How Risk of Kidney Transplant Rejection May be Mitigated by Differential Exposure to Mycophenolate?

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List of Abbreviations

ABMR: Antibody-Mediated Rejection

AbVer: Antibody-Verified

AcMPAG: Acyl MPA Glucuronide

APC: Antigen-Presenting Cell

$AUC_{0\text{-}12}$: Area Under the Curve 0 to 12 hours

BMI: Body Mass Index

CES1/CES2: Carboxylesterase Enzymes 1/2

CI: Confidence Interval

CMV: Cytomegalovirus

CNI: Calcineurin Inhibitor

CYP: Cytochrome

DCD: Donation after Circulatory Death

DnDSA: De Novo Donor-Specific Antibodies

DNA: Deoxyribonucleic Acid

DSA: Donor-Specific Antibodies

ECD: Expanded Criteria Donor

EC-MPS: Enteric-coated Mycophenolate Sodium

eGFR: Estimated Glomerular Filtration Rate

EMM: Eplet Mismatch

ESKD: End-Stage Kidney Disease

GI: Gastrointestinal

HLA: Human Leukocyte Antigens

HR: Hazard Ratio

IL-6: Interleukin-6

IL-2: Interleukin-2

IMPDH: Inosine 5-Monophosphate Dehydrogenase

IPTW: Inverse Probability of Treatment Weighting

IQR: Inter-Quartile Range

IVIG: Intravenous Immunoglobulin

MFI: Mean Fluorescence Intensity

MG: Milligrams

MHC: Major Histocompatibility Complex

MMF: Mycophenolate Mofetil

MPA: Mycophenolic Acid

MPAG: MPA Glucuronide

MSM: Marginal Structural Models

MUHC: McGill University Health Centre

NC: Negative Control

NDD: Neurological Determination of Death

OR: Odds Ratio

PC: Positive Control

PD: Pharmacodynamics

PLEX: Plasmapheresis

PK: Pharmacokinetics

PPI: Proton-Pump Inhibitor

PRA: Panel Reactive Antibodies

RR: Risk Ratio

SCD: Standard Criteria Donor

SSO: Sequence-Specific Oligonucleotides

T_{max}: Peak Concentration

TacSD: Tacrolimus Standard Deviation

TDM: Therapeutic Drug Monitoring

Tx: Transplant

UGT: Uridine 5'- Diphospho-Glucuronosyltransferase

Abstract (English)

Background/Rationale: Antibody-mediated rejection (ABMR) is the leading cause of kidney transplant loss and is driven by molecular incompatibility in human leukocyte antigens (HLA) between donors and recipients. To address the risk of rejection as a result of HLA incompatibility, recipients are prescribed a maintenance regimen including 2 to 3 immunosuppressive agents (calcineurin inhibitor (e.g., tacrolimus), anti-metabolite (e.g., mycophenolate), steroid (e.g., prednisone)). Modifications based on individual patient characteristics are seldom made to existing regimens but rather, occur due to recipient intolerance, side effects or concurrent comorbidities. Being that the level of immune suppression of kidney transplant recipients is a modifiable risk factor, estimations of the relationship between specific immunosuppressive regimens and the risk of rejection can help inform personalized therapies capable of mitigating this risk. We aimed to determine how ABMR risk associated with molecular HLA incompatibility may be modified by exposure to mycophenolate.

Methods: We conducted a nested case-control study including first-time kidney transplant recipients transplanted from January 1st, 2012, to June 30st, 2019, at the McGill University Health Centre (MUHC). Re-transplant and multi-organ transplant recipients, as well as patients in whom donor and recipient HLA typing could not be ascertained, were excluded. Cases were defined as patients experiencing their first ABMR event. Controls matched for transplant year, time post-transplant and donor source (living vs. deceased donor) were randomly selected from the remaining cohort using incidence density sampling. HLA eplet mismatches (EMMs) were estimated from allele-level donor and recipient HLA-A, -B, -C, DRB1,3,4,5 and DQB1 types. Multivariable conditional logistic regression models were fit to estimate the independent association of EMMs and ABMR lagged by 3-months while adjusting for cumulative

mycophenolate dose modelled as a time-varying exposure, recipient age and sex. Interactions between EMMs, mycophenolate exposure, recipient age and sex in relation to ABMR were also assessed. Sensitivity analyses were conducted to verify the robustness of observations.

Results: A total of 48 eligible ABMR cases were matched with 48 controls. With the implementation of a multivariable conditional logistic regression model considering class II eplets, each additional EMM was associated with an odds ratio (OR) of 1.07 (95% CI: 1.02, 1.13). We observed an OR of 0.55 (95% CI: 0.19, 1.58) to experience ABMR per 1000mg increases in mycophenolate dose during the 7-days preceding the index event minus a 3-month lag. ORs for ABMR of 0.41 (95% CI: 0.15, 1.17) and 0.43 (95% CI: 0.14, 1.33) were observed for female recipients (vs. males) and kidney transplant recipients > 60 years old (vs. ≤ 60 years), respectively. For class II antibody-verified (AbVer) EMMs, an OR of 1.11 (95% CI: 1.01, 1.21) to experience ABMR was observed with each additional EMM. Associations between mycophenolate dose, recipient age, and sex with ABMR did not reach the threshold for statistical significance. Also, we did not observe statistically significant interactions between class II and class II AbVer EMMs, mycophenolate dose, and recipient characteristics (age, sex). Sensitivity analyses modelling mycophenolate exposure as cumulative, 14- and 30-day doses preceding the index date minus 3-months, as well as considering 1-, and 2-month lag periods, generated results consistent with our main analysis.

Conclusion: We observed an independent association between ABMR and class II, as well as class II AbVer EMMs. Exposure to mycophenolate was not found to be an independent predictor of ABMR, nor was it a statistically significant modifier of the effect of ascending EMMs on ABMR risk. Exposure to mycophenolate depends on patient adherence, as well as drug pharmacokinetics and pharmacodynamics, which may vary as a function of patient characteristics,

protein binding, and renal elimination. Larger multicenter studies are required to shed further light on how the risk for ABMR may benefit from personalized immunosuppression regimens including mycophenolate.

Abstract (French)

Contexte/Raisonnement : Le rejet médié par les anticorps (RMA) est la principale cause de perte de greffons rénaux. Ceci est dû à une incompatibilité moléculaire entre les antigènes leucocytaires humains (HLA) du donneur et du receveur. Pour diminuer les risques de rejet résultant d'une incompatibilité HLA, les receveurs se voient prescrire des doses standard de 2 à 3 agents immunosuppresseurs (inhibiteur de la calcineurine (ex. tacrolimus), antimétabolite (ex. mycophenolate), stéroïde (ex. prednisone)). Les modifications médicamenteuses basées sur les caractéristiques individuelles des patients sont rarement apportées aux schémas thérapeutiques existants. Leurs modifications sont plutôt apportées en réponse à l'intolérance du receveur, des effets secondaires et aux comorbidités concomitantes. Étant donné que le niveau de suppression immunitaire des greffés rénaux est un facteur de risque modifiable, les estimations de la relation entre les régimes immunosuppresseurs spécifiques et le risque de rejet peuvent aider à informer les thérapies personnalisées capables d'atténuer ce risque. Nous avons cherché à déterminer comment le risque de RMA associé à l'incompatibilité HLA peut être modifié par l'exposition au mycophénolate.

Méthodes : Nous avons mené une étude cas-témoin nichée dans une cohorte incluant des receveurs d'une première greffe rénale reçut entre le 1er janvier 2012 et le 30 juin 2019 au Centre Universitaire de Santé McGill. Les receveurs d'un second rein, d'organes multiples ainsi que les patients pour lesquels le typage moléculaire HLA complet du donneur et du receveur n'étaient pas disponible ont été exclus. Les cas ont été définis comme des patients ayant reçu leur premier

diagnostic de le RMA. Les témoins appariés pour l'année de transplantation, le temps après transplantation, le centre et l'origine du donneur (donneur vivant par rapport au donneur décède) ont été sélectionnés au hasard dans la cohorte restante à l'aide d'un échantillonnage de densité d'incidence. Les mésappariements d'épitopes entre les HLA du donneur et du receveur ont été calculé à partir des types allèles HLA-A, -B, -C, DRB1,3,4,5 et DQB1. Des modèles de régression logistique conditionnelle multivariés ont été ajustés pour estimer l'association indépendante des mésappariements des épitopes du HLA et de le RMA. Un décalage de 3 mois entre le diagnostic et le traitement a été introduit, tout en ajustant la dose cumulée de mycophénolate modélisée comme une variable d'exposition dépendante du temps, l'âge et le sexe du receveur. Les interactions entre les mésappariements d'épitopes, le mycophénolate, l'âge et le sexe du receveur ont également été évaluées. Des analyses de sensibilité ont été réalisées pour vérifier la robustesse de nos observations.

Résultats : Un total de 48 cas RMA éligibles ont été appariés avec 48 témoins. Selon la mise en œuvre d'un modèle de régression logistique conditionnelle multivariable prenant en compte les mésappariements d'épitopes de classe II, chaque mésappariement supplémentaire était associé à un rapport de cotes (OR) de 1,07 (IC à 95 % : 1,02, 1,13). Nous avons observé un OR de 0,55 (IC à 95 % : 0,19, 1,58) pour faire un rejet par augmentation de chaque 1000 mg de la dose de mycophénolate au cours des 7 jours précédant l'événement index moins un décalage de 3 mois. Des ORs pour le RMA de 0,41 (IC 95 % : 0,15, 1,17) et de 0,43 (IC 95 % : 0,14, 1,33) ont été observés pour les receveurs de sexe féminin (par rapport aux hommes) et les receveurs de greffe rénale âgés de >60 ans (par rapport à ≤ 60 ans), respectivement. Pour les épitopes de classe II vérifiés par des anticorps (AbVer), un OR de 1,11 (IC à 95 % : 1,01, 1,21) par rapport à une épisode de RMA a été observé avec chaque mésappariement d'épitopes supplémentaire. Les associations entre la dose de mycophénolate, l'âge et le sexe du receveur avec le RMA n'ont pas atteint le seuil

de signification statistique. De plus, nous n'avons pas observé d'interactions statistiquement significatives entre les mésappariements d'épitopes de classe II ou bien seulement des épitopes de classe II AbVer, la dose de mycophénolate et les caractéristiques du receveur (âge, sexe). Les analyses de sensibilité modélisant l'exposition au mycophénolate sous forme de doses cumulatives, de doses 14 et 30 jours précédant l'événement moins 3 mois ainsi que la prise en compte de périodes de décalage de 1 et 2 mois, ont généré des résultats cohérents avec notre analyse principale.

Conclusion : Nous avons observé une association indépendante entre le RMA, les mésappariements d'épitopes de classe II ainsi que la classe II AbVer. L'exposition au mycophénolate ne s'est pas avérée être un prédicteur indépendant de le RMA, ni un modificateur statistiquement significatif de l'effet de mésappariement d'épitopes ascendant sur le risque de RMA. L'exposition au mycophénolate dépend de l'adhérence du patient ainsi que de la pharmacocinétique et de la pharmacodynamique du médicament, qui peuvent varier en fonction des caractéristiques du patient, de la liaison aux protéines et de l'élimination rénale. Des études multicentriques sont nécessaires pour mieux comprendre comment le risque de RMA peut bénéficier de régimes d'immunosuppression personnalisés, incluant le mycophénolate.

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Contributions of Authors

Alexia Della Vecchia, MSc. Candidate, was responsible for protocol development, receiving research ethics board approval, data extraction, validation of cases and prescription data, data management, exploring statistical models, statistical analyses, and interpretation of study findings. All tasks were performed under the supervision of Dr. Ruth Sapir-Pichhadze.

Dr. Sahar Parto assisted in the identification of cases and matched controls using a computer-based matching algorithm. The MSc. Candidate also helped Dr. Sahar Parto and Dr. Amelie Bourdiec with HLA typing imputations for donors and recipients.

Alexia Della Vecchia and Yao Chen, Ph.D. Candidate, were responsible for implementing a literature search, screening title/abstract and full-text manuscripts for eligibility, data extraction, data synthesis, interpretation of findings and manuscript writing for a review on mycophenolate. All roles were guided by the supervision of Dr. Harvey Wong and Dr. Ruth Sapir-Pichhadze. Findings in accordance with this thesis objective are presented in the literature review chapter.

Chapter 1- Introduction

1.1 Kidney Transplantation

End-stage kidney disease (ESKD) is defined as the irreversible decline in a patient's kidney function to where the kidney is unable to meet the body's needs of filtering waste and excess fluids[1]. In the presence of persistent prerenal, intrinsic or postrenal causes for kidney injury and chronic kidney disease, ESKD may develop[1]. Main causes include diabetes, hypertension as well as genetic (e.g., polycystic kidney) and autoimmune diseases[1-3]. In the absence of proper treatment, ESKD can be fatal[1]. Available therapies for ESKD include dialysis and kidney transplantation[1]. Kidney transplantation is the preferred treatment in ESKD as it offers a better quality of life, significantly improved survival, and decreased health care costs in comparison to dialysis[4,5]. The demand for organ donation has grown considerably with the recent decade seeing a 33% increase in patients with ESKD[6]. According to the annual report published by the Canadian Organ Replacement Register, 40,734 Canadians (excluding Quebec) were living with ESKD at the end of 2019, 56.8% of whom received dialysis[6]. A total of 1,789 individuals (including Quebec) received a kidney transplant, whilst 1,902 were on the waiting list for kidney transplantation[6].

Kidney transplants are made possible thanks to living and deceased donors. Deceased donors are deemed eligible for transplantation following neurological determination of death (NDD) or donation after circulatory death (DCD)[6]. While NDD donors make up most of the donor pool, in recent years, the number of DCD donors has been steadily increasing[6,7,8].

