Acquired Low Immunoglobulin Levels and Risk of Clinically Relevant Infection in Adult Patients with Systemic Lupus Erythematosus Cohort Study

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Infection is a leading cause of mortality in systemic lupus erythematosus (SLE). Low immunoglobulin levels might be a potential risk for infection. We aim to examine the effect of acquired hypogammaglobulinemia on risk of infection in adults with SLE.

Methods: We used Toronto Lupus Cohort to compare adult SLE patients who had acquired any low immunoglobulin levels to those who did not with respect to infection using time to event analysis. Several propensity score methods were used to improve group balance.

Results: Patients with hypogammaglobulinemia had longer disease duration, more proteinuria and history of lupus nephritis. Low IgA levels increased the risk of infection. Recovery of immunoglobulins occurred in 64 percent of acquired hypogammaglobulinemia over four years.

Conclusion: The majority of acquired hypogammaglobulinemia in adults with SLE is transient. Only low acquired IgA increases the risk of infection among adult patients with SLE. Mechanism of this association requires further investigation.
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List of Abbreviations:

1. SLE: Systemic lupus erythematosus.
2. EBV: Epstein–Barr virus.
3. ANA: Antinuclear antibodies.
4. APA: Anti-phospholipid antibodies.
7. SDI: The SLICC/ACR Damage Index.
8. SMR: Standardized mortality ratio.
10. CVID: Common variable immune deficiency.
12. BAFF: B cell-activating factors.
14. MCAR: missing completely at random.
15. MAR: missing at random.
16. MNAR: missing not at random.
17. VIF: variance inflation factor.
18. PS: Propensity score.
Chapter 1
Literature review

Introduction

In this introduction, I cover several subjects that will be encountered in our study of the relationship between acquired low immunoglobulins and the risk of clinically relevant infection in adult patient with systemic lupus erythematosus (SLE). I first review SLE as disease, including important demographic factors, immunological alterations and clinical phenotypes. Then, I review the literature for infection in the context of SLE, including risk factors and outcomes of infection in the vulnerable population of SLE. I finish my introduction by reviewing our exposure variable (low immunoglobulins) with respect to structure and function in the available literature in the context of SLE.

Systemic Lupus Erythematosus

Disease overview

Systemic lupus erythematosus (SLE) is a chronic multi-system disease in which an underlying aberrant immune system leads to inflammation in various organs, and possibly consequent damage (Tsokos 2011). Incidence and prevalence of the disease varies significantly – from 1 to 7 per 10,000 patients to 19 to 159 per 10,000 patients, respectively, in the United States (Kaul et al. 2016; Lim et al. 2014; Somers et al. 2014). Such variation is likely due to the use of different definitions, as well as studies’ standardization of age and racial background (Kaul et al. 2016). Ethnicity is an important variable in disease occurrence, severity, phenotypic presentation and prognosis (Gonzalez, Toloza, and Alarcon 2014; Johnson et al. 2006). For example, African, Hispanic and Native American populations tend to have more severe disease outcomes and,
consequently, increased mortality rates (Flower et al. 2012; Gonzalez, Toloza, and Alarcon 2014; Mok, Kwok, and Yip 2013; Johnson et al. 2006). Sex is another influential factor for disease occurrence and activity. Women are more likely to be affected by the SLE, with an estimated prevalence of 9:1 and up to 15:1 in premenopausal period (with lower prevalence in post-menopausal period (Pons-Estel et al. 2010). This skewed risk can also be appreciated in patients with Klinefelter’s syndrome (XXY karyotype). Men with this syndrome have a 14-fold increased risk of SLE compared to those without the syndrome (Scofield et al. 2008). This indicates, at least partially, the importance of hormonal effect on the disease pathogenesis and the impact of the X chromosome in the pathogenesis (Kaul et al. 2016; Scofield et al. 2008). However, the complexity of the disease is not limited to the demographics of the patient, but it can also be influenced by the polygenic nature of the disease – and resulting multiple immunological alterations – as well as by various environmental triggers.

