proportion of late-onset PTLDs are EBV-

positive.[45;50] One study found that nearly 100% of early-onset PTLDs were EBV-positive, while only 60% of late-onset PTLDs were EBV-positive.[50] Late-onset PTLDs tend to be monomorphic, monoclonal, and have a poorer prognosis than those that occur shortly after transplant.[32] Late-onset PTLDs more commonly develop in sites that are unusual when compared to lymphomas that develop in the general population, including the gastrointestinal tract and central nervous system.[60] Early-onset PTLDs are more likely than late-onset PTLDs to develop in the transplanted organ.[50;52;56;61;62] Previous research has shown that early-onset PTLDs are more likely than late-onset PTLDs to be of donor origin.[63]

There has been limited large-scale epidemiologic research into the specific risk factors that impact the timing of PTLD development. It has been observed that patients that are EBV seropositive at the time of transplantation are more likely to develop late-onset PTLD than early-onset PTLD.[60] One possible explanation is that these late-onset PTLDs are the result of EBV latent infection reactivating in these patients after prolonged exposure to immunosuppressive agents. Persons that are EBV seronegative at the time of transplant, by contrast, are more prone to develop early-onset PTLD than late-onset PTLD. This is largely explained by the risk of primary infection with EBV in the period immediately following transplant when the intensity of immunosuppression is highest.[34] The median time of PTLD onset tends to be earlier in children than in adults, likely because a high proportion of children are EBV seronegative at transplantation.[53] One study examined risk factors for early-onset and late-onset NHL following transplantation.[51] Risk factors for early-onset NHL were EBV seronegativity and receipt

of T-cell depleting antibodies, while late-onset NHL risk was associated with older age and current use of calcineurin inhibitors.[51]

Earlier studies have indicated that the type of organ transplanted can be related to the timing of subsequent PTLD development. Early-onset PTLD has been shown to be more common than late-onset PTLD following lung, pancreas, and multi-organ transplantation.[50] Late-onset PTLD was more common than early-onset after liver and heart transplantation. Early and late onset PTLD occurred with equal frequency in kidney transplant recipients.[50;52]

The studies that previously compared early and late-onset PTLD have provided important clues about possible etiological differences between these two disease entities.[50;52;61;62;64] However, these studies had limited numbers of cases of PTLD and post-transplant follow-up time since they were often constrained to a single transplant center[50;52;61;62] or geographic region,[51;64] or focused only on one component of the PTLD spectrum.[51] The maximum number of PTLD cases in the studies referenced above was 230[64], whereas the remaining studies had fewer than 150 cases to analyze.[50;52;61;62] The small number of cases made it difficult to study the differences between early-onset and late-onset PTLD in great detail.

Justification of Hypotheses

The information above emphasizes the importance and relevance of the specific hypotheses of this project. First, any form of immune disturbance has been shown to increase the risk of HM development. These immune disturbances include HIV/AIDS as well as prolonged exposure to immunosuppressive medications following solid organ

transplantation. The increase in risk has been observed to be most profound for the higher-grade subtypes of NHL, but may also be present for other NHL subtypes and other HMs. Therefore, we would expect that there will be an association between solid organ transplant and later HM development and the association will be strongest for high-grade NHL subtypes. The proposed mechanism for this association is that the aggressive (high-grade) lymphomas that develop under conditions of immunosuppression are the result of uncontrolled proliferation of EBV-infected lymphocytes. We expect that other subtypes of HM will be associated with solid organ transplant to varying degrees, depending on the role played by EBV or other oncogenic viruses in their etiology. This project will allow us to explore these associations in some detail.

Secondly, previous studies have suggested distinct differences between early-onset and late-onset PTLDs in terms of histology, presentation, and other factors. These differences support the hypothesis that early-onset and late-onset PTLD are distinct disease entities and would be expected to have different etiologies associated with their development. Therefore, it would be expected that the specific risk factors would also differ between early-onset and late-onset PTLD.

Importance of Current Project

With the increasing number of transplants performed each year, as well as the improvements in short-term patient and allograft survival, it is important to develop a better understanding of the long-term health consequences of solid organ transplantation. Malignancy is one of the most important risks for transplant recipients. Earlier studies have shown an increase in the incidence of NHL in transplant recipients. This project

will build on this by exploring which particular NHL subtypes are most strongly associated with a history of solid organ transplantation, as well as to explore other HM subtypes, such as leukemia and multiple myeloma, as well as extranodal and central nervous system lymphomas, to determine if they are also associated with solid organ transplantation. The information needed to conduct analyses such as these was often lacking in earlier studies due to a limited number of HM cases or the lack of detailed and accurate information on the cases.

Earlier studies have demonstrated distinct differences between PTLD cases that develop shortly after transplant and those that develop later. This project will explore this further by assessing differences in pathology and risk factors between early-onset and late-onset PTLD.

Taken together the results of these two projects will provide a more enhanced understanding of the risk of HM development following solid organ transplantation and will provide a clearer picture of the spectrum of HM impacted by solid organ transplantation. Understanding the subtypes of HM most strongly associated with solid organ transplantation may provide further clues to the mechanisms of carcinogenesis. In addition, by examining the risk factors that impact the timing of lymphoma development following organ transplantation, we will gain a better understanding of the etiology of early-onset and late-onset PTLD.

Chapter 4: Results

Increased Risk for Lymphoid and Myeloid Neoplasms Following Solid Organ Transplant

Authors: Scott C. Quinlan[1], Lindsay M. Morton[1], Ruth M. Pfeiffer[1], Lesley A. Anderson[1,2], Ola Landgren[1], Joan L. Warren[3], and Eric A. Engels[1]

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD

[2] Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland.

[3] Division of Cancer Control and Population Sciences, National Cancer Institute, Rockville, MD

* This research was supported by the Intramural Research Program of the National Cancer Institute.

Address correspondence to:

Eric A. Engels, MD MPH
Infections and Immunoepidemiology Branch
Division of Cancer Epidemiology and Genetics
National Cancer Institute, DHHS
6120 Executive Blvd, EPS 7076
Rockville, MD 20892, USA

Phone: 301-496-8115
Fax: 301-402-0817
Email: engelse@exchange.nih.gov

Keywords: transplantation; lymphoma; myeloma; leukemia; United States

Category: Epidemiology

Running Title: Lymphoid/Myeloid Neoplasms Following Organ Transplant

Abstract

Purpose: By assessing the spectrum of hematologic malignancies associated with solid organ transplantation, we provide information on the pathogenesis of lymphoid and myeloid neoplasms and the clinical manifestations of immunosuppression.

Methods: Using data from the U.S. Surveillance, Epidemiology, and End Results (SEER) Medicare database, we identified 83,016 cases with a hematologic malignancy (age 66-99 years) and 166,057 population-based controls matched to cases by age, sex, and calendar year. Medicare claims were used to identify a history of solid organ transplantation. We utilized polytomous logistic regression to calculate odds ratios (ORs) comparing transplantation history among cases with various hematologic malignancy subtypes and controls, adjusting for the matching factors and race.

Results: A prior solid organ transplant was identified in 216 (0.26%) cases and 204 (0.12%) controls. Transplantation was associated with increased risk for non-Hodgkin lymphomas (NHLs) (OR=2.13, 95%CI 1.67-2.72), especially diffuse large B-cell lymphoma (DLBCL, OR=3.29, 95%CI 2.28-4.76), marginal zone lymphoma (OR=2.48, 95%CI 1.17-5.22), lymphoplasmacytic lymphoma (OR=3.32, 95%CI 1.41-7.81), and T-cell lymphoma (OR=3.07, 95%CI 1.56-6.06). Transplantation was also associated with elevated risk of Hodgkin lymphoma (OR=2.53, 95%CI 1.01-6.35) and plasma-cell neoplasms (OR=1.91, 95%CI 1.24-2.93). Risks for myeloid neoplasms were also elevated (OR=1.99, 95%CI 1.41-2.81).

Conclusion: Solid organ transplantation is associated with a wide spectrum of hematologic malignancies in the elderly. Risk was increased for four specific NHL

subtypes for which a viral agent has been implicated, supporting an added role for immunosuppression. Our results support monitoring for a wide spectrum of hematologic malignancies after transplant.

Introduction

Hematologic malignancies are a diverse group of leukemias, lymphomas, and related proliferative disorders characterized by heterogeneity in clinical presentation, pathology and molecular characteristics, and treatment. While the etiology of many subtypes is not fully understood, one factor that appears to play an etiologic role in multiple hematologic malignancy subtypes is immunodeficiency. Persons infected with the human immunodeficiency virus (HIV) are at increased risk of these malignancies, particularly non-Hodgkin lymphomas (NHLs), which may result from uncontrolled proliferation of Epstein Barr virus (EBV) infected lymphocytes.[3;31] Nonetheless, recent research has demonstrated etiologic heterogeneity among NHL subtypes, and immune dysfunction appears to impact some subtypes more than others.[10;11] In addition, the role of immunodeficiency in the development of other hematologic malignancy subtypes, such as myeloid neoplasms, is less well understood.

Solid organ transplantation is a life-saving treatment for people with end-stage organ disease. The number of solid organ transplants performed annually in the United States has increased steadily over the past two decades, with 28,291 transplants performed in 2006.[65] In addition, the number of persons living with a functioning transplanted organ has increased due to improved post-transplant survival.[65] Solid organ transplant recipients have an elevated risk of NHL due to immunosuppressive medications that they receive to prevent rejection of the allograft.[3-5;7;8;66] NHLs represent one component of a spectrum of lymphoid proliferations arising in transplant recipients, termed post-transplant lymphoproliferative disorder (PTLD).[22;24;44] These disorders also include

Hodgkin lymphoma and multiple myeloma, as well as non-malignant lymphoproliferations. Most NHLs in transplant recipients appear to be high-grade subtypes such as diffuse large B cell lymphoma (DLBCL)[32;67], and many are EBV-positive.[24] Nonetheless, due to inclusion of small numbers of NHL cases with limited information, studies have been unable to delineate which NHL subtypes are most strongly increased in incidence. In addition, very little is known about the impact of transplant-related immunosuppression on myeloid neoplasms and lymphoid neoplasms other than NHL.

A systematic evaluation of hematologic malignancy risk following solid organ transplantation will be helpful in better understanding the role of immunosuppression in lymphomagenesis and leukemogenesis. Furthermore, demonstration of an elevated risk for specific subtypes of hematologic malignancies after transplantation may affect follow-up and treatment of transplant recipients. The Surveillance, Epidemiology and End Results (SEER)-Medicare dataset offers a unique resource to expand on prior research in this area, since medical data are available on more than 80,000 patients with hematologic malignancies. We used these data to conduct a case-control study examining associations between specific hematologic malignancy subtypes and prior solid organ transplantation in the elderly.

Methods

SEER-Medicare dataset

The National Cancer Institute's SEER cancer surveillance program provides population-based data on incident malignancies diagnosed in 1973-2002 for multiple state and metropolitan areas, currently covering approximately 26% of the U.S. population (http://seer.cancer.gov/). Medicare is a federally funded program providing health insurance for approximately 97% of persons aged 65 years or older in the U.S.[68] Ninety-eight percent of Medicare beneficiaries receive Part A coverage for inpatient hospital stays, nursing facilities, and home health and hospice care, and 95% of beneficiaries pay for Part B coverage, which covers physician and outpatient services.[68] Medicare also provides health insurance for individuals under the age of 65 years with end-stage renal disease (ESRD) or disability.

Details of the SEER-Medicare linked dataset are provided elsewhere.[68] In summary, incident cancers reported to SEER registries were linked to Medicare enrollment and claims files. The SEER-Medicare database includes demographic and clinical information on all newly diagnosed cancer cases living in SEER areas during 1973-2002. Inpatient hospitalization claims are available for 1986-2002 and physician and outpatient claims for 1991-2002. In addition, Medicare claims data for persons who do not have cancer are available from a 5% random sample of Medicare beneficiaries living in SEER areas.

Selection of cases and controls

As previously described, the SEER-Medicare Assessment of Hematopoietic Malignancy Risk Traits (SMAHRT) Study is a population-based, case-control study of hematologic malignancies using SEER-Medicare data.[69] Individuals diagnosed with a SEER-registered hematologic malignancy as a first malignancy during 1987-2002, aged 66-99 years, and with at least 12 months of non-health maintenance organization (HMO) Medicare coverage prior to diagnosis were included as cases. We required at least 12 months of coverage to ensure that cases had sufficient time to accrue Medicare claims prior to diagnosis. Non-HMO Medicare coverage was required because HMOs do not routinely submit separate claims for individual diagnoses and procedures.

Lymphoproliferative malignancies were grouped according to a hierarchical system based on the World Health Organization classification.[70] NHLs were classified as either nodal or extranodal using International Classification of Diseases for Oncology topography codes.[71] Myeloproliferative malignancies were classified as acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, or chronic myeloproliferative disorder.[69]

For each case, two controls were randomly selected from the 5% random sample of Medicare recipients living in SEER areas. Controls were alive and cancer-free as of July 1 of the calendar year of selection, and had at least 12 months of non-HMO Medicare coverage prior to selection. Controls were frequency matched to cases on calendar year of selection, age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), and gender.

Under this sampling scheme, the same person could be selected as a control in different calendar years, or as a control in years prior to being diagnosed with a hematologic malignancy. After selection, cases and controls with Medicare claims evidencing HIV infection were excluded (n = 97 cases and n=169 controls).

Ascertainment of history of solid organ transplant

We reviewed Medicare claims data for the period prior to diagnosis/selection to determine whether cases and controls had had a history of solid organ transplantation, based on three types of evidence (i.e., transplant procedure, history of transplant, or complication of transplant). Specifically, a subject was considered to have had a solid organ transplantation based on a hospital claim indicating the diagnosis-related group code for a transplant procedure (kidney: 302, heart: 103, lung: 495, liver: 480). In addition, we identified people with prior transplants who had at least one hospital, physician, or outpatient claim indicating a prior history or unspecified complication of transplantation (International Classification of Diseases, version 9 codes: kidney V42.0, 996.81; heart V42.1, 996.83; lung V42.6, 996.84; liver V42.7, 996.82) [http://www.cdc.gov/nchs/icd9.htm]. We included Medicare claims prior to age 65 years, which could have been present if the person was covered at that time due to ESRD or disability.

Statistical analysis

We used polytomous logistic regression to compute odds ratios (ORs) and 95% confidence intervals (CIs) to assess the association of solid organ transplant with specific

subtypes of hematologic malignancies. If no cases of a hematologic malignancy subtype had a transplant history, we used the one-sided Fisher's exact test to examine the statistical significance of the association. We also examined the association with solid organ transplant separately for nodal and extranodal NHL. Additional models evaluated associations with specific organ transplants (kidney, liver, lung, and heart) and the type of Medicare claim evidencing transplant history (claims for transplant procedure, history, or complication). We tested for heterogeneity to determine whether the association with solid organ transplant varied significantly by hematologic malignancy subtype, NHL topography, or type of transplanted organ using Wald chi-square tests. We also evaluated associations for specified hematologic malignancy subtypes as a function of time since transplantation for those subjects where the date of the transplant procedure was indicated by a Medicare claim. All analyses were adjusted for the three matching factors (age, sex, calendar year) and race. We accommodated the repeated selection of controls, and the possibility of a control later becoming a case, in the variance computations (see Statistical Appendix). Observations in which the number of subjects was between 1 and 10 are reported as "< 11" to preserve subject confidentiality in accordance with the SEER-Medicare data use agreement.

Results

The study included 83,016 hematologic malignancy cases and 166,057 controls (127,397 unique control individuals, with repeat sampling). Cases and controls were similar with respect to sex, age, and calendar year of selection (Table 1). Although differences were

small, cases were more likely than controls to be non-Hispanic white and had more claims for hospitalization, physician visits, and outpatient care (Table 1).

A total of 216 (0.26%) hematologic malignancy cases and 204 controls (0.12%) had at least one Medicare claim indicating a prior solid organ transplant (OR 2.16, 95%CI 1.75-2.65). In analyses by hematologic malignancy subtype, solid organ transplant was associated with statistically significant increased risk for lymphoid neoplasms overall (OR 2.17) and more specifically for NHL (OR 2.13), plasma-cell neoplasms (OR 1.91), and Hodgkin lymphoma (OR 2.53) (Table 2). Among NHL subtypes, strong associations were observed for DLBCL (OR 3.29), the most common lymphoma subtype, as well as for lymphoplasmacytic lymphoma (OR 3.32), marginal zone lymphoma (OR 2.48), and T-cell lymphoma (OR 3.07). No significant association was observed with follicular lymphoma or chronic lymphocytic leukemia (Table 2). The association between solid organ transplant and DLBCL risk was significantly stronger than the association for follicular lymphoma (p=0.02) or for chronic lymphocytic leukemia (p=0.0001). Most plasma cell neoplasms (94.9%) were multiple myelomas, and solid organ transplant was associated specifically with increased risk of multiple myeloma (OR 1.91, 95%CI 1.29-2.83). In addition, transplant history was significantly associated with elevated risk for myeloid neoplasms overall (OR 1.99). Although we examined specific myeloid neoplasms (Table 2), the association with transplant did not vary significantly across these subtypes (p=0.33).

Thirty-six percent of DLBCLs were extranodal. A stronger association with transplantation (p=0.04) was observed for extranodal DLBCL (OR 4.58, 95%CI 3.02-6.97) than for nodal DLBCL (OR 2.59, 95%CI 1.70-3.94). Among DLBCLs arising in transplant recipients, fewer than 11 (<1%) were diagnosed in the transplanted organ (e.g., a kidney DLBCL in a kidney recipient). DLBCLs located in the central nervous system (CNS) were rare (3.1% of cases), and none had had a transplant.

We examined associations for the most common hematologic malignancy subtypes with specific transplanted organs (Table 3). DLBCL risk varied by transplanted organ (p=0.0006) and was significantly higher following liver transplant (OR 6.58) than all other organ transplants (p=0.02). Risk of plasma cell neoplasms did not vary by transplanted organ, either overall (p=0.79) or specifically for kidney transplants compared with all other organ transplants (p=0.53). Risk of myeloid neoplasm did not vary by transplanted organ (p=0.11). We also examined associations for the most common hematologic malignancy subtypes by type of Medicare evidence for transplant (Table 3). DLBCL risk estimates were higher if a claim for the transplant procedure was present (OR 11.81) compared with other forms of evidence (p<0.0001). The association with transplantation did not vary by type of Medicare evidence for either plasma cell neoplasms (p=0.38) or myeloid neoplasm (p=0.76).

There was a suggestion of a "U-shaped" pattern for DLBCL risk as a function of time since transplantation (Figure 1A), with the strongest associations occurring within 2 years following the procedure or more than 10 years after the procedure. For plasma cell

neoplasms, we found no cases within the first 2 years of the procedure, but strong associations were present more than 5 years after transplant (Figure 1B). For myeloid neoplasms, associations were observed both within 2 years of the procedure and, more strongly, greater than 10 years post-transplant (Figure 1C).

Discussion

This large population-based investigation is the first study to systematically examine associations between solid organ transplant and specific hematologic malignancy subtypes in the elderly. Among NHLs, three-fold increased risks were observed for DLBCL, lymphoplasmacytic lymphoma, and T-cell lymphoma, and lower but still elevated risk for marginal zone lymphoma. We also found that transplantation was associated with significantly elevated risk for plasma-cell neoplasms and Hodgkin lymphoma. Finally, the association between transplant and myeloid neoplasms was also notable, adding to limited prior evidence suggesting an increased risk of these malignancies in transplant recipients.

Among solid organ transplant recipients, DLBCL was the most common NHL subtype, and the association with transplantation was especially strong (OR 3.29). The increased risk for DLBCL was highest following liver transplantation (OR 6.58), which is consistent with previous studies showing the risk of lymphoma is higher after liver transplant compared to kidney transplant.[32] Based on data for a limited number of cases, we observed a "U-shaped" pattern of DLBCL risk after transplant, with the strongest associations apparent within 2 years or more than 10 years post-transplant. This finding is

consistent with previous reports for PTLD overall.[49;72] The majority of PTLDs occurring shortly after transplantation are EBV-positive, and EBV-induced lymphoproliferation secondary to intense immunosuppression is implicated.[24] Recent work has described that some early DLBCLs in transplant recipients are of donor origin[63], but we could not examine that possibility in our study. In contrast, the etiology of PTLDs late after transplant is less well understood.[24] We also found a particularly strong increase in risk for extranodal DLBCL risk associated with transplantation. Although other researchers have reported that extranodal lymphomas tend to arise within the transplanted organ[32;52], we did not have sufficient numbers of cases to evaluate associations by extranodal site. We did not find a transplant history among any CNS NHLs, although these lymphomas are increased in incidence among people with AIDS in relation to immunosuppression[10] and have been reported in the setting of transplantation.[32;73;74] Nonetheless, CNS NHL can also occur without obvious immunosuppression, particularly in older adults.[75;76]

Our study documents elevated risk for certain other NHL subtypes. Prior case reports have described the occurrence of T-cell lymphoma and marginal zone lymphoma following transplant.[77-79] The observed increases among transplant recipients could be explained by loss of immune control of oncogenic viruses implicated in these lymphoma subtypes (hepatitis C virus for lymphoplasmacytic and marginal zone lymphoma, EBV for T-cell lymphoma).[80-82] In contrast, we did not observe increased risks for two common lymphoma subtypes, follicular lymphoma or chronic lymphocytic leukemia. Of note, we found elevated risk of Hodgkin lymphoma associated with transplantation, which is consistent with previous studies in transplant recipients[3;44] and likely reflects the

important etiologic role of EBV. Elevated risk of Hodgkin lymphoma is also reported in people with AIDS.[3;29]

Plasma-cell neoplasms are not common after transplant, leading to variability of risk estimates in previous studies.[3;4;7;9] Although multiple myeloma is a cause of ESRD and thus kidney transplantation, we did not find significantly higher risk for plasma-cell neoplasms related to kidney transplants over other organ types. Also, the risk for plasma-cell neoplasms was highest more than 5 years after transplantation, suggesting that prolonged disturbances in immune function could account for late development of plasma-cell neoplasms among transplant recipients. A modest elevation in multiple myeloma risk is also observed among people with AIDS.[3;83]

The increased risk that we observed for myeloid neoplasms as a group is somewhat supported by prior research pointing to elevated risk for myelodysplastic syndrome and acute myeloid leukemia following transplantation.[23;41;84] DNA damage resulting from prolonged exposure to azathioprine has been implicated [22;23], which would be consistent with our observation that risk is greatest more than 10 years after the transplantation. Nonetheless, we also observed increased risk earlier following transplantation, and cases of myeloid neoplasms have also been reported in transplant recipients not receiving azathioprine.[41]

We note that our relative risk estimates for some hematologic malignancies following transplant appeared lower than in prior reports. In comparison to results in a recent meta-

analysis[3], we found a more modest association for NHL (OR of 2.13 in our study vs. a standardized incidence ratio of 8.07 in the meta-analysis) and Hodgkin lymphoma (2.53 vs. 3.89), but results were more similar for multiple myeloma (1.91 vs. 3.12) and myeloid neoplasms (1.99 vs. 2.38 for leukemia). One possible explanation for these differences is that our study was restricted to elderly adults. The effect of transplantation on risk of some hematologic malignancies may be smaller in elderly adults than in younger individuals, perhaps reflecting the age-related rise in incidence of these malignancies in the general population, changes in the immune system with age, or differences in the immunosuppressive protocols used in older transplant recipients. Alternatively, the weaker associations in our study could arise because our use of Medicare claims data likely resulted in some underascertainment of transplantation, particularly for procedures performed before age 65 years. Associations were strongest when a procedure claim for transplant was present, perhaps relating to a greater specificity in these claims. Nonetheless, we believe that the sensitivity of our overall approach was improved by also considering claims indicating either a history or complication of prior transplantation. In the end, any misclassification of transplantation status would likely have been non-differential between hematologic malignancy cases and controls, which would have conservatively biased measures of association towards the null. However, the similar results in our study and the Grulich meta-analysis for plasma cell neoplasms and myeloid neoplasms argue against a large artifact.