Kidney allocation algorithms can vary across nations and organ procurement organizations. Standard donor-recipient pairing typically ensures blood group (ABO) matching and compatibility of human leukocyte antigens (HLA)[9,10]. Some of the other factors considered in

kidney allocation schemes include medical urgency, time spent on dialysis, donor and recipient age, and the need for a combined organ transplant (e.g., kidney/pancreas)[9,10].

Despite the overall success of kidney transplants and gradual improvements in graft survival, transplant outcomes have not reached their full potential of serving all patients for their entire lifetime. Importantly, kidney transplant loss is associated with a 3-fold increase in patient mortality, a decrease in quality-of-life and a 4-fold increase in healthcare costs[4,5].

1.2 Human Leukocyte Antigens

The major histocompatibility complex (MHC) genes encode a group of highly polymorphic cell surface proteins known as human leukocyte antigens (HLA)[11,12]. Among the various loci, class I (HLA-A, B, C) and class II (HLA-DR, DQ, DP) are considered when assessing donor and candidate compatibility. Class I HLA are expressed on all nucleated cells and their structure is composed of polymorphic α chains and a non-polymorphic β2 microglobulin chain[11,12]. Class II HLA are expressed on antigen-presenting cells (APCs) and are also comprised of α and β chains. HLA-DQ and -DP proteins have polymorphic α and β chains, while only the β chain of HLA-DR is polymorphic[11,12]. **(Figure.1)**

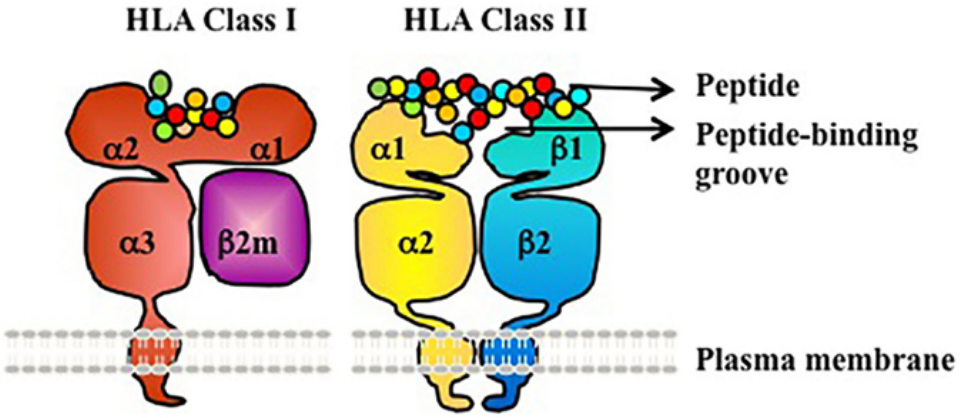

Figure 1. Class I and Class II HLA Structures[12]

Functionally, HLA comprise an essential part of the host immune defence mechanism and are responsible for identifying foreign peptides (antigens) resulting in an immune response, antibody production, and destruction of the affected cell[11,12]. HLA class I molecules present degraded fragments of foreign antigens on the surface of nucleated cells. They are then recognized by and primarily bind to CD8+ on T-lymphocytes resulting in cell death[13]. In contrast, HLA class II molecules present foreign antigens on the surface of APCs which then bind to CD4+ on T-

lymphocytes[13]. This results in a cascade of immune-mediated reactions, CD8+ activation and production of antibodies against foreign antigens[13]. By recognizing structural differences in foreign antigens, unique HLA have evolved to be highly efficient at discriminating self from non-self[14]. Consequentially, much of the extensive genetic diversity observed in HLA molecules has been attributed to the continuous fight against new pathogens[14].

HLA are at the forefront of our immunological barrier against the non-self[14,15]. As such, HLA compatibility between donors and recipients greatly dictates the success of the transplanted kidney[15]. Donor HLA may provoke a recipient's immune response when considered different than self-HLA and can lead to kidney loss[15]. To prevent immune-mediated injuries, kidney transplant candidates undergo screening for anti-HLA antibodies[15]. HLA types of donors and candidates are then verified to assess for HLA compatibility[15]. A candidate is deemed eligible to receive a transplant from a given donor, in the absence of donor-specific pre-formed anti-HLA antibodies[15]. However, given the high degree of polymorphism at 11 relevant HLA loci, it is challenging to achieve perfect HLA matching between donors and recipients, except in the case of identical twins[15]. This is especially problematic for highly sensitized recipients with a wide selection of pre-formed antibodies from previous exposure to foreign HLA[15]. HLA sensitization occurs through blood transfusions, pregnancy, and prior transplantation. In consequence, highly sensitized recipients have access to a significantly lower number of compatible donors in comparison to other transplant candidates[15].

Historically, compatibility at the level of a limited number of HLA (-A, -B, -DR) were primarily studied and found to be greatly associated with the success of the transplanted kidney[16-18]. More specifically, donor and recipient compatibility was assessed based on serological HLA typing, with greater incompatibility of donor and recipient HLA antigens associated with an

increased risk of experiencing a rejection episode, graft failure, and death[16,18,19]. With the development of molecular typing, HLA compatibility is now assessed at the allele level, and the degree of HLA compatibility between donors and recipients is defined by mismatches in class I (HLA-A, HLA-B, and HLA-C) and class II (HLA-DRB1/3/4/5, HLA-DQA1/B1, and HLA-DPA1/B1) loci[20].

In the effort to improve the assessment of HLA compatibility between donors and recipients, there has been a growing interest in determining structural and molecular configuration differences between HLA proteins[21-23]. HLA proteins may be encoded by several HLA alleles, each with their own respective nucleotide sequence[20]. Variations in the immune response to donor HLA have been attributed to HLA B-cell epitopes, which represent polymorphic sequences of amino acids on the surface of HLA antigens that are recognized by specific anti-HLA antibodies[22,23]. HLA epitopes can be specific to one HLA molecule (private epitopes), whilst others can be shared by more than one antigen (public epitopes), thus demonstrating the ability for cross-reactivity of antibodies with different HLA molecules[23,24]. Moreover, within an HLA epitope, there are functional sequences of two to five amino acids, which bind specifically to anti-HLA antibodies[22,23].

Computer matching algorithms have been created to assess donor: recipient histocompatibility in HLA class I and II. For instance, Professor Rene Duquesnoy developed an algorithm called *HLAMatchmaker*, which identifies potential functional epitopes, or "eplets" when using the Matchmaker nomenclature[23,24]. Eplets are then characterized by their ability to react to antibodies as well as their capacity to elicit an immune response[22,23].

To date, 27,000 HLA alleles and 515 eplets have been identified[25,26]. Given sharing of eplets by alleles of similar loci and across HLA loci, matching on HLA eplets is expected to reduce

complexity in comparison to allele-level matching[26,27]. It has also been proposed that HLA eplet mismatches (EMMs) provide a more accurate assessment of the immunological risk for each donor-recipient pair[27]. Comparisons between donor and recipient eplets allow for estimations of the sum of EMMs or EMM load[24]. Greater mismatch load has been associated with adverse transplant outcomes such as rejection, de novo donor-specific antibodies (dnDSA), and graft loss [21,22,28,29,30]. Furthermore, it is expected that not all EMMs infer the same immunological risk[30,31]. Certain EMMs are considered to result in greater immune risk than others and thus, are more likely to impact transplant outcomes[30,31]. A better understanding of EMM immunogenicity could minimize the potential for developing dnDSA post-transplant[24,28,29,31].

1.3 Pathophysiology of Antibody-Mediated Rejection

Antibody-mediated rejection (ABMR) is defined as graft rejection driven by antibodies of the recipient directed against donor HLA[32,33]. ABMR has been shown to explain at least 64% of late graft failure events and is said to be the leading cause of premature graft loss in kidney transplant recipients[33,34]. While pre-formed donor-specific antibodies (DSA) are avoided at the time of transplantation, dnDSA may develop over the post-transplant course[33]. Immune suppression reduction either as a result of medication nonadherence or changes to prescribed doses caused by adverse effects, can give rise to the development of dnDSA[33]. As high as 25% of first-time kidney transplant recipients are likely to develop DSA within 10 years post-transplant[35]. Notably, although not as common, there do exist cases of ABMR without documentation of DSA[36]. Similarly, some kidney transplant recipients may develop DSA. Yet, this may not manifest as rejection when conducting a kidney biopsy[37].

Kidney biopsies, often conducted for cause, upon rises in creatinine levels and/or development of proteinuria, remain the gold standard for diagnosing ABMR[37,38]. Histological evidence provided by the biopsy is examined by a pathologist and using the Banff criteria, rejection diagnoses along with the relevant subtype(s) are assigned. There are several subtypes of ABMR that vary in severity and prognosis, ranging from acute, chronic-active and chronic rejection[13,30,32,38]. Each subtype has its own diagnostic criteria guided by the Banff classification, an ever-evolving standardized schema pertaining to kidney allograft pathology[13,30,32,38]. The Banff classification is a universally accepted classification system with rejection phenotypes including antibody-mediated and T-cell mediated rejection[38]. Since its inception in 1991, the Banff classification system has undergone considerable revisions to better diagnose kidney rejection[38]. Only in 2011, was the diagnosis of ABMR introduced into the classification for the first time[38].

Modern HLA typing and avoidance of transplantation in the presence of pre-formed DSA, has largely prevented the occurrence of hyperacute rejection (occurring within minutes to hours post-transplantation)[13,30,32]. Acute rejection typically occurs within days up to several months post-transplantation and most often involves the interaction of dnDSA or pre-formed DSA with foreign HLA through complement binding of IgG1 and IgG3 antibodies[13,30,32]. Chronic rejection can arise several years into the post-transplant course and occurs most frequently with non-complement binding of dnDSA (IgG2, IgG4) and foreign HLA[13,30,32].

An episode of acute ABMR with serological evidence of DSA can result in activation of the complement cascade causing inflammation of the microcirculation and endothelial cell damage leading to tissue injury and potentially, chronic dysfunction of the transplanted kidney[30,34,37]. Chronic ABMR is characterized by the presence of DSA, morphological evidence of transplant glomerulopathy as a result of chronic tissue injury, and persistent microvascular inflammation[32,34,37,39,40]. Finally, chronic-active ABMR presents characteristics of both acute and chronic ABMR and can be categorized as an intermediate step in the development of late-stage acute to chronic rejection[37,39]. However, a recipient with acute rejection may not progress to chronic rejection[37]. If not prevented or detected and treated early, an episode of ABMR can result in irreversible damage to the transplanted kidney, and a 4-fold increased risk of experiencing premature kidney transplant loss[13,33,41].

Despite being increasingly prevalent and detrimental to the survival of the transplanted kidney, ABMR currently lacks a highly effective treatment[37,42]. In the treatment of acute ABMR, maintenance immune suppression is typically increased and combinations of anti-rejection therapies such as plasmapheresis (PLEX), intravenous immunoglobulin (IVIG), and rituximab are frequently prescribed[37,39,42-45]. Tocilizumab has also been proposed for use in recipients with

chronic-active ABMR[46]. Dosing and prescription intervals for such therapies are dependent on transplant and centre practices.

Plasmapheresis is effective at removing circulating DSA and reducing DSA load[41,43], whereas IVIG regulates T- and B-lymphocyte activity by blocking the binding of DSA to target Fc receptors, among others[13,44]. Rituximab is a chimeric monoclonal antibody against CD20 which depletes B-lymphocytes and reduces donor antibody production[30,45]. Nevertheless, several studies have failed to demonstrate an effect on ABMR outcome with rituximab use[30,39, 42]. Lastly, tocilizumab reduces DSA load by inhibiting interleukin-6 (IL-6), a pro-inflammatory cytokine involved in the production and stimulation of T- and B-lymphocytes[46].

Chronic ABMR tends to result in irreversible injury to the transplanted kidney, making current therapies ineffective. Unlike acute ABMR, cases of chronic ABMR are predominately caused by dnDSA interacting with class II HLA which tend to be more persistent than class I HLA[30,39]. Attempts to reduce class II dnDSA may put recipients at risk of being over-immunosuppressed and more likely to experience complications of over-immune suppression[39]. The development of transplant glomerulopathy as a result of dnDSA is also a key contributor to poor graft survival in recipients with chronic ABMR[39,40]. In consequence, prevention of chronic ABMR through early detection and treatment of acute rejection, as well limiting exposure to highly immunogenic HLA EMMs at the time of transplantation, remain some of the more promising management strategies[13,30,44].

1.4 Immune Suppression

To address the risk of rejection as a result of HLA incompatibility, transplant recipients are obliged to receive immunosuppressive therapy[31]. In common practice, at the time of transplantation, kidney recipients are given induction agents, which are highly potent biologic immunosuppressants, to prevent early graft rejection and loss[45,47]. Recipients are then prescribed a standard maintenance regimen typically including a combination of 2 to 3 immunosuppressive agents (calcineurin inhibitor, anti-metabolite, and prednisone)[45,47]. The choice of immunosuppressive therapy used follows transplant centre practices and may vary over time amongst kidney transplant recipients.

Induction Therapy

Induction agents administered at the time of transplantation are mainly categorized into two groups: lymphocyte-depleting and non-lymphocyte depleting[47]. Frequently administered lymphocyte-depleting agents include rabbit anti-thymocyte globulin (Thymoglobulin) and alemtuzumab (Campath), a monoclonal antibody targeted against CD52[47]. Alemtuzumab suppresses the immune system by binding to CD52 present on B- and T-lymphocytes, resulting in cell lysis; rabbit anti-thymocyte globulin does so by triggering complement-dependent cell lysis leading to the depletion of T-lymphocytes[45,47,48]. Non-lymphocyte depleting agents include basiliximab (Simulect) and daclizumab (Zenapax). They are both monoclonal antibodies targeted against CD25 on interleukin 2 (IL-2) receptors, causing inhibition of T-lymphocyte activity[45,47]. The use of newer lymphocyte-depleting agents such as alemtuzumab has been steadily increasing in comparison to more conventional agents (e.g., basiliximab, and daclizumab), due to their greater efficacy in reducing the risk of early rejection[45,49]. However, the use of specific induction agents remains dependent on centre practices and a recipient's immunological risk[45].