**Disease pathogenesis**

Pathogenesis of SLE is intricate. Typically, disease is polygenic, with multiple variants associated with disease located in genes related to important pathways in our immune system (James 2014; Ding et al. 2018). The strongest association is for C1q deletion mutation. C1q is a component of the complement cascade that facilitates waste removal and pathogen elimination. Hence, the deletion of C1q can result in accumulation of nuclear material and render a patient exposed to antibody production and, consequently, autoimmunity (Truedsson, Bengtsson, and Sturfelt 2007). Other genes include HLA-B8 and HLA-DR3, which are involved predominantly in antigen presentation. Others, which are related to enzymatic break of nuclear material, include TREX1 or type 1 interferon production like IRF5 and IRF7 (Graham et al. 2007; Price et al. 1999; Namjou et al. 2011; Niewold et al. 2008). All these variations (and many others) represent genetic
tendency that may predispose to SLE development or potentiate its activity. Yet, genetic tendency alone does not explain the pathogenesis of the disease (Gergianaki and Bertsias 2018). This can be appreciated from concordance rate between monozygotic twins, which lies anywhere between 30 to 50 percent (Deapen et al. 1992; Niewold 2015). Contribution from environmental factors might provide the potential trigger for the disease (Kaul et al. 2016). These factors might include viral infection, such as Epstein–Barr virus (EBV), the use of certain medications, like hydralazine, or even toxin exposure, such as from smoking (Kaul et al. 2016). The mechanism by which these factors influence the immune system varies. For example, EBV infection potentiates type 1 interferon production and stimulates autoantibody production (Yadav et al. 2011; Larsen et al. 2011). Hydralazine, on the other hand, inhibits DNA methylation. This inhibition renders areas of the DNA exposed to recognition by the immune system, as well as modifies genes transcription (Gorelik et al. 2007). These examples show how internal factors, such as genetic mutations, or external factors, like infection, can potentiate important pathways involved in an imbalanced immune system and highlight the importance of understanding such systems in SLE (Crispin et al. 2010). To understand this further, we can generally categorize the immune dysregulations in SLE into: altered waste clearance; changes in the innate immune system; changes in adaptive immune system; autoimmunity in the form of autoantibodies production; and changes in cytokines milieu (Kaul et al. 2016; La Paglia et al. 2017). Unfortunately, the initial tipping point of homeostatic immune system in SLE patients is not very clear. However, clearly the interactions between these categories – including both internal and external factors – may lead to various clinical phenotypes (ranging in severity). The interaction between these categories also provides a basis for risk modifications and drug-targeted therapies in this unique disease (Touma and Gladman 2017).
**Disease presentation and clinical phenotypes**

Although SLE can involve almost any organ in the body, certain organs are more likely to be affected by the disease. Mucocutaneous, musculoskeletal, hematological, respiratory and kidney involvement represent commonly involved organs (Cervera et al. 2003). Interestingly, organ involvement varies in terms of significance. For example, kidney involvement in SLE is estimated to affect up to 45 percent of patients in their lifetime compared to mucocutaneous involvement, which occurs in up to 80 percent of patients (Watanabe and Tsuchida 1995; To and Petri 2005). The importance of kidney involvement comes from its prognostic impact. Lupus nephritis is an important cause of increased morbidity and mortality in patients with SLE (Yurkovich et al. 2014). The hallmark of lupus nephritis is usually proteinuria, which reflects ongoing inflammation in the kidney parenchyma (particularly glomeruli). Unfortunately, such involvement may result in loss of several important serum proteins, such as albumin, protein C and S, lipoprotein and immunoglobulins (Gyamlani et al. 2017). Consequences of these losses may have deleterious effects on patients, including increased risk of thrombosis, altered lipids profiles, and possibly increased risk of infection (Kronenberg 2005; Gyamlani et al. 2017; Wilfert and Katz 1968; Alwadhi, Mathew, and Rath 2004). Lung involvement has also been attributed to significant morbidity and mortality in lupus patients. This was evident in a population-based study which looked at the risk of mortality in SLE patients admitted with infection. In this study, inpatient mortality was higher in bacterial infection requiring mechanical ventilation compared to the general population (Tektonidou et al. 2015). Furthermore, lung involvement due to SLE seems to be a risk factor of major infection. This was demonstrated in a nested case-control study after adjustment of appropriate confounders. Both studies highlight the potential vulnerability of SLE patients with lung involvement – particularly to infection risk (Jeong et al. 2009). However, not
all types of lung involvement are equally important. Pleural inflammation is a common presentation in lupus patients, but it may not carry similar risk compared to pulmonary hemorrhage, which carries a high risk of mortality (Schwab et al. 1993). Furthermore, lung involvement can be mimicked by lung infection (or overlap with it), which makes it hard to differentiate both – especially in observational studies – unless strict definitions of different types of lung involvement and infection are used.