Our study has several important strengths. First, the large number of hematologic malignancy cases and systematic information on histology allowed us to examine specific

subtypes of hematologic malignancy, and NHL in particular, in greater detail than in previous research in this area. Further, our population-based sampling ensured that hematologic malignancy cases and controls were representative of the general elderly population in the U.S. Limitations to our study also need to be considered. As noted above, our study was restricted to elderly adults, and the results may not be generalizable to other age groups. A further limitation of our study was the rarity of solid organ transplant history (0.12% among control subjects), which limited our ability to detect associations for less common hematologic malignancy subtypes. Finally, we did not formally adjust for multiple comparisons, and some associations could have been due to chance. Nonetheless, the associations between transplantation and DLBCL, T-cell lymphoma, and myelodysplastic syndrome remained significant even after applying a Bonferroni correction for ~25 comparisons (p-values <0.002, Table 2).

From a clinical perspective, our results suggest that there are a wide variety of hematologic malignancies linked to immunosuppression. Following solid organ transplantation, recipients should be closely monitored for symptoms and findings consistent with these neoplasms, even though these events are rare. Some subtypes (e.g., extranodal DLBCL) can have a quite aggressive clinical course, and as our data show, risk for these malignancies persists for years following a transplant. Given that immunosuppression may play a role in their etiology, reduction of the intensity of the immunosuppressive regimen might be considered as part of the clinical management of patients with these malignancies. These possibilities need to be further evaluated in clinical studies.

In conclusion, our results provide a comprehensive picture of the risk of hematologic malignancy following solid organ transplantation for elderly transplant recipients. We found an association between solid organ transplantation for three specific NHL subtypes (DLBCL, lymphoplasmacytic, and T-cell lymphoma) as well as for myeloid neoplasms. We also observed increased risks of Hodgkin lymphoma and plasma cell neoplasms following transplantation. Additional research on the etiologic roles of disturbed immunity, viral infections, and medications in the development of these malignancies is warranted.

Acknowledgements

The authors thank Winnie Ricker (Information Management Services, Rockville, MD) for assistance with database management.

Statistical Appendix

Let $Y=(Y_0, Y_1, Y_2, \ldots, Y_K)$ denote the outcome variable in a nested case-control study comprised of one control group and K case groups. We use indicator notation, that is $Y_0=1$ if the person is a control and 0 otherwise and $Y_i=1$ is the person is a case of type i and 0 otherwise, i=1,…,K. We use polytomous logistic regression to compare each case group to the controls, by modeling

$$P(Y_i = 1 \mid X) = p(X, \theta_i) = \exp(X'\theta_i) / \sum_{s=1}^{K} \{1 + \exp(X'\theta_s)\},$$

for the covariate vector $X=[1, X_1, ..., X_m]$, that includes a one for the intercept term. As $\sum_{i=1}^{K} P(Y_i = 1) = 1,$ we assume $\theta_0 = [0, \ldots, 0]$. We then use maximum likelihood estimation we obtain the log odds ratio estimates $\theta_j = [\theta_{j1}, \theta_{j2}, \ldots, \theta_{jm}]$, j=1, …K, for the j^{th} outcome in the polytomous logistic model.

While the corresponding co-variance estimator accounts for the fact that the same control group is used for each disease subtype comparison, we additionally need to consider that due to constraints in our cohort a substantial number of healthy individuals were sampled multiple times as controls, and that some case individuals were sampled as controls prior to developing disease. We accommodate this issue adapting an approach by Anderson[69] as follows. Let the covariance matrix of the maximum likelihood estimates of the log odds ratio parameters be denoted by Σ. For each study subject, we obtain the scores $S_i = (S_{i1}, ..., S_{iK})$, from each of the *K* polytomous logistic regression models. For example

for subject *i* the score for model j, or equivalently, θ_j, is given by

$S_{ij} = -X_{ij}[Y_{ij} - P(Y_{ij} = 1 | X_{ij}, \theta_j)]$. We define the matrix of scores for n subjects as

$$S = \begin{pmatrix} S_{11} & S_{12} & ... & S_{1K} \\ S_{21} & S_{22} & .. & S_{2K} \\ S_{(n-1)1} & .. & S_{(n-1)(K-1)} & S_{(n-1)K} \\ S_{n1} & S_{n2} & S_{n(K-1)} & S_{nK} \end{pmatrix}$$

Control subjects have entries in every column of the score matrix, as they contribute to all logistic models. Some individuals served as controls before they were selected as cases and thus can also contribute to several logistic models. If there is no overlap between cases and controls, S simplifies to

$$S = \begin{pmatrix} S_1 & 0 & ... & 0 \\ 0 & S_2 & .. & 0 \\ 0 & .. & S_{K-1} & 0 \\ S_1 & S_2 & S_{K-1} & S_K \end{pmatrix}$$

Using the above notation, the asymptotic variance of the estimates $(\theta_1, ..., \theta_K)$ is given by $\Sigma B \Sigma$. B is estimated by

$$\hat{B} = \sum_i (\sum S_{ik})(\sum S_{ik})'$$

where i denotes the sum over individuals and the second sum inside refers to the repeated measurements on the same person.

Table 1. Hematologic malignancy cases and controls in the SMAHRT Study (1987-2002)

Characteristic	Hematologic malignancy cases N=83,016 (%)	Controls N=166,057 (%)	P-value*
Sex †			
Male	40,983 (49.4)	81,996 (49.4)	0.96
Female	42,033 (50.6)	84,061 (50.6)	
Age, years †			
65-69	12,544 (15.1)	25,106 (15.1)	1.00
70-74	20,295 (24.5)	40,611 (24.5)	
75-79	20,864 (25.1)	41,724 (25.1)	
80-84	16,043 (19.3)	32,091 (19.3)	
85-99	13,270 (16.0)	26,525 (16.0)	
Median age in years	77.0	76.0	
Race			
Non-Hispanic white	72,850 (87.7)	140,250 (84.5)	<0.0001
Non-Hispanic black	5,123 (6.2)	11,379 (6.9)	
Asian/Pacific Islander	1,541 (1.9)	5,818 (3.5)	
Hispanic	1,114 (1.3)	3,539 (2.1)	
American Indian/Alaskan Native	141 (0.2)	469 (0.3)	
Other/unknown	2,247 (2.7)	4,602 (2.7)	
Selection/diagnosis year †			
1987-1990	12,071 (14.5)	24,148 (14.5)	1.00
1991-1994	16,284 (19.6)	32,566 (19.6)	
1995-1998	18,186 (21.9)	36,387 (21.9)	
1999-2002	36,475 (43.9)	72,956 (43.9)	
Hospital claims, no. ‡			
0	43,456 (52.3)	92,789 (55.9)	<0.0001
1-2	23,831 (28.7)	44,736 (26.9)	
3+	15,729 (19.0)	28,532 (17.2)	
Median	0	0	
Physician claims, no. ‡			
0-4	24,383 (29.4)	51,642 (31.1)	<0.0001
5-40	18,650 (22.5)	41,050 (24.7)	
41-125	21,569 (26.0)	42,259 (25.5)	
126+	18,414 (22.2)	31,106 (18.7)	
Median	37	29	
Outpatient claims, no. ‡			
0-1	37,874 (45.6)	81,661 (49.2)	<0.0001
2-5	15,199 (18.3)	31,125 (18.7)	
6-15	15,709 (18.9)	29,802 (18.0)	
16+	14,234 (17.2)	23,469 (14.1)	
Median	2	2	

Notes

* P-values are based on chi-square test.

† Gender, age, and selection year were matching factors.

‡ The number of Medicare claims is for the period prior to diagnosis/selection, excluding the 12 months immediately prior to diagnosis/selection.

Table 2. Associations of hematologic malignancies with solid organ transplantation

	N	Transplant history (%)	OR (95%CI)*	P-value
Controls	166,057	204 (0.12)	Reference	Reference
Lymphoid neoplasm	65,897	169 (0.26)	2.17 (1.75-2.70)	<0.0001
NHL (overall)	45,824	115 (0.25)	2.13 (1.67-2.72)	<0.0001
DLBCL	13,330	52 (0.39)	3.29 (2.28-4.76)	<0.0001
Burkitt lymphoma	221	<11 (<5)	2.78 (0.38-20.20)	0.32
Marginal zone lymphoma	1,989	<11 (<1)	2.48 (1.17-5.22)	0.02
Follicular lymphoma	6,142	11 (0.18)	1.50 (0.77-2.92)	0.23
Chronic lymphocytic leukemia†	13,124	16 (0.12)	1.08 (0.62-1.89)	0.78
Lymphoplasmacytic lymphoma	1,434	<11 (<1)	3.32 (1.41-7.81)	0.006
B-cell NHL, NOS	2,013	<11 (<1)	0.36 (0.05-2.62)	0.32
T-cell lymphoma	2,362	<11 (<1)	3.07 (1.56-6.06)	0.001
Unknown lineage NHL	3,330	<11 (<1)	2.36 (1.00-5.53)	0.05
Mantle cell lymphoma	1,171	<11 (<1)	1.72 (0.53-5.58)	0.37
Precursor B-cell lymphoma	267	<11 (<5)	2.65 (0.36-19.31)	0.34
Hairy cell leukemia/lymphoma	441	0 (0)	0	0.58§
Plasma cell neoplasm‡	14,000	32 (0.23)	1.91 (1.24-2.93)	0.003
Hodgkin lymphoma	1,692	<11 (<1)	2.53 (1.01-6.35)	0.05
Lymphoid neoplasm, NOS	4,381	17 (0.39)	3.72 (2.14-6.48)	<0.0001
Myeloid neoplasm	15,116	41 (0.27)	1.99 (1.41-2.81)	<0.0001
Acute myeloid leukemia	8,055	15 (0.19)	1.51 (0.85-2.68)	0.16
Chronic myeloid leukemia	2,250	<11 (<1)	2.04 (0.81-5.15)	0.13
Myelodysplastic syndrome	3,366	15 (0.45)	2.75 (1.54-4.88)	0.0006
Chronic myeloproliferative disorder	1,099	<11 (<1)	2.99 (1.28-6.99)	0.01
Myeloid leukemia, NOS	346	0 (0)	0	0.65§
Hematologic malignancy, NOS	2,003	<11 (<1)	3.10 (1.31-7.32)	0.01

Notes

Abbreviations: OR odds ratio, CI confidence interval, NOS not otherwise specified, NHL non-Hodgkin lymphoma,

DLBCL diffuse large B-cell lymphoma

Observations in which the number of exposed cancer cases was between 1 and 10 are reported as <11, to preserve subject confidentiality in accordance with the SEER-Medicare data use agreement.

Significant associations ($p < 0.05$) are underlined.

* Odds ratios were adjusted for age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), sex, race (white, non-white), and calendar year of diagnosis/selection (1987-1990, 1991-1994, 1995-1998, and 1999-2002).

† This category also includes small lymphocytic lymphoma.

‡ This category includes multiple myeloma (n=13,291), plasmacytoma (n=647), and plasma cell leukemia (n=62)

§ P-value was calculated using one-sided Fisher's exact test.

Table 3 – Associations of hematologic malignancies with specific types of solid organ transplant and Medicare evidence

	Control (N=166,057)	DLBCL (N=13,300)		Plasma cell neoplasm (N=14,000)		Myeloid neoplasm (N=15,116)	
	n (%)	n (%)	OR (95%CI)*	n (%)	OR (95%CI)*	n (%)	OR (95%CI)*
Organ transplanted							
Kidney	113 (0.07)	34 (0.26)	4.00 (2.68-5.96)	20 (0.14)	2.13 (1.31-3.48)	15 (0.10)	1.35 (0.78-2.34)
Liver	31 (0.02)	16 (0.12)	6.58 (3.48-12.45)	<11 (<1)	1.62 (0.56-4.69)	11 (0.07)	3.44 (1.68-7.01)
Heart	52 (0.03)	14 (0.11)	3.45 (1.90-6.29)	<11 (<1)	1.49 (0.63-3.51)	16 (0.11)	2.88 (1.63-5.11)
Lung	25 (0.02)	<11 (<1)	1.43 (0.41-4.97)	<11 (<1)	1.48 (0.44-4.99)	<11 (<1)	1.61 (0.55-4.70)
Medicare evidence of transplant							
Procedure	29 (0.02)	25 (0.19)	11.81 (6.69-20.85)	<11 (<1)	3.75 (1.72-8.19)	<11 (<1)	2.79 (1.28-6.10)
History	177 (0.11)	51 (0.38)	3.76 (2.71-5.20)	27 (0.19)	1.88 (1.24-2.85)	37 (0.24)	2.07 (1.43-2.97)
Complication	72 (0.04)	31 (0.23)	5.68 (3.65-8.83)	14 (0.10)	2.41 (1.35-4.31)	14 (0.09)	1.91 (1.07-3.41)

Notes

Abbreviations: OR odds ratio, CI confidence interval, NHL non-Hodgkin lymphoma, DLBCL diffuse large B-cell lymphoma

Observations in which the number of exposed cancer cases was between 1 and 10 are reported as "<11" in accordance with the SEER-Medicare data use agreement.

* Odds ratios were adjusted for age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), sex, race (white, non-white), and calendar year of diagnosis/selection (1987-1990, 1991-1994, 1995-1998, and 1999-2002). Odds ratios significantly different from 1.00 are underlined.

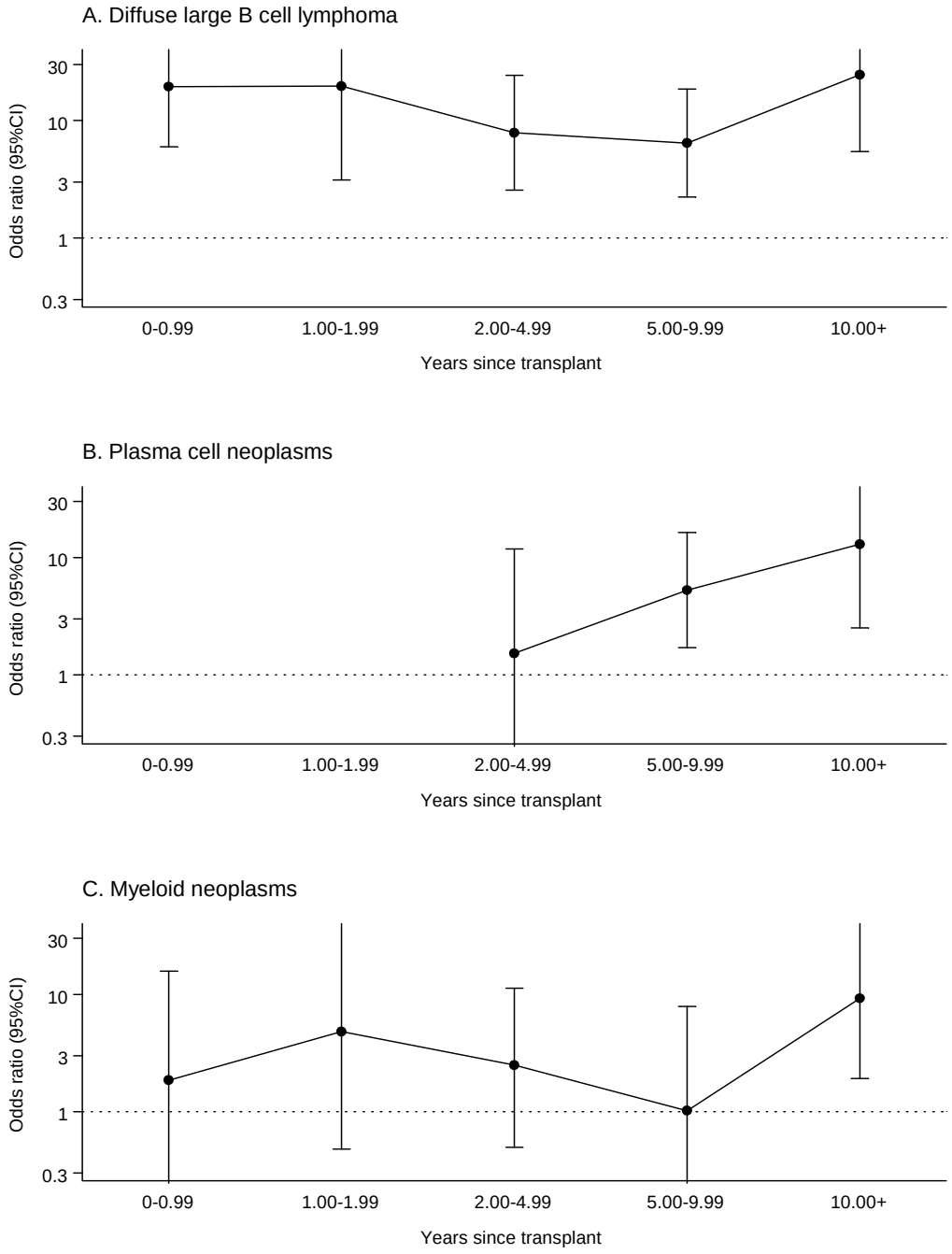


Figure 1 Legend

Risk of diffuse large B-cell lymphoma (A), plasma-cell neoplasms (B), and myeloid neoplasms (C) associated with solid organ transplantation as a function of time since transplantation. The figure presents odds ratios and 95% confidence intervals for 0-0.99, 1.00-1.99, 2.00-4.99, 5.00-9.99, and more than 10 years after a transplant procedure. For plasma-cell neoplasms (B), the odds ratios were 0 for the first two time points and are not presented.

Risk Factors for Early-onset and Late-onset Post-transplant Lymphoproliferative Disorder in U.S. Kidney Recipients

Quinlan S.C.[1], Pfeiffer R.M.[1], Morton L.M.[1], and Engels E.A.[1]

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD

This work was supported by the Intramural Research Program of the National Cancer Institute, National Institutes of Health.

Address correspondence to:

Eric A. Engels, MD MPH Phone: 301-496-8115
Infections and Immunoepidemiology Branch Fax: 301-402-0817
Division of Cancer Epidemiology and Genetics Email: engelse@exchange.nih.gov
National Cancer Institute, DHHS
6120 Executive Blvd, EPS 7076
Rockville, MD 20892, USA

Running Title: Risk factors for early-onset and late-onset PTLD

Word Count: Abstract – 200; Main Body – 3,478

Abstract

Incidence of post-transplant lymphoproliferative disorder (PTLD) is bimodal, suggesting distinct early-onset and late-onset subtypes. We evaluated differences in risk factors for early-onset and late-onset PTLD in a retrospective cohort study of U.S. kidney transplant recipients using data from the Scientific Registry of Transplant Recipients (N=156,740, 1999-2007). Multivariate hazard ratios (HRs) for risk factors were estimated using proportional hazards models. For early-onset PTLD, significantly increased risk was associated with young age at transplantation (HR 3.97 for <20 vs. 20-50 years) and non-Hispanic white race/ethnicity (HR 1.82), while Epstein Barr virus (EBV) and cytomegalovirus seropositivity at transplantation decreased risk (HR 0.32 and 0.67, respectively). By comparison, younger and older age (HRs 2.68 and 1.28 for <20 and >50 respectively, vs. 20-50 years) and non-Hispanic white race/ethnicity (HR 1.77) increased late-onset PTLD risk. The association with young age was weaker for late-onset than early-onset PTLD (p=0.06), and EBV and cytomegalovirus serostatus were not associated with late-onset PTLD risk. These results support different etiologies for early-onset and late-onset PTLD. For early-onset PTLD, associations with young age and EBV seronegativity highlight the etiologic role of EBV primary infection. For late-onset PTLD, higher risk with older age is consistent with lymphoma patterns in the general population.

Introduction

Solid organ transplantation is a life-saving medical procedure for persons suffering from end-stage organ failure. Improvements in medical procedures and immunosuppressive therapies have increased short-term patient and graft survival, leading to greater importance of the long-term complications of solid organ transplantation.[22] Malignancy is one of the key long-term complications, arising in excess among solid organ transplant recipients due in part to immunosuppression.[3;8] After skin cancer, non-Hodgkin lymphoma (NHL) is the second most common malignancy in transplant recipients [3], and NHL contributes the greatest cancer-related mortality.[22]

NHL comprises one end of a spectrum of post-transplant lymphoproliferative disorder (PTLD) ranging from benign hyperplasia to lymphoid malignancy.[85] PTLD risk is influenced by a number of factors, including the type of organ transplanted, the age and Epstein Barr virus (EBV) serostatus of the transplant recipient, and the intensity of immunosuppression.[24;34;45-47;51;86] The incidence pattern of PTLD is bimodal, with high incidence immediately after transplantation, followed by decreasing incidence, and then a rise again 4-5 years from transplantation.[49;72] This incidence pattern suggests the presence of two subtypes of PTLD: early-onset PTLD occurring in the period immediately following transplantation, and late-onset PTLD occurring some years later.

Differences in early-onset PTLD and late-onset PTLD have been previously reported. Early-onset PTLDs tend to be EBV-positive and are more likely than late-onset PTLDs to be localized to the transplanted organ and of polymorphic pathology.[50;52] Late-onset

PTLD is less likely to be associated with EBV, and is more likely than early-onset PTLD to be monomorphic and extranodal.[50;87] The prognosis for early-onset PTLD tends to be more favorable than for late-onset PTLD, largely because early-onset PTLD responds better to reductions in immunosuppression.[64;87]

While previous studies have identified biological and clinical differences between early-onset PTLD and late-onset PTLD, little research has been done on differences in risk factors between these two disease entities. Previous results were based on small numbers of PTLD cases [50;87], or were focused only on malignant lymphoproliferations.[51] A better understanding of the differences in risk factors between early-onset PTLD and late-onset PTLD would provide additional clues to their respective etiologies. The Scientific Registry of Transplant Recipients (SRTR) includes data on a large number of U.S. solid organ transplant recipients and information on malignancies diagnosed post-transplant. We used these data to conduct a retrospective cohort study among kidney transplant recipients to examine differences in risk factors between early-onset PTLD and late-onset PTLD.

Methods

Study design and subjects

We conducted a retrospective cohort study of U.S. kidney transplant recipients using SRTR data. The SRTR contains data from all kidney transplants performed in the U.S. since 1986, provided by the transplantation centers and organ procurement organizations that together comprise the Organ Procurement and Transplantation Network (OPTN). Baseline data are collected at the time of registration and at transplantation, and follow-

up data are collected 6 and 12 months after transplantation and annually thereafter. Recipients of first kidney transplants conducted between October 1, 1987, and August 31, 2007 were eligible for the present study. Included recipients had no evidence of human immunodeficiency virus infection and had at least 30 days of follow-up after transplantation.

Exposure assessment

For each transplant recipient, data were obtained from the SRTR baseline file regarding demographic characteristics, indication for transplantation, and number of human leukocyte antigen (HLA) mismatches with the donor at the A, B, and DR loci. We calculated an overall HLA mismatch score (range 0-6) by summing mismatches at the A, B, and DR loci. Serology results were included for EBV (EBV IgG) and cytomegalovirus (CMV IgG).

Information on the initial immunosuppressive regimen prescribed prior to hospital discharge was obtained from the SRTR immunosuppression file. The data on initial immunosuppressive regimen included medications prescribed to induce or maintain immunosuppression, or to treat initial rejection episodes. We created variables for categories of medication, including antibody induction therapy, steroid-based maintenance therapy, and anti-rejection therapy. Indications for kidney transplantation were categorized according to broad OPTN groupings (Table 1).