Maintenance Therapy

Calcineurin Inhibitors

Cyclosporine (Neoral) and tacrolimus (Advagraf/Prograf) are classified as calcineurin inhibitors (CNIs). CNIs selectively inhibit calcineurin, a protein involved in the activation of T-lymphocytes[45,47,50]. Tacrolimus binds to immunophilin FKBP-12 and cyclosporine to cyclophilins creating complexes that inhibit calcineurin[47,50]. This results in the suppression of T-lymphocyte activity and IL-2 transcription[47,50]. Once orally administered, tacrolimus and cyclosporine are absorbed and metabolized in the small intestine and liver by cytochrome (CYP) P450 enzymes (CYP3A4/CYP3A5)[50]. Unmetabolized tacrolimus and cyclosporine then enter the systemic circulation and carry out their immunosuppressive effect[50]. Metabolites generated from the metabolized drugs are then excreted into the bile and urine[50].

There are two bioequivalent tacrolimus formulations currently available. The first is immediate-release tacrolimus, the brand name being Prograf, and extended-release tacrolimus (Advagraf)[51]. Prograf is administered twice daily, whereas Advagraf is taken once daily[51,52]. Depending on tacrolimus concentrations prescribed, Advagraf half-life is approximately 48.4 (± 12.3) hours and peak concentration (T_{max}) is achieved within 2.0 (1.0-6.0) hours after ingestion[52]. Prograf concentrations peak within 1.5 to 3.0 hours depending on dosage and has a half-life of 12.0 (3.5-40.5) hours[53,54]. Lastly, cyclosporine has a half-life of roughly 8.4 (5.0-18.0) hours and a T_{max} of 1.5 to 2.0 hours[55]. Standard tacrolimus dosage for kidney transplant recipients is dependent on body weight and varies from 0.1mg/kg/day to 0.2mg/kg/day depending on co-medications used[53,43]. For low dose maintenance cyclosporine, typical trough levels range from 50.0 to 100.0 ng/ml[45].

Tacrolimus remains the primary immunosuppressive agent prescribed in the prevention of rejection due to its greater potency, fewer rejection episodes, and improved graft survival when compared to cyclosporine[45,56]. In spite of the success of CNIs in improving graft and patient outcomes, both cyclosporine and tacrolimus may result in nephrotoxicity, leading to an increased risk of kidney injury[57-59]. To mitigate the risk of CNI nephrotoxicity, dose minimization, and simultaneous treatment with non-nephrotoxic agents such as anti-metabolites are used[58-60].

Prednisone

Prednisone is both an anti-inflammatory, and an immunosuppressive agent that may be prescribed to transplanted recipients as part of a triple agent maintenance regimen[61,62]. Prednisone is a synthetic glucocorticoid that is derived from cortisone[61,62]. It induces its anti-inflammatory and immunosuppressive effect by first entering the cell through the cell membrane, binding to glucocorticoid receptors, interacting with glucocorticoid response elements in the nucleus, and finally, acting on gene expression. This results in the reduction of cytokines, and other signalling molecules involved in producing an immune response[61-64].

Prednisone is converted in the liver to prednisolone, its active compound. Prednisolone is metabolized to inactive glucuronide, and sulphate metabolites by CYP3A4 which are then predominantly excreted in the urine[61,62]. Prednisone is typically administered at 5mg per day and has a half-life of 2.6 (± 0.3) hours and a T_{max} of approximately 2.0 hours[63,65].

Despite being highly effective at reducing episodes of acute rejection, there are a plethora of side effects associated with long-term exposure to prednisone. Some of these include an increased risk of developing post-transplant diabetes, hyperlipidemia, osteoporosis, and hypertension[61,63, 66]. To minimize prednisone-related side effects, there have been efforts in current

practice to wean off and discontinue maintenance prednisone treatment in kidney transplant recipients[45,63,66].

Anti-Metabolites

Azathioprine

Azathioprine (Imuran) is a prodrug for 6-mercaptopurine and is a non-selective purine inhibitor[67-69]. By interfering with deoxyribonucleic acid (DNA) synthesis needed for the proliferation of B- and T-lymphocytes, azathioprine induces its immunosuppressive effect[67-69]. Once prescribed as a first-line immunosuppressive treatment for kidney transplant recipients, azathioprine has since been largely replaced by mycophenolate[67,68]. Although being mechanistically similar, mycophenolate is believed to be the more potent immunosuppressive agent and more effective at reducing the incidence of acute rejection, and graft loss in comparison to azathioprine[67,68].

Mycophenolate

Mycophenolate is an immunosuppressive agent commonly prescribed to kidney transplant recipients. There are currently two therapeutically equivalent formulations of mycophenolate. They include mycophenolate mofetil (MMF, Cellcept) and enteric-coated mycophenolate sodium (EC-MPS, Myfortic)[47,70]. Mycophenolate mofetil is prescribed at a standard fixed dose of 1000mg twice daily. The equivalent dosage of enteric-coated mycophenolate sodium would be 720mg twice daily[70].

Both formulations of mycophenolate are mechanistically equivalent except for their absorption[70]. The enteric coating of mycophenolate sodium delays the absorption of mycophenolate, causing the drug to be released in the small intestine rather than in the stomach,

as with MMF[70]. Following oral administration, EC-MPS reaches its T_{max} within 1.5 to 2.7 hours and has a half-life ranging from 8.0 to 16.0 hours[71]. In comparison, MMF has an apparent half-life of 17.9 (±6.5) hours and has a T_{max} between 0.5 and 1.0 hours[72].

Both MMF and EC-MPS are generally well tolerated; however, as with any treatment, there are adverse events associated with their use. Gastrointestinal (GI) and hematological (leukopenia, thrombocytopenia, anemia) complications, as well as opportunistic infections, have been the cause of mycophenolate dosage reduction and/or discontinuation leading to an increased risk of rejection and graft loss[73-77]. GI events, most frequently being persistent diarrhea, remain the primary adverse event induced by mycophenolate exposure[78]. In consequence, delayed absorption of mycophenolate due to the enteric coating of MPS has attempted to reduce the incidence of GI events[79]. Considering the important role of mycophenolate in maintenance immunosuppressive regimens, a literature review on mycophenolate was conducted, and the findings are summarized in Chapter.2.

Therapeutic Drug Monitoring of Immune Suppression Agents

The use of immunosuppressive agents in the context of kidney transplantation has been heavily centred on tacrolimus. Given its narrow therapeutic index, variability in exposure and potential for nephrotoxicity, tacrolimus is subject to routine therapeutic drug monitoring (TDM)[47,80]. In doing so, TDM maximizes the efficacy of tacrolimus (mitigating risk of graft rejection) all while minimizing toxicity[81,82].

Tacrolimus is monitored most frequently by measured trough concentrations (i.e., drug concentration reached before next administration) in whole blood[80]. Depending on centre practices and co-medication with other immunosuppressive agents, target trough levels for tacrolimus range between 4.0 and 11.0 ng/ml[45]. Interestingly, while achieving trough concentrations within the

desired therapeutic window, wide variations in intra-patient tacrolimus trough levels have been observed[83-88]. More specifically, given an unchanged tacrolimus dose, increasing standard deviations in intra-patient trough variability have been shown to increase the risk of acute rejection, transplant glomerulopathy, and total graft loss[86]. The observed intra-patient fluctuations in trough levels have been attributed to medication non-adherence, variability in tacrolimus pharmacokinetics (PK), and drug-drug interactions with other prescribed medications[83-88]. Consequently, prescribed tacrolimus therapy is under constant surveillance by the treating physician[83-88].

Mycophenolate doses are monitored and measured using an area under the curve 0 to 12 hours (AUC_{0-12})[89]. The area under the curve is a measure of plasma drug concentration over time and is directly proportional to the rate of elimination of that drug[89]. That said, an elevated AUC translates to decreased drug clearance. Despite not being subject to routine TDM, the recommended target AUC_{0-12} for mycophenolate is 30 to 60 ng/ml, with sub- and supratherapeutic levels being strongly associated with transplant outcomes[82,90,91].

1.5 Rationale and Objectives

Advances in immunosuppressive therapies have been instrumental in improving short-term transplant survival[45,92]. Long-term transplant survival, on the other hand, has not been as successful; with many patients not enjoying the benefits of transplantation for their lifetime[33,92]. Donor-recipient incompatibility, limited donor pools, inadequate immune suppression, graft injuries, and adverse transplant outcomes are said to affect long-term kidney survival[82,92,93].

Rejection is the leading cause of kidney transplant loss and is driven by molecular incompatibility in HLA eplets between donors and recipients[15]. To mitigate immune-mediated injuries stemming from HLA incompatibility, kidney transplant recipients receive standardized immune suppression regimens informed by centre practices. However, there is a gap in our ability to estimate the immune suppression needs of individual kidney transplant recipients.

While contributing to the decreased risk of rejection, immunosuppressive agents also give rise to various adverse effects requiring monitoring, and adjustment of therapy[93,76]. To prevent adverse effects, tacrolimus undergoes routine TDM. In contrast, mycophenolate is often administered at a standard dose equivalent to 2000mg per day of mycophenolate mofetil and is not as closely monitored[93,80]. Mycophenolate absorption, distribution, metabolism, and excretion may vary across patients, potentially modifying its intended and unintended immune effects, including rejection and complications of over-immune suppression, respectively[73-78]. For example, the metabolism of immunosuppressive agents varies by sex[94,95]. Also, females of childbearing age are at greater risk of experiencing rejection and graft failure[95]. Thus, suggesting that females have greater immune reactivity in comparison to males[95]. In addition to sex, immune responses are said to also differ with increasing age[96]. Yet, how the risk of rejection is modified by differential exposure to immune suppression by sex, and increasing age remains largely unknown.

Being that the level of immune suppression that kidney transplant recipients are exposed to is a modifiable risk factor, estimations of the relationship between specific immunosuppressants and the risk of ABMR can help inform personalized immune suppression regimens and decrease its incidence. This thesis will review the determinants of mycophenolate pharmacokinetics (PK) and pharmacodynamics (PD) as reported in the literature. This thesis will also evaluate how, in a contemporary Canadian cohort, exposure to mycophenolate over the post-transplant course may modify the risk of ABMR given each donor and recipient pair's HLA EMMs.

Chapter 2 - Scoping Review of the Literature: Mycophenolate PK, PD and Association with Kidney Transplant Outcomes

Mycophenolate Pharmacology

Mycophenolate exerts its antiproliferative effect through inhibition of inosine 5-monophosphate dehydrogenase (IMPDH) type I and II, a rate-limiting step in the pathway involved in the production of guanine nucleotides essential for DNA synthesis[47]. The reversible inhibition of IMPDH depletes the synthesis of guanine nucleotides and impedes the proliferation of T- and B-lymphocytes which are exclusively dependent on this pathway[47,97,98].

The PK of mycophenolate is complex. This is mostly attributed to mycophenolate undergoing enterohepatic recirculation[97,98]. It is a prodrug that, after oral administration, is hydrolyzed to its active form, mycophenolic acid (MPA), by carboxylesterase enzymes (CES1, CES2). Once absorbed and activated, MPA then is metabolized and rendered inactive by a reaction catalyzed by uridine 5'- diphospho-glucuronosyltransferases (UGTs)[98,99]. The enzyme UGT1A9 converts MPA into MPA glucuronide (MPAG) and UGT2B7 converts MPA to acyl MPA glucuronide (AcMPAG)[98,99]. These metabolites are then excreted into the bile and urine[98]. Before being excreted, MPAG then undergoes enterohepatic recirculation to MPA by multidrug-resistant protein 2 (ABC22) and anion-transporting polypeptides (SLCO1B1)[98,99].

Unlike tacrolimus, MPA is often administered at a standard dose equivalent to 2000mg per day of mycophenolate mofetil and does not undergo routine TDM[87,88]. Dose modifications based on individual patient characteristics are seldom made to existing regimens but rather, occur due to recipient intolerance of the immunosuppressive agent, and concurrent complications[47,90,100]. This strategy makes transplant recipients more vulnerable to experiencing toxicity, infection and cancer[47]. On the other hand, dose reductions caused by such complications expose patients to risks

of breakthrough rejection[47]. Intra and inter-patient variability in MPA PK attributed to patient and transplant characteristics have also been described[73-78,100]. However, quantifying their effects on transplant outcomes remains inconsistent and not fully understood[100]. Still, routine TDM of mycophenolate is not being implemented in clinical practice. This can be largely attributed to the inconvenience incurred by patients, technical difficulties, costs associated with collecting multiple samples to compute a 12- hour MPA AUC, as well as lack of consistency in clinical data supporting the efficacy of implementing widespread MPA TDM[82,91,100].

Objectives of Scoping Review

To inform personalized treatment with mycophenolate and maximize effectiveness in preventing rejection, while simultaneously minimizing adverse effects such as infection or cancer, a better understanding of the determinants of variability in the PK and PD of mycophenolate is required. This literature review aims to (1) determine patient characteristics and laboratory variables affecting MPA PK, and (2) assess how MPA PK informs pertinent patient and transplant outcomes.