Unfortunately, solid organs may not be the only target of SLE. Hematological changes are key features of the disease as well. Various cell lines can be affected, including leukopenia (and particularly lymphopenia), anemia, and thrombocytopenia (Fayyaz et al. 2015). This drop in the levels might be related to consumption of these cells through antibody-mediated processes or a decrease in bone marrow production due to ongoing inflammation and associated inflammatory cytokines (Giannouli et al. 2006). Several other serological changes take place in SLE patients, including consumption of complements and autoantibodies production, such as antinuclear antibodies (ANA), anti-DNA, and anti-phospholipids antibodies. Some of these abnormalities have clinical implications and prognostic value, while others are of primary value in the diagnosis of a patient. To explain further, let’s take common hematological changes as an example. Although leukocytes, neutrophils and lymphocytes are essential components of our immune system, drops in their counts (as part of SLE disease phenotype) do not strongly correlate with disease activity or predispose patients to a higher risk of infection unless the counts are significantly low (Martinez-Banos et al. 2006; Lertchaisataporn et al. 2013b; Vananuvat et al. 2011; Velo-Garcia, Castro, and Isenberg 2016). In contrast, anti-DNA and complement levels have better correlation with disease activity – which may suggest a stronger role in pathogenesis (Petri et al. 2013). Anti-phospholipid antibodies (APA), on the other hand, play many roles in disease phenotypes and
prognosis. APAs are a group of autoantibodies that are directed against phospholipid binding proteins (Corban et al. 2017). The importance of these antibodies is through their role in the pathogenesis of the antiphospholipid syndrome. This syndrome is characterized by arterial and venous thrombosis, recurrent miscarriages, and the presence of these antibodies. The condition carries significant morbidity and mortality in affected patients who may have the disease secondary to SLE (usually referred to as secondary antiphospholipid syndrome) (Ruiz-Irastorza et al. 2004). Patients with antiphospholipid syndrome have increased risk of cardiovascular, cerebrovascular and valvular heart diseases (Corban et al. 2017). In addition, patients with secondary antiphospholipid syndrome tend to have increased frequency of thrombosis and fetal losses compared to primary antiphospholipid syndrome (Danowski et al. 2009). Patients with SLE may also have these antibodies in the absence of clinical thrombosis or pregnancy losses. This was demonstrated in a study from the Toronto Lupus Cohort – with an estimation of 4 percent of all patients with SLE (Abu-Shakra et al. 1995). Unfortunately, the presence of these antibodies carries an independent risk of morbidity and mortality in SLE patients. In a cohort of SLE patients, antiphospholipid syndrome was associated with increased mortality of patients after adjustment of other variables (Drenkard et al. 1994). Antiphospholipid antibodies have been associated with increased risk of pulmonary hemorrhage, pregnancy complications in SLE patients, and even risk of infection (Espinosa et al. 2002). The latter was investigated in a nested case-control study that examined predictors of major infections in SLE patients. In that study, the presence of antiphospholipid antibody was associated with major infection in univariate analysis. However, in final logistic regression model – after adjustment of appropriate confounders – it was not associated with infection (Ruiz-Irastorza et al. 2009).
This discrepancy between aberrant phenotypes and clinical implications of such phenotypes undermines the use of extrapolated data from other diseases to understand immunological changes in the context of SLE.