Outcome ascertainment

We identified transplant recipients with PTLD using the SRTR follow-up files. At the specified follow-up intervals, OPTN transplant center providers were required to report

any malignancies diagnosed since transplantation. If a recipient was diagnosed with PTLD, additional data were collected on date of diagnosis and clinical features. Occurrences of death, graft failure, re-transplantation, and loss to follow-up were also identified using SRTR follow-up files.

For this analysis, recipients with PTLD had diagnoses of a *de novo* lymphoproliferative disease or lymphoma that were not characterized as multiple myeloma or Hodgkin lymphoma. PTLD cases were categorized according to pathology (polymorphic, including hyperplasia, monomorphic, or unknown) and cell type (B-cell, T-cell, or unknown). Reporting of PTLD to the SRTR changed over time. OPTN did not routinely collect PTLD data on kidney transplant recipients until March 1, 1997. In 1999, the SRTR transitioned from a paper-based to a web-based system for filing follow-up reports.

Statistical analysis

Recipients were followed from 30 days after transplant until the earliest of PTLD diagnosis, graft failure, re-transplantation, death, loss to follow-up, or 10 years post-transplantation. To ensure uniform reporting of PTLD throughout the follow-up period, we included only follow-up time and PTLD events from January 1, 1999 onwards.

We used the Breslow method to estimate cumulative incidence of PTLD. Risk factors for early-onset PTLD (within 2 years of transplantation) and late-onset PTLD (2 or more years from transplantation) were evaluated using separate proportional hazards regression models, with time since transplantation as the time metric. In these analyses, recipients

contributed to each model during their person-time within the window of interest. PTLD risk factors examined in univariate analyses included demographic characteristics, indication for transplantation, HLA mismatch, baseline viral serologies, and initial immunosuppressive regimen. We tested all models for deviations from the proportional hazards assumption by incorporating an interaction term between each risk factor and follow-up time. To determine if the strength of association differed for each risk factor between early-onset and late-onset PTLD, we tested the significance of an interaction term between the variable of interest and a time-dependent indicator variable that distinguished early and late follow-up periods.

Baseline EBV and CMV data were missing for 62.1% and 41.9% of recipients, respectively. We therefore used a multiple imputation approach. Using data from all recipients with non-missing EBV serology, we developed a logistic regression model to predict EBV seropositivity based on age at transplantation, race, sex, use of induction therapy, number of HLA mismatches, and indication for transplantation. We used this model to compute the probability of EBV seropositivity for recipients with missing results. For each recipient, EBV serostatus was then drawn from a Bernoulli distribution with probability given by the logistic model. The imputed data were combined with data from recipients who had non-missing EBV serology information to create a full dataset. A similar process was utilized to impute CMV serostatus for recipients with missing results. This full dataset was used to estimate hazard ratios for EBV and CMV serostatus. The imputation and estimation was repeated 10 times and the results were

combined using the Rubin method in the MIANALYZE procedure (SAS, Version 9.1, Cary, NC).[88]

We constructed separate multivariate proportional hazards regression models for early-onset and late-onset PTLD. Two sets of multivariate models were created, one that did not include EBV and CMV serostatus as covariates (model 1) and another that included these covariates (model 2). We conducted sensitivity analyses to examine the impact of restricting to transplants performed in 1999 or later, as well as restricting to transplant recipients with complete EBV and CMV data.

Results

The study included 156,740 kidney transplant recipients (Table 1). Recipients accrued a total of 532,342 person-years of follow-up during 1999-2007 (mean 3.4 years per recipient). The majority of recipients were male (59.9%), non-Hispanic white (58.6%), 20-50 years old at transplant (52.4%), and transplanted in calendar years 1999-2007 (64.9%). EBV and CMV serology results were frequently missing, but most recipients with known results were seropositive for both. Almost all recipients (87.6%) received steroid-based maintenance therapy, and the majority (54.5%) received antibody induction therapy. Few (8.3%) required anti-rejection therapy in the period immediately following transplant. Most recipients (69.8%) had at least 3 HLA allele mismatches with the kidney donor. The most common medical conditions leading to kidney transplantation were glomerular disease (28.4%), diabetes mellitus (16.0%), and hypertensive nephrosclerosis (16.5%).

Cumulative incidence of PTLD at 5 and 10 years after transplantation was 0.7% and 1.4%, respectively. As shown in Figure 1, a "U-shaped" pattern of incidence with time since transplantation was observed, with high PTLD incidence shortly after transplantation, decreasing until approximately 4 years from transplantation, and rising thereafter. Early-onset PTLD (i.e., within the first two years after transplant) was more likely to be monomorphic than polymorphic (48.2% vs. 41.6%, with 10.2% of unknown pathology), and late-onset PTLD (more than two years after transplant) was even more likely to be of monomorphic pathology (55.9% vs. 31.4%, 12.7% unknown). Early-onset PTLD was predominantly of B-cell origin (72.3% B-cell versus 4.2% T-cell, 23.6% unknown). Late-onset PTLD was also predominantly of B-cell origin, but with a slightly higher proportion of T-cell PTLD (64.3% B-cell versus 9.7% T-cell, 25.9% unknown).

We examined PTLD risk factors stratified by onset time (Table 2). Gender was not associated with early-onset PTLD risk, but males had significantly higher late-onset PTLD risk than females (hazard ratio [HR] 1.23). Young age was more strongly associated with risk of early-onset PTLD than late-onset PTLD (HRs 6.59 and 2.98, respectively, compared to age 20-50 years at transplant; $p<0.0001$ for difference in HRs), while older age (>50 years) was significantly associated only with late-onset PTLD risk (HR 1.29). Non-Hispanic whites were at significantly higher risk of early-onset and late-onset PTLD than other racial/ethnic groups (HRs 2.09 and 1.76, respectively). EBV seropositivity was associated with significantly decreased risk of both early-onset and late-onset PTLD, although the association was stronger for early-onset PTLD (HRs 0.21 vs. 0.66, $p<0.0001$). CMV seropositivity was also associated with decreased early-onset

PTLD risk more strongly than late-onset PTLD risk (HRs 0.41 vs. 0.80, p=0.0001). Steroid maintenance therapy did not impact early-onset PTLD risk, but significantly decreased the risk of late-onset PTLD (HR 0.64). Among specified medical indications for transplantation, kidney transplants for a congenital, rare familial, or metabolic disorder (HR 7.01), glomerular disease (HR 2.32), tubular/interstitial disease (HR 3.38), or malignant neoplasm (HR 8.74) significantly increased early-onset PTLD risk compared to kidney transplants for hypertensive nephrosclerosis. Kidney transplants for tubular/interstitial disease (HR 2.00) and congenital, rare familial, or metabolic disorder (HR 2.93) increased late-onset PTLD risk compared to transplants for hypertensive nephrosclerosis. Use of antibody induction or anti-rejection therapies and the degree of HLA mismatch were not statistically significantly associated with PTLD risk, regardless of the timing of PTLD onset.

Results of separate multivariate models for early-onset and late-onset PTLD are shown in Table 3. When EBV and CMV serostatus were not included in the model (model 1), young age was significantly associated with both early-onset and late-onset PTLD risk (HRs 6.47 and 2.92, respectively, compared to age 20-50 years), and non-Hispanic whites were at significantly increased risk of both early-onset PTLD and late-onset PTLD, compared to other racial/ethnic groups (HRs 2.11 and 1.73, respectively). The association with young age was stronger for early-onset than for late-onset PTLD ($p<0.0001$).

With addition of information for EBV and CMV serostatus (model 2), young age remained significantly associated with both early-onset and late-onset PTLD risk (HRs

3.97 and 2.68, respectively, compared to age 20-50 years), although the association was attenuated for early-onset PTLD compared to the results of model 1. Non-Hispanic whites continued to be at significantly increased risk of both early-onset PTLD and late-onset PTLD compared to other racial/ethnic groups (HRs 1.82 and 1.77, respectively). EBV seropositivity and CMV seropositivity were significantly associated with decreased risk of early-onset PTLD only (HRs 0.32 and 0.67, respectively).

In both multivariate models, gender was no longer associated with risk of either early-onset or late-onset PTLD. When added to model 2, glomerular disease, tubular/interstitial disease, and malignant neoplasms leading to kidney transplant remained significant risk factors for early-onset PTLD (HR 1.57, 1.71, and 4.38, respectively, compared to hypertensive nephrosclerosis), while steroid maintenance therapy remained a significant predictor of decreased late-onset PTLD risk (HR 0.66). Congenital, rare familial, or metabolic disorders no longer had an impact on early-onset PTLD risk, and no medical indications impacted late-onset PTLD risk (data not shown). Finally, restricting analysis to transplants performed in 1999 or later had no impact on the significance of the reported risk factors. The protective effect of EBV and CMV seropositivity remained apparent when analyses were restricted to recipients with complete EBV and CMV serology data (data not shown).

Discussion

This large retrospective cohort study of kidney transplant recipients showed distinct differences in pathology and risk factors between early-onset PTLD and late-onset PTLD. The onset of PTLD was clearly bimodal, and a greater proportion of late-onset PTLDs

than early-onset PTLDs were of monomorphic pathology. Independent risk factors for early-onset PTLD included young age at transplantation, EBV and CMV seronegativity, and the medical indication leading to transplantation. By comparison, independent risk factors for late-onset PTLD included older age at transplantation, and non-Hispanic white race/ethnicity. Steroid-based maintenance therapy decreased late-onset PTLD risk. Our results demonstrate differing features of early-onset and late-onset PTLD, suggesting that these entities may have different etiologies.

The overall incidence of PTLD in our study (cumulative 5-year and 10-year incidence of 0.7% and 1.4%, respectively) was similar to what has been reported previously.[32;49;51;64] As in prior studies [49;72], we also observed a "U-shaped" pattern of PTLD incidence with time since transplantation. This pattern of PTLD incidence identifies two subtypes of PTLD: early-onset PTLD, occurring in the 1-2-year period immediately following transplantation, and late-onset PTLD, occurring some years later. In our study, a greater proportion of late-onset PTLDs than early-onset PTLDs were of monomorphic pathology and of T-cell origin. Other studies have found similar biological differences between early-onset PTLD and late-onset PTLD.[50;61] While early-onset PTLD is more likely than late-onset PTLD to be localized in the transplanted organ [50;52], we were unable to examine this pattern in our study due to insufficient data on tumor location.

Young age, EBV seronegativity, and CMV seronegativity at the time of transplant were strong risk factors for early-onset PTLD. Previous studies have also shown that young transplant recipients are at highest risk of PTLD development, particularly in the period

immediately following transplantation.[51;89] The increased risk is likely due to the high percentage of young solid organ transplant recipients who are EBV naive at the time of transplantation, leaving them susceptible to primary EBV infection in the period immediately following transplantation. Supporting this interpretation, we found that the association of young age at transplant with early-onset PTLD became weaker when we included EBV serostatus in a multivariate model (model 2 vs. model 1, Table 3), suggesting that the effect of young age was partly mediated by EBV infection. Primary infection during the period of intense immunosuppression immediately following transplantation allows for uncontrolled EBV-driven lymphoproliferation.[34;46;56] The vast majority of early-onset PTLDs express EBV proteins, providing further evidence of the virus's etiologic role.[50] The role of CMV infection in PTLD development is less well understood. An earlier study pointed to a possible synergistic role of CMV disease with primary EBV infection in causing PTLD.[58] Nonetheless, the association that we observed between CMV serostatus and risk of early-onset PTLD could be the result of uncontrolled confounding, since CMV serostatus is related to age, EBV serostatus, and perhaps other unmeasured factors.

We also found evidence that the medical indication for kidney transplantation impacted subsequent early-onset PTLD risk. The finding that glomerular disease increased early-onset PTLD risk has not been reported previously. Many glomerular diseases are autoimmune-related, which could explain the elevated risk, since chronic immune stimulation associated with certain autoimmune diseases has been linked to increased lymphoma risk.[90;91] The most common glomerular diseases leading to kidney

transplantation were chronic glomerulonephritis (26.6%), focal glomerular sclerosis (21.5%), and immunogloblulin A (IgA) nephropathy (15.7%). We also found that malignancy resulting in kidney transplantation was associated with increased early-onset PTLD risk. The most common such malignancy was renal cell carcinoma (59.8% of transplants with this indication). Although the association with prior malignancy was also noted in another study [56], the mechanism has not been elucidated.

We did not find an association between antibody induction therapy and early-onset PTLD risk. In contrast, earlier reports indicated that antibody induction increased PTLD risk up to 9-fold.[51;55-57] The reason for the discrepancy could relate to calendar period differences between our study and previous research. We focused on a later calendar period (1999-2007), well after widespread introduction of antibody induction therapy during the mid to late 1990s.[16] When new therapies are introduced, the rate of PTLD has been observed to increase as transplant centers work to establish the appropriate dosing regimen to ensure a balance of efficacy and safety ("learning-curve" effect).[33] We hypothesize that transplant centers had had sufficient time by 1999 to gain experience with antibody-based induction therapies, leading to an attenuation of associated PTLD risk.

Finally, our study found that late-onset PTLD risk was increased in association with both younger and older age at transplantation, and non-Hispanic white race/ethnicity, while steroid-based maintenance therapy was observed to decrease late-onset PTLD risk. The increased risk for young transplant recipients was not as strong for late-onset PTLD as for

early-onset PTLD (HRs 2.68 vs. 3.97, compared to age 20-50 years). As for early-onset PTLD, the association between young age and late-onset PTLD risk could be related to primary EBV infection following transplantation. However, in terms of pathology and clinical features, compared to early-onset PTLDs, late-onset PTLDs more closely resemble lymphomas seen in the general population.[24] In the general population, NHL incidence is higher among non-Hispanic whites than other races/ethnicities and increases monotonically with age [92;93], which may explain the observed associations of these two factors with late-onset PTLD. While the association with increasing age has been described previously [51;94], the protective effect of steroid-based maintenance therapy on late-onset PTLD risk is a new finding. In comparison, research on NHLs in the general population has yielded inconsistent results, with some studies indicating that use of steroid-based medications increases NHL risk, while other studies show no relationship.[95]

Our study had several important strengths. Most notably, all kidney transplants performed in the U.S. during the study interval were included, allowing us to evaluate a large number of cases and facilitating the generalizability of our results. We studied both malignant and non-malignant PTLDs, overcoming a limitation of previous studies that used cancer registry data and were thus limited to malignant proliferations.[51] As a result, our study is the first large study to examine differences in pathology and risk factors for the full spectrum of early-onset and late-onset PTLD.

Limitations to our study also need to be considered. We likely did not have complete ascertainment of PTLD, especially for diagnoses that occurred late after transplantation.

Transplant centers are typically able to follow a very high percentage of recipients in the first 1-2 years after transplantation, but this percentage decreases with time, and by 3 years post-transplant, more than 10% of kidney transplant recipients have been lost to follow-up.[96] While incomplete reporting could have led us to underestimate PTLD incidence, it is less likely that it would have biased the associations with risk factors that we examined. Missing data on EBV and CMV serology for a large percentage of transplant recipients required that we impute the data using demographic and other characteristics, so that these findings need to be interpreted with some caution. However, a sensitivity analysis showed that the protective effect of EBV and CMV seropositivity on early-onset PTLD risk remained after restricting to recipients with complete EBV and CMV serology data. In addition, our data on immunosuppressive medications were limited to baseline and did not include specific dosing information. Thus, we could not assess the effect of immunosuppressive intensity or changes in the immunosuppressive regimen on PTLD risk.

In conclusion, the bimodal timing of PTLD after transplant and the observed differences in pathology and risk factors provide evidence that early-onset PTLD and late-onset PTLD are distinct disease entities with different etiologies. We found that late-onset PTLD was more likely than early-onset PTLD to be of monomorphic pathology. Young age and EBV seronegativity increased early-onset PTLD risk, providing support for an important etiologic role of EBV infection in PTLDs arising shortly after transplantation. The risk of late-onset PTLD was increased in older and non-Hispanic white transplant recipients, suggesting that compared to early-onset PTLDs, late-onset PTLDs are more

similar to the lymphomas that occur in the general population. PTLD remains an important source of morbidity associated with solid organ transplantation, and additional research on the risk factors and clinical features of PTLD, particularly late-onset PTLD, is required to better understand the role of prolonged immunosuppression and immune dysfunction in lymphomagenesis.

Acknowledgments

The Scientific Registry of Transplant Recipients (SRTR) is supported by contract 231–00-0116 from the U.S. Department of Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services.

The data reported here have been supplied by the contractor for the SRTR. The interpretation and reporting of these data are the responsibility of the authors and should not be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

Table 1 - Demographic and transplant characteristics of kidney recipients followed during 1999-2007 in the United States (N=156,740)

Characteristic	Recipients, n (%)
Gender	
Male	93,830 (59.9%)
Female	62,910 (40.1%)
Age at transplant, years	
0-19	10,711 (6.8%)
20-50	82,186 (52.4%)
>50	63,843 (40.7%)
Median age	47.0
Race/ethnicity	
Non-Hispanic white	91,867 (58.6%)
Other	64,873 (41.4%)
Calendar year of transplant	
1987-1995	29,081 (18.6%)
1996-1998	25,902 (16.5%)
1999-2007	101,757 (64.9%)
EBV serostatus	
Positive	49,790 (31.8%)
Negative	9,666 (6.2%)
Missing/not done	97,284 (62.1%)
CMV serostatus	
Positive	56,877 (36.3%)
Negative	34,250 (21.8%)
Missing/not done	65,613 (41.9%)
Antibody induction*	
Yes	84,458 (54.5%)
No	70,373 (45.5%)
Steroid maintenance*	
Yes	135,635 (87.6%)
No	19,196 (12.4%)
Anti-rejection therapy*	
Yes	12,780 (8.3%)
No	142,051 (91.7%)
HLA mismatch , number of alleles	
0-2	46,838 (30.1%)
3-4	66,269 (42.6%)
5-6	42,300 (27.2%)
Reason for transplant§	
Glomerular disease	44,461 (28.4%)
Diabetes mellitus	25,022 (16.0%)
Hypertensive nephrosclerosis	25,873 (16.5%)
Polycystic kidney	15,444 (9.9%)
Tubular/interstitial disease	8,667 (5.5%)
Vascular disease	6,771 (4.3%)
Congenital, rare familial, or metabolic disorder	4,922 (3.2%)
Malignant neoplasm	557 (0.4%)
Other	24,668 (15.8%)

Notes

Abbreviations: EBV Epstein Barr virus, CMV cytomegalovirus, HLA human leukocyte antigen

* Medication information was missing for 1,909 kidney recipients.

† HLA mismatch information was missing for 1,333 kidney recipients.

§ Reason for transplant was missing for 355 kidney recipients.

Table 2 - PTLD risk factors among U.S. kidney transplant recipients during 1999-2007

Characteristic	Early-onset PTLD HR (95% CI)*	Late-onset PTLD HR (95% CI)*	P-value†
Gender			
Male	1.00 (0.81-1.24)	1.23 (1.01-1.51)	0.16
Female	1.0 (ref)	1.0 (ref)	
Age at transplant, years			
0-19	6.59 (5.12-8.48)	2.98 (2.26-3.92)	<0.0001
20-50	1.0 (ref)	1.0 (ref)	
>50	1.04 (0.81-1.33)	1.29 (1.04-1.61)	0.19
Race/ethnicity			
Non-Hispanic white	2.09 (1.66-2.64)	1.76 (1.41-2.21)	0.30
Other	1.0 (ref)	1.0 (ref)	
EBV serostatus			
Positive	0.21 (0.17-0.27)	0.66 (0.48-0.89)	<0.0001
Negative	1.0 (ref)	1.0 (ref)	
CMV serostatus			
Positive	0.41 (0.32-0.53)	0.80 (0.62-1.03)	0.0001
Negative	1.0 (ref)	1.0 (ref)	
Antibody induction			
Yes	1.08 (0.87-1.34)	1.23 (1.00-1.50)	0.39
No	1.0 (ref)	1.0 (ref)	
Steroid maintenance			
Yes	1.33 (0.96-1.84)	0.64 (0.44-0.95)	0.005
No	1.0 (ref)	1.0 (ref)	
Anti-rejection therapy			
Yes	0.68 (0.40-1.14)	1.15 (0.84-1.57)	0.09
No	1.0 (ref)	1.0 (ref)	
HLA mismatch, number of alleles			
0-2	1.0 (ref)	1.0 (ref)	
3-4	1.12 (0.88-1.44)	1.02 (0.82-1.28)	0.56
5-6	0.85 (0.64-1.13)	1.02 (0.78-1.34)	0.36
Reason for transplant			
Hypertensive nephrosclerosis	1.0 (ref)	1.0 (ref)	
Glomerular disease	2.32 (1.57-3.44)	1.35 (0.96-1.91)	0.04
Diabetes	1.29 (0.80-2.08)	1.17 (0.80-1.72)	0.75
Polycystic kidney	1.36 (0.81-2.30)	1.06 (0.68-1.66)	0.48
Tubular/interstitial disease	3.38 (2.06-5.53)	2.00 (1.30-3.08)	0.12
Vascular disease	1.50 (0.79-2.86)	1.02 (0.55-1.89)	0.40
Congenital, rare familial, or metabolic disorder	7.01 (4.42-11.14)	2.93 (1.85-4.65)	0.009
Malignant neoplasm	8.74 (3.65-20.90)	0.99 (0.14-7.21)	0.05
Other	2.00 (1.30-3.08)	1.31 (0.85-2.00)	0.17

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus, HLA human leukocyte antigen

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratio is based on a univariate proportional hazards regression model.

† P-value compares hazard ratios for early-onset and late-onset PTLD and is based on the significance of an interaction term between the variable of interest and an indicator variable that distinguished early and late follow-up periods.

Table 3 – Multivariate analysis of risk factors for PTLD among U.S. kidney transplant recipients during 1999-2007

	Model 1			Model 2		
Characteristic	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**P-value†**	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**P-value†**
Gender						
Male	1.00 (0.81-1.23)	1.20 (0.98-1.47)	0.21	0.93 (0.75-1.15)	1.19 (0.97-1.47)	0.10
Female	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
Age at transplant, years						
<20	6.47 (5.02-8.33)	2.92 (2.21-3.84)	<0.0001	3.97 (3.02-5.22)	2.68 (1.97-3.64)	0.06
20-50	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
>50	0.98 (0.77-1.26)	1.26 (1.02-1.57)	0.14	1.09 (0.85-1.41)	1.28 (1.02-1.60)	0.37
Race/ethnicity						
Non-Hispanic white	2.11 (1.68-2.67)	1.73 (1.38-2.16)	0.22	1.82 (1.43-2.32)	1.77 (1.39-2.25)	0.87
Other	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
EBV serostatus						
Positive	--	--		0.32 (0.24-0.41)	0.79 (0.56-1.10)	<0.0001
Negative				1.0 (ref)	1.0 (ref)	
CMV serostatus						
Positive	--	--		0.67 (0.51-0.87)	1.01 (0.77-1.34)	0.02
Negative				1.0 (ref)	1.0 (ref)	

Notes

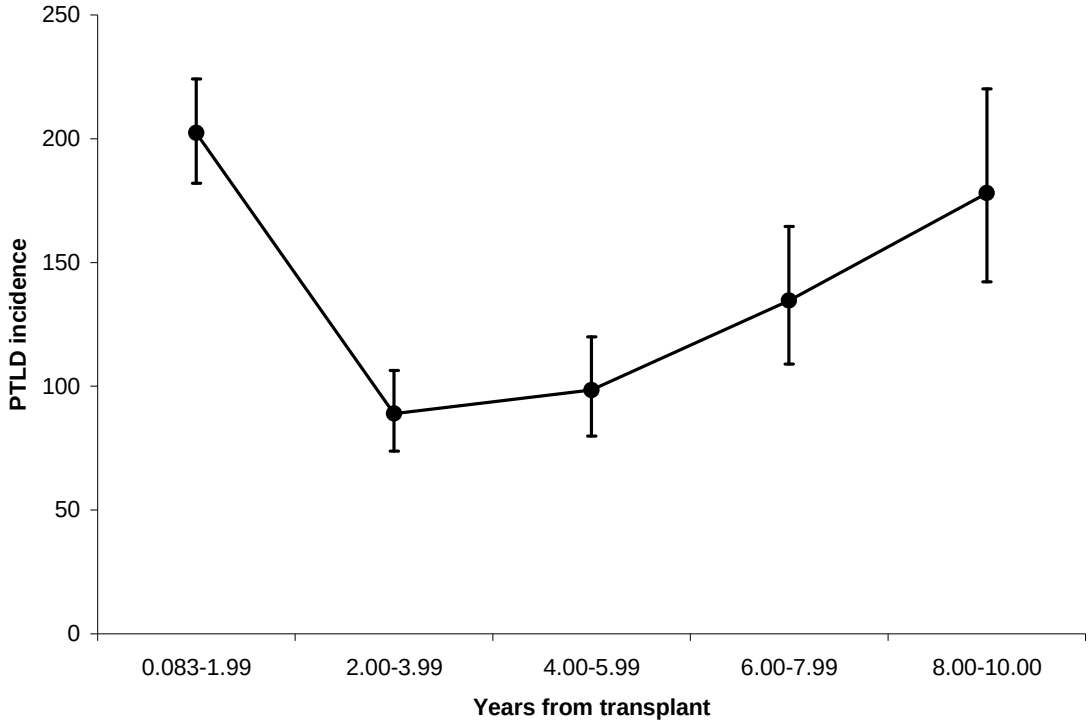
Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratios are based on a multivariate proportional hazards regression model controlling for gender, age at transplant, race/ethnicity, EBV status, and CMV status.