Literature Search, Study Selection, and Data Collection

We conducted a scoping review of the literature. The electronic database, Medline, was searched to identify manuscripts published between January 1, 2009, and March 16, 2020. Titles and abstracts were screened for eligibility for inclusion by two independent reviewers, Alexia Della Vecchia (A.D.V), and Yao Chen (Y.C). Disagreements were resolved by consensus or by a third reviewer. Manuscripts were selected if they described clinical research on kidney-only transplant recipients with ≥20 participants and mentioned mycophenolate PK, PD or related terms in the title and/or abstract. Then, the two reviewers, (A.D.V), and (Y.C), independently verified each eligible manuscript at the full-text level. Full-text manuscripts were deemed suitable to

inform on patient characteristics and laboratory variables affecting MPA PK and/or PD if they (1) were mycophenolate centred, (2) introduced quantifiable covariates as determinants of mycophenolate PK and/or PD, and (3) did not focus on methodology validation but rather, on the association of mycophenolate PK with transplant outcomes. The same two reviewers extracted descriptive data on study design, participant demographics, sample size, immunosuppressant(s) prescribed, and outcomes using a standardized questionnaire. Furthermore, the effect of each variable along with the size and direction of impact on PK (if mentioned) were collected for each eligible study pertaining to the determinants of MPA PK. A detailed report was generated for studies addressing mycophenolate exposure-outcome models, and PD. PK parameters and non-PK covariates along with their effects on outcome(s), methods for modelling main exposure and covariates, as well as, statistical analyses implemented, were collected. Given significant qualitative heterogeneity between studies, a narrative synthesis was conducted on the effect of patient characteristics and laboratory measures on MPA trough level and/or AUC, as well as the association of these MPA PK parameters with clinical transplant outcomes.

Mycophenolate PK

Several studies assessed the effect of patient characteristics on mycophenolate PK. Characteristics commonly introduced in PK models included recipient age, sex, and body mass index (BMI). When considering the impact of recipient age on MPA AUC, studies conducted by Kaplan et al., and Miura et al., demonstrated a decrease in MPA AUC_{0-12} with increasing recipient age[101,102]. More recently, a prospective study conducted by Velickovic-Radovanovic and colleagues also demonstrated that with similar doses of mycophenolate, elderly kidney transplant recipients (> 70 years) had up to a 14% decrease in MPA AUC_{0-12} compared with recipients who

were 40 years of age[103]. In contrast, studies by Tang and Romano showed seemingly no impact on MPA AUC_{0-12} with increased age[104,105].

To date, sex differences in mycophenolate PK have not been extensively studied. Yet, evidence is mounting on differential metabolism and elimination of MPA, as well as distinct adverse effects observed in males and females. Studies by Tornatore et al., determined that males had a 24% higher BMI-adjusted MPA clearance and experienced less severe mycophenolate-induced GI effects in comparison to females[106-108]. In addition, males had lower concentrations of MPAG, an inactive metabolite of MPA[106-108]. Meaney et al., also demonstrated increased mycophenolate-related GI effects in females[109]. Similarly, Morissette and colleagues, concluded that male recipients appeared to have increased concentrations of UGT-mediated glucuronidation, which is responsible for converting MPA to its inactive form (MPAG)[110].

While the recipient's weight is not often considered for adjustment of mycophenolate dosage, evidence suggests that MPA AUC_{0-12} decreases with increasing recipient BMI. Several studies have shown that recipients with a lower BMI (< 70 kg) had an elevated MPA AUC_{0-12}, whereas those with higher BMIs (> 70kg), had reduced AUC_{0-12} levels[101,111-113]. The greatest absolute differences in MPA AUC_{0-12} were observed at either end of the body weight spectrum.

Several studies also assessed the effect of various laboratory tests on MPA PK. Mycophenolate is mainly protein bound with approximately 97% of active MPA binding reversibly to albumin. In consequence, mycophenolate is highly dependent on albumin concentrations for MPA to undergo enterohepatic recirculation and exert its immunosuppressive effect. De Winter and colleagues determined that recipients with a 10g/L increase in albumin concentrations had up to a 35% increase in MPA AUC_{0-12}[114,115]. A study by Guillet et al., also demonstrated a rise in MPA AUC_{0-12} with increasing albumin concentrations[111].

Renal function plays a vital role in mycophenolate elimination. When exploring the effect of estimated glomerular filtration rate (eGFR) on mycophenolate PK, studies have demonstrated that reduced eGFR resulted in reduced clearance and increased MPA AUC_{0-12}[116,117]. With an eGFR as low as 29 ml/min (reference eGFR being ≥ 90 mL/min), dose-normalized MPA AUC_{0-12} increased by up to 48%[116]. This effect was also observed in a study conducted by Kaplan and colleagues[111].

Lastly, as mycophenolate is often prescribed alongside a CNI, it may be subject to drug-drug interactions with co-administered treatments. Studies considering the impact of CNIs on mycophenolate PK demonstrated that co-administration with cyclosporine resulted in decreased MPA AUC_{0-12}[118-120]. However, co-administration with tacrolimus did not appear to similarly impact mycophenolate PK[118-120].

PD Biomarkers

The literature on mycophenolate also reported on IMPDH activity as a PD marker of MPA exposure. Of the few studies addressing IMPDH activity and transplant outcomes, lower IMPDH activity following treatment with mycophenolate appeared to be associated with a decreased risk of rejection. Studies by Raggi et al., and Chiarelli et al., showed significant decreases in post-transplant IMPDH activity, by 114% and 42%, respectively, in recipients who did not experience kidney rejection versus those who did[121,122].

MPA Exposure-Outcome Models

Studies focusing on MPA exposure-outcome models assessed the relationship between the degree to which a recipient is exposed to mycophenolate and the risk of experiencing adverse graft and/or patient outcomes. Increasing MPA exposure, whether it be represented by dose, AUC_{0-12}, or trough level, resulted in a reduced risk of experiencing rejection and increased graft/patient

survival[123-129]. At the same time, increased MPA exposure resulted in an increased risk of breakthrough infections[130]. **Table.1** presents the association between MPA PK and non-PK variables on rejection risk.

Heterogeneity in the Literature on Mycophenolate PK and PD

We found the literature on the impact of MPA PK on transplant outcomes to be sparse and highly heterogeneous. Heterogeneity was observed in relation to the patient populations studied (i.e., ethnicity, recipient age/sex), MPA exposure definitions, outcomes considered, and covariates adjusted for in multivariable models. This made it challenging to discern consistent patterns in recipient, and transplant characteristics, which significantly impact mycophenolate PK and PD.

Single MPA trough or AUC_{0-12} measurements, most often modelled as a categorical variable, were used to define MPA exposure, as opposed to using several PK measurements to account for fluctuations in MPA PK over time[125,126,128]. Few studies also modelled MPA exposure using mycophenolate prescribed doses over fixed intervals post-transplant[124,127,129]. Furthermore, studies differed by the MPA trough/AUC_{0-12} and dosage thresholds considered to define the main exposure. The thresholds used were informed by the median value of the MPA PK parameter in the population studied[125,126,129]. Alternatively, studies applied thresholds that were used in prior publications[124,127].

Significant heterogeneity was also observed in the transplant outcomes studied. Given temporal changes in Banff classifications, outcomes such as kidney rejection were defined differently across studies. Some manuscripts (e.g., by Rhu, Sanchez-Fructuoso and Knorr) provided no Banff classification[125,126,129]. Hence, it was not possible to account for changes in rejection definitions over time.

Models assessing the association between MPA, and rejection often adjusted for baseline characteristics such as recipient/donor age, sex and number of HLA mismatches. Differing associations between MPA exposure and rejection were observed depending on the choice of model specification and non-PK covariates included.

Knowledge Synthesis

This review sought to identify and quantify the determinants of mycophenolate PK, as well as the impact of PK parameters alongside pertinent demographic, clinical and laboratory variables on important kidney transplant outcomes. Acknowledging heterogeneity across studies in patient populations, MPA exposure models, outcomes studied, and covariates included, we were able to note that recipients with lower BMIs were observed to have an elevated MPA $AUC_{0\text{-}12}$. Female kidney transplant recipients tended to experience mycophenolate-induced GI effects and had reduced MPA clearance in comparison to males. Many studies have shown a decrease in $AUC_{0\text{-}12}$ with increasing age, whilst others showed no impact of age on MPA $AUC_{0\text{-}12}$. Increasing albumin concentrations and decreasing eGFR appeared to increase MPA $AUC_{0\text{-}12}$. Co-administration with cyclosporine decreased MPA $AUC_{0\text{-}12}$. When evaluating mycophenolate PD, decreased IMPDH activity was observed in recipients who did not experience rejection. Finally, in relation to MPA exposure-outcome models, increasing MPA exposure reduced the risk of experiencing rejection and graft failure, as well as increased the risk of breakthrough infection.

Inter-patient variability in MPA PK was observed in patient characteristics such as recipient BMI, sex, and age. Several studies were suggestive of members of the female sex having lower MPA clearance and an increased risk of adverse effects in comparison to males[106-110]. To account for these observed sex differences, and to reduce the risk of over-immune suppression and frequency of adverse side effects, sex must be considered when determining mycophenolate

dosage. Increasing recipient BMI resulted in decreasing MPA AUC_{0-12}[101,111-113]. Being that mycophenolate dosage is seldom guided by recipients' weight, dosage adjustment by weight may reduce the risk of over- immunosuppression in those with lower body weight and under-immunosuppression in recipients with higher body weight. Also, considering both BMI and sex differences, prescribing adjusted doses for females with lower BMIs could reduce the incidence of undesirable outcomes. Lastly, several studies were suggestive of increasing age resulting in decreased MPA AUC_{0-12}[101-103], whilst other studies showed seemingly no impact of increasing age[104,105]. Those studies suggestive of no impact of age on MPA PK varied in relation to their included cohort age distributions and follow-up times considered. Thus, rather than supporting the notion that recipient age is not an important determinant of MPA PK, the discrepancy in results may be attributed more so to the heterogeneity observed in these studies.

Kidney function, measured by eGFR, is seldom considered when kidney transplant recipients are prescribed mycophenolate. Yet, our literature review suggested an increase in MPA AUC_{0-12} with decreasing eGFR[116,117]. Furthermore, MPA primarily binds to albumin to exert its immunosuppressive effect[114,115]. We observed that increasing albumin concentrations resulted in increased MPA AUC_{0-12}[114,115]. Taken together, these findings suggest that albumin levels and eGFR could help guide modifications in mycophenolate dosage to avoid inadequate immune suppression.

Recent studies have focused on the impact of co-administered tacrolimus and mycophenolate in relation to transplant outcomes. Tacrolimus appeared to have an insignificant effect on MPA AUC_{0-12}[118-120]. In contrast, MPA AUC_{0-12} decreased with increasing cyclosporine doses[118-120]. Nonetheless, depending on the transplant era and centre, most recipients are now

prescribed mycophenolate and tacrolimus with or without steroid use. Thus, drug-drug interactions with cyclosporine are largely avoided.

IMPDH activity has been identified as a potential biomarker for MPA PD. Decreased IMPDH activity was observed in recipients who did not experience rejection[121,122]. Still, the limited studies demonstrating a relationship between IMPDH activity, and transplant outcomes often did not consider patient characteristics. Failing to account for these covariates potentially inhibits our ability to assess the true strength of IMPDH as a biomarker. Demonstration of how, with any given prescribed mycophenolate dose, patient characteristics may influence IMPHD activity, could also inform on strategies for adjusting mycophenolate dose over follow-up.

A clear relationship was demonstrated in studies examining the effect of MPA exposure on the risk of rejection. As MPA exposure increased, the risk of rejection decreased[124-129]. However, MPA exposure is not the sole contributor to this risk. Inferior transplant outcomes are observed as a result of HLA incompatibility between donors and recipients, with exposure to the immunosuppressive agent, MPA, likely acting as an effect modifier. Eligible studies modelled HLA incompatibility as the number of HLA mismatches between donors and recipients and showed an increase in the risk of experiencing rejection the higher the number of mismatches[124,127,129]. In contrast, recent literature suggests that incompatibility is more complex than simply the number of antigen mismatches and that the risk of rejection and graft loss is largely attributed to molecular mismatches between donors and recipients at the level of HLA eplets[21-24]. Oversimplification of this instrumental variable might provide an inaccurate assessment of a recipient's true immunological risk. In addition to MPA exposure and HLA incompatibility, the risk of rejection may be further modified by recipient characteristics such as age and sex.

Importantly, evidence suggests that patients' sex and age are imperative predictors of kidney transplant survival and have been shown to affect the risk of experiencing adverse outcomes such as rejection and graft failure[94,126,129]. Several studies have suggested that females are at greater risk of experiencing rejection[94,131-133]. This may be attributed to differences in immune reactivity, sensitization, and variability in MPA clearance observed between the sexes[94, 131-133]. The current literature has also proposed that in comparison to younger kidney recipients, older recipients have a reduced incidence of rejection due to reduced B- and T- lymphocyte immune responses with increasing age[96,134]. However, the construction of many of the regression models assessing the relationship between MPA exposure and outcome did not consider these predictors[123-125,129]. When these variables were introduced in multivariable models, the impact of the female sex and increasing age were most often insignificant[127,130]. These statistically insignificant results may be explained by a case-mix (or cohort characteristics beyond population age and sex) that were not assessed in exposure-outcome models, choice of arbitrary age thresholds rather than biologically relevant age groups (e.g., 18-24, 25-44, 45-64, > 65)[94,131-133], and variability in model specifications.