**Disease classification**

Given the diversity in clinical presentation, outcomes and complications, clinical ascertainmetn of the disease and disease activity become much more crucial in conducting research in SLE. This requires the use of validated classification criteria and disease indices to confirm appropriate classification of patients, and to objectively measure disease activity (Kaul et al. 2016; Touma, Urowitz, and Gladman 2013).

Several approaches to the development of classification criteria have been devised. These criteria aim to define homogenous groups of patients by sets of clinical and laboratory findings (including clinical, serological and pathological results) (Wallace, Hahn, and Dubois 2013; Johnson et al. 2007). The two most commonly used criteria are the revised American College of Rheumatology (ACR) classification criteria and the Systemic Lupus International Collaborating Clinics (SLICC) criteria (Amezcua-Guerra et al. 2015; Hochberg 1997; Petri et al. 2012). It should be noted that variations between criteria sets affects the sensitivity and specificities associated with them (Amezcua-Guerra et al. 2015). Recently, for instance, a joint effort of the American College of Rheumatology and European League Against Rheumatism developed new classification criteria aimed at achieving higher sensitivity and specificity. These proposed criteria require the presence of positive ANA and components of 11 domains with differently weighted components. The criteria are now undergoing further validation (Tedeschi et al. 2018; Johnson, Khanna, et al. 2018; Tedeschi SK 2019; Aringer et al. 2016; Leuchten, Hoyer, et al. 2018; Leuchten, Milke, et al. 2018; Schmajuk et al. 2018; Mosca et al. 2019). Several validated indices have been used widely in
multiple clinical trials, including the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (Mikdashi and Nived 2015). This composite index measures disease activity by giving a weighted score to each of 24 descriptors which reflect the spectrum of clinical and laboratory features of the disease. The total score ranges from 0 to 105, with a score more than or equal to 6 considered clinically active disease (Gladman, Ibanez, and Urowitz 2002). Using such an activity index allows us to assess disease activity in a standardized fashion, and also use a common language when comparing patients across different centres. It also holds important prognostic values for SLE patients. For example, a SLEDAI score of 11 to 19 has an increased relative mortality risk of 4.74 compared to a score of 0, while a score >20 carries a relative risk of 14 (Cook et al. 2000).

Patients with SLE face several challenges other than their disease activity and organ involvement, including a list of comorbidities that may result either from the disease itself, the medications used to control the disease, or accrual damage related to one or both of these factors over time (Bruce et al. 2015). The SLICC/ACR Damage Index (SDI) is a validated index that was developed to measure accumulated damage from the onset of SLE (Gladman et al. 1996; Gladman et al. 1997). The SDI scores 12 different organs for the presence of irreversible damage – regardless of the cause – occurring after SLE diagnosis and usually lasting at least six months(Gladman et al. 1996). High damage scores predict further damage and mortality in SLE patients (Gladman et al. 1996; Gladman et al. 1997; Rahman et al. 2001). This was perfectly demonstrated in a multicentre international cohort study that examined the factors associated with damage in a multi-state model. The study showed that utilization of SDI allowed the estimation of progression to higher score or death (Bruce et al. 2015). This is interesting because SDI scores can then be utilized as a decent outcome serving as a surrogate of mortality in short-term studies.
Infection risk in SLE