† P-value compares hazard ratios for early-onset and late-onset PTLD and is based on the significance of an interaction term between the variable of interest and an indicator variable that distinguished early and late follow-up periods.

Figure 1 Legend

Incidence of post-transplant lymphoproliferative disorder (PTLD) among kidney recipients during 1999-2007. Incidence and 95% confidence intervals are shown as a function of time since transplantation. PTLD incidence is displayed as PTLD events per 100,000 person-years. Follow-up for all recipients began 30 days (0.083 years) after transplantation.



Hodgkin Lymphoma among U.S. Solid Organ Transplant Recipients

Scott C. Quinlan[1], Ola Landgren[2], Lindsay M. Morton[1], and Eric A. Engels[1]

[1] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD
[2] Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD

Keywords: Hodgkin lymphoma; transplantation; Epstein-Barr virus; United States

Word Count: Abstract – 250; Text - 2,124

Tables: 1

Figures: Total – 1; Color - 0

Address correspondence to:

Eric A. Engels, MD MPH Phone: 301-496-8115
Infections and Immunoepidemiology BranchFax: 301-402-0817
Division of Cancer Epidemiology and Genetics Email: engelse@exchange.nih.gov
National Cancer Institute, DHHS
6120 Executive Blvd, EPS 7076
Rockville, MD 20892, USA

Author Contributions

O.L. and L.M.M. participated in research design; S.C.Q. and E.E. participated in research design, data analysis, and writing of the manuscript.

This work was supported by the Intramural Research Program of the National Cancer Institute, National Institutes of Health. The Scientific Registry of Transplant Recipients (SRTR) is supported by contract 231–00-0116 from the U.S. Department of Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services.

Conflicts of Interest

The authors declare no conflicts of interest.

Abbreviations

CI: Confidence interval

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

HIV: Human immunodeficiency virus

HL: Hodgkin lymphoma

HLA: Human leukocyte antigen

HR: Hazard ratio

NHL: non-Hodgkin lymphoma

OPTN: Organ Procurement and Transplantation Network

PTLD: post-transplant lymphoproliferative disorder

SEER: Surveillance, Epidemiology and End Results

SIR: Standardized incidence ratio

SRTR: Scientific Registry of Transplant Recipients

Abstract

Background: To assess the risk and identify risk factors of Hodgkin lymphoma (HL) in solid organ transplant recipients. Prior research has been limited by the rarity of HL and the requirement for extended follow-up after transplantation.

Methods: Using data from the Scientific Registry of Transplant Recipients (SRTR), we conducted a retrospective cohort study of U.S. solid organ transplant recipients (1997-2007). We estimated hazard ratios (HRs) for HL risk factors using proportional hazards regression. Standardized incidence ratios (SIRs) compared HL risk in the transplant cohort with the general population.

Results: The cohort included 283,190 transplant recipients (average follow-up 3.7 years after transplantation). Based on 73 cases, HL risk factors included male gender (HR 2.1, 95%CI 1.2-3.7), young age (4.0, 2.3-6.8), and EBV seronegativity at the time of transplantation (3.1, 1.2-8.1). Among tumors with EBV status information, 79% were EBV positive, including all tumors in recipients who were initially seronegative. Overall, HL risk was higher than in the general population (SIR 2.2) and increased monotonically over time following transplantation (SIR 4.1 at 8-10 years post-transplant). Excess HL risk was especially high following heart and/or lung transplantation (SIR 3.2).

Conclusion: HL is a late complication of solid organ transplantation. The high HL risk in recipients who were young or EBV seronegative at the time of transplant, and the fact that most HL tumors were EBV positive, highlight the role of primary EBV infection and

poor immune control of this virus. The occurrence of HL may rise with improved long-term survival in transplant recipients.

Introduction

The long-term health consequences of solid organ transplantation have taken on greater importance due to improvements in patient and graft survival.[22] Due in large part to long-term immunosuppression, solid organ transplant recipients are at greatly elevated risk of a number of malignancies, including non-melanoma skin cancer and non-Hodgkin lymphoma (NHL).[3;8;22;97;98] NHL and Hodgkin lymphoma (HL) both comprise part of a spectrum of post-transplant lymphoproliferative disease (PTLD) arising in transplant recipients.[85]

The impact of solid organ transplantation on the incidence of HL has not been extensively evaluated, but earlier studies have demonstrated that solid organ transplantation is associated with increased HL risk compared to the general population.[3;8;22;97;98] Previous studies, which have typically been limited to small case series, describe post-transplant HL as an aggressive, late complication of solid organ transplantation, with tumors typically manifesting mixed cellularity pathology and almost uniform Epstein-Barr virus (EBV) positivity.[35-37;99] The HLs described in transplant recipients are thus similar to those arising in the setting of human immunodeficiency virus (HIV) infection, providing evidence that disturbances in immune function play an important etiologic role in this malignancy.[29]

A better understanding of HL risk following solid organ transplantation will provide additional clues to the role of immunosuppression and EBV infection in the etiology of this malignancy. The U.S. Scientific Registry of Transplant Recipients (SRTR) is a

unique resource for evaluating the epidemiology of post-transplant HL, because detailed follow-up data are available on a large number of transplant recipients. We used these data to conduct a retrospective cohort study examining the risk factors and timing of HL following solid organ transplantation.

Methods

Study design and subjects

We conducted a retrospective cohort study of U.S. transplant recipients using data provided to the SRTR by transplantation centers and organ procurement organizations that together comprise the Organ Procurement and Transplantation Network (OPTN). Baseline and follow-up data are available on all solid organ transplants performed in the U.S. since 1986. Follow-up data are available at 6 and 12 months after transplantation, and annually thereafter. In our cohort we included all recipients of first organ transplants conducted between October 1, 1987, and August 31, 2007, who had no evidence of HIV infection and had at least 30 days of post-transplant follow-up.

Exposure assessment

For each transplant recipient we obtained data from the SRTR baseline file regarding demographic and transplant characteristics. The transplant characteristics included the type of organ transplanted (kidney and/or pancreas, liver, heart and/or lung, other) and the total number of HLA mismatches with the donor at the A, B, and DR loci (range: 0-6). We also obtained baseline viral serology data for EBV (EBV IgG) and

cytomegalovirus (CMV IgG). Updated EBV serostatus was obtained from SRTR follow-up files.

The SRTR immunosuppression file was used for data on the initial immunosuppressive regimen prescribed for each transplant recipient prior to hospital discharge. The data included medications prescribed to induce or maintain immunosuppression, or to treat initial rejection episodes. We created variables for categories of medication, including antibody induction therapy, steroid-based maintenance therapy, and anti-rejection therapy.

Outcome ascertainment

We used the SRTR follow-up files to identify recipients with HL. At each follow-up visit, transplant center providers were required to report any malignancies diagnosed since transplantation. If a recipient was diagnosed with HL, the date of diagnosis was recorded, along with the EBV status of the tumor. The SRTR follow-up files were also used to identify occurrence of death, graft failure, re-transplantation, and loss to follow-up.

Reporting of HL to the SRTR changed over time. HL diagnosis information was not collected on lung transplant recipients until April 1, 1994, and on all other recipients until March 1, 1997.

Statistical analysis

Follow-up for all recipients started 30 days after transplantation and continued until they developed HL or were censored due to PTLD other than HL, graft failure, re-transplantation, death, loss to follow-up, or 10 years post-transplantation. We included only follow-up time and HL events starting on July 1, 1997 to allow for uniform ascertainment for transplants of all organs.

To estimate hazard ratios and 95% confidence intervals for risk factors for HL, we developed univariate proportional hazards regression models with time since transplantation as the time metric. HL risk factors examined in the analyses included demographic characteristics, organ transplanted, overall number of HLA mismatches, baseline viral serologies, and initial immunosuppressive regimen. By evaluating an interaction between each risk factor and follow-up time, we demonstrated that all of the models that we present satisfied the proportional hazards assumption.

We utilized population-based incidence data from the Surveillance, Epidemiology, and End Results (SEER) network of U.S. cancer registries (http://seer.cancer.gov/) to calculate the expected numbers of HL cases in our cohort using rates specific to gender, race/ethnicity (non-Hispanic white, non-Hispanic black, and Hispanic), age, and calendar year. We then calculated standardized incidence ratios (SIRs) as the ratio of the observed number of HL cases to the expected number. For this analysis, we included only transplant recipients of white, black, or Hispanic race/ethnicity, since incidence data were available from SEER to calculate expected case numbers.

Results

The cohort included 283,190 recipients followed on average for 3.7 years after transplantation (Table 1). The majority of transplant recipients were male (61.5%), non-Hispanic white (66.6%), and age 20 years or older (91.9%). The most common organ transplanted was kidney and/or pancreas (63.7%), followed by liver (20.6%).

During follow-up, 73 HL cases were diagnosed. As shown in Figure 1, HL incidence was low in the first two years after transplant (1.8 cases per 100,000 person-years) and increased steadily thereafter, reaching 13.8 cases per 100,000 person-years 8-10 years after transplant. Risk factors for HL are presented in Table 1. Risk factors included male gender (hazard ratio [HR] 2.1, 95% CI 1.2-3.7) and young age at transplantation (HR 4.0, 95% CI 2.3-6.8, for age 0-19 vs. 20-50 years at transplant). Based on limited data, EBV seronegativity at transplant was associated with elevated subsequent HL risk (HR 3.1, 95% CI 1.2-8.1). Recipient race/ethnicity, CMV serostatus, type of organ transplanted, HLA mismatch, and immunosuppressive regimen were not significantly associated with HL risk.

Of the 73 HL cases, 7 were diagnosed in transplant recipients who were EBV seronegative at the time of transplantation. Information on EBV status of the tumors was available in 6 of these 7 cases, of which all were EBV positive. Also, of the 7 HL cases who were EBV seronegative at the time of transplantation, 3 had follow-up EBV serology data, and all showed evidence of EBV seroconversion. Finally, EBV status was available for 32 of the remaining 66 HL tumors, of which 24 (75%) were EBV positive. Overall, 79% of tumors with available information were EBV positive.

Overall, HL risk was doubled in solid organ transplant recipients compared to the general population (SIR 2.2, 95%CI 1.7-2.7). The excess risk compared to the general population was greatest in children and adolescents (SIRs 93.7 and 11.1 for ages 0-9 and 10-19 years at transplant, respectively) and recipients who were EBV seronegative at transplant (SIR 4.7, 95% CI 1.9-9.6). Heart and/or lung transplant recipients experienced especially high HL risk compared to the general population (SIR 3.2, 95% CI 1.9-5.0), whereas the excess risk associated with kidney and/or pancreas or liver transplantation was more modest (SIRs 1.8 and 2.3, respectively). While the risk was not elevated in the first two years post-transplant (SIR 0.6, 95% CI 0.2-1.2), by 8-10 years post-transplant HL risk was increased 4-fold compared with the general population (SIR 4.1 95% CI 2.2-7.0).

Discussion

In this large cohort study of transplant recipients, the incidence of HL was twice the incidence in the general population. The excess HL risk was especially high for recipients who were young or EBV seronegative at the time of transplant, and most HL tumors were EBV positive. HL incidence increased steeply over time following transplant, highlighting the importance of HL as a late complication of solid organ transplantation.

Previous research on this malignancy has been limited by the rarity of HL and the requirement for a long period of post-transplant follow-up. Our risk estimate appears lower than reported previously (SIR 2.2 in our study, vs. 3.9 in a recent meta-analysis).[3]

Losses to follow-up may have partly contributed to underascertainment of HL in our study, or some cases of HL could have been reported incorrectly as other types of PTLD (e.g., NHL). Unfortunately, we could not retrieve tumor tissue from reported cases of HL or other PTLD to perform additional pathological review.

The monotonic increase of HL incidence with time since transplantation contrasts with the bimodal incidence pattern of PTLD overall (mostly NHL).[49;72] The highest incidence of PTLD is typically observed in the period immediately following transplantation [32;49], and is believed to be the result of primary infection or reactivation of EBV in this period of intense immunosuppression.[47;86]

Several observations in our study support the importance of EBV in development of post-transplant HL. First, although EBV serostatus was missing for most recipients, we found that seronegative recipients had an elevated HL risk. This observation is consistent with a model that primary EBV infection following transplant, when recipients are unable to mount an effective initial immune response, greatly increases HL risk. Second, young transplant recipients were at especially high risk of HL, likely because a large proportion of young recipients are EBV seronegative at the time of transplantation. In western countries, many people acquire EBV during adolescence, and an elevated risk of HL following primary EBV infection in adolescence is seen in the general population.[100] Third, as reported in previous studies[35-37;99], we found that a large proportion of HL tumors were EBV positive. Most HLs in the setting of HIV infection are also EBV positive.[29] Within HL tumors, EBV can be detected in the malignant Hodgkin Reed Sternberg cells [31], where it has been shown to inhibit apoptosis.[101] Additionally, a high

frequency of tumors following transplantation and in the setting of HIV infection manifest mixed cellularity pathology.[29;35;36] These characteristics of transplant-related HL differ from HL cases in the U.S. general population, within which the most common subtype is nodular sclerosis and many cases are EBV-negative.[27;31] Overall, the late timing of HL in transplant recipients and the associations with EBV infection suggest that the impact of EBV primary infection on the development of HL may be delayed compared to its effect on transplant-associated NHL.

Additional findings deserve some brief comments. HL risk was more than twice as high in male compared to female transplant recipients. This gender difference was greater than observed in the general population, where HL incidence is only slightly higher in males than females.[102] The especially high HL risk following heart and/or lung transplantation likely reflects the greater intensity of immunosuppression following heart and lung transplantation, and it mirrors the increased risk of NHL following heart transplantation compared to kidney transplantation.[32] We did not find any other significant risk factors for post-transplant HL. One previous large-scale study that used data from the United States Renal Data System found few risk factors for post-transplant HL, but this study was limited to 3 years of post-transplant follow-up and focused only on adult transplant recipients.[44]

Our study had a number of strengths. It is the first large-scale study to examine HL incidence following all types of solid organ transplantation. All recipients of first organ transplants performed in the U.S. during the study interval were included, allowing us to evaluate a large number of cases and facilitating the generalizability of our results. The

large size was especially important since HL is a rare outcome and has been observed to occur late after transplantation.

Limitations also need to be considered. As noted above, the occurrence of HL may have been underascertained. Previous research indicates that losses to follow-up are minimal in the first 1-2 years post-transplant but can exceed 10% three or more years from transplantation.[96] This pattern could have contributed to underascertainment of HL late after transplantation. Incomplete reporting by transplant providers may also have contributed. However, underascertainment of HL incidence is unlikely to have biased the observed associations with risk factors that we examined. In addition, we had limited data on viral serostatus of transplant recipients and the EBV status of HL tumors, making it difficult to assess the impact of EBV on post-transplant HL etiology in detail.

To conclude, our results add to limited previous research indicating that HL is a rare but notable late complication associated with solid organ transplantation. Because HL incidence increases monotonically with time since transplantation, it is likely that the occurrence of HL will continue to rise as post-transplant survival improves. Our results highlight the importance of EBV infection and immunosuppression in the development of this cancer, but additional research is needed to further clarify its etiology.

Acknowledgments

The data reported here have been supplied by the contractor for the SRTR. The interpretation and reporting of these data are the responsibility of the authors and do not reflect official policy of or interpretation by the SRTR or the U.S. Government.

Table 1 – Hodgkin lymphoma risk factors among U.S. transplant recipients during 1997-2007

	Recipients, n (%)	Hodgkin lymphoma cases, n	Hazard ratio (95% CI)
Total	283,190 (100%)	73	--
Gender			
Male	174,194 (61.5%)	56	2.1 (1.2-3.7)
Female	108,996 (38.5%)	17	1.0 (ref)
Age at transplant, years			
0-19	22,870 (8.1%)	21	4.0 (2.3-6.8)
20-50	139,430 (49.2%)	32	1.0 (ref)
>50	120,890 (42.7%)	20	0.9 (0.5-1.5)
Median age	48.0		
Race/ethnicity			
White, non-Hispanic	188,648 (66.6%)	57	1.5 (0.9-2.6)
Other	94,542 (33.4%)	16	1.0 (ref)
EBV serostatus at transplant			
Positive	87,291 (30.8%)	10	1.0 (ref)
Negative	17,612 (6.2%)	7	3.1 (1.2-8.1)
Missing/unknown	178,287 (63.0%)	56	1.0 (0.5-2.1)
CMV serostatus at transplant			
Positive	93,726 (33.1%)	9	1.0 (ref)
Negative	57,213 (20.2%)	9	1.6 (0.6-4.0)
Missing/unknown	132,251 (46.7%)	55	1.6 (0.8-3.5)
Type of organ transplanted			
Kidney and/or pancreas	180,449 (63.7%)	40	1.0 (ref)
Liver	58,235 (20.6%)	15	1.1 (0.6-2.0)
Heart and/or lung	42,447 (15.0%)	18	1.6 (0.9-2.9)
Other	2,059 (0.7%)	0	0
HLA mismatch, number of alleles*			
0-2	54,948 (22.4%)	17	1.0 (ref)
3-4	103,708 (42.2%)	28	0.9 (0.5-1.7)
5-6	87,106 (35.4%)	19	0.8 (0.4-1.6)
Antibody induction†			
Yes	119,505 (43.1%)	25	1.0 (0.6-1.7)
No	157,549 (56.9%)	46	1.0 (ref)
Steroid maintenance†			
Yes	246,799 (89.1%)	67	1.0 (0.4-2.8)
No	30,255 (10.9%)	4	1.0 (ref)
Anti-rejection therapy†			
Yes	34,628 (12.5%)	13	1.2 (0.7-2.2)
No	242,426 (87.5%)	58	1.0 (ref)

Notes

Abbreviations: CI confidence interval, HLA human leukocyte antigen, EBV Epstein-Barr virus, CMV cytomegalovirus.
* HLA information was missing for 37,428 (13.2%) transplant recipients.
† Medication information was missing for 6,136 (2.2%) transplant recipients.

Figure 1

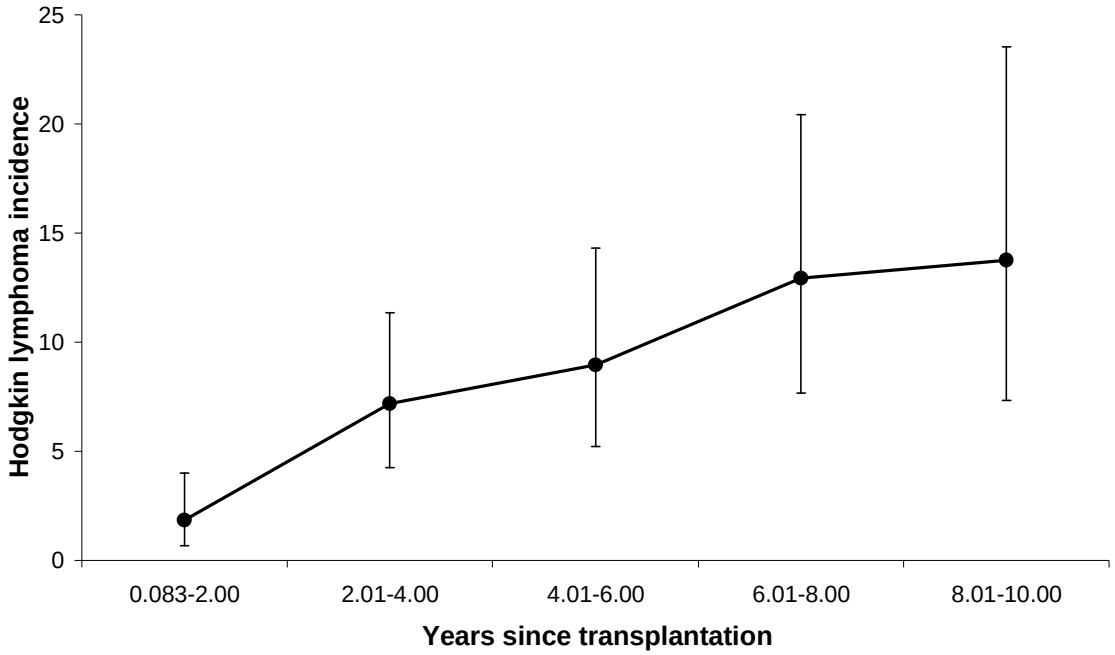


Figure 1 Legend

Incidence of Hodgkin lymphoma among U.S. transplant recipients during 1997-2007. Incidence rates and their 95% confidence intervals are shown as a function of time since transplantation and are displayed as Hodgkin lymphoma cases per 100,000 person-years. Follow-up for all recipients began 30 days (0.083 years) after transplantation.

Chapter 5: Conclusion

Our results further the understanding of the long-term health consequences of solid organ transplantation by providing a clearer picture of the risk and timing of hematologic malignancy (HM) following solid organ transplantation. As short-term patient and allograft survival have improved, long-term complications of solid organ transplantation have taken on greater importance. Malignancy is one of the most important long-term complications of transplantation, and HM contributes the greatest amount of cancer-related post-transplant mortality.[3;22;98] We have shown that solid organ transplantation is associated with increased risk of a wide spectrum of HMs in the elderly. Previous research had shown that the risk of non-Hodgkin lymphoma (NHL) was significantly increased following transplantation.[3] We expanded on this by showing that the increased risk was specific to NHL subtypes associated with oncogenic viruses. In addition, our results show that the impact of solid organ transplantation on HM risk is not limited to NHL, but solid organ transplantation is also associated with increased risk of Hodgkin lymphoma (HL), multiple myeloma, and myeloid neoplasms

In addition to demonstrating the wide spectrum of HMs associated with solid organ transplantation, we showed that the timing of development of post-transplant lymphoproliferative disorder (PTLD) was bimodal, identifying two subtypes of PTLD. Early-onset PTLD, occurring in the period immediately following transplantation; and late-onset PTLD occurring some years later. While there is likely some overlap in the presentation of early-onset and late-onset PTLD, our work highlighted that there are aspects of early-onset and late-onset PTLD that are somewhat distinct. We reported

differences in pathology and risk factors between early-onset PTLD and late-onset PTLD. Ours is the first large-scale study to report key differences in risk factors for the full spectrum of early-onset PTLD and late-onset PTLD. We also showed that post-transplant HL behaves very differently from PTLD, and tends to be a later occurring complication of transplantation that is almost uniformly associated with EBV infection. Research in this area had previously been limited by small case numbers and limited follow-up time, but we demonstrated that HL is a rare, but important late complication of transplantation that will take on greater importance as post-transplant survival continues to improve.