Several limitations must be noted. There is currently no tool for quality assessment of PK, PD, and exposure-outcome studies. Given challenges in measuring MPA AUC_{0-12}, studies estimated MPA AUC using trapezoidal methods and limited sampling strategies. Also, being that mycophenolate does not undergo TDM, some manuscripts relied on prescribed mycophenolate doses or single MPA trough/AUC measurements; both of which may inaccurately estimate MPA exposure. Furthermore, the timing of MPA level measurements in reference to the date of transplant differed by study. In the case of exposure-outcome models, studies varied in their selection criteria, standard induction, and choice of co-medications alongside mycophenolate for

maintenance immunosuppression. The likelihood of detecting rejection also varied whereby some studies relied on biopsies for cause and some on surveillance biopsies. Lastly, missing information on the sample size and/or power calculations performed was a recurrent issue in both the PK and PD studies included. This was particularly problematic in studies with smaller sample sizes as they may not have had enough power to detect significant results.

Future Directions for Studies Assessing Mycophenolate Exposure

Our review suggests that despite the prevalence of mycophenolate within maintenance immunosuppression regimens, the literature considering its PK and PD is sparse. Opportunities for personalized mycophenolate prescriptions based on patient characteristics require verification of determinants of mycophenolate exposure in large cohort studies and randomized controlled trials. There is a need to construct MPA exposure models illustrating how pertinent demographic, clinical and laboratory variables modify MPA PK, as well as clinical outcomes. Such tools may be used to personalize regimens but also guide modifications in mycophenolate prescribing over the post-transplant course.

Lack of standardized modelling of MPA exposure rendered it difficult to both compare observations from one study to another, as well as synthesize the available evidence on this topic. Future studies should ensure a sufficiently large sample size, availability of MPA TDM parameters, clear and consistent outcome definitions, and a standardized set of covariates, including information on HLA incompatibility, to allow the development of accurate systems pharmacology models. Also, as determinants of mycophenolate PK depend on patient characteristics (e.g., age) and laboratory tests (e.g., eGFR and albumin) that may change over time, future studies may consider including time-varying MPA PK and covariate data into exposure-outcome models. This will allow for a better understanding of how these variables may influence

both exposure to MPA, and kidney transplant outcomes. The findings of this literature review were considered in the construction of our exposure-outcome model assessing the modifying effect of mycophenolate exposure on ABMR risk (Chapter.3).

Table.1 Impact of MPA PK and Non-PK Covariates on the Risk of Kidney Transplant Rejection

Reference	Population, Sample Size; Follow-Up	MPA Type, Co-Medication	Statistical Analysis	Outcome; Definition	Risk Factors	Effect; 95% CI	Main MPA Exposure	Effect; 95% CI
Sánchez-Fructuoso et al., 2009	Spanish Kidney Transplant Recipients (n=314); 1-year post-Tx	MMF, Tacrolimus, Steroid	Multivariable Logistic Regression Model	Acute Vascular Rejection (Grade II or III); Banff Classification †	Donor age (> 60 years vs. ≤ 60) Female Recipient (vs. Male) Delayed Graft Function (Yes vs. No)	OR: 2.50 [0.80, 8.00] OR:1.60 [1.00, 2.70] OR: 1.70 [1.00, 2.80]	MPA 12-hour Trough (μg/mL) (< 1.6 vs. ≥ 1.6)	↑ OR: 2.60 [1.60, 4.30] *
Gourishankar et al., 2010	Canadian Kidney Transplant Recipients (n=126); 6 months post-Tx	MMF, Tacrolimus, Prednisone	Univariable Logistic Regression Model	Suspicious or Acute Rejection; Defined as (1) including borderline with Banff Grade 1, (2) all suspected and treated acute rejection episodes (Banff Classification 2005)	N/A	N/A	MMF Dose (3000mg/day) vs. 2000mg/day) MPA AUC at day 5 (incr. of 10 units) MPA AUC at day 3 (incr. of 10 units)	↓ OR:0.35 [0.13, 0.96] * ↓OR: 0.71 [0.54, 0.93] * OR: 0.87 [0.70, 1.08]
Vanhove et al., 2013	Belgian Kidney Transplant Recipients (n=749); 400 days post-Tx	MMF, Tacrolimus, Cyclosporine, Methyl-Prednisone	Multivariable Cox Proportional Hazards Model	Acute Rejection; Deterioration of graft function and histology findings (Banff Classification 2007)	Recipient Age at Tx (incr.) Donor Age (incr.) Delayed Graft Function (Yes vs. No) HLA Mismatches (incr.) Living Donor (vs. Deceased) PRA ≥ 20 % (vs. < 20%)	↓HR:0.98 [0.97, 0.99] * ↑HR:1.01 [1.00, 1.01] * ↑HR:2.13 [1.47, 3.08] * ↑ HR: 1.16 [1.03, 1.31] * ↑HR: 2.21 [1.17, 4.15] * ↑HR: 2.66 [1.62, 4.40] *	MMF Dose Reduction ≥50% from Initial Dose MMF Dose Reduction < 50% from Initial Dose	↑HR:1.47 [1.06, 2.03] * HR: 0.84 [0.48, 1.46]

Table.1 Continued

Daher et al., 2014	French Kidney Transplant Recipients (n=222); 2 years post-Tx	MMF, Tacrolimus, Cyclosporine	Time to Event Model (TTE)	Biopsy-Proven Acute Rejection; (Banff Classification 2007)	CMV+ vs. CMV−	↑HR: 10.90 [6.50, 21.70] *	MPA AUC (Per 1-mg.h/l increase)	↓HR: 0.96 [0.93, 0.99] *
					Cyclosporine C_{2h} (Per 1-ng/ml increase)	HR: 1.00 [0.99, 1.00]		
					Tacrolimus C_0 (Per 1-ng/ml increase)	HR: 1.10 [0.98, 1.30]		
Knorr et al., 2014	American Kidney Transplant Recipients (n=597); 1-year post-Tx	MMF, Tacrolimus, Cyclosporine, Methyl-Prednisone	Multivariable Generalized Linear Model	Biopsy-Proven Acute Cellular or Antibody-Mediated Rejection; Banff Grade I or higher as well as borderline rejections that were treated. †	Recipient Age (incr.)	RR: 0.99 [0.97, 1.01]	MMF Dosage at Discharge (incr.)	RR: 1.00 [0.99, 1.00]
					Donor Age (incr.)	RR: 1.00 [0.98, 1.02]		
					Black Race (vs. Others)	↑RR: 2.38 [1.41, 4.03] *		
					HLA Mismatch (incr.)	RR: 1.08 [0.92, 1.27]		
					Female Sex (vs. Males)	↓RR: 0.55 [0.32, 0.94] *		
					Prior Transplantation (Yes vs. No)	RR: 1.01 [0.49, 2.10]		
					PRA > 20% (vs. ≤ 20%)	RR: 1.51 [0.75, 3.02]		
					CMV Seromismatch(D+/R-)	RR: 1.65 [0.99, 2.74]		
					PPI Exposure (Yes/No)	RR: 1.41 [0.88, 2.23]		
					Thymoglobulin Induction dose (incr.)	RR: 0.95 [0.83, 1.08]		
					Cold Ischemia Time (incr. hours)	RR: 1.00 [0.96, 1.03]		

Table.1 Continued

					Standard Criteria Donor (vs. Living Donor) ECD (vs. Living Donor) DCD (vs. Living Donor) ECD/DCD (vs. Living Donor)	RR: 0.99 [0.42, 2.32] RR: 1.47 [0.52, 4.13] RR: 1.11 [0.41, 3.05] RR: 0.49 [0.07, 3.71]		
Rhu et al., 2017	Korean Kidney Transplant Recipients (n=268); > 1-year post-Tx	MMF, Tacrolimus, Methyl-Prednisone	Multivariable Cox Proportional Hazards Model	Biopsy Proven Acute Cellular Rejection; Banff Classification †	Recipient BMI ≥21 (vs. < 21)	↓HR:0.46 [0.24, 0.89] *	Mean MPA Trough (mg/L) ≥ 0.7 vs. < 0.7	↓HR: 0. 21 [0.07, 0.60] *

*P < 0.05

† Banff Classification Year Not Provided

Abbreviations: AUC: Area Under the Curve, BMI: Body Mass Index, CI: Confidence Interval, CMV: Cytomegalovirus, D+: Positive Donor, DCD: Donation after Circulatory Death, ECD: Expanded Criteria Donor, HLA: Human Leukocyte Antigens, HR: Hazard Ratio, MMF: Mycophenolate Mofetil, MPA: Mycophenolic Acid, PPI: Proton-Pump Inhibitor, PRA: Panel Reactive Antibodies, R-: Negative Recipient, RR: Risk Ratio, Tx: Transplant.

Chapter 3 – Research Project

This thesis aimed to determine how ABMR risk associated with HLA EMM incompatibility may be mitigated by exposure to mycophenolate, and further modified by recipient characteristics (age, sex).

3.1 Methodology

Study Design, Population, Case Ascertainment, and Control Selection

We conducted a nested case-control study using the McGill University Health Centre (MUHC) CanPREVENT AMR cohort to investigate the role of exposure to mycophenolate on the risk of ABMR as a function of HLA EMM incompatibility. First-time kidney transplant recipients transplanted from January 1st, 2012, to June 30th, 2019, were eligible to participate. Re-transplant and multi-organ transplant recipients, participants in whom donor and recipient HLA typing could not be ascertained, and recipients with missing immune suppression prescription data, were excluded.

The primary endpoint was the time to the first ABMR diagnosis. Cases were identified as patients with ABMR (across its continuum and including active, chronic active and chronic rejection) detected on biopsies for cause and defined according to the Banff 2017 classification. In collaboration with MUHC pathologists, biopsy reports and slides preceding the publication of the 2017 Banff classification, underwent review, extraction of the required minimum set of variables as recommended by the Banff Knowledge Dissemination Group, and then were reclassified according to the Banff 2017 classification[135].

Patients who had not experienced any rejection event or developed DSA, from transplantation to the date of case diagnosis, were eligible as controls. Controls matched on transplant year, time-post transplant (to case diagnosis), and donor type (living vs. deceased donor)

were randomly selected from the remaining cohort using incidence density sampling[136]. Ethics approval was obtained from the MUHC research ethics board.

Data Sources

Recipient, donor, and transplant characteristics including immune suppression data, laboratory tests, and pathology data from kidney transplant biopsies were obtained from the MUHC transplant database and the patient's electronic medical records. DSA assay results and HLA molecular genotyping by sequence-specific oligonucleotide (SSO) strings were obtained from the MUHC histocompatibility laboratory.

HLA Eplet Mismatch Estimation

EMM's were estimated from allele-level donor and recipient HLA-A, -B, -C, DRB1,3,4,5 and DQB1 types. To verify the most likely HLA genotype, molecular HLA types provided by the HLA laboratory were entered into the HaploStats application[137]. The resultant HaploStats output for each recipient and donor included the frequency and likelihood of the genotypes provided by HaploStats in the entire population, as well as by self-reported ethnicity. Donor and recipient genotypes congruent with the measured SSO strings were then transformed into epitypes with the most frequent donor and recipient epitypes considered for the estimation of EMMs. Molecular HLA incompatibilities as represented by the EMM load for the complete epitype (class I and II), antibody-verified (AbVer) epitype (class I and II), class II epitype, and AbVer class II epitype were modelled as a continuous variable (i.e., by 1 and 10 EMM increments).

DSA Verification

During the study period, kidney transplant recipients at the MUHC underwent routine monitoring for HLA class I and class II DSA at intervals of 3-, 6-, and 12-months post-transplant, and annually thereafter. Additionally, DSA was also verified when blood samples were drawn for

cause in recipients who were suspected of experiencing rejection at any time point over the post-transplant course.

Assignment of DSA in our cohort was verified by a single HLA laboratory director. Using One Lambda's single antigen assays (LABScreen^{TM} single antigen HLA Class I and Class II)[138], HLA assay beads were coated with recipient serum and tagged with a fluorescent agent (R-phycoerythrin conjugated anti-IgG). The Fusion software was used to provide mean fluorescence intensity (MFI). The baseline normalized fluorescence value for each HLA-coated bead equalled the value of that bead minus the value of the negative control (NC) bead. For antibody identification, the positive control (PC) and NC were recorded first and verified to have > 10,000 MFI and <500 MFI, respectively, and a PC/NC ratio to be >2 (ideally > 10). Patients with a raw NC >1500 MFI underwent treatment by Adsorb Out^{TM,} or, alternatively, by fetal calf serum, dithiothreitol, and /or dilution before further testing[139]. Generally, a specificity above cut-off level 6x or 1000 MFI was deemed consistent with DSA. Additionally, changes in bead ranking (lowest to highest) pre-and post-transplantation, eplet sharing across beads, degree of reactivity against recipient's antigens, and previous sensitization events, were also considered in the process of DSA verification.

Exposure to Mycophenolate

Standard immune suppression regimens for kidney transplant recipients at the MUHC adhere to a steroid-sparing protocol, which includes a biologic induction agent administered at the time of transplantation (typically alemtuzumab), and oral maintenance immunosuppressive agents, including a CNI (tacrolimus) and an anti-metabolite (mycophenolate), which were administered in all cases and controls. Computer-recorded prescription data in the MUHC transplant database and electronic prescription records allowed direct calculation of the daily dose of each medication as

pill strength (in milligrams (mg)) times the number of pills divided by the number of days supplied. Days supplied and consecutive prescription dates confirmed the duration of each dispensing and allowed the identification of gaps between the end of a prescription and the start of the next one. Patients were considered unexposed to mycophenolate until the time of the first prescription. Mycophenolate dosage was averaged across each prescription period and converted to a daily equivalent dosage.