One of the most common and persistent threats to SLE patients during their lifetime is risk of infection. Several studies have demonstrated the persistent nature of infection risk – both early and late in the course of the disease (Gladman et al. 2002; Goldblatt et al. 2009; Petri and Genovese 1992; Duffy, Duffy, and Gladman 1991). This was demonstrated in the literature in several ways. First, evidence was highlighted by a multicentre international study in 2006 for patients with SLE that examined standardized mortality ratio (SMR) among SLE patients from the 1970s to 2001. A total of 9,547 patients from 23 centres were included in the study, which demonstrated that infection increased mortality rate five-fold (SMR=5). It demonstrated also that pneumonia significantly contributed to such an increase (SMR=2). The increase in mortality seems to decline over calendar-years (from SMR of 32 from the period 1970 to 1979 to a SMR of 5 in the period between 1990 to 2001. It was also found that all-cause mortality SMR was highest among younger patients and early in the disease course (Bernatsky et al. 2006). However, it was not clear if this was true for infection-specific mortality. This issue was examined again in several population studies, including a population-based study from the United Kingdom from 1999 to 2012 which used a health administrative database. In this study, the overall SMR was higher in SLE compared to controls. Infection, however, was not the most common cause of death – in contrast to what had been suggested in previous studies. This fact could be related to a misclassification of respiratory infection under respiratory causes of infection, which was the fourth-highest cause of mortality in this study. What was interesting about this study is the different frequency of infection-related mortality when patients were stratified according to the time of SLE diagnosis. Infection-related mortality was most commonly seen in the first 10 years of diagnosis of SLE (Rees et al. 2016). More recently, an abstract presented at the American college of Rheumatology from the Toronto
Lupus cohort studied age-specific mortality. In this study, the authors examined SMR in a cohort that was established since 1970 and compared it to national data (Statistics Canada for a matching period) for all-cause and cause-specific mortality. Several interesting observations were noted, including that the SMR related to infection significantly dropped over the years. This trend was also seen among other causes of mortality, such as cardiovascular and malignancy. When infection-specific mortality was stratified by age, most of the risk occurred among younger patients below 40 years of age (SMR= 30.2, 95% CI: 14.4-46) compared to those over 40 (SMR= 3.5, 95% CI: 2.5-4.5) (Tselios K 2018). These findings highlight the persistent nature of infection, with special emphasis on its effect on younger patients and those in early disease course.

**Nature of infection in SLE patients**

The nature of infections in patients with SLE varies significantly. While common bacterial infections contribute the most to infection risk, other pathogens – such as viral, fungal or opportunistic organisms – have also been described in the literature (Noël et al. 2002; Ramos-Casals et al. 2008; Zonana-Nacach et al. 2001; Godeau et al. 1994; Chen et al. 2007). Data from the Toronto Lupus Cohort that examined 363 patients with SLE, from 1987 until 1992, revealed 148 infections recorded among 93 patients. Although 64 percent of the infections were bacterial, the other 36 percent were divided among viral (27 percent), fungal, and protozoal infections (9 percent). Infection with multiple organisms occurred in only seven patients. What is important about this study is that it included both milder infections (which are managed in outpatient settings) and those requiring inpatient hospitalization. One-third of the infections in this study required hospitalization. Interestingly, mortality was higher in patients with infections (20.4 percent) compared to those without infection from the same cohort (8.6 percent) – matched for age and duration of follow-up (Gladman et al. 2002). It was not clear if the increase in mortality was driven
by a specific type of infection or not. However, bacterial infections continue to be the most common type of infection and are thus a major cause of morbidity and mortality (Danza and Ruiz-Irastorza 2013; Ruiz-Irastorza et al. 2009). Unfortunately, the identification of bacterial organism is not always possible. This fact can easily be appreciated in previously published studies. For example, in a study by Ruiz-Irastorza et al. which looked for predictors of infection in SLE patients, the type of infection was only identified in half of the patients (Ruiz-Irastorza et al. 2009). Most of the ascertained infections in the literature are gram positive organisms, including staphylococcus and streptococcus species. They are followed by gram negatives, represented largely by E. coli species, and then atypical such as mycobacterium species (Gladman et al. 2002). This distribution changes if we stratify infection according to the site or severity, although it does not seem to vary greatly from the general population (Danza and Ruiz-Irastorza 2013). In fact, the distribution of infection may actually reflect prevalence of local pathogens. For example, in a study of the nature of infections in an Indian SLE cohort, the most common pathogen was tuberculosis, which is much more endemic in India than in Western populations (Shyam and Malaviya 1996). This finding was further reflected in other international SLE cohorts (Al-Rayes et al. 2007; Yun et al. 2002).