Our first study reported on the wide spectrum of HMs associated with solid organ transplantation in the elderly. As expected, NHL was found to be strongly associated with solid organ transplantation. DLBCL, the most common NHL subtype, was found to be especially strongly associated with transplantation. The strong association is likely a reflection of the etiologic role of EBV infection, as the majority of post-transplant DLBCLs have been reported to be EBV positive.[37] We also found that other NHL subtypes associated with viruses were increased in risk following solid organ transplantation, including lymphoplasmacytic lymphoma (hepatitis C), T-cell lymphoma (EBV), and marginal zone lymphoma (hepatitis C). In addition, we reported an association between solid organ transplantation and HL risk, likely due to the known role of EBV infection in the etiology of HL in immunosuppressed populations and consistent with results of our third study.[31] Our results provide further evidence for the crucial role of oncogenic viruses in the etiology of post-transplant malignancies. We also reported that myeloid neoplasms were associated with transplantation, a finding that has not been

widely reported and merits further investigation. Although our results provide evidence of the wide-spectrum of HMs associated with transplantation, the associations that we reported were generally weaker than what has been reported previously.[3] There are a number of possible explanations for this discrepancy. We focused on the elderly population, and the effect of transplantation on risk of some HMs may be smaller in elderly adults than in younger individuals, perhaps reflecting the age-related rise in incidence of these malignancies in the general population, changes in the immune system with age, or differences in the immunosuppressive protocols used in older transplant recipients. Additionally, the use of Medicare claims likely lead to the underascertainment of transplantation history, particularly for transplants occurring prior to age 65 years. This underascertainment was likely non-differential between cases and controls and would have biased our results towards the null.

In our second paper we report differences in pathology and risk factors between early-onset PTLD (< 2 years from transplantation) and late-onset PTLD (2 or more years from transplantation). Previous studies had highlighted important differences between these two entities, and we confirmed these differences and expanded upon them by highlighting differences in risk factors between early-onset PTLD and late-onset PTLD. We showed that early-onset PTLD is strongly associated with young age and EBV seronegativity at transplantation, providing support for the etiologic role of primary EBV infection in PTLDs arising shortly after transplantation. By comparison, late-onset PTLD was shown to be associated with older age at transplantation and non-Hispanic white race/ethnicity, demonstrating that compared to early-onset PTLD, late-onset PTLDs are more similar to the lymphomas that occur in the general population. Our finding that

CMV seronegativity increases early-onset PTLD risk has not been consistently reported. It is difficult to know whether our results regarding CMV reflect a true association, or possible uncontrolled confounding by age, EBV serostatus, or other unmeasured factors. One study reported that severe CMV disease increased PTLD risk, but CMV serostatus had no impact on PTLD risk.[34] The additional finding that steroid-based maintenance therapy at baseline is associated with lower late-onset PTLD risk is new and merits further investigation. The mechanism by which steroid-based maintenance therapy at baseline would reduce late-onset PTLD risk is unknown, but steroid-based medications have been shown to have an inconsistent effect on NHL risk in the general population.[95] Our study was limited by incomplete ascertainment of PTLD cases, due in large part to losses to follow-up. The losses to follow-up that were recorded by SRTR would not have impacted our PTLD incidence estimates since these recipients would have been censored in our analyses. Previous studies have shown that up to 10% of kidney transplant recipients are known to be lost to follow-up at 3 years post-transplantation.[96] The greater concern is with losses to follow-up or PTLD events that were not accurately recorded by SRTR. It is difficult to determine the proportion of kidney transplant recipients with inaccurate follow-up status, but any unrecorded PTLD events would lead to an underestimate of PTLD incidence. However, our PTLD incidence estimates were in-line with previous reports.[45] In addition, the implementation of a web reporting system in 1999 likely reduced the proportion of recipients with inaccurate follow-up status information, and restricting our analyses to transplants occurring in 1999 or later did not impact the significance of our findings. While incomplete reporting could have led us to underestimate PTLD incidence, it is less likely that it would have biased the associations

with risk factors that we examined. Additionally, we had very limited data on EBV and CMV serology at transplantation and the EBV status of the reported tumors. Our data on viral serostatus and immunosuppressive regimen were limited to baseline, so we were unable to examine the impact of changes in these factors on PTLD risk. We also did not have access to the actual doses of immunosuppressive medication, which prevented us from assessing the impact of immunosuppressive intensity on PTLD risk.

In our third paper we report on the risk factors and timing of post-transplant HL. The association of HL with transplantation has been reported previously, but typically only in small case series with limited follow-up time.[35-37] We demonstrated that post-transplant HL is a late developing complication of transplantation that is strongly associated with EBV infection. The role of EBV infection in post-transplant HL appears to be delayed compared to PTLD, where the incidence is highest immediately following transplantation when infection risk is typically highest. The mechanistic explanation for this delay is currently unknown. Our results showed that few demographic or transplant characteristics were associated with post-transplant HL risk, which is similar to what was reported by one large-scale study that examined post-transplant HL risk factors.[44] We found that the prescribed immunosuppressive regimen at baseline had no impact on subsequent HL risk. Our conclusions were somewhat limited, however, since our data on immunosuppressive regimen were limited to baseline and HL was shown to be a late-developing complication of transplantation. It would be informative to expand on our results by examining the immunosuppressive regimen at a time that is concurrent with HL development. In addition, similar to our analysis of PTLD, losses to follow-up and

other factors likely led to the underascertainment of HL in our cohort. This underascertainment may have reduced the incidence of HL in our cohort, but was unlikely to impact the associations with risk factors that we examined.

One of the factors that impacted all of our analyses was underascertainment. This limitation is especially important due to two difficulties of studying malignancy outcomes in solid organ transplant recipients. First, solid organ transplantation is a rare exposure and less than 1% of the general population has undergone a transplant. Therefore, when solid organ transplant is the exposure of interest in a case-control study, any degree of underascertainment can bias observed results towards the null, masking the true strength of association between transplantation and malignancy. Secondly, even though transplant recipients are at increased risk of HM compared to the general population, the absolute risk is still small. Typically, less than 10% of transplant recipients will experience an HM, with the proportion being even lower in kidney transplant recipients, the most common type of solid organ transplant.[24] As a result, in a cohort study of transplant recipients where malignancy is the outcome of interest, any degree of underascertainment will reduce the magnitude of the observed incidence. These two limitations make it imperative that when studying malignancy risk in transplant recipients significant efforts should be made to limit underascertainment as much as possible. In a case-control setting these efforts might include using multiple data sources to verify the transplant history of cases and controls, perhaps by linking to a transplant registry. In cohort studies, efforts should be made to limit losses to follow-up as much as possible and to also consider using multiple data sources to gather as much

outcome information as possible, particularly for individuals no longer being actively followed by transplant centers.

One disadvantage of the large-scale studies that we conducted was our inability to verify the histology and pathology information of the malignancies that were analyzed. This limitation could have resulted in some misclassification of the HM subtypes that we investigated. This is unlikely to have played a major role in our SEER-Medicare analyses, since SEER registries take great efforts to ensure the accuracy of the malignancy information reported. For the analyses that used SRTR data, this limitation needs to be considered. The malignancy information is reported by transplant centers, and some centers may be better equipped than others to accurately report this information. This could have resulted, for example, in cases of post-transplant HL being mistakenly reported as NHL. Unfortunately, we could not retrieve tumor tissue from reported cases of HL or other PTLD to perform additional pathological review. We did review the limited data provided by the transplant centers for each case of HL or other PTLD to ensure that it was classified correctly. However, this approach did not ensure that all HLs or PTLDs were classified correctly, and this could have contributed to underascertainment of HL. Smaller studies focused on a single transplant center or geographic region could have offered the opportunity to verify these diagnoses, however, at the expense of smaller case numbers and less generalizability.

Taken together our results provide a clearer picture of the risk of HM following solid organ transplantation. We have demonstrated that solid organ transplantation is

associated with a wide spectrum of HMs and the increased risk extends for many years after the actual transplant procedure. The characteristics of malignancies occurring immediately following transplantation are generally different from those that occur later. These later developing malignancies are only now being fully realized as post-transplant survival has improved. Our results support monitoring for a wide spectrum of HMs for many years after transplantation. The different characteristics of early and late occurring malignancies should be built into these monitoring plans so that developing malignancies can be recognized more quickly.

Future Directions

Our results have furthered the understanding of HM risk following solid organ transplantation. Future research could expand on our findings in a number of ways. While we demonstrated that solid organ transplantation was associated with a wide spectrum of HMs in the elderly, it would be informative to recreate our results in a younger population. It is possible that the HM risk profile could be very different in a younger population of transplant recipients compared to the elderly. The advantage to focusing on the elderly was the availability of Medicare claims data to assess solid organ transplant history. A comparable national database for younger populations is not readily available, but perhaps data from private health insurance plans could be used for such a purpose. Since the majority of transplantations occur prior to age 65 years, and are thus covered by private insurance and not Medicare, the use of such data might provide more complete and accurate transplantation history information. A case-control design would be most appropriate for examining the full spectrum of HMs in a younger population.

Many HM subtypes are rare, especially certain NHL subtypes, making a cohort study prohibitive in terms of the requisite sample size and follow-up time.

Another approach would be to conduct a large-scale study that directly links a transplant registry to state or national cancer registries. This approach would ensure nearly complete ascertainment of post-transplant malignancy outcomes, even for transplant recipients no longer being actively followed by the transplant centers. The incidence of cancer in transplant recipients could then be compared to general population incidence rates by the calculation of standardized incidence ratios (SIRs). Since all transplant recipients included in the registry would be available for analysis, this approach would also allow for an accurate assessment of the impact of age, or other demographic or transplant characteristics, on the excess risk of malignancy in transplant recipients. One limitation to such an approach would be that the rarity of certain subtypes of post-transplant malignancy would require that the linkage take place for a large enough sample of transplant recipients to ensure adequate statistical power.

Our finding that solid organ transplantation is associated with the risk of myeloid neoplasms has not been widely reported and merits further study. Our results, as well as limited previous reports[23;41], indicate that myeloid neoplasms are a late-developing complication of transplantation. As post-transplant survival continues to improve, reports of myeloid neoplasms will become more widespread. Previous research has linked the development of myeloid neoplasms to the use of specific immunosuppressive medications, in-particular, prolonged exposure to azathioprine.[23;41] Future research

should attempt to confirm or refute these findings by examining the association between solid organ transplantation and myeloid neoplasms in larger cohorts of transplant recipients with extended follow-up time. It will be important to include information on the specific immunosuppressive regimen in these investigations, particularly exposure to azathioprine. Information on the actual dose of azathioprine would also be valuable, since it would allow for dose-response relationships to be evaluated. Additionally, azathioprine is often prescribed in settings other than solid organ transplantation, such as in the treatment of refractory ulcerative colitis.[103] It would be interesting to investigate the risk of myeloid neoplasms in these populations to see if it is also elevated. Finding an excess of myeloid neoplasm risk in a different population of patients taking azathioprine would strengthen the evidence for an etiologic role of prolonged exposure to azathioprine in the development of these malignancies. Transplant recipients typically take a number of different immunosuppressive medications simultaneously, making it difficult to examine the independent etiologic role of any single medication.[21] In other conditions, such as ulcerative colitis, only one immunosuppressive medication is taken at a time, making it easier to evaluate the independent association with myeloid neoplasm risk.[103]

Our first study also reported an association between solid organ transplantation and multiple myeloma. This association has not be consistently reported in previous studies, partly due to the known role of myeloma in causing end stage renal disease and subsequent need for kidney transplantation, leading to the concern that any observed associations could be explained by reverse causality.[8] Our finding that the observed association was no stronger following kidney transplantation than following the

transplantation of other organs increases the likelihood that we observed a true association. In addition, we found the association to be strongest more than 5 years after transplantation, further decreasing the likelihood that reverse causality explains the associations we observed. Additional studies are needed to further confirm the association between multiple myeloma risk and solid organ transplantation. These studies should focus on non-kidney solid organ transplant recipients to remove the possibility of reverse causality explaining any observed associations.

The results of our PTLD and HL analyses highlight the importance of pre-transplant EBV serology, and to a lesser extent CMV serology, in determining subsequent malignancy risk. Other studies have also demonstrated the importance of pre-transplant EBV serology in determining PTLD risk.[34] Despite these results, pre-transplant viral serologies are not uniformly collected. In 2006, for example, nearly 30% of transplant recipients had unknown EBV serology at the time of transplantation. In the future, transplant centers should attempt to collect EBV serology information on as many transplant recipients as possible. This information is vital to establishing a recipient's malignancy risk profile following transplantation, and potentially identifying the most appropriate post-transplant malignancy monitoring plan. EBV seronegative transplant recipients require closer monitoring than EBV seropositive recipients, particularly if the recipient received an organ from an EBV seropositive donor. Since the majority of donors are EBV positive (>90% in some reports[104]), EBV seronegative recipients need to be monitored very carefully. A monitoring strategy might include longitudinal measurement of EBV viral load in the peripheral blood. Previous research has shown

that low peripheral EBV viral load has a high negative predictive value of subsequent PTLD development.[105] However, high peripheral EBV viral load poorly predicts subsequent PTLD development, making the monitoring of EBV viral load alone an inefficient measure of PTLD risk.[105]

Our results add to previous research indicating that solid organ transplant recipients are at dramatically increased risk of HM compared to the general population. However, despite this increased risk compared to the general population, the absolute risk of HM in transplant recipients is still relatively low. Previous research has estimated that less than 10% of transplant recipients will experience a PTLD.[24] While we have identified important risk factors for malignancy development, such as young age and EBV seronegativity at the time of transplantation, other factors likely play an important role in determining a transplant recipient's PTLD risk. Limited previous research has examined the role of genetic susceptibility to PTLD.[106;107] This research has identified certain polymorphisms in cytokine genes that are associated with increased PTLD risk. One study found that polymorphisms in genes for interleukin-10 and tumor growth factor (TGF)-beta increased susceptibility to EBV-associated PTLD.[106] Another study showed that polymorphisms in a tumor necrosis factor gene increased PTLD risk.[107] Future research should expand on these findings in an attempt to clarify these associations and to perhaps identify new polymorphisms associated with increased risk of malignancy in transplant recipients. If certain polymorphisms that increase PTLD risk are identified they could be built into post-transplant monitoring plans. This would allow transplant centers with limited resources to focus intensive monitoring on recipients at highest risk of PTLD. In addition, a monitoring strategy that includes simultaneous consideration of

both genetic polymorphisms and EBV viral load was shown to improve the PTLD positive predictive ability beyond the ability of either approach alone.[105] Such robust approaches should be considered to further refine the PTLD risk profile of transplant recipients.

Additionally, there are currently no population-based analyses that examine the risk of PTLD or other malignancies in relation to biomarkers that measure the extent of immunosuppression. Unlike the routine measurement of CD4 counts in the setting of HIV infection, transplant centers typically do not directly measure the degree of immunosuppression in transplant recipients. The dose and type of immunosuppressive medication are typically used as proxies for immunosuppressive intensity, however, it is likely that the effect of these therapies varies from individual to individual. One study reported a wide range of biomarkers that have been used at different transplant centers with little consistency or standardization.[108] This study highlighted the importance of conducting multi-center clinical trials to determine which biomarkers are most accurate and reliable for measuring immune function in transplant recipients.[108] An additional study described an assay that measures CD4 activity in transplant recipients and used the results to establish the appropriate therapeutic ranges for immunosuppressive medications that prevent organ rejection but do not excessively increase the risk of infection.[109] In the future, it will be important to further develop biomarkers that measure immune function in transplant recipients and to test them in large multi-center studies. Established biomarkers could be helpful in tailoring immunosuppressive therapy to maximize benefit, while minimizing the potentially harmful side effects of over immunosuppression such as infection and malignancy. In addition, reliable measures of immunosuppressive intensity

would inform post-transplant malignancy monitoring strategies by identifying when recipients are maximally immunosuppressed and thus at greatest risk of malignancy development. One consideration will be the cost of measuring biomarkers in a large number of transplant recipients. The cost of measuring a wide range of biomarkers will likely be prohibitive, which increases the importance of identifying a small number of key biomarkers that can be measured and interpreted in a standardized fashion from one transplant center to the next.

In summary, future research should focus on better identifying transplant recipients at highest risk of malignancy development. Transplant centers can then focus their limited resources on closely monitoring high-risk recipients to prevent malignancy development, or to diagnose malignancies at an early enough stage that treatment can be successful. Our results and previous research have identified a constellation of factors that impact post-transplant malignancy risk, including the duration and intensity of immunosuppression, infection by viral agents, and other host factors such as age, genetic susceptibility, and medical history. Developing a better understanding of how these different components fit together to form the malignancy risk profile for a given transplant recipient will be vital to ensuring that gains in post-transplant survival continue to be realized.

References

Reference List

(1) Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225-249.

(2) Ries LAG, Melbert D, Krapcho M, Mariotto A, Miller BA, Feuer EJ et al. SEER Cancer Statistics Review, 1975-2004. 2009. Bethesda, MD, National Cancer Institute.
Ref Type: Report

(3) Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007;370:59-67.

(4) Adami J, Gabel H, Lindelof B et al. Cancer risk following organ transplantation: a nationwide cohort study in Sweden. *Br J Cancer* 2003;89:1221-1227.

(5) Aseni P, Vertemati M, De CL et al. De novo cancers and post-transplant lymphoproliferative disorder in adult liver transplantation. *Pathol Int* 2006;56:712-715.

(6) Kyllonen L, Salmela K, Pukkala E. Cancer incidence in a kidney-transplanted population. *Transpl Int* 2000;13 Suppl 1:S394-S398.

(7) Serraino D, Piselli P, Busnach G et al. Risk of cancer following immunosuppression in organ transplant recipients and in HIV-positive individuals in southern Europe. *Eur J Cancer* 2007;43:2117-2123.

(8) Vajdic CM, McDonald SP, McCredie MR et al. Cancer incidence before and after kidney transplantation. *JAMA* 2006;296:2823-2831.

(9) Villeneuve PJ, Schaubel DE, Fenton SS, Shepherd FA, Jiang Y, Mao Y. Cancer incidence among Canadian kidney transplant recipients. *Am J Transplant* 2007;7:941-948.

(10) Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA. AIDS-related cancer and severity of immunosuppression in persons with AIDS. *J Natl Cancer Inst* 2007;99:962-972.

(11) Morton LM, Wang SS, Cozen W et al. Etiologic heterogeneity among non-Hodgkin lymphoma subtypes. *Blood* 2008;112:5150-5160.

(12) Wolfe RA, Merion RM, Roys EC, Port FK. Trends in organ donation and transplantation in the United States, 1998-2007. *Am J Transplant* 2009;9:869-878.

(13) Gutierrez-Dalmau A, Campistol JM. Immunosuppressive therapy and malignancy in organ transplant recipients: a systematic review. *Drugs* 2007;67:1167-1198.

(14) Bloom RD, Goldberg LR, Wang AY, Faust TW, Kotloff RM. An overview of solid organ transplantation. *Clin Chest Med* 2005;26:529-43, v.

(15) 2007 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients: Transplant Data 1997-2006. 2009. Rockville, MD, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation.
Ref Type: Report

(16) Meier-Kriesche HU, Li S, Gruessner RW et al. Immunosuppression: evolution in practice and trends, 1994-2004. *Am J Transplant* 2006;6:1111-1131.

(17) Smith SL. Immunosuppressive therapies in organ transplantation. Medscape . 6-25-2002.
Ref Type: Generic

(18) Chan M, Pearson GJ. New advances in antirejection therapy. *Curr Opin Cardiol* 2007;22:117-122.

(19) Knoll G. Trends in kidney transplantation over the past decade. *Drugs* 2008;68 Suppl 1:3-10.

(20) Campistol JM. Minimizing the risk of posttransplant malignancy. *Transplant Proc* 2008;40:S40-S43.

(21) Guba M, Graeb C, Jauch KW, Geissler EK. Pro- and anti-cancer effects of immunosuppressive agents used in organ transplantation. *Transplantation* 2004;77:1777-1782.

(22) Buell JF, Gross TG, Woodle ES. Malignancy after transplantation. *Transplantation* 2005;80:S254-S264.

(23) Offman J, Opelz G, Doehler B et al. Defective DNA mismatch repair in acute myeloid leukemia/myelodysplastic syndrome after organ transplantation. *Blood* 2004;104:822-828.

(24) Taylor AL, Marcus R, Bradley JA. Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. *Crit Rev Oncol Hematol* 2005;56:155-167.

(25) *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press, 2001.

(26) Hartge P, Wang SS, Bracci PM, Devesa SS, Holly EA. Non-Hodgkin Lymphoma. In: Schottenfeld D, Fraumeni JF, eds. *Cancer Epidemiology and Prevention*. 3 ed. Oxford University Press; 2006;898-911.

(27) Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. *Blood* 2006;107:265-276.

(28) Muller AM, Ihorst G, Mertelsmann R, Engelhardt M. Epidemiology of non-Hodgkin's lymphoma (NHL): trends, geographic distribution, and etiology. *Ann Hematol* 2005;84:1-12.

(29) Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. *Blood* 2006;108:3786-3791.

(30) Engels EA. Infectious agents as causes of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* 2007;16:401-404.

(31) Hjalgrim H, Engels EA. Infectious aetiology of Hodgkin and non-Hodgkin lymphomas: a review of the epidemiological evidence. *J Intern Med* 2008;264:537-548.

(32) Opelz G, Dohler B. Lymphomas after solid organ transplantation: a collaborative transplant study report. *Am J Transplant* 2004;4:222-230.

(33) Penn I. Post-transplant malignancy: the role of immunosuppression. *Drug Saf* 2000;23:101-113.

(34) Opelz G, Daniel V, Naujokat C, Dohler B. Epidemiology of pretransplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma. *Transplantation* 2009;88:962-967.

(35) Bierman PJ, Vose JM, Langnas AN et al. Hodgkin's disease following solid organ transplantation. *Ann Oncol* 1996;7:265-270.

(36) Garnier JL, Lebranchu Y, Dantal J et al. Hodgkin's disease after transplantation. *Transplantation* 1996;61:71-76.

(37) Knight JS, Tsodikov A, Cibrik DM, Ross CW, Kaminski MS, Blayney DW. Lymphoma after solid organ transplantation: risk, response to therapy, and survival at a transplantation center. *J Clin Oncol* 2009;27:3354-3362.

(38) Müller AM, Ihorst G, Mertelsmann R, Engelhardt M. Epidemiology of non-Hodgkin's lymphoma (NHL): trends, geographic distribution, and etiology. *Annals of Hematology* 2005;84:1-12.

(39) Xie Y, Davies SM, Xiang Y, Robison LL, Ross JA. Trends in leukemia incidence and survival in the United States (1973-1998). *Cancer* 2003;97:2229-2235.

(40) Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer* 2006;107:2099-2107.

(41) Menes M, Vakiani E, Keller CE et al. The spectrum of myelodysplastic syndromes post-solid organ transplantation: a single institutional experience. *Leuk Res* 2007;31:59-65.

(42) Devenney B, Erickson C. Multiple myeloma: an overview. *Clin J Oncol Nurs* 2004;8:401-405.