Given the frequency of physician evaluation occurred at a minimum of 3-month intervals over the post-transplant follow-up, and, similarly, one would expect changes in prescriptions, as well as investigation for possible rejection events to have occurred at such intervals, a lag period of 3 months, was applied between the last administered dose of mycophenolate and the index date. Mycophenolate dosage was modelled as a time-varying covariate with a cumulative exposure estimated over a period of 7- days preceding the index date minus 3 months **(Figure.2).**

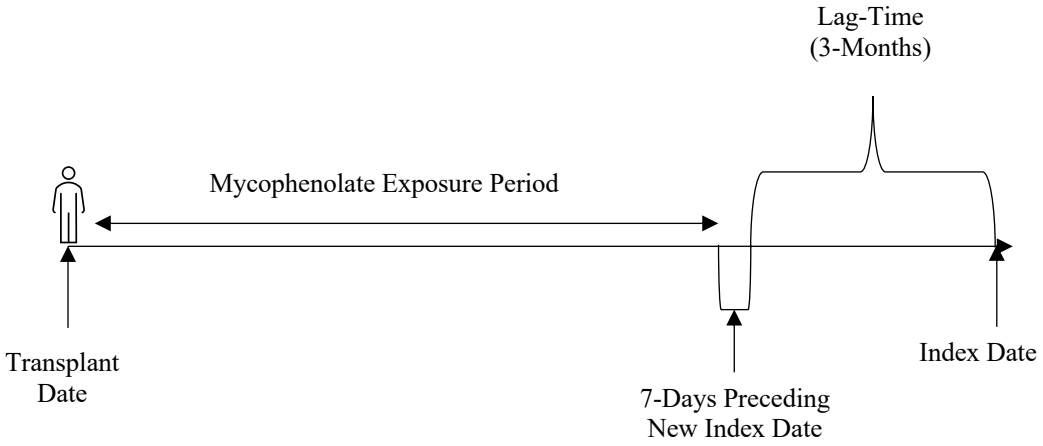

Figure.2 Diagram of the mycophenolate exposure period in relation to the index event detected, minus a 3-month lag.

Other Variables

Recipient characteristics included age, sex, self-reported ethnicity, cause of ESKD, panel-reactive antibodies (PRA) pre-transplantation, dialysis modality, and dialysis vintage. Donor

characteristics included age, sex, donor type (living vs. deceased) and deceased donor type (standard criteria donor, expanded criteria donor). Transplant characteristics included the prescribed induction agent (none, non-lymphocyte-depleting, lymphocyte-depleting), HLA EMMs, and cold-ischemia time. Prescription data on tacrolimus and prednisone were also collected for the duration of follow-up. Finally, to assess the effect of intra-patient variability in tacrolimus trough levels while on a stable tacrolimus regimen, tacrolimus standard deviation (TacSD) from individual trough tacrolimus blood levels (indexed by i) for the j^{th} time interval were generated. As TacSD was expected to change in proximity to modifications in tacrolimus dosing, TacSD was measured during each of j intervals when tacrolimus dose was unchanged[86].

Statistical Analysis

The distribution of recipient, donor and transplant characteristics at the time of transplantation were assessed for both cases and matched controls and compared using a paired t-test for continuous (e.g., age, HLA EMMs) variables. The Chi-square and Fisher's exact test were used for categorical variables (e.g., prednisone use, sex). We fit univariable conditional logistic regression models to compare the risk of ABMR by donor: recipient HLA EMM as well as multivariable models adjusting for recipient age (> 60 vs. ≤ 60; median age in our cohort), sex (female vs. male), and mycophenolate exposure (7-days preceding the index date minus a 3-month lag). Whether the prescribed mycophenolate dose modified the effect of EMMs on ABMR, was assessed using an interaction term between the main exposures (EMMs) and the prescribed mycophenolate dose. Additional sensitivity analyses were conducted using varying lag times (1- and 2-months) and mycophenolate exposure periods (cumulative, 14-days and 30-days preceding the index date minus 3 months) aligning with the changes in the frequency of physician visits over the post-transplant course. Finally, tacrolimus exposure was modelled as TacSD, generated from

the last prescribed tacrolimus dose preceding the index date minus 3 months. Sequentially nested conditional logistic regression models considering class II EMMs, mycophenolate dosage (7-days preceding the index date minus 3 months) and, lastly, TacSD were fit. To avoid model overfitting, recipient age, and sex were not included in the nested models. All statistical analyses were conducted using RStudio (Version 4.0.2). A two-tailed p-value of 0.05 was deemed significant.

3.2 Research Findings

A total of 816 kidney transplant recipients underwent transplantation at the MUHC during the study period. After the implementation of exclusion criteria, 675 recipients were eligible and included in the analytic cohort. Of the 141 kidney transplant recipients excluded, 75.2% were re-transplant recipients. The median (inter-quartile range (IQR)) follow-up time for the entire cohort was 2.17 (2.97) years.

Incidence of DSA and ABMR in the MUHC Cohort

Of the 675 recipients in our cohort, 165 developed DSA. The incidence of positive DSA in the eligible cohort was 7.14 (95% CI: 6.10, 8.31) per 100 person-years. The median (IQR) time to diagnosis of DSA was 1.05 (1.65) years. In female recipients (n=231), the incidence of DSA was 5.95 (95% CI: 4.41, 7.84) and in male recipients (n=444), the incidence was 7.81 (95% CI: 6.45, 9.37) per 100 person-years. For recipients aged 18-24 years, 25-44 years, 45-64 years, and ≥ 65 years, DSA incidence was 11.41 (95% CI: 2.35, 33.34), 8.94 (95% CI: 6.36, 12.22), 7.23 (95% CI: 6.36, 8.94), and 12.60 (95% CI: 10.06, 15.60) per 100 person-years, respectively. When assessing DSA specificities, we predominantly observed antibodies against HLA class I (52.8%), with the most frequent DSA specificities to HLA-B (65.0%) including B76. We also observed HLA class II antibodies (47.2%), with specificities including DR7, DQ2, DQ7, DQ 8, and DQ9.

The incidence rate of ABMR in the analytic cohort (n=675), was 3.35 (95% CI: 2.63,4.21) per 100 person-years. The median (IQR) time to diagnosis of ABMR in cases was 1.10 (1.42) years. In female kidney transplant recipients (n=231), an ABMR incidence of 2.76 (95% CI:1.73, 4.18) per 100 person-years was observed, whereas, in males (n=444), the incidence was 3.68 (95% CI: 2.75, 4.83). When assessing ABMR incidence by age, recipients aged 18-24 years, 25-44 years, 45-64 years, and ≥ 65 years had incidence rates of 7.83 (95% CI: 0.85, 28.30), 3.30 (95% CI: 1.87, 5.36), 3.04 (95% CI: 2.11, 4.22), and 3.85 (95% CI 2.40, 5.89) per 100-person years, respectively.

Nested Case-Control Study Construction

Of the 63 ABMR events observed in the analytic cohort during the study period, 8 were excluded due to missing donor and/or recipient DNA. Thus, resulting in a total of 55 eligible ABMR cases. Consideration of a 3-month lag period between the last administered dose of mycophenolate and the index date resulted in the exclusion of 7 cases who had shorter follow-up from the transplantation date. Consequently, the final cohort of nested case-control study participants consisted of 48 cases and 1:1 randomly selected controls matched for transplant type, year of transplantation, and follow-up time post-transplant. **Figure.3** presents a study flow diagram outlining the case/control selection process.

Sensitivity analyses applying various lag periods and modelling of mycophenolate exposure resulted in slightly different numbers of eligible nested cases and matched controls from the analytical cohort. For example, sensitivity analyses considering mycophenolate dosage 14-, and 30-days preceding the index date minus a 3-month lag, included a total of 47 and 46 ABMR cases, respectively. The analysis considering cumulative mycophenolate dosage over follow-up minus a 3-month lag had the same number of cases as our primary analysis (n=48). Sensitivity analyses applying 1-, and 2-month lags in reference to the index date and mycophenolate exposure

over the 7-days preceding the index date, included 50 and 49 ABMR cases, respectively, as a larger number of participants had a sufficiently long follow-up time from transplantation. Lastly, consideration of TacSD as a confounder in sequentially nested multivariable models had to be restricted to kidney transplant recipients with enough trough tacrolimus level measurements to allow its calculation. Thus, 27 pairs of cases and matched controls had to be excluded.

Study Population

Our study cohort was rather diverse. Most kidney transplant recipients were Caucasian; however, Black, Asian, and First Nations were also among the self-reported ancestries represented. There was a higher proportion of male recipients (72.9%), and a higher proportion of female donors (56.3%). Cases more frequently had PRA > 0% in comparison to controls (45.8% vs. 27.1%). As for immunosuppression regimens, almost all kidney transplant recipients (99.0%) received lymphocyte-depleting induction agents. The median number of class II HLA EMMs was higher among cases than in controls (Median (IQR): 27.50 (19.25) vs. 22.00 (12.00), respectively). A similar trend was observed for class II AbVer EMMs (p=0.04). A detailed table of baseline characteristics of both cases and controls is presented in **Table.2**.

Results of Conditional Logistic Regression Models

Table.3 presents the results of univariable and multivariable conditional logistic regression models examining the association between HLA EMMs, mycophenolate dosage 7-days preceding the index data minus 3 months, age and sex in relation to ABMR risk. When assessing the independent association of EMMs with time to ABMR, we observed an increased risk by ascending EMM load for class II HLA (OR: 1.06 (95% CI: 1.01, 1.11) and OR: 1.76 (95% CI: 1.10, 2.84), per 1 and 10 additional EMMs, respectively). HLA class II AbVer EMMs were also associated with ABMR risk (OR: 1.09 (95% CI:1.00, 1.18) and OR: 2.31 (95% CI: 1.01, 5.30), per

1 and 10 additional EMMs, respectively; $p < 0.05$). A similar trend was observed for increments of 1 and 10 EMMs for the entire epitype (class I and II) and AbVer epitype (class I and II), albeit not reaching the threshold for statistical significance. Aligned with several studies suggestive of ascending class II EMMs having a higher ABMR risk, our multivariable conditional logistic regression models considered class II EMMs exclusively[30,32,39]. Mycophenolate dose increments of 1000mg of MPA demonstrated a protective effect on the risk of ABMR. The female sex and recipient age (>60 years) also appeared protective. Although, neither of these variables reached the threshold of statistical significance.

Multivariable conditional logistic regression models confirmed an independent association between HLA class II or AbVer class II EMMs and the risk of ABMR (OR: 1.07 (95% CI: 1.02, 1.13) and OR: 1.11 (95% CI: 1.01,1.21), respectively) when adjusting for recipient age, sex, and mycophenolate dosage 7-days preceding the index date minus 3 months. Point estimates suggested a protective effect of mycophenolate exposure (by increments of 1000mg), the female sex (vs. male sex), and recipient age (> 60 vs. ≤ 60 years). Yet, the confidence intervals crossed one, suggesting no statistically significant association with ABMR for these variables. We did not observe a statistically significant interaction between class II or class II AbVer EMMs and recipient sex, as well as age modelled as a categorical (>60 vs. ≤ 60 years) or as a continuous variable. The same held true when assessing an interaction between class II or class II AbVer EMMs and mycophenolate dosage in relation to ABMR.

Post hoc sensitivity analyses to evaluate the impact of varying lag times (1-, 2-months) between exposure to mycophenolate and index date (**Table.4**), showed a similar pattern to that observed in the main analysis (OR:1.07 (95% CI: 1.02, 1.13), OR: 1.07 (95% CI: 1.01, 1.13), respectively) per each additional class II EMM. Similar findings were observed for sensitivity

analyses considering AbVer class II EMMs as an exposure. We did not observe a statistically significant protective effect of mycophenolate on ABMR risk in these analyses.

Table.5 presents several sensitivity analyses assessing the association of ABMR with differing MPA exposure models including cumulative exposure, as well as 14-day- and 30-day exposure to mycophenolate preceding the index date minus a 3-month lag. The independent effect of HLA class II EMMs on the risk of ABMR persisted across models. In the same models, point estimates were suggestive of a protective effect of ascending MPA exposure (by 1000mg), female (vs. male sex), and recipient age (>60 vs. ≤ 60 years); however, there was no statistically significant association with ABMR.

Table.6 presents a series of sequentially nested conditional logistic regression models using the main exposure (class II EMMs), and indicators of exposure to mycophenolate and tacrolimus (dosage 7-days preceding the index date and TacSD, respectively). There was an increased risk of ABMR by ascending numbers of class II EMMs across nested models and this risk was independent of MPA exposure and TacSD. Point estimates were suggestive of an increased ABMR risk with increasing TacSD; however, this variable did not reach the threshold of statistical significance.