**Risk factors for infection in SLE patients**

Several factors have been associated with infection risk in SLE populations. These factors can be grouped into the following categories: SLE inherent factors; immunosuppressive medications; and accrued damage.

**SLE inherent risk factors**

Immunological alterations are important factors in risk of infection among SLE patients (Cuchacovich and Gedalia 2009). Impairment of innate response, including phagocytosis,
chemotaxis and oxidative metabolism, as well as a defective complement system, have been detected in SLE patients (Alarcon 2006; Marquart et al. 1995). Furthermore, T-cell defects have also been observed and seem to correlate with disease activity (Bermas et al. 1994). However, the causes of such alterations could be related to the disease itself or be associated with exposure to medications such as prednisone (Bermas et al. 1994). Several other clinical variables have been identified as associated factors with infection risk in SLE. Disease activity is the most commonly reported – and the most consistent of all the variables in the literature (Danza and Ruiz-Irastorza 2013). An SLEDAI score greater than 8 was found to be of significance in infection requiring hospitalization among the Toronto Lupus Cohort (Duffy, Duffy, and Gladman 1991). This was further confirmed in another study from the Johns Hopkins cohort, which examined factors associated with infection requiring hospitalization. In this study, disease activity in the form of CNS lupus and previous hospitalization were risk factors, along with prednisone and immunosuppressive use (Petri and Genovese 1992). Even for infections which are managed in outpatient settings, this variable was associated with infection risk (Zonana-Nacach et al. 2001).

Not all organ involvement with active disease is equally important for predisposition to infection in SLE. Patients with lupus nephritis, for instance, seem to be particularly vulnerable to such an outcome. This was demonstrated by several case series and international cohorts of SLE and was confirmed in a large population-based study in the United States (U.S.). In a study by Feldman et al., the incidence rate of serious infection among patients with SLE and lupus nephritis patients was determined. The authors used previously validated SLE cohorts that utilize Medicare-extracted health claims. A total of 33,565 patients with SLE (of whom 7,113 had lupus nephritis) were included. There were 9,078 events of infection (including bacterial, fungal, viral or opportunistic) in the total SLE cohort, and 3,494 events in the lupus nephritis cohort. The incidence
of infection was much higher in the lupus nephritis cohort versus the total SLE cohort (23.9 versus 10.8 per 100 person-year) after adjustment of relevant confounders which support the vulnerability of such a group of SLE patients. In both cohorts, the author used a cox-regression model to examine risk factors for serious infection. Risk factors for serious infection in SLE patients included: being male; being older than 50; being Black or Native American; the use of prednisone or immunosuppressive medications. Meanwhile, the use of Plaquinil was protective (Feldman et al. 2015). Of course, the results of this predictive model are limited because of the lack of a major important confounder, which is disease activity. However, the identified factors were commonly reported in several other academic cohorts. Ruiz-Irastorza et al. conducted a nested case-control study aimed at identifying predictors of major infections among adult patients with SLE. In this particular study, 83 patients with major infections were matched on age at diagnosis and follow-up time to 166 controls. ‘Major infection’ was defined as infections that caused dissemination, deep organ involvement, hospitalization, or death. The logistic regression model was used to examine predictors of major infection. Several factors were found to be associated with increased odds of major infections – after adjustment of all potential confounders. These included lung involvement at the index date, prednisone dose, and anti-phospholipids antibodies. In contrast, antimalarial use was associated with reduced odds of major infection and a protector of infection in the form of effect modifier to prednisone dose (Ruiz-Irastorza et al. 2009). All of these findings suggest that disease activity overall, or specific domains of activity such as lung or kidney involvement, predispose adult patients with SLE to major infection that likely requires hospitalization.