(43) Grogan TM, Van Camp B, Kyle RA, Muller-Hermelink HK, Harris NL. Plasma cell neoplasms. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics - Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001;142-156.

(44) Caillard S, Agodoa LY, Bohen EM, Abbott KC. Myeloma, Hodgkin disease, and lymphoid leukemia after renal transplantation: characteristics, risk factors and prognosis. *Transplantation* 2006;81:888-895.

(45) LaCasce AS. Post-transplant lymphoproliferative disorders. *Oncologist* 2006;11:674-680.

(46) Andreone P, Gramenzi A, Lorenzini S et al. Posttransplantation lymphoproliferative disorders. *Arch Intern Med* 2003;163:1997-2004.

(47) Epstein-Barr virus and lymphoproliferative disorders after transplantation. *Am J Transplant* 2004;4 Suppl 10:59-65.

(48) Everly MJ, Bloom RD, Tsai DE, Trofe J. Posttransplant lymphoproliferative disorder. *Ann Pharmacother* 2007;41:1850-1858.

(49) Faull RJ, Hollett P, McDonald SP. Lymphoproliferative disease after renal transplantation in Australia and New Zealand. *Transplantation* 2005;80:193-197.

(50) Ghobrial IM, Habermann TM, Macon WR et al. Differences between early and late posttransplant lymphoproliferative disorders in solid organ transplant patients: are they two different diseases? *Transplantation* 2005;79:244-247.

(51) van Leeuwen MT, Grulich AE, Webster AC et al. Immunosuppression and other risk factors for early and late non-Hodgkin lymphoma after kidney transplantation. *Blood* 2009;114:630-637.

(52) Bakker NA, van Imhoff GW, Verschuuren EA et al. Early onset post-transplant lymphoproliferative disease is associated with allograft localization. *Clin Transplant* 2005;19:327-334.

(53) Jain A, Nalesnik M, Reyes J et al. Posttransplant lymphoproliferative disorders in liver transplantation: a 20-year experience. *Ann Surg* 2002;236:429-436.

(54) Bakker NA, van Imhoff GW, Verschuuren EA et al. HLA antigens and post renal transplant lymphoproliferative disease: HLA-B matching is critical. *Transplantation* 2005;80:595-599.

(55) Bustami RT, Ojo AO, Wolfe RA et al. Immunosuppression and the risk of post-transplant malignancy among cadaveric first kidney transplant recipients. *Am J Transplant* 2004;4:87-93.

(56) Caillard S, Dharnidharka V, Agodoa L, Bohen E, Abbott K. Posttransplant lymphoproliferative disorders after renal transplantation in the United States in era of modern immunosuppression. *Transplantation* 2005;80:1233-1243.

(57) Swinnen LJ, Costanzo-Nordin MR, Fisher SG et al. Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac-transplant recipients. *N Engl J Med* 1990;323:1723-1728.

(58) Manez R, Breinig MC, Linden P et al. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. *J Infect Dis* 1997;176:1462-1467.

(59) Funch DP, Walker AM, Schneider G, Ziyadeh NJ, Pescovitz MD. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. *Am J Transplant* 2005;5:2894-2900.

(60) Hopwood P, Crawford DH. The role of EBV in post-transplant malignancies: a review. *J Clin Pathol* 2000;53:248-254.

(61) Nelson BP, Nalesnik MA, Bahler DW, Locker J, Fung JJ, Swerdlow SH. Epstein-Barr virus-negative post-transplant lymphoproliferative disorders: a distinct entity? *Am J Surg Pathol* 2000;24:375-385.

(62) Paranjothi S, Yusen RD, Kraus MD, Lynch JP, Patterson GA, Trulock EP. Lymphoproliferative disease after lung transplantation: comparison of presentation and outcome of early and late cases. *J Heart Lung Transplant* 2001;20:1054-1063.

(63) Capello D, Rasi S, Oreste P et al. Molecular characterization of post-transplant lymphoproliferative disorders of donor origin occurring in liver transplant recipients. *J Pathol* 2009;218:478-486.

(64) Caillard S, Lelong C, Pessione F, Moulin B. Post-transplant lymphoproliferative disorders occurring after renal transplantation in adults: report of 230 cases from the French Registry. *Am J Transplant* 2006;6:2735-2742.

(65) Port FK, Merion RM, Roys EC, Wolfe RA. Trends in organ donation and transplantation in the United States, 1997-2006. *Am J Transplant* 2008;8:911-921.

(66) Jiang Y, Villeneuve PJ, Fenton SS, Schaubel DE, Lilly L, Mao Y. Liver transplantation and subsequent risk of cancer: findings from a Canadian cohort study. *Liver Transpl* 2008;14:1588-1597.

(67) Morrison VA, Dunn DL, Manivel JC, Gajl-Peczalska KJ, Peterson BA. Clinical characteristics of post-transplant lymphoproliferative disorders. *Am J Med* 1994;97:14-24.

(68) Warren JL, Klabunde CN, Schrag D, Bach PB, Riley GF. Overview of the SEER-Medicare data: content, research applications, and generalizability to the United States elderly population. *Med Care* 2002;40:IV-18.

(69) Anderson LA, Pfeiffer R, Warren JL et al. Hematopoietic malignancies associated with viral and alcoholic hepatitis. *Cancer Epidemiol Biomarkers Prev* 2008;17:3069-3075.

(70) Morton LM, Turner JJ, Cerhan JR et al. Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). *Blood* 2007;110:695-708.

(71) Fritz A, Percy C, Jack A, Sobin LH, Parkin MD. *International Classification of Diseases for Oncology*. 3rd ed. 2000.

(72) Morton LM, Landgren O, Chatterjee N et al. Hepatitis C virus infection and risk of posttransplantation lymphoproliferative disorder among solid organ transplant recipients. *Blood* 2007;110:4599-4605.

(73) Castellano-Sanchez AA, Li S, Qian J, Lagoo A, Weir E, Brat DJ. Primary central nervous system posttransplant lymphoproliferative disorders. *Am J Clin Pathol* 2004;121:246-253.

(74) Phan TG, O'Neill BP, Kurtin PJ. Posttransplant primary CNS lymphoma. *Neuro Oncol* 2000;2:229-238.

(75) Batchelor T, Loeffler JS. Primary CNS lymphoma. *J Clin Oncol* 2006;24:1281-1288.

(76) Remick SC, Diamond C, Migliozzi JA et al. Primary central nervous system lymphoma in patients with and without the acquired immune deficiency

syndrome. A retrospective analysis and review of the literature. *Medicine (Baltimore)* 1990;69:345-360.

(77) Hanson MN, Morrison VA, Peterson BA et al. Posttransplant T-cell lymphoproliferative disorders--an aggressive, late complication of solid-organ transplantation. *Blood* 1996;88:3626-3633.

(78) Lok C, Viseux V, Denoeux JP, Bagot M. Post-transplant cutaneous T-cell lymphomas. *Crit Rev Oncol Hematol* 2005;56:137-145.

(79) Ueda Y, Chiba T. Helicobacter pylori in solid-organ transplant recipient. *Curr Opin Organ Transplant* 2008;13:586-591.

(80) de Sanjose S, Benavente Y, Vajdic CM et al. Hepatitis C and non-Hodgkin lymphoma among 4784 cases and 6269 controls from the International Lymphoma Epidemiology Consortium. *Clin Gastroenterol Hepatol* 2008;6:451-458.

(81) Giordano TP, Henderson L, Landgren O et al. Risk of non-Hodgkin lymphoma and lymphoproliferative precursor diseases in US veterans with hepatitis C virus. *JAMA* 2007;297:2010-2017.

(82) International Agency for Research on Cancer. Epstein-Barr virus and Kaposi's sarcoma herpesvirus/human herpesvirus 8. [70]. 1997. Lyon, France, IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans.
Ref Type: Serial (Book,Monograph)

(83) Frisch M, Biggar RJ, Engels EA, Goedert JJ. Association of cancer with AIDS-related immunosuppression in adults. *JAMA* 2001;285:1736-1745.

(84) Thalhammer-Scherrer R, Wieselthaler G, Knoebl P et al. Post-transplant acute myeloid leukemia (PT-AML). *Leukemia* 1999;13:321-326.

(85) Harris NL, Swerdlow SH, Frizzera G, Knowles DM. Post-transplant lymphoproliferative disorders. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics - Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001;264-269.

(86) Cockfield SM. Identifying the patient at risk for post-transplant lymphoproliferative disorder. *Transpl Infect Dis* 2001;3:70-78.

(87) Leblond V, Davi F, Charlotte F et al. Posttransplant lymphoproliferative disorders not associated with Epstein-Barr virus: a distinct entity? *J Clin Oncol* 1998;16:2052-2059.

(88) Rubin DB. *Multiple Imputation for Nonresponse in Surveys*. New York: J. Wiley & Sons, 1986.

(89) Dharnidharka VR, Tejani AH, Ho PL, Harmon WE. Post-transplant lymphoproliferative disorder in the United States: young Caucasian males are at highest risk. *Am J Transplant* 2002;2:993-998.

(90) Anderson LA, Gadalla S, Morton LM et al. Population-based study of autoimmune conditions and the risk of specific lymphoid malignancies. *Int J Cancer* 2009;125:398-405.

(91) Ekstrom SK, Vajdic CM, Falster M et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. *Blood* 2008;111:4029-4038.

(92) Alexander DD, Mink PJ, Adami HO et al. The non-Hodgkin lymphomas: a review of the epidemiologic literature. *Int J Cancer* 2007;120 Suppl 12:1-39.

(93) Grulich AE, Vajdic CM. The epidemiology of non-Hodgkin lymphoma. *Pathology* 2005;37:409-419.

(94) Caillard S, Lelong C, Pessione F, Moulin B. Post-transplant lymphoproliferative disorders occurring after renal transplantation in adults: report of 230 cases from the French Registry. *Am J Transplant* 2006;6:2735-2742.

(95) Ekstrom-Smedby K. Epidemiology and etiology of non-Hodgkin lymphoma--a review. *Acta Oncol* 2006;45:258-271.

(96) Levine GN, McCullough KP, Rodgers AM, Dickinson DM, Ashby VB, Schaubel DE. Analytical methods and database design: implications for transplant researchers, 2005. *Am J Transplant* 2006;6:1228-1242.

(97) Kasiske BL, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. *Am J Transplant* 2004;4:905-913.

(98) Vajdic CM, van Leeuwen MT. Cancer incidence and risk factors after solid organ transplantation. *Int J Cancer* 2009;125:1747-1754.

(99) Semakula B, Rittenbach JV, Wang J. Hodgkin lymphoma-like posttransplantation lymphoproliferative disorder. *Arch Pathol Lab Med* 2006;130:558-560.

(100) Hjalgrim H, Askling J, Rostgaard K et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. *N Engl J Med* 2003;349:1324-1332.

(101) Ambinder RF. Epstein-Barr virus and Hodgkin lymphoma. *Hematology Am Soc Hematol Educ Program* 2007;204-209.

(102) Landgren O, Caporaso NE. New aspects in descriptive, etiologic, and molecular epidemiology of Hodgkin's lymphoma. *Hematol Oncol Clin North Am* 2007;21:825-840.

(103) Rogler G. Medical management of ulcerative colitis. *Dig Dis* 2009;27:542-549.

(104) Lazda VA. Evaluation of Epstein-Barr virus (EBV) antibody screening of organ donors for allocation of organs to EBV serostatus matched recipients. *Transplant Proc* 2006;38:3404-3405.

(105) Lee TC, Savoldo B, Barshes NR et al. Use of cytokine polymorphisms and Epstein-Barr virus viral load to predict development of post-transplant lymphoproliferative disorder in paediatric liver transplant recipients. *Clin Transplant* 2006;20:389-393.

(106) Babel N, Vergopoulos A, Trappe RU et al. Evidence for genetic susceptibility towards development of posttransplant lymphoproliferative disorder in solid organ recipients. *Transplantation* 2007;84:387-391.

(107) McAulay KA, Haque T, Crawford DH. Tumour necrosis factor gene polymorphism: a predictive factor for the development of post-transplant lymphoproliferative disease. *Br J Cancer* 2009;101:1019-1027.

(108) Millan O, Urtasun N, Brunet M. Biomarkers of the immunomodulatory effect of immunosuppressive drugs in transplant recipients. *Transplant Rev (Orlando)* 2009;23:120-128.

(109) Kowalski RJ, Post DR, Mannon RB et al. Assessing relative risks of infection and rejection: a meta-analysis using an immune function assay. *Transplantation* 2006;82:663-668.

(110) Fisher ES, Baron JA, Malenka DJ, Barrett J, Bubolz TA. Overcoming potential pitfalls in the use of Medicare data for epidemiologic research. *American Journal of Public Health* 1990;80:1487-1490.

(111) MacKinnon DP, Krull JL, Lockwood CM. Equivalence of mediation, confounding, and suppression effect. *Prevention Science* 2000;1:173.

Appendices

Appendix 1 - Additional Analyses for Increased Risk for Lymphoid and Myeloid Neoplasms Following Solid Organ Transplant

Narrative

The impact of controlling for Medicare claim history on the association between hematologic malignancy (HM) subtypes and solid organ transplantation is displayed in Table 1. Controlling for Medicare claims attenuated the strength of the observed associations, but did not change their statistical significance. We chose not to control for Medicare claim history in our analyses since transplant recipients would be expected to have more Medicare claims than individuals who have no transplant history. By controlling for Medicare claims we would be controlling for a corollary of our exposure of interest.

Table 1. Associations of hematologic malignancies with solid organ transplantation controlling for Medicare claims history

	Model 1	Model 2
	OR (95%CI)*	OR (95%CI)**
Controls	Reference	Reference
Lymphoid neoplasm	2.17 (1.75-2.70)	2.01 (1.64-2.47)
NHL (overall)	2.13 (1.67-2.72)	1.97 (1.56-2.48)
DLBCL	3.29 (2.28-4.76)	3.06 (2.25-4.16)
Burkitt lymphoma	2.78 (0.38-20.20)	2.62 (0.36-18.89)
Marginal zone lymphoma	2.48 (1.17-5.22)	2.24 (1.10-4.56)
Follicular lymphoma	1.50 (0.77-2.92)	1.39 (0.75-2.55)
Chronic lymphocytic leukemia†	1.08 (0.62-1.89)	1.01 (0.61-1.69)
Lymphoplasmacytic lymphoma	3.32 (1.41-7.81)	2.87 (1.27-6.48)
B-cell NHL, NOS	0.36 (0.05-2.62)	0.32 (0.04-2.26)
T-cell lymphoma	3.07 (1.56-6.06)	2.70 (1.43-5.11)
Unknown lineage NHL	2.36 (1.00-5.53)	2.22 (0.98-5.02)
Mantle cell lymphoma	1.72 (0.53-5.58)	1.64 (0.52-5.15)
Precursor B-cell lymphoma	2.65 (0.36-19.31)	2.37 (0.33-17.06)
Hairy cell leukemia/lymphoma	0	0
Plasma cell neoplasm‡	1.91 (1.24-2.93)	1.78 (1.22-2.59)
Hodgkin lymphoma	2.53 (1.01-6.35)	2.44 (1.00-5.94)
Lymphoid neoplasm, NOS	3.72 (2.14-6.48)	3.42 (2.08-5.64)
Myeloid neoplasm	1.99 (1.41-2.81)	1.67 (1.19-2.35)
Acute myeloid leukemia	1.51 (0.85-2.68)	1.28 (0.76-2.17)
Chronic myeloid leukemia	2.04 (0.81-5.15)	1.80 (0.74-4.38)
Myelodysplastic syndrome	2.75 (1.54-4.88)	2.19 (1.29-3.72)
Chronic myeloproliferative disorder	2.99 (1.28-6.99)	2.70 (1.19-6.11)
Myeloid leukemia, NOS	0	0
Hematologic malignancy, NOS	3.10 (1.31-7.32)	2.50 (1.11-5.67)

Notes

Abbreviations: OR odds ratio, CI confidence interval, NOS not otherwise specified, NHL non-Hodgkin lymphoma,
DLBCL diffuse large B-cell lymphoma

Significant associations ($p < 0.05$) are underlined.

* Odds ratios were adjusted for age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), sex, race (white, non-white), and calendar year of diagnosis/selection (1987-1990, 1991-1994, 1995-1998, and 1999-2002).

** Odds ratios were adjusted for age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), sex, race (white, non-white), calendar year of diagnosis/selection (1987-1990, 1991-1994, 1995-1998, and 1999-2002), and total number of Medicare claims up to 1 year prior to diagnosis/selection (0-2, 3-36, 37-115, and 116+).

† This category also includes small lymphocytic lymphoma.

‡ This category includes multiple myeloma (n=13,291), plasmacytoma (n=647), and plasma cell leukemia (n=62)

Appendix 2 - Additional Analyses for Risk Factors for Early-Onset and Late-onset Post-transplant Lymphoproliferative Disorder in U.S. Kidney Recipients

Narrative

Multivariate analyses of risk factors for both monomorphic and polymorphic PTLD are presented in Table 1. The results are similar to what was observed for PTLD overall, with a few notable exceptions. The impact of young age at transplantation on early-onset PTLD risk was much stronger for polymorphic PTLD than monomorphic PTLD (multivariate HR 6.46 vs. 2.33, compared to age 20-50 years). For late-onset PTLD, the associations with young age were more similar for polymorphic and monomorphic pathology (multivariate HR 3.64 vs. 2.43). Non-Hispanic white race/ethnicity was a significant risk factor for both early-onset and late-onset monomorphic PTLD (HR 2.45 and 2.16, respectively), but was not a significant risk factor for polymorphic PTLD.

The impact of restricting the multivariate analyses to transplants occurring in 1999 and later is displayed in Table 2 (model 2). Compared to the original model (model 1), there were no major changes in the risk factors associated with either early-onset PTLD or late-onset PTLD.

The results of multivariate models that include either CMV serostatus (model 1) or EBV serostatus (model 2), but not both, are displayed in Table 3. The attenuation of the association between young age at transplant and early-onset PTLD risk is much stronger when just EBV serostatus is included in the model compared to a model including just CMV serostatus. This indicates that part of the observed impact of young age at

transplantation on subsequent early-onset PTLD risk is explained by the large proportion of young transplant recipients who are EBV seronegative at the time of transplantation.

The results of a multivariate model that was restricted to transplant recipients with non-missing EBV and CMV serostatus (model 2) are presented in Table 4. This restriction reduced the size of the cohort by more than 50%, but did not alter the significance of the reported risk factors for early-onset PTLD and late-onset PTLD. The protective effect of EBV seropositivity was stronger in the model restricted to recipients with non-missing EBV and CMV serostatus (model 2).

Baseline viral serology information for hepatitis B and C is presented in Table 5. The majority of kidney transplant recipients were negative for both hepatitis B and C at the time of transplantation. Hepatitis C seropositivity at baseline reduced the risk of early-onset PTLD (HR 0.34, 95% CI 0.14-0.84), but had no impact on late-onset PTLD risk. Hepatitis B serostatus did not impact the risk of either early-onset or late-onset PTLD. When hepatitis C serostatus was added to a multivariate model including gender, age at transplant, race/ethnicity, EBV serostatus, and CMV serostatus, it no longer had an impact on early-onset PTLD risk.

The impact of baseline EBV serostatus on early-onset PTLD risk, stratified by age at transplantation, is shown in Table 6. The reduced risk of early-onset PTLD in recipients who were EBV seropositive at baseline did not vary by age at transplantation.

The association between antibody induction therapy and early-onset PTLD risk, stratified by age at transplantation, is presented in Table 7. Antibody induction therapy had no impact on early-onset PTLD risk, regardless of age at transplantation.

Table 1 – Multivariate analysis of risk factors for polymorphic PTLD and monomorphic PTLD among U.S. kidney transplant recipients during 1999-2007

	Polymorphic (271 PTLD events)			Monomorphic (392 PTLD events)		
Characteristic	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**P-value†**	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**P-value†**
Gender						
Male	0.97 (0.70-1.35)	1.13 (0.78-1.64)	0.54	0.93 (0.69-1.27)	1.20 (0.91-1.59)	0.23
Female	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
Age at transplant, years						
<20	6.46 (4.25-9.82)	3.64 (2.20-6.03)	0.09	2.33 (1.53-3.57)	2.43 (1.61-3.67)	0.89
20-50	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
>50	1.13 (0.74-1.74)	1.25 (0.83-1.89)	0.75	1.15 (0.82-1.62)	1.24 (0.92-1.67)	0.75
Race/ethnicity						
Non-Hispanic white	1.40 (0.98-2.02)	1.43 (0.94-2.17)	0.94	2.45 (1.68-3.56)	2.16 (1.54-3.04)	0.63
Other	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
EBV serostatus						
Positive	0.34 (0.22-0.52)	0.77 (0.44-1.35)	0.03	0.28 (0.20-0.40)	0.77 (0.51-1.15)	0.0002
Negative	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	
CMV serostatus						
Positive	0.59 (0.38-0.91)	0.99 (0.56-1.73)	0.16	0.69 (0.48-1.00)	1.00 (0.71-1.41)	0.11
Negative	1.0 (ref)	1.0 (ref)		1.0 (ref)	1.0 (ref)	

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratios are based on multivariate Cox proportional hazards regression models controlling for gender, age at transplant, race/ethnicity, EBV status, and CMV status.

† P-value compares hazard ratios for early-onset and late-onset PTLD and is based on the significance of an interaction term between the variable of interest and an indicator variable that captured early and late follow-up periods.

Table 2 – Multivariate analysis of risk factors for PTLD among U.S. kidney transplant recipients during 1999-2007

	Model 1 – All Recipients†		Model 2 – Transplants 1999 and later‡	
Characteristic	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***
Gender				
Male	0.93 (0.75-1.15)	1.19 (0.97-1.47)	0.88 (0.71-1.10)	1.16 (0.81-1.64)
Female	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Age at transplant, years				
<20	3.97 (3.02-5.22)	2.68 (1.97-3.64)	3.87 (2.90-5.18)	2.99 (1.78-5.02)
20-50	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
>50	1.09 (0.85-1.41)	1.28 (1.02-1.60)	1.22 (0.94-1.59)	1.59 (1.08-2.34)
Race/ethnicity				
Non-Hispanic white	1.82 (1.43-2.32)	1.77 (1.39-2.25)	1.88 (1.46-2.43)	2.10 (1.39-3.17)
Other	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
EBV serostatus				
Positive	0.32 (0.24-0.41)	0.79 (0.56-1.10)	0.29 (0.22-0.37)	0.48 (0.31-0.73)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
CMV serostatus				
Positive	0.67 (0.51-0.87)	1.01 (0.77-1.34)	0.66 (0.50-0.86)	0.94 (0.63-1.39)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratios are based on a multivariate Cox proportional hazards regression models controlling for gender, age at transplant, race/ethnicity, EBV status, and CMV status.

† Model 1 includes all transplant recipients who contributed follow-up time during 1999 to 2007 (749 PTLD events).

‡ Model 2 is restricted to transplant recipients who underwent transplantation in 1999 or later (461 PTLD events).

Table 3 – Multivariate analysis of risk factors for PTLD among U.S. kidney transplant recipients during 1999-2007

	Model 1 – No EBV†		Model 2 – No CMV‡	
Characteristic	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***
Gender				
Male	0.96 (0.77-1.18)	1.20 (0.98-1.48)	0.96 (0.77-1.18)	1.19 (0.97-1.47)
Female	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Age at transplant, years				
<20	5.89 (4.55-7.62)	2.89 (2.17-3.86)	4.27 (3.26-5.59)	2.67 (1.97-3.61)
20-50	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
>50	1.06 (0.83-1.37)	1.27 (1.02-1.59)	1.03 (0.80-1.32)	1.28 (1.03-1.59)
Race/ethnicity				
Non-Hispanic white	1.87 (1.46-2.38)	1.78 (1.40-2.27)	2.00 (1.58-2.53)	1.76 (1.40-2.22)
Other	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
EBV serostatus				
Positive	--	--	0.30 (0.23-0.39)	0.79 (0.56-1.10)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
CMV serostatus				
Positive	0.61 (0.47-0.79)	1.01 (0.76-1.33)	--	--
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratios are based on a multivariate Cox proportional hazards regression models.