Figure.3 Study Flow Diagram

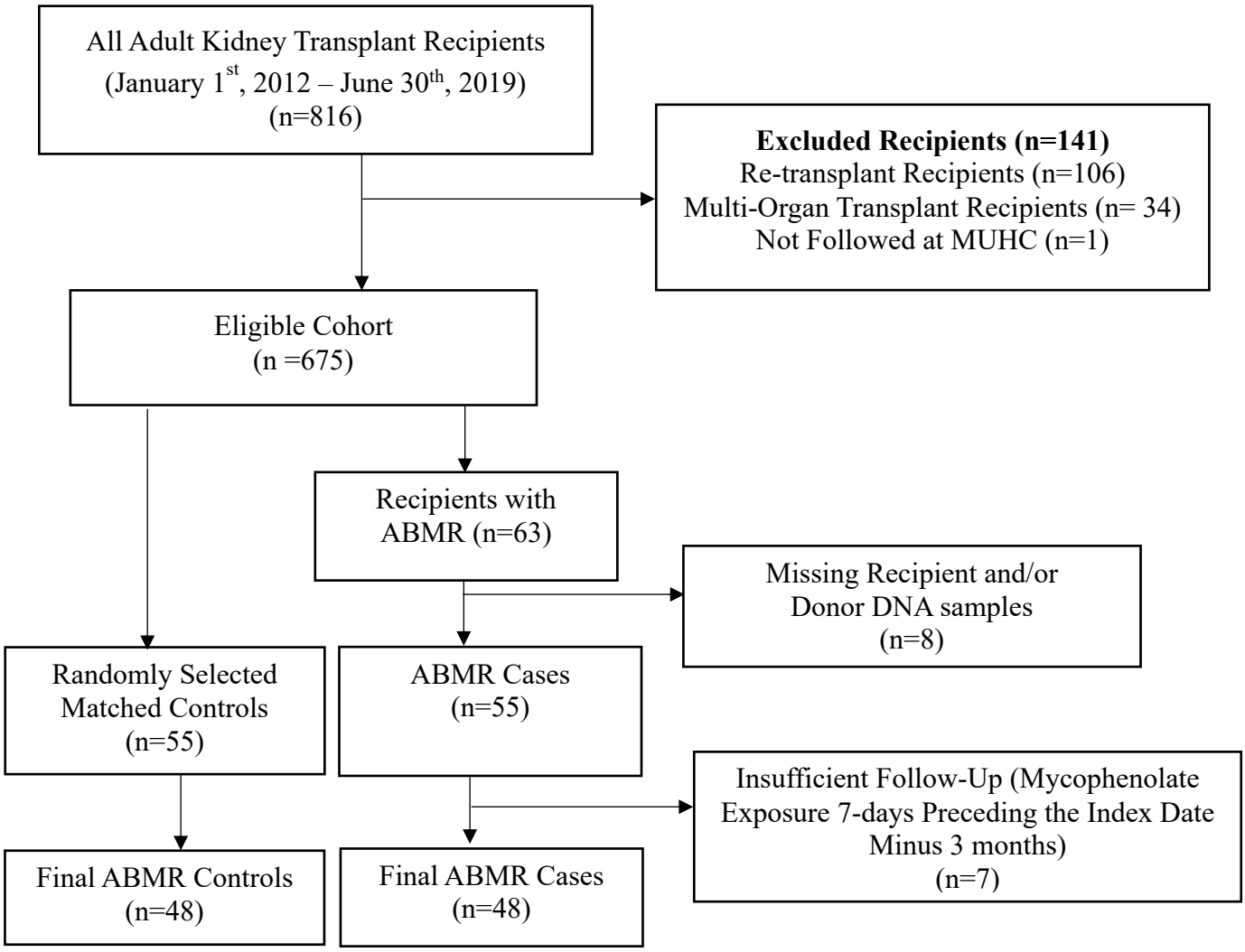

Table.2 Baseline Characteristics of ABMR Cases, and Randomly Matched Controls

	Cases (n=48) %	Controls (n=48) %	p-Value
Recipient			
Recipient Age (Years)			
Median (IQR)	57.40 (18.08)	60.55 (12.23)	0.24
Recipient Sex			
Female	10 (20.8%)	16 (33.3%)	
Male	38 (79.2%)	32 (66.7%)	0.25
Self-Reported Recipient Ethnicity			
Caucasian	29 (60.4%)	34 (70.8%)	
Black	3 (6.2%)	5 (10.4%)	0.34
Asian	8 (16.7%)	6 (12.5%)	
Other	8 (16.7%)	3 (6.3%)	
Cause of ESKD			
Diabetes	15 (31.2%)	12 (25.0%)	0.65
Non-Diabetes	33 (68.8%)	36 (75.0%)	
Dialysis Vintage (Years)			
Median (IQR)	2.59 (2.34)	3.80 (2.37)	0.10
Dialysis Modality			
Hemodialysis	34 (70.8%)	25 (52.1%)	
Peritoneal Dialysis	8 (16.7%)	11 (22.9%)	0.15
No Dialysis	6 (12.5%)	12 (25.0%)	
Panel Reactive Antibodies (PRA) Pre-transplantation			
PRA > 0	22 (45.8%)	13 (27.1%)	0.09
PRA ≤ 0	26 (54.2%)	35 (72.9%)	
Donor			
Donor Age (Years)			
Mean ± SD	60.50 (20.25)	62.00 (15.50)	0.84
Donor Sex			
Female	29 (60.4 %)	25 (52.1%)	0.54
Male	19 (39.6 %)	23 (47.9%)	

Donor Type			
SCD	13 (27.1%)	12 (25.0%)	
ECD	25 (52.1 %)	26 (54.2%)	0.97
Living Donor	10 (20.8%)	10 (20.8 %)	
Transplant			
Induction Agent			
Lymphocyte-depleting	48 (100%)	47 (97.9%)	
Non-lymphocyte depleting	0	1 (2.1%)	1.00
None	0	0	
Cold Ischemia Time (Hours)			
Median (IQR)	12.45 (13.99)	11.98 (10.80)	0.42
HLA Eplet Mismatches			
Median (IQR)			
Epitype*	58.50 (20.25)	55.00 (18.00)	0.26
AbVer Epitype*	24.00 (8.50)	21.00 (6.00)	0.10
Class II eplets	27.50 (19.25)	22.00 (12.00)	**0.01**
Class II AbVer eplets	10.50 (10.25)	7.00 (6.00)	**0.04**
Maintenance Prednisone			
Yes	14 (29.2%)	12 (25.0%)	0.82
No	34 (70.8%)	36(75.0%)	

*****Class I and II HLA

Abbreviations: AbVer: Antibody-Verified, ECD: Expanded Criteria Donor, ESKD: End-Stage Kidney Disease, HLA: Human Leukocyte Antigens, IQR: Inter-Quartile Range, PRA: Panel Reactive Antibodies, SCD: Standard Criteria Donor

Table.3 Conditional Logistic Regression Models Evaluating Determinants of ABMR in the MUHC Nested Case-Control Study

Univariable Analysis			**Multivariable Analysis**	
Covariates	OR [95% CI]		OR [95% CI]	
			Class II Eplets (Per EMM)	**Class II AbVer Eplets (Per EMM)**
Recipient Characteristics				
Recipient Sex (Female vs. Male)	0.54 [0.22, 1.35]		0.41 [0.15, 1.17]	0.45 [0.17, 1.23]
Recipient Age (> 60 vs. ≤ 60 years)	0.55 [0.20, 1.48]		0.43 [0.14, 1.33]	0.46 [0.15, 1.38]
Transplant Characteristics				
HLA EMM Compatibility Strategy	**Per 10 EMM**	**Per 1 EMM**		
Epitype***	1.17 [0.89, 1.54]	1.02 [0.99, 1.04]	-	-
AbVer Epitype***	1.61 [0.90, 2.87]	1.05 [0.99, 1.11]	-	-
Class II Eplets	1.76 [1.10, 2.84] *	1.06 [1.01, 1.11] *	1.07 [1.02, 1.13] *	-
Class II AbVer Eplets	2.31 [1.01, 5.30] *	1.09 [1.00, 1.18] *	-	1.11 [1.01, 1.21] *
Mycophenolate Dose** (Per 1000mg)	0.65 [0.25, 1.68]		0.55 [0.19,1.58]	0.54 [0.19, 1.55]

*P< 0.05
** Mycophenolate exposure 7-days preceding the index date minus 3-months
*** Class I and II HLA

Abbreviations: CI: Confidence Interval, EMM: Eplet Mismatch, OR: Odds Ratio

Table.4 Sensitivity Analysis – Multivariable Conditional Logistic Regression Models Assessing Risk of ABMR (1- and 2- Month Lag)

	OR [95% CI]	
	Model 1: 1-Month Lag **	Model 2: 2-Month Lag**
Class II Eplets (Per 1 EMM)	1.07 [1.02, 1.13] *	1.07 [1.01, 1.13] *
Mycophenolate Dose (Per 1000mg)	0.68 [0.28, 1.65]	0.51 [0.20, 1.31]
Recipient Sex (Female vs. Male)	0.47 [0.17, 1.29]	0.42 [0.15, 1.21]
Recipient Age (> 60 vs. ≤ 60 years)	0.51 [0.17, 1.52]	0.42 [0.14, 1.32]

* $P<0.05$

** Mycophenolate exposure 7-days preceding the index date

Abbreviations: CI: Confidence Interval, EMM: Eplet Mismatch, OR: Odds Ratio

Table.5 Sensitivity Analysis – Multivariable Conditional Logistic Regression Models Assessing Risk of ABMR (3-Month Lag and Various Strategies to Model Exposure to Mycophenolate)

	OR [95% CI]		
	Model 3: Cumulative mycophenolate exposure	Model 4: Mycophenolate exposure 14-days preceding the index date	Model 5: Mycophenolate exposure 30-days preceding the index date
Class II Eplets (Per 1 EMM)	1.07 [1.02 ,1.13] *	1.07 [1.02, 1.13] *	1.07 [1.02, 1.13] *
Mycophenolate Dose (Per 1000mg)	0.65 [0.29, 1.45]	0.54 [0.19, 1.54]	0.53 [0.19, 1.52]
Recipient Sex (Female vs. Male)	0.37 [0.12, 1.09]	0.42 [0.15, 1.17]	0.44 [0.15, 1.25]
Recipient Age (> 60 vs. ≤ 60 years)	0.43 [0.14 ,1.35]	0.49 [0.15,1.58]	0.53 [0.16, 1.74]

* $P<0.05$

Abbreviations: CI: Confidence Interval, EMM: Eplet Mismatch, OR: Odds Ratio

Table.6 Sequentially Nested Conditional Logistic Regression Models for the Risk of ABMR (3-Month Lag)

	OR [95% CI]		
	Nested Model 1:	**Nested Model 2:**	**Nested Model 3:**
Class II Eplets (Per 1 EMM)	1.07 [0.94, 1.14]	1.08 [1.01, 1.17] *	1.10 [1.00, 1.20] *
Mycophenolate Dose** (Per 1000mg)	-	0.25 [0.04, 1.70]	0.14 [0.01, 1.86]
TacSD	-	-	3.25 [0.77, 13.73]

* P<0.05

**Mycophenolate exposure 7-days preceding the index date

Abbreviations: CI: Confidence Interval, EMM: Eplet Mismatch, OR: Odds Ratio, TacSD: Standard deviation of trough tacrolimus levels

Chapter 4- A Comprehensive Discussion of the Findings

A nested case-control study was conducted to assess whether the risk for ABMR as a function of HLA eplet incompatibility was independent of exposure to mycophenolate or modified by this treatment. Univariable and multivariable conditional logistic regression models demonstrated an increased risk of ABMR by ascending class II, as well as AbVer class II EMMs. This independent association persisted in multiple sensitivity analyses considering different periods of exposure to mycophenolate and lag periods between exposure and outcome. Despite observing protective point estimates with increasing doses (i.e., by 1000mg increments), the confidence interval bounds for mycophenolate crossed 1. Thus, mycophenolate was not found to be an independent predictor of ABMR risk. Moreover, the interaction term between mycophenolate dosage and HLA EMMs did not meet the threshold for statistical significance. Significant interactions between EMMs, age and sex, were also not observed. Sequentially nested models adjusting for intra-patient variability in trough tacrolimus levels (TacSD) and mycophenolate dose, yielded similar results.

In this single-centre study, we found that the degree of incompatibility in HLA class II and AbVer class II EMMs were significant predictors of ABMR, regardless of mycophenolate exposure. In the various multivariable models we fit, an increased risk of up to 7% was observed per each additional class II EMM and up to 11% for each additional AbVer class II EMM. Our findings are in accordance with several studies establishing a relationship between class II EMMs and the risk of ABMR[29,140-142]. These findings also align with publications suggesting EMM load is associated with DSA, transplant glomerulopathy, and graft failure[29,31,143-145]. The independent effect of EMMs on ABMR risk, despite adjustment for contemporary immune suppression exposure, highlights the importance of minimizing HLA incompatibility as a strategy to prevent

immune-mediated injuries like ABMR. Proactive matching of donors and recipients on class II and AbVer class II eplets at the time of transplantation may improve long-term graft survival. It has been recently shown that not all EMMs between donors and recipients infer similar risk for transplant outcomes and specifically death-censored graft failure[31]. As proposed by Zahran et al., a better understanding of the risks associated with individual EMMs may guide priorities for matching and avoid the potential development of DSA, immune-mediated injuries, and graft failure[31].