Many other clinical and serological variables, including leukopenia, low complements and elevated anti-DNA, have also been reported (Danza and Ruiz-Irastorza 2013). All of these
variables are captured in the SLEDAI disease measurement index (Bombardier et al. 1992), but the effect of these variables individually on the infection risk is confounded by other associated variables. For example, leukopenia is a common feature of disease activity. However, the impact of such abnormality in general is not necessarily significant for infection risk (Carli et al. 2015). In a systematic review that examined published papers on leukopenia, including its subsets (neutropenia and lymphopenia), the authors found 17 papers addressing this issue. Unfortunately, the heterogeneity and low quality of the studies made it hard to make appropriate inference (Carli et al. 2015). The variation of these studies and reports, in terms of their findings, are likely related to definitions used for infection, severity, settings (inpatients or outpatients), length of follow up and study designs, included variables in the analysis, method of inclusion, and model used for analysis.

*Immunosuppressive medications*

Immunosuppressive medications have always been regarded as potential contributing factors for the risk of infection. Prednisone, in particular, has been deemed to be problematic (Ruiz-Irastorza, Danza, and Khamashta 2012). The mechanism of this effect involves alteration at multiple components of the immune system, including reduction in monocytes, prolonged T-cell suppression, modified fibroblast and endothelial cell function (Cutolo et al. 2008). Furthermore, the impact of steroid on infection risk seems to follow a dose response pattern (Dixon et al. 2011). Many studies, including small and large academic cohorts, highlight the potential risk of prednisone. Several definitions of prednisone are used in the literature, which may explain the slight variations in the findings of these studies. The two most common definitions are prednisone use (which might be at ‘index date’ or ‘ever use’) and prednisone dose (which is usually treated as a continuous variable at index date or as a cumulative dose). In the Ruiz-Irastorza et al. study,
which examined predictors of major infection in SLE using nest case-control design, the authors defined prednisone in the following two ways: prednisone use at index and prednisone dose treated as continuous variable at index. Both definitions were associated with major infection in univariate analysis. However, in the final logistic regression model, only prednisone dose was significant (Ruiz-Irastorza et al. 2009). In another study of the nature and outcome of infection in SLE patients by Gladman et al, using the Toronto Lupus Cohort, prednisone was also examined using two definitions: steroid use (ever and at index) and steroid dose at index. In this nested case-control study, all definitions of prednisone were associated with infection in univariate analysis. However, in the final logistic regression model, only the definition ‘prednisone use ever’ increased the odds of infection after adjustment of appropriate confounders (Gladman et al. 2002). Furthermore, in the large population-based study of predictors of major infection in SLE by Feldman et al., prednisone as defined as ‘ever use’ was found to increase the hazard of major infection in the SLE population and in those with lupus nephritis after adjustment of all potential confounders (Feldman et al. 2015). All of these findings support the role of prednisone in infection predisposition among SLE patients. The variations in the definition of prednisone can indeed influence the results, and hence use of more than one definition might be important when conducting studies in the field to capture different angles of an association.

SLE patients are usually started on other immunosuppressive medications in addition to prednisone. Many of these medications contribute to infection risk as well. These include Methotrexate, Azathioprine, Mycophenolate Mofetil, Cyclophosphamide and Biologics (including Rituximab and Belimumab) (Danza and Ruiz-Irastorza 2013). Most of the observational studies that examined the risk of infection in adult patients with SLE defined immunosuppressive collectively as ‘ever use.’ This reason is because most patients are treated with more than one
immunosuppressive medication at the same time (or often switch from one medication to another over the course of their disease). As a result, some discrepancy emerges between different studies about the impact of immunosuppressive medication and the risk of infection in SLE. This can be appreciated in several well-conducted studies that examined the