† Model 1 controls for gender, age at transplant, race/ethnicity, and CMV status (749 PTLD events).

‡ Model 2 controls for gender, age at transplant, race/ethnicity, and EBV status (749 PTLD events).

Table 4 – Multivariate analysis of risk factors for PTLD among U.S. kidney transplant recipients during 1999-2007

	Model 1 – Imputed EBV and CMV†		Model 2 – Non-missing EBV and CMV‡	
Characteristic	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***	**Early-onset PTLD HR (95% CI)***	**Late-onset PTLD HR (95% CI)***
Gender				
Male	0.93 (0.75-1.15)	1.19 (0.97-1.47)	0.85 (0.64-1.13)	1.17 (0.74-1.86)
Female	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Age at transplant, years				
<20	3.97 (3.02-5.22)	2.68 (1.97-3.64)	2.93 (2.06-4.16)	2.40 (1.28-4.51)
20-50	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
>50	1.09 (0.85-1.41)	1.28 (1.02-1.60)	1.12 (0.79-1.58)	1.43 (0.86-2.38)
Race/ethnicity				
Non-Hispanic white	1.82 (1.43-2.32)	1.77 (1.39-2.25)	1.95 (1.40-2.72)	2.48 (1.40-4.40)
Other	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
EBV serostatus				
Positive	0.32 (0.24-0.41)	0.79 (0.56-1.10)	0.15 (0.11-0.20)	0.33 (0.20-0.53)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
CMV serostatus				
Positive	0.67 (0.51-0.87)	1.01 (0.77-1.34)	0.70 (0.51-0.95)	1.01 (0.63-1.63)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratios are based on a multivariate Cox proportional hazards regression models controlling for gender, age at transplant, race/ethnicity, EBV status, and CMV status.

† Model 1 uses imputed data for EBV and CMV serostatus for recipients initially missing this information (749 PTLD events).

‡ Model 2 is restricted to recipients with complete EBV and CMV serostatus information (282 PTLD events).

Table 5 - Hepatitis viral serologies and associations with early-onset and late-onset PTLD risk for kidney transplant recipients followed during 1999-2007 in the United States (N=156,740)

Characteristic	Recipients, n (%)	Early-onset PTLD HR (95% CI)	Late-onset PTLD HR (95% CI)
HCV serostatus			
Positive	5,453 (3.5%)	0.34 (0.14-0.84)	1.04 (0.57-1.90)
Negative	118,045 (75.3%)	1.0 (ref)	1.0 (ref)
Missing/not done	33,342 (21.2%)	0.83 (0.58-1.17)	1.19 (0.93-1.51)
HBV surface antigen status			
Positive	2,158 (1.4%)	0.71 (0.26-1.89)	0.62 (0.20-1.94)
Negative	137,888 (88.0%)	1.0 (ref)	.1.0 (ref)
Missing/not done	16,694 (10.6%)	0.91 (0.66-1.26)	0.99 (0.69-1.43)
HBV core antibody serostatus			
Positive	9,129 (5.8%)	0.98 (0.64-1.49)	0.53 (0.28-1.00)
Negative	94,561 (60.3%)	1.0 (ref)	1.0 (ref)
Missing/not done	53,050 (33.9%)	1.11 (0.88-1.41)	1.10 (0.89-1.36)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, HCV hepatitis C virus, HBV hepatitis B virus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation.

Hazard ratios are based on univariate Cox proportional hazards regression models.

Table 6 - Impact of EBV serology on early-onset PTLD risk stratified by age at transplantation for kidney transplant recipients followed during 1999-2007 in the United States (N=156,740)

Age at transplant (years)	0-19	20-50	>50
Characteristic	**Early-onset PTLD HR (95% CI)**	**Early-onset PTLD HR (95% CI)**	**Early-onset PTLD HR (95% CI)**
EBV serostatus			
Positive	0.31 (0.18-0.54)	0.27 (0.18-0.42)	0.29 (0.20-0.44)
Negative	1.0 (ref)	1.0 (ref)	1.0 (ref)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus

* Early-onset PTLD is defined as occurring less than 2 years after transplantation

Hazard ratios are based on univariate Cox proportional hazards regression models.

Table 7 - Impact of antibody induction therapy on early-onset PTLD risk stratified by age at transplantation for kidney transplant recipients followed during 1999-2007 in the United States (N=156,740)

Age at transplant (years)	0-19	20-50	>50
Characteristic	**Early-onset PTLD HR (95% CI)**	**Early-onset PTLD HR (95% CI)**	**Early-onset PTLD HR (95% CI)**
Antibody induction			
Yes	0.82 (0.56-1.20)	1.11 (0.77-1.58)	1.17 (0.80-1.72)
No	1.0 (ref)	1.0 (ref)	1.0 (ref)

Notes

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval

* Early-onset PTLD is defined as occurring less than 2 years after transplantation;

Hazard ratios are based on univariate Cox proportional hazards regression models.

Appendix 3 - Additional Analyses for Hodgkin Lymphoma among U.S. Solid Organ Transplant Recipients

Narrative

Overall, HL risk was doubled in solid organ transplant recipients compared to the general population (SIR 2.2, 95%CI 1.7-2.7). The excess risk compared to the general population was greatest in children (SIRs 93.7 and 11.1 for ages 0-9 and 10-19 years at transplant, respectively) and recipients who were EBV seronegative at transplant (SIR 4.7, 95% CI 1.9-9.6). Non-Hispanic white transplant recipients experienced especially high HL risk compared to the general population (SIR 2.3, 95% CI 1.7-2.9). Heart and/or lung transplant recipients experienced especially high HL risk compared to the general population (SIR 3.2, 95% CI 1.9-5.0), whereas the excess risk associated with kidney and/or pancreas or liver transplantation was more modest (SIR 1.8 and 2.3, respectively). Transplants that occurred in 1987-1995 resulted in especially high HL risk compared to the general population (SIR 3.8, 95% CI 2.5-5.4), while the excess risk associated with transplantation was more modest in more recent transplants. While the risk was not elevated in the first two years post-transplant (SIR 0.6, 95% CI 0.2-1.2), by 8-10 years post-transplant HL risk was increased 4-fold compared with the general population (SIR 4.1, 95% CI 2.2-7.0).

Significant univariate HL risk factors were included in a multivariate model and the results are presented in Table 2. Male gender (HR=2.2) and young age at transplantation (HR=3.8, compared to age 20-50 years) remained significant HL risk factors. EBV seronegativity was no longer a significant HL risk factor, however, this could be a –

reflection of the small number of HL cases who were EBV seronegative at the time of transplantation.

Table 1 - Observed and expected numbers of Hodgkin lymphoma cases and standardized incidence ratios among U.S. transplant recipients during 1997-2007

	Observed	Expected	SIR (95% CI)	Observed Incidence (events/100,000 py)
Overall	72	33.5	2.2 (1.7-2.7)	7.2
Gender				
Male	55	23.2	2.4 (1.8-3.1)	9.0
Female	17	10.3	1.6 (1.0-2.6)	4.4
Age at transplant, years				
0-9	8	0.0854	93.7 (40.4-184.6)	25.3
10-19	9	0.81	11.1 (5.1-21.0)	24.0
20-29	9	3	3.0 (1.4-5.7)	15.1
30-39	7	5.1	1.4 (0.6-2.8)	5.5
40-49	11	7	1.6 (0.8-2.8)	5.0
50-59	12	7.9	1.5 (0.8-2.6)	4.3
60-69	12	7.5	1.6 (0.8-2.8)	6.0
70+	4	2.2	1.8 (0.5-4.6)	8.3
Race/ethnicity				
Non-Hispanic white	57	25.1	2.3 (1.7-2.9)	7.9
Non-Hispanic black	9	5.4	1.7 (0.8-3.2)	5.3
Hispanic	6	3.1	1.9 (0.7-4.2)	5.4
Organ type				
Heart and/or lung	18	5.7	3.2 (1.9-5.0)	10.9
Kidney and/or pancreas	39	21.2	1.8 (1.3-2.5)	6.3
Liver	15	6.5	2.3 (1.3-3.8)	7.2
Other	0	0.15	0.0 (0.0-24.6)	0.0
Year of Transplant				
1987-1995	30	8	3.8 (2.5-5.4)	12.5
1996-2002	39	20.3	1.9 (1.4-2.6)	6.4
2003-2007	3	5.2	0.6 (0.1-1.7)	1.9
EBV Status				
Positive	10	8	1.2 (0.6-2.3)	4.2
Negative	7	1.5	4.7 (1.9-9.6)	13.8
Missing/Not Done	55	24	2.3 (1.7-3.0)	7.7
Time since transplant, years				
0-2	6	10.8	0.6 (0.2-1.2)	1.8
2-4	18	8.4	2.1 (1.3-3.4)	7.2
4-6	17	6.4	2.7 (1.5-4.3)	9.0
6-8	18	4.7	3.8 (2.3-6.0)	12.9
8-10	13	3.2	4.1 (2.2-7.0)	13.8

Notes

Abbreviations: SIR standardized incidence ratio, CI confidence interval, EBV Epstein-Barr virus, py person-years

* Expected numbers of Hodgkin lymphoma cases are based on Surveillance, Epidemiology, and End Results (SEER 13 Registries Limited-Use, Nov 2008 Sub (1992-2006) Katrina/Rita Population Adjustment) incidence rates applied to the total person-years of follow-up, stratified by cohort distributions of gender, race/ethnicity, age, and calendar year of follow-up.

Table 2 – Univariate and multivariate analysis of risk factors for Hodgkin lymphoma among U.S. transplant recipients during 1997-2007

	Hodgkin lymphoma cases, n	Univariate Hazard ratio (95% CI)	Multivariate Hazard ratio* (95% CI)
Gender			
Male	56	2.1 (1.2-3.7)	2.2 (1.3-3.9)
Female	17	1.0 (ref)	1.0 (ref)
Age at transplant, years			
0-19	21	4.0 (2.3-6.8)	3.8 (2.1-6.6)
20-50	32	1.0 (ref)	1.0 (ref)
>50	20	0.9 (0.5-1.5)	0.8 (0.5-1.5)
EBV serostatus at transplant			
Positive	10	1.0 (ref)	1.0 (ref)
Negative	7	3.1 (1.2-8.1)	2.1 (0.8-5.6)
Missing/unknown	56	1.0 (0.5-2.1)	1.0 (0.5-2.0)

Notes

Abbreviations: CI confidence interval, EBV Epstein-Barr virus

* Hazard ratios are based on a multivariate Cox proportional hazards regression model controlling for gender, age at transplant, and EBV status.

Appendix 4 - Additional Considerations in Response to Committee Comments

Use of Medicare Data

In our first study we utilized the SEER-Medicare linked dataset to examine the association between solid organ transplantation and specific subtypes of hematologic malignancy (HM). We identified incident cases of HM reported to SEER registries and cancer-free controls living in SEER areas. We then used Medicare claims data to determine the history of transplantation for all cases and controls. The use of Medicare claims data for epidemiologic research needs to be approached with some caution. These data are not designed for research purposes but instead to ensure payment for medical services. The accuracy of claims indicating a history or complication of prior transplantation is difficult to establish. As a result, there was likely some misclassification of transplant history, but any misclassification was likely to be non-differential and would bias any observed associations towards the null. The claims for the transplant procedure were assumed to be highly accurate since the complexity and expense of transplantation makes it unlikely to be classified incorrectly. Although based on a small number of claims, we observed the associations between solid organ transplantation and common HM subtypes to be strongest when a claim for the transplant procedure was present.

Medicare coverage is predominantly restricted to persons aged 65 years and older. Less than 12% of annual transplants occur in persons over the age of 65 years, therefore, Medicare does not provide coverage for the majority of U.S. transplants. Regardless of age, Medicare does provide coverage for any kidney transplants that occur due to end stage renal disease (ESRD), or any transplants that occur in disabled persons. However, if the patient had concurrent private insurance coverage at the time of transplantation it is

unlikely that a claim for the procedure would be submitted to Medicare. These limitations likely led us to miss some transplant procedures. By including history and complication claims in our exposure definition we likely increased our sensitivity to capture transplant history, but any misclassification of transplant history was likely be non-differential and would have biased any observed associations towards the null. This limitation may explain why the associations we observed were generally weaker than has been reported previously.

Although more than 95% of the elderly population is covered by Medicare, some studies have identified differences between the Medicare population and the overall elderly population in the United States.[110] The Medicare population tends to be slightly older than the overall elderly population as Medicare coverage increases with increasing age. Medicare coverage tends to more complete for females than males, and non-Hispanic white race/ethnicity compared to other races. For example, approximately 92% of the overall population aged 65-70 years is covered by Medicare. The proportion is smaller for races/ethnicities other than non-Hispanic white, with less than 80% of non-Hispanic blacks covered by Medicare at age 65-70 years.[110] These differences could have impacted the generalizability of our results to the elderly population in the United States, particularly for minority groups, but since the great majority of elderly people regardless of age or race have coverage, it is unlikely to have had a large effect.

Outcome Misclassification

In our first study we identified incident cases of HM using SEER registry data. The data that are included in SEER are known to be highly accurate and reliable.[68] We could not retrieve tumor tissue to confirm the assignment of HM subtype so it is possible that some

of the subtypes were classified incorrectly, but this is unlikely to have been a large artifact.

In our second two studies we used data from the Scientific Registry of Transplant Recipients (SRTR) to establish post-transplant lymphoproliferative disorder (PTLD) and Hodgkin lymphoma (HL) outcomes. For the analyses that used SRTR data, it is possible that some of the outcomes were misclassified. The malignancy information is reported by transplant centers, and some centers may be better equipped than others to accurately gather and report this information. This could have resulted, for example, in cases of post-transplant HL being mistakenly reported as NHL. Unfortunately, we could not retrieve tumor tissue from reported cases of HL or other PTLD to perform additional pathological review. We did review the limited data provided by the transplant centers for each case of HL or other PTLD to ensure that it was classified correctly. However, this approach did not ensure that all HLs or PTLDs were classified correctly. It is possible that some cases of PTLD and HL were actually not PTLD or HL and could have been some other form of cancer. This misclassification is likely to be non-differential across the different covariates that were examined and therefore would have biased any observed associations towards the null.

Exposure Misclassification

In our first study the use of Medicare claims data to establish exposure to solid organ transplantation likely led to non-differential misclassification between cases and controls, as discussed previously. In our second two studies we used data from SRTR to establish transplant recipient demographics and exposure to different immunosuppressive medications and viruses, as well as factors related to the transplant itself such as the

degree of human leukocyte antigen (HLA) mismatch and the medical indication for transplantation. These data are recorded by transplant centers prior to transplantation. The accuracy of these data is difficult to establish and it is possible that some misclassification could have occurred. It is unlikely that the degree of misclassification varied between recipients that developed PTLD and those that did not develop PTLD since the information was recorded prior to the transplantation. We chose to use baseline data only since we felt that these data were likely to be more accurate than information recorded during follow-up. The data at baseline are collected very carefully as the candidate is being prepared for transplantation and it is therefore important to ensure the health and eligibility of the candidate. During follow-up, collection of data is not as consistent and transplant centers may be more focused on providing medical care than recording information. There have not been any studies to confirm the accuracy of data recorded by transplant centers prior to transplantation, but due to the importance of such factors as age, race, gender, and HLA mismatch in establishing the appropriate organ donor for a given transplant recipient it is unlikely that the information would be recorded incorrectly. Any misclassification of these different variables is likely to be non-differential between PTLD cases and non-cases and would have biased any observed associations towards the null.

One of the important limitations of our two studies was the large amount of missing data on Epstein-Barr virus (EBV) and cytomegalovirus (CMV) serology. Different transplant centers have different protocols regarding the measurement of viral serology prior to transplantation, and therefore the information is not collected uniformly. The missing data could have contributed to bias if the data were not missing at random, i.e., if the

likelihood of missing data was higher for EBV positive than EBV negative serology conditional on the available predictors. There was no way to formally test if the data were missing at random, but we did find that the best predictor of missing EBV was the year of transplantation, as the likelihood of missing EBV data decreased with increasing transplant year. We did conduct a multiple imputation for EBV serology, but due to the large amount of missing data the results of the imputation need to be interpreted with some caution. However, our imputed EBV results had a similar distribution by age to another population of transplant recipients which indicates that our imputation was likely accurate.[34] When using our imputed data we found that EBV seronegativity at the time of transplantation was a strong risk factor for early-onset PTLD risk, which is consistent with previous research. In addition, when we restricted our analyses to transplant recipients with complete EBV and CMV data we found that our results were very similar to the results obtained when using imputed data.

Confounding vs. Mediation

When examining the role of a third variable on the association between an independent variable and dependant variable, it is often not possible to know if the third variable serves as a confounding variable or a mediating variable. A mediating variable exists in the causal pathway between the independent variable and dependent variable. The mediating variable is associated with the dependent variable, but is also caused to vary by the independent variable and is therefore also associated with the independent variable. A confounding variable lies outside the causal pathway between the independent variable and dependent variable, but is associated with both the independent and dependent

variables and therefore may explain any observed associations between the independent and dependent variables.

An example of the difficulty in distinguishing between confounding and mediation is observed when examining the impact of young age at transplantation on early-onset PTLD risk before and after controlling for EBV serostatus. When examined in a univariate model, young age at transplantation was a very strong risk factor for early-onset PTLD (HR~6). When EBV serostatus at the time of transplantation is included in the model the effect of young age on early-onset PTLD risk is significantly attenuated. However, it is not possible to know whether EBV serostatus is a confounder or a mediating variable in the relationship between young age and early-onset PTLD risk based purely on the attenuation of the age effect.

There are typically three criteria used to establish mediation; the first is that the independent variable must be significantly associated with the dependent variable; the second is that the independent variable must be associated with the mediating variable; and last is the mediating variable must be significantly associated with the dependent variable when included in a model that includes the independent variable.[111] While these three criteria are necessary for a variable to be considered a mediating variable, they are not sufficient. To establish whether a variable is a mediating variable requires an understanding of the causal pathway between the independent and dependent variables and whether the mediating variable acts within this causal pathway. The criteria for mediation are met for EBV serostatus in the example above, since young age is a significant predictor of early-onset PTLD risk, young age is associated with EBV seronegativity, and EBV serostatus remained a significant predictor of early-onset PTLD

risk when included in a multivariate model that also included age. One proposed causal mechanism that links young age at transplantation and early-onset PTLD risk is that young transplant recipients are more likely to be EBV seronegative and therefore at risk of primary EBV infection at the time of transplantation. EBV primary infection then leads to the development of PTLD in the period immediately following transplantation. In this scenario, EBV seronegativity explains the increased risk of PTLD in young transplant recipients and could therefore be considered a mediating variable as it lies within the proposed causal pathway between young age and early-onset PTLD risk. While this scenario is consistent with the well documented causal effects of EBV in PTLD, there are other possible causal pathways that could link young age at transplantation and early-onset PTLD risk that are independent of EBV serostatus and therefore EBV would not be considered a mediating variable. For example, young transplant recipients typically have more lymphoid tissue than older transplant recipients, and young transplant recipients are generally more severely immunosuppressed than older transplant recipients. In these cases, EBV would be considered a confounder since it lies outside the causal pathway, but is associated with both young age and early-onset PTLD risk.

In summary, it is not possible to confirm whether a given variable is a confounder or a mediating variable using statistical analysis techniques alone. Distinguishing between confounding and mediation requires an understanding of the causal pathway that links the independent and dependent variables and then being able to determine if the third variable lies within or outside of the proposed pathway. As the exact causal pathway that

links a dependent and independent variable is often not known, it is therefore important to keep both possibilities in mind

Type 1 Error

In our first study we did not formally adjust for multiple comparisons and some of the observed associations between solid organ transplantation and HM could have been due to chance. Nonetheless, the associations between transplantation and DLBCL, T-cell lymphoma, and myeloid neoplasms remained significant even after applying a Bonferroni correction for ~25 comparisons (p-values <0.002). The associations that remained significant after applying the Bonferroni correction are summarized in Table 1.

In our second study, we tested a large number of covariates to assess their impact on both early-onset and late-onset PTLD risk. Due to the large number of covariates that were examined it is possible that some of the observed associations were due to chance alone. When we applied a Bonferroni correction for ~ 40 comparisons (p-value <0.0125) we observed that some of the characteristics associated with early-onset or late-onset PTLD risk were no longer significant (Table 2). We did find that young age and EBV seronegativity at the time of transplantation and malignant neoplasm leading to kidney transplant remained strong risk factors for early-onset PTLD risk. Older age at the time of transplantation was no longer a significant risk factor for late-onset PTLD risk. These findings indicate that young age and EBV seronegativity at the time of transplantation are very strong risk factors for early-onset PTLD risk and likely represent true associations. When we applied the Bonferroni correction to our Hodgkin lymphoma analyses (~20 comparisons, p-value<0.0033) we found that young age at the time of transplantation remained the only significant risk factor for Hodgkin lymphoma (Table 3). This finding

makes it very unlikely that observed association between young age at transplantation and Hodgkin lymphoma is due to chance alone.

Type II Error

In our first study we had a large number of cases of HM, but due to the rarity of solid organ transplantation we likely had limited statistical power to detect associations for rarer HM subtypes. We estimated the statistical power to detect associations of different strengths, assuming a type I error rate of 0.05 and an exposure prevalence among controls of 0.12%, using all controls (N=166,057) and the indicated number of cases (Table 4). For the more common subtypes of HM we had high statistical power to detect even moderate associations with solid organ transplantation. However, for less common HM subtypes such as mantle cell lymphoma and chronic myeloid leukemia we only had statistical power to detect strong associations with an odds ratio of 5 or more. For very rare subtypes of HM, such as Burkitt lymphoma and precursor B-cell lymphoma we had extremely limited power to detect even strong associations with odds ratios of 5 or greater. This limitation may be one explanation for why we did not observe an association between solid organ transplantation and less common HM subtypes.

In our examination of risk factors for early-onset and late-onset PTLD we had a large number of kidney transplant recipients. However, PTLD was a rare outcome and this may have limited our statistical power to detect significant associations between the different covariates and PTLD risk. We estimated the power to detect associations of different strengths for early-onset PTLD assuming 120,000 kidney transplant recipients, an average follow-up of 1.4 years, and cumulative incidence of PTLD at 2 years of 0.4%

(Table 5a). We had good statistical power to detect moderate associations with PTLD risk for exposures with a prevalence ranging from 0.33 to 0.50. For rarer exposures, we had statistical power to detect strong associations only. For late onset PTLD (Table 5b), we estimated statistical power by assuming 114,000 kidney transplant recipients with follow-up in this period, an average follow-up of 3.1 years, and cumulative incidence of PTLD at 10 years of 1.0%. Compared to early-onset PTLD, we had greater statistical power to detect moderate associations for rare exposures. For rare exposures, such as exposure to anti-rejection medications (prevalence 8.3% overall), we may not have had sufficient statistical power to detect anything except for very strong associations, which may be one explanation why no association was observed between anti-rejection medications and PTLD risk. We did not observe significant associations between the use of antibody induction medications and PTLD risk, but due to the high prevalence of antibody induction use in our cohort (54.5% overall), it is unlikely that the lack of an association can be explained by limitations in statistical power. Overall, due to the large number of transplant recipients in our cohort it is unlikely that statistical power limitations can explain the lack of association observed between such factors as HLA mismatch and steroid maintenance therapy and early-onset PTLD risk.

For Hodgkin lymphoma, we estimated statistical power by assuming 290,000 transplant recipients with an average follow-up of 3.7 years and a cumulative incidence of 0.08% at 10 years post-transplant (Table 6). Due the rarity of Hodgkin lymphoma after transplantation we had very limited statistical power to detect anything other than very strong associations. This may explain why we only observed significant associations between Hodgkin lymphoma and young age and EBV seronegativity at transplantation.

Incidence Data

The incidence of PTLD by time since transplantation, age at transplantation, and baseline EBV serostatus is presented in Figure 1. The incidence of PTLD immediately following transplantation increases rapidly but then decreases after approximately 1 year after transplantation. The incidence immediately following transplantation is higher in younger transplant recipients (<40 years of age at transplantation) than older transplant recipients (40 years and older), but the incidence does not vary greatly by age one year or more after transplantation (Figure 1A). Although based on a small number of recipients with known EBV serostatus at baseline, the incidence of early-onset PTLD was especially high in EBV seronegative transplant recipients (Figure 1B). Due to the small number of Hodgkin lymphoma cases the pattern of incidence post-transplant is difficult to interpret (Figure 2), but the incidence generally increases with time since transplantation.

The Breslow estimates of cumulative incidence of PTLD by time since transplantation, and age at transplantation are presented in Figure 3A. The cumulative incidence of PTLD increases more rapidly in younger transplant recipients compared to older transplant recipients, driven by the high incidence in the period immediately following transplantation. Approaching 10 years post-transplantation the cumulative incidence of PTLD is still slightly higher in younger transplant recipients, but the cumulative incidence is less than 2% in both age groups. The Breslow estimates of cumulative incidence of PTLD by time since transplantation and EBV serostatus are presented in Figure 3B. Although based on small numbers of recipients with known EBV serostatus, the cumulative incidence of PTLD is noticeably higher in EBV seronegative transplant recipients compared to EBV seropositive recipients, again driven by the high incidence in

the early-onset period. The cumulative incidence of PTLD in EBV seronegative transplant recipients approaches 3% at 10 years post-transplantation. Overall, PTLD was a rare outcome in our cohort and affected less than 2% of kidney transplant recipients. This finding is consistent with previous studies which indicated that transplant recipients are at increased risk for lymphoproliferative disorders compared to the general population, but the absolute risk is still small.

The Breslow estimate of cumulative incidence of Hodgkin lymphoma by time since transplantation is presented in Figure 4. The incidence of Hodgkin lymphoma is noticeably lower than PTLD, with cumulative incidence less than 0.1% at 10 years post-transplantation.

Table 1. Associations of hematologic malignancies with solid organ transplantation after applying Bonferroni correction (p <0.002).

	N	Transplant history (%)	OR (95%CI)*	P-value
Controls	166,057	204 (0.12)	Reference	Reference
Lymphoid neoplasm	65,897	169 (0.26)	2.17 (1.75-2.70)	<0.0001
NHL (overall)	45,824	115 (0.25)	2.13 (1.67-2.72)	<0.0001
DLBCL	13,330	52 (0.39)	3.29 (2.28-4.76)	<0.0001
T-cell lymphoma	2,362	<11 (<1)	3.07 (1.56-6.06)	0.001
Lymphoid neoplasm, NOS	4,381	17 (0.39)	3.72 (2.14-6.48)	<0.0001
Myeloid neoplasm	15,116	41 (0.27)	1.99 (1.41-2.81)	<0.0001
Myelodysplastic syndrome	3,366	15 (0.45)	2.75 (1.54-4.88)	0.0006

Notes

Abbreviations: OR odds ratio, CI confidence interval, NOS not otherwise specified, NHL non-Hodgkin lymphoma,

DLBCL diffuse large B-cell lymphoma

Observations in which the number of exposed cancer cases was between 1 and 10 are reported as <11, to preserve subject confidentiality in accordance with the SEER-Medicare data use agreement.

Significant associations ($p < 0.05$) are underlined.

* Odds ratios were adjusted for age in five strata (66-69, 70-74, 75-79, 80-84, and 85-99 years), sex, race (white, non-white), and calendar year of diagnosis/selection (1987-1990, 1991-1994, 1995-1998, and 1999-2002).

Table 2– PTLD risk factors among U.S. kidney transplant recipients during 1999-2007

Characteristic	Early-onset PTLD HR (95% CI)*	Late-onset PTLD HR (95% CI)*
Gender		
Male	1.00 (0.81-1.24)	1.23 (1.01-1.51)
Female	1.0 (ref)	1.0 (ref)
Age at transplant, years		
0-19	**6.59 (5.12-8.48)**	**2.98 (2.26-3.92)**
20-50	1.0 (ref)	1.0 (ref)
>50	1.04 (0.81-1.33)	1.29 (1.04-1.61)
Race/ethnicity		
Non-Hispanic white	**2.09 (1.66-2.64)**	**1.76 (1.41-2.21)**
Other	1.0 (ref)	1.0 (ref)
EBV serostatus		
Positive	**0.21 (0.17-0.27)**	0.66 (0.48-0.89)
Negative	1.0 (ref)	1.0 (ref)
CMV serostatus		
Positive	**0.41 (0.32-0.53)**	0.80 (0.62-1.03)
Negative	1.0 (ref)	1.0 (ref)
Antibody induction		
Yes	1.08 (0.87-1.34)	1.23 (1.00-1.50)
No	1.0 (ref)	1.0 (ref)
Steroid maintenance		
Yes	1.33 (0.96-1.84)	0.64 (0.44-0.95)
No	1.0 (ref)	1.0 (ref)
Anti-rejection therapy		
Yes	0.68 (0.40-1.14)	1.15 (0.84-1.57)
No	1.0 (ref)	1.0 (ref)
HLA mismatch, number of alleles		
0-2	1.0 (ref)	1.0 (ref)
3-4	1.12 (0.88-1.44)	1.02 (0.82-1.28)
5-6	0.85 (0.64-1.13)	1.02 (0.78-1.34)
Reason for transplant		
Hypertensive nephrosclerosis	1.0 (ref)	1.0 (ref)
Glomerular disease	**2.32 (1.57-3.44)**	1.35 (0.96-1.91)
Diabetes	1.29 (0.80-2.08)	1.17 (0.80-1.72)
Polycystic kidney	1.36 (0.81-2.30)	1.06 (0.68-1.66)
Tubular/interstitial disease	**3.38 (2.06-5.53)**	2.00 (1.30-3.08)
Vascular disease	1.50 (0.79-2.86)	1.02 (0.55-1.89)
Congenital, rare familial, or metabolic disorder	**7.01 (4.42-11.14)**	**2.93 (1.85-4.65)**
Malignant neoplasm	**8.74 (3.65-20.90)**	0.99 (0.14-7.21)
Other	2.00 (1.30-3.08)	1.31 (0.85-2.00)

Abbreviations: PTLD post-transplant lymphoproliferative disorder, HR hazard ratio, CI confidence interval, EBV Epstein-Barr virus, CMV cytomegalovirus, HLA human leukocyte antigen

* Early-onset PTLD is defined as occurring less than 2 years after transplantation; late-onset PTLD is defined as occurring 2 or more years after transplantation. Hazard ratio is based on a univariate proportional hazards regression model.

Highlighted associations remained significant at the 0.00125 level.

Table 3 – Hodgkin lymphoma risk factors among U.S. transplant recipients during 1997-2007

	Recipients, n (%)	Hodgkin lymphoma cases, n	Hazard ratio (95% CI)
Total	283,190 (100%)	73	--
Gender			
Male	174,194 (61.5%)	56	2.1 (1.2-3.7)
Female	108,996 (38.5%)	17	1.0 (ref)
Age at transplant, years			
0-19	22,870 (8.1%)	21	**4.0 (2.3-6.8)**
20-50	139,430 (49.2%)	32	1.0 (ref)
>50	120,890 (42.7%)	20	0.9 (0.5-1.5)
Median age	48.0		
Race/ethnicity			
White, non-Hispanic	188,648 (66.6%)	57	1.5 (0.9-2.6)
Other	94,542 (33.4%)	16	1.0 (ref)
EBV serostatus at transplant			
Positive	87,291 (30.8%)	10	1.0 (ref)
Negative	17,612 (6.2%)	7	3.1 (1.2-8.1)
Missing/unknown	178,287 (63.0%)	56	1.0 (0.5-2.1)
CMV serostatus at transplant			
Positive	93,726 (33.1%)	9	1.0 (ref)
Negative	57,213 (20.2%)	9	1.6 (0.6-4.0)
Missing/unknown	132,251 (46.7%)	55	1.6 (0.8-3.5)
Type of organ transplanted			
Kidney and/or pancreas	180,449 (63.7%)	40	1.0 (ref)
Liver	58,235 (20.6%)	15	1.1 (0.6-2.0)
Heart and/or lung	42,447 (15.0%)	18	1.6 (0.9-2.9)
Other	2,059 (0.7%)	0	0
HLA mismatch, number of alleles*			
0-2	54,948 (22.4%)	17	1.0 (ref)
3-4	103,708 (42.2%)	28	0.9 (0.5-1.7)
5-6	87,106 (35.4%)	19	0.8 (0.4-1.6)
Antibody induction†			
Yes	119,505 (43.1%)	25	1.0 (0.6-1.7)
No	157,549 (56.9%)	46	1.0 (ref)
Steroid maintenance†			
Yes	246,799 (89.1%)	67	1.0 (0.4-2.8)
No	30,255 (10.9%)	4	1.0 (ref)
Anti-rejection therapy†			
Yes	34,628 (12.5%)	13	1.2 (0.7-2.2)
No	242,426 (87.5%)	58	1.0 (ref)

Abbreviations: CI confidence interval, HLA human leukocyte antigen, EBV Epstein-Barr virus, CMV cytomegalovirus.

* HLA information was missing for 37,428 (13.2%) transplant recipients.

† Medication information was missing for 6,136 (2.2%) transplant recipients.

Highlighted associations remained significant at the 0.003 level.

Table 4. Power for Detecting an Association with Solid Organ Transplantation by Hematologic Malignancy Subtype

		Odds Ratio		
	N	**1.5**	**2.5**	**5.0**
Controls	166,057			
Lymphoid neoplasm	65,897	0.92	1.00	1.00
NHL (overall)	45,824	0.85	0.99	0.99
DLBCL	13,330	0.48	0.99	0.99
Burkitt lymphoma	221	0.12	0.27	0.55
Marginal zone lymphoma	1,989	0.18	0.58	0.97
Follicular lymphoma	6,142	0.30	0.90	0.99
Chronic lymphocytic leukemia†	13,124	0.47	0.99	0.99
Lymphoplasmacytic lymphoma	1,434	0.16	0.50	0.93
B-cell NHL, NOS	2,013	0.18	0.59	0.97
T-cell lymphoma	2,362	0.19	0.63	0.98
Unknown lineage NHL	3,330	0.22	0.73	0.99
Mantle cell lymphoma	1,171	0.15	0.46	0.89
Precursor B-cell lymphoma	267	0.12	0.28	0.58
Hairy cell leukemia/lymphoma	441	0.12	0.32	0.68
Plasma cell neoplasm‡	14,000	0.49	0.99	1.00
Hodgkin lymphoma	1,692	0.17	0.54	0.95
Lymphoid neoplasm, NOS	4,381	0.25	0.81	1.00
Myeloid neoplasm	15,116	0.51	1.00	1.00
Acute myeloid leukemia	8,055	0.35	0.95	1.00
Chronic myeloid leukemia	2,250	0.18	0.62	0.98
Myelodysplastic syndrome	3,366	0.22	0.74	1.00
Chronic myeloproliferative disorder	1,099	0.15	0.44	0.88
Myeloid leukemia, NOS	346	0.12	0.30	0.63
Hematologic malignancy, NOS	2,003	0.18	0.59	0.97

Notes

Abbreviations: NOS not otherwise specified, NHL non-Hodgkin lymphoma, DLBCL diffuse large B-cell lymphoma

Power calculations assumed a two-sided alpha of 0.05, exposure prevalence among controls of 0.12%, all controls (N=166,057), and the indicated number of cases.

† This category also includes small lymphocytic lymphoma.

‡ This category includes multiple myeloma (n=13,291), plasmacytoma (n=647), and plasma cell leukemia (n=62)

Table 5a. Power to detect early-onset PTLD risk by the proportion exposed to a risk factor and expected hazard ratio compared to unexposed.

	Hazard Ratio				
Proportion exposed	**1.25**	**1.50**	**2.00**	**2.50**	**5.00**
0.50	0.48	0.91	1.00	1.00	1.00
0.33	0.44	0.88	1.00	1.00	1.00
0.25	0.38	0.82	0.99	1.00	1.00
0.10	0.21	0.51	0.87	0.97	0.999

Notes

Assumed 120,000 transplant recipients, an average follow-up time of 1.4 years, cumulative incidence of PTLD at 2 years of 0.4%, and a two-sided alpha of 0.05.

Table 5b. Power to detect late-onset PTLD risk by the proportion exposed to a risk factor and expected hazard ratio compared to unexposed.

	Hazard Ratio				
Proportion exposed	**1.25**	**1.50**	**2.00**	**2.50**	**5.00**
0.50	0.51	0.93	1.00	1.00	1.00
0.33	0.46	0.89	1.00	1.00	1.00
0.25	0.40	0.84	1.00	1.00	1.00
0.10	0.24	0.58	0.92	0.98	1.00

Notes

Assumed 114,000 transplant recipients, an average follow-up time of 3.1 years, cumulative incidence of PTLD at 10 years of 1.0%, and a two-side alpha of 0.05.

Table 6. Power to detect Hodgkin Lymphoma risk by the proportion exposed to a risk factor and expected hazard ratio compared to unexposed.

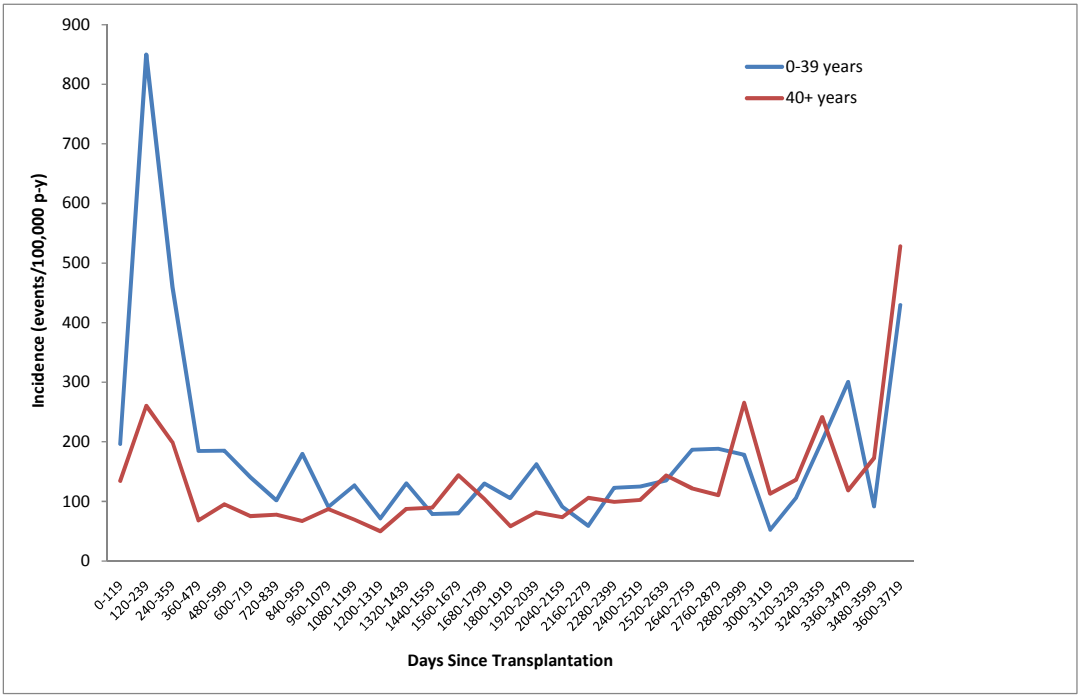
	Hazard Ratio			
Proportion	**1.50**	**2.00**	**2.50**	**5.00**
0.50	0.41	0.77	0.91	0.99
0.33	0.37	0.72	0.88	0.99
0.25	0.32	0.65	0.82	0.97
0.10	0.18	0.37	0.51	0.76

Notes

Assumed 290,000 transplant recipients, an average follow-up time of 3.5 years, cumulative incidence of Hodgkin lymphoma at 10 years post-transplant of 0.08%, and a two-sided alpha of 0.05.

Figure 1. PTLD incidence by time since transplantation.

A. Age at transplantation.



B. Epstein-Barr virus (EBV) serology.

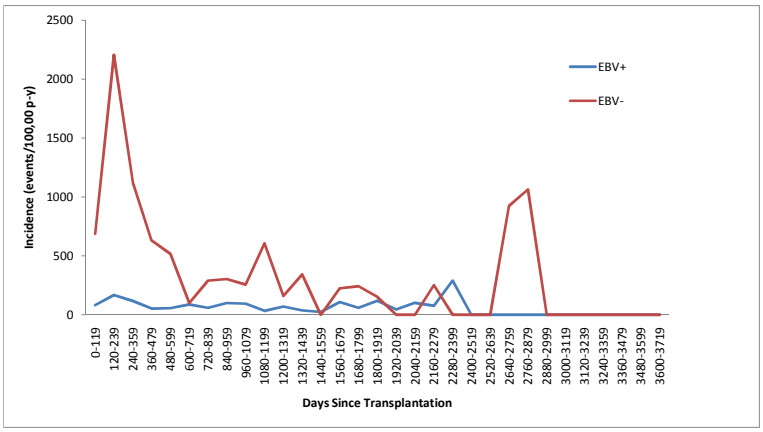


Figure 2. Hodgkin lymphoma incidence by time since transplantation.

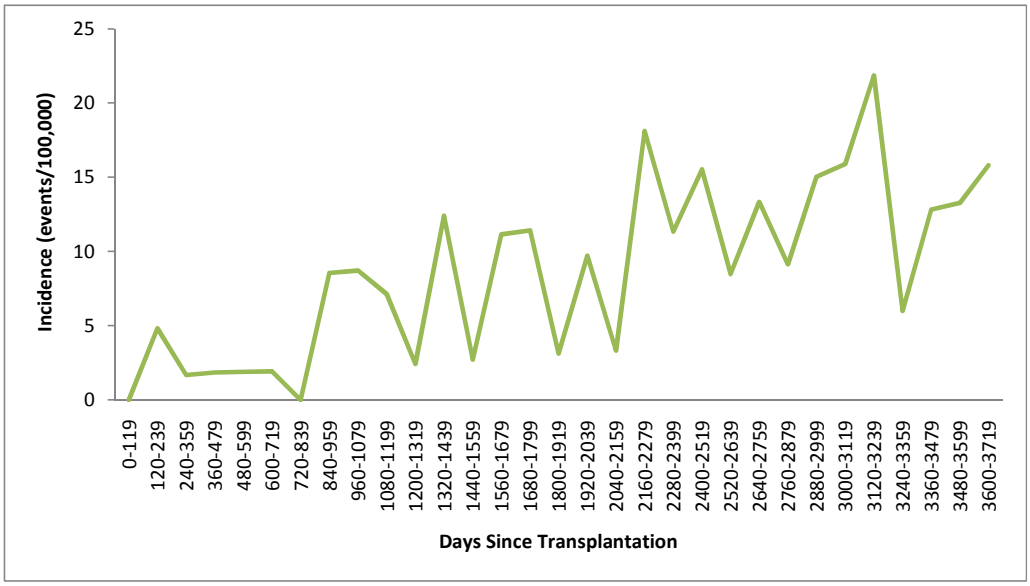
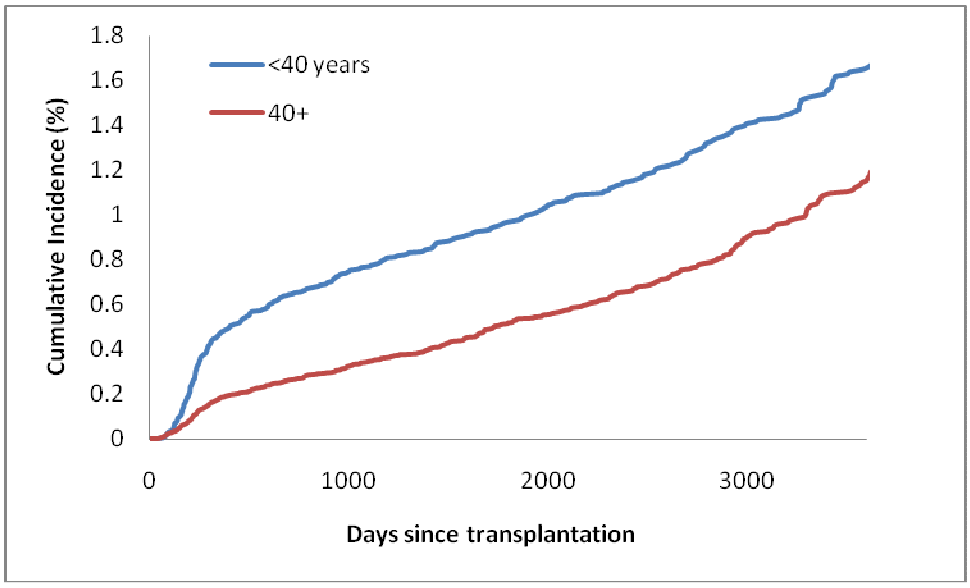


Figure 3. Cumulative incidence of PTLD by time since transplantation

A. Age at transplantation.



B. EBV serostatus

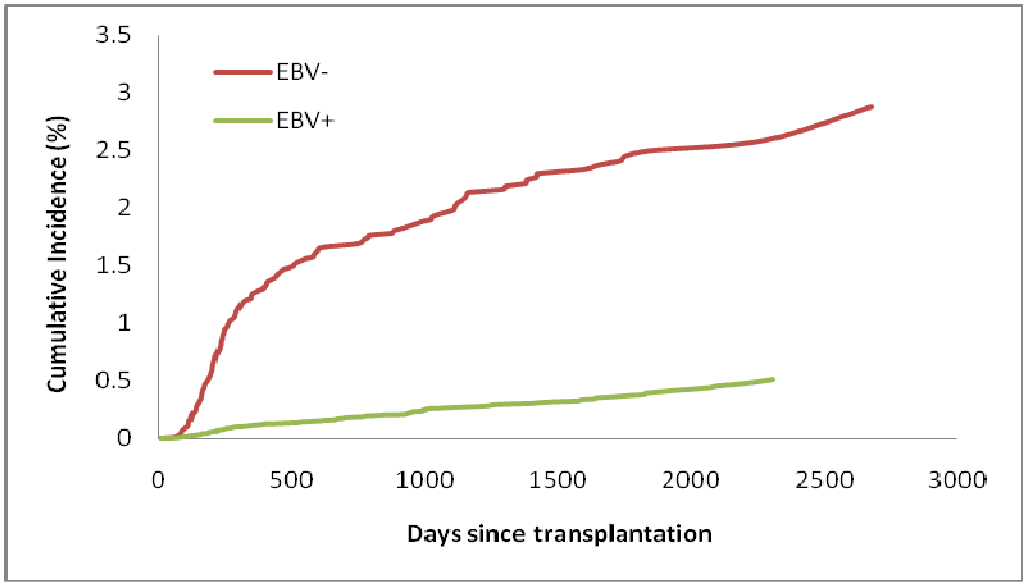


Figure 4. Cumulative incidence of Hodgkin lymphoma by time since transplantation.