The optimal strategy for modelling mycophenolate exposure and how it may modify the risk for ABMR in the presence of HLA incompatibility is unknown. Evidence suggests that in a closely monitored context, even short periods of mycophenolate dose reduction or altogether interruption, are associated with the risk of rejection[126,129]. With this in mind, we fit a time-varying mycophenolate exposure model (exposure over 7-days preceding the index date minus 3 months). We also conducted several sensitivity analyses with various time-varying mycophenolate exposure periods (cumulative, 14-days, and 30-days preceding the index date minus 3 months). Prior studies demonstrated a dose-response relationship between exposure to mycophenolate and the risk of rejection[124-129,146]. For example, a Canadian study by Gourishankar et al., reported a 65% reduction in the risk of acute rejection with a 1000mg mycophenolate dose increase over follow-up[127]. However, contrary to prior publications, while point estimates were still suggestive of a protective effect against the risk of ABMR, we did not observe a statistically significant protective effect with increasing doses of mycophenolate (i.e., by 1000mg increments). Furthermore, we hypothesized that HLA EMMs mediated their effect on ABMR risk through the development of DSA. By suppressing the immune response to molecular HLA incompatibility, mycophenolate was expected to serve as an effect measure modifier[147-149]. As such, reductions in mycophenolate

dose were expected to precede an episode of ABMR[147-149]. Importantly, neither the initially prescribed mycophenolate dose nor adjustments in dose that preceded rejection events were informed by baseline molecular HLA mismatches[147-149]. Instead, as described in the current literature, changes in mycophenolate dose typically occurred in response to decreased white blood cell count and neutropenia as well as complications like opportunistic infections [37,126,129,146]. Thus, rather than refute the protective role of mycophenolate in relation to immune-mediated injury, it is possible that the relatively small sample size resulted in our study being underpowered to observe a statistically significant association between mycophenolate dose and ABMR. In addition to mycophenolate, given multiple publications suggestive of an increased risk of immune-mediated injury and graft failure as a function of intra-patient variability in trough tacrolimus levels, we assessed the impact of TacSD on ABMR risk[86,88]. In line with the published literature, our point estimates were suggestive of an increased risk of ABMR with ascending intra-patient variability in trough tacrolimus levels (TacSD) while tacrolimus dose remained unchanged. Still, the association did not meet the threshold of statistical significance. Unlike tacrolimus, mycophenolate does not undergo routine TDM. Thus, future studies should consider TDM of mycophenolate (PK parameters such as MPA trough or AUC_{0-12}) to better inform exposure to this immunosuppressive agent. Also, non-adherence to immunosuppressive agents has been shown to be an important predictor of dnDSA development[33,150,151]. The synergistic effect between nonadherence and HLA incompatibility was illustrated in a retrospective study conducted by Wiebe et al., in which non-adherent kidney transplant recipients with ≥ 10 EMMs in class II DR loci had a 35% increased risk of graft loss versus 8% in adherent recipients with fewer EMMs[151]. Evaluation of non-adherence, however, was not routinely documented in medical health records of eligible participants from our retrospective cohort. Furthermore, as described in our literature

review, when prescribed similar doses of mycophenolate, MPA PK varied by patients' BMI and as a function of albumin levels and eGFR. Consequently, intra- and inter-patient variability in mycophenolate exposure as a function of these parameters can further modify the association between mycophenolate dose and transplant outcomes. To quantify the true effect of mycophenolate exposure on the risk of rejection, implementation of systems pharmacology models that consider mycophenolate PK and PD, and how they are influenced by patient characteristics, as well as drug-drug interactions, will help inform how personalized MPA prescriptions can improve transplant outcomes.

Lastly, when modelling prescription data, it is also important to consider how best to handle treatment gaps, medication switching, and time-varying confounders[152-154]. Observational studies using conventional time-varying models such as cox proportional hazards and random effects models might produce biased effect estimates because of their inability to adjust for time-varying confounders (e.g., tacrolimus trough levels, eGFR)[152-155]. To better address such confounders, future cohort studies could apply marginal structural models (MSM)[155-158]. By assigning an inverse probability of treatment weighting (IPTW) corresponding to the likelihood of being exposed given a set of covariates (confounders) at that specific time point, MSM creates a "pseudo-population" whereby the impact of confounders is removed and the association of treatment with the outcome of interest can be ascertained[155-158]. However, it is important to note that weighted covariates in MSM are restricted to sufficiently large sample sizes[155-158].

The number of elderly patients requiring kidney transplants has been steadily increasing over the recent years, making age a significant factor in determining recipient and graft outcomes. Several studies have demonstrated that the risk of rejection decreases with age[96,159-161]. This has been suggested to be a consequence of decreased immunity in elderly recipients resulting from

age-related immune senescence[161-164]. This effect was observed in a study by von Moos and colleagues, showing that older recipients (≥ 60 years) had up to a 5.6 times lower risk of developing dnDSA when compared to pediatric (< 10 years) kidney transplant recipients[162]. Our study did not demonstrate a statistically significant association between recipient age and ABMR risk. Yet, our point estimates, in accordance with the published literature, suggested a protective effect, whereby older kidney transplant recipients (> 60 years) were less likely to experience ABMR. Importantly, despite the suggested reduced risk, increased comorbidities, polypharmacy, and variability in the PK and PD of immunosuppressive agents attributed to hypoalbuminemia, and reduced drug clearance, have been proposed to make rejection episodes more detrimental both to the elderly patients themselves, as well as their grafts, in comparison to their younger counterparts[159,161,164,165]. To mitigate risks, and account for changes in immunological activity, it has been proposed that elderly recipients require less aggressive immune suppression regimens, and that tailored age-dependent dosing should be considered[161,163-165]. Future larger studies using both observational and experimental design may allow evaluation of how age may affect the risk of ABMR in a more granular fashion. This can further guide changes to prescribed immune suppression regimens and optimize graft outcomes beyond the initial diagnosis of rejection.

Recipient sex is also an important predictor of transplant and survival outcomes. However, the current literature on recipient sex differences in kidney transplantation remains conflicting. A large study of 73,477 kidney transplant recipients from the US Registry of Transplant Recipients, showed that women had 10% increased odds of experiencing rejection early post-transplant and were at an increased risk for earlier transplant loss in comparison to men[166]. This has been attributed to women having stronger immune reactivity driven by differences in sex hormones, pregnancy-induced sensitization, and variability in the PK of prescribed immune suppression such

as those observed with lower BMI and decreased MPA metabolism/clearance[94,95,131-133,166]. This risk may also be mitigated by gender-related adherence to immunosuppressants. Bocquemont et al., found that through monitoring of electronic pillboxes, young female recipients demonstrated better adherence to immunosuppressive agents than males of the same age (OR: 3.26 (95% CI:1.43, 7.45))[167]. Despite an overall small number of participants (n=26 female recipients) in our study, we found point estimates to be suggestive that females may have a protective effect in relation to ABMR in both univariable and multivariable models, albeit the associations were not statistically significant. Notably, previous studies evaluating the association between sex and rejection, have demonstrated inconsistent results, which may be attributed to heterogeneity in the composition of female recipients represented in the studied populations[94,167,168]. This is in relation to access to kidney transplantation, patient post-transplant care, and the prevalence of highly sensitized female kidney transplant recipients[165,169]. Also, previous studies may not have modelled HLA incompatibility at the level of the epitope or proceeded with transplantation only in the context of negative virtual crossmatch results. Importantly, women would be at greater risk for ABMR when proceeding with transplantation in the presence of pre-formed DSA, which women are more likely to exhibit due to pregnancies[12,168,163,169,170].

Finally, it is important to note that immune response may vary as a function of both age and sex. An analysis conducted by Lepeytre and colleagues demonstrated that female recipients (≥45 years) had a reduced risk of death-censored graft failure when compared to males of an equivalent age (HR: 0.95 (95% CI: 0.91,0.99)), while younger females (15-24 years) had an increased risk of graft failure compared to their male counterparts (HR: 1.28 (95% CI:1.06, 1.53))[94]. Thus, suggesting that a protective effect on the risk of death-censored graft failure observed in older females be in part, a result of the interplay between age, and sex. This complex

interaction may be the cause of several factors such as differing sex hormones, age-related immune reactivity, and gender-related medication adherence[94,167,168]. Aligned with these assumptions, the mean (± SD) age for female recipients in our cohort was 54.42 (± 11.98) years with most females presumed to either be peri- or post-menopausal. Unlike the study by Lepeytre et al., our sample size was too small to allow the evaluation of interactions between multiple variables[94].

Our analysis is the first attempt at estimating the mitigating effect of mycophenolate on HLA eplet incompatibility and ABMR risk. It intended to consider the time-varying effect of mycophenolate exposure and overcome limitations of prior studies that considered only baseline exposure to types of immunosuppression rather than the type and dose of exposure over the post-transplant course. It may be plausible, however, that clinicians having a high index of suspicion of rejection might have augmented immunosuppression (including mycophenolate), compromising the capacity to demonstrate the association between mycophenolate exposure and outcome. The implementation of a 3-month lag between ABMR event and last prescription of mycophenolate considered, aimed to address this issue. To ensure internal validity, the case-control study design is dependent on an unbiased selection of controls. Being that matched controls were selected from a clearly defined source population (MUHC cohort), the concern around bias in control selection is minimized. Another concern arising when relying on *for-cause* biopsies relates to the underdiagnosis of subclinical ABMR. However, current practices of routine DSA monitoring at the MUHC and verification of ABMR by biopsies upon detection of DSA, increase the likelihood of ABMR case ascertainment. Moreover, DSA status assignment was done by a single HLA laboratory director and ABMR ascertainment was also standardized to a single Banff classification (Banff 2017) across the cohort years. Although relying on HLA molecular typing to infer HLA eplet incompatibility, not all 11 HLA loci were considered. Thus, we cannot rule out the possibility

of EMMs related to loci, for which typing was not available like HLA-DP, for example. A major limitation of this study was the relatively small number of cases and matched controls included in our cohort. Given the small sample size, our study may have been underpowered to evaluate the association with ABMR and effect measure modification related to mycophenolate dose. Also, as with any retrospective cohort study, our study is vulnerable to residual confounding. Given the relatively small number of events, and to avoid model overfitting, we were limited by the number of covariates in addition to HLA EMMs and mycophenolate exposure that could be included in our multivariable models. For example, recipient and donor self-reported ancestries may be deemed relevant confounders in the relationship between the main exposure (HLA EMMs) and ABMR risk. However, the key aspect in which ancestry likely informs immune-mediated injury is already addressed by the main exposure. Based on a sample size calculation of prior OR estimates for risk of transplant glomerulopathy as a function of HLA-DR EMM load with a β of 0.8 and α of 0.05, 186 participants (cases and matched controls) would have been needed to reject the null hypothesis. A larger nested case-control study with unambiguous allele-level typing from a multicentre Quebec cohort is underway that may better address some of the mentioned concerns. Lastly, our cohort represented a single North American transplant centre using alemtuzumab and maintained on a dual agent maintenance immune suppression regimen (mycophenolate, tacrolimus) functioning within a publicly funded health care system. Consequentially, the findings of this single-centre study may not be generalizable to other centres and populations.

Chapter 5 - Conclusions and Summary

Summary

Advances in immunosuppressive regimens have been instrumental in improving patient and graft outcomes. Despite their success, decreased long-term kidney transplant survival has left many kidney recipients without the benefits of transplantation for their lifetime. Contributing factors include donor-recipient HLA incompatibility, inadequate immune suppression, graft injury, and adverse transplant outcomes. ABMR is said to be one of the leading causes of premature graft loss in kidney recipients and is the result of recipient pre-formed or dnDSA directed against donor HLA. To mitigate immune-mediated injuries as a result of HLA incompatibility, kidney transplant recipients are prescribed standard mycophenolate dosing regimens, with doses being reduced temporarily and at times indefinitely, when complications are encountered. This management strategy, however, does not account for intra and inter-patient variability in mycophenolate PK and PD, leaving recipients vulnerable to inferior graft outcomes such as rejection, and transplant complications including infections and cancer, all of which are associated with inadequate immune suppression. The primary objective of this thesis was to assess the impact of HLA eplet incompatibility on the risk of experiencing ABMR, whether mycophenolate exposure mitigates this risk, and how this risk is further modified by recipient age and sex. Our findings demonstrated that each additional EMM in class II and class II AbVer HLA independently increased the risk of ABMR. Cumulative and time-varying mycophenolate doses (7-,14-,30-days preceding the index date), the female sex and recipient age (> 60 years), showed a trend in being protective against the risk of ABMR. Significant interactions between recipient age, sex and the risk of ABMR were not observed.

Our literature review on mycophenolate identified key determinants of mycophenolate PK and assessed the impact of these PK parameters along with pertinent recipient and laboratory variables on important clinical outcomes in kidney transplant research. Literature review findings were then used to guide the construction of mycophenolate exposure models and the choice of covariates included. Fluctuating albumin concentrations, recipient BMI, and eGFR demonstrated an effect on MPA pharmacokinetics while associations with recipient age and sex varied across studies. Studies also demonstrated that increasing MPA exposure was associated with a reduced risk of rejection and graft failure, as well as an increased risk of breakthrough infection. Significant heterogeneity in patient populations, mycophenolate exposure modelling, outcome definitions, and covariates included were observed. These findings may partially explain our inability to observe a significant impact of mycophenolate exposure on ABMR risk.

Future Directions

This thesis summarizes findings from a single-centre study. A larger multicentre study is underway, that should have greater power to assess the modifying effect of dynamic exposure to mycophenolate as well as recipient sex, and age on the risk of ABMR by ascending HLA incompatibility at the eplet level. A larger sample size will also allow for a better evaluation of the interaction between age and sex on ABMR risk. Future studies will also consider dynamic exposure to mycophenolate, and tacrolimus all while simultaneously accounting for various clinical and laboratory measures that are likely to affect exposure and adherence to mycophenolate. To achieve a more accurate assessment of true mycophenolate exposure, and the risk of ABMR in kidney transplant recipients, future studies should incorporate mycophenolate TDM. Tools informing on how MPA exposure models may be modified by patient characteristics will permit for the development of personalized mycophenolate prescription patterns and potentially, prevent

or minimize inferior transplant outcomes. Lastly, this thesis has focused exclusively on ABMR. With the increasing number of elderly patients requiring kidney transplants and being that post-transplant infections are now the leading cause of graft loss and all-cause mortality; future studies should investigate additional transplant outcomes such as graft failure and complications of immune suppression like cancer and infections.

Conclusion

Our study identified HLA class II and AbVer class II EMMs as being independent predictors of ABMR risk. A significant protective effect of mycophenolate exposure, recipient age, and sex on the risk of ABMR was not observed. This may in part be explained by the small sample size, limitations of relying on mycophenolate prescription data to inform exposure, and inability to control for time-varying covariates known to affect mycophenolate PK. To confirm these findings, future studies assessing exposure to mycophenolate should address the complexity of dynamic mycophenolate exposure along with its determinants in greater depth. As kidney transplant recipients are obliged to adhere to an immune suppression regimen to sustain their transplanted kidney, a better understanding of individual immunosuppression needs and the dynamic relationship between mycophenolate dose and its modifying effect on HLA eplet incompatibility is needed. This may help inform personalized modifications in mycophenolate prescribing patterns and reduce the risk of inferior transplant outcomes associated with inadequate immune suppression for the most vulnerable recipients.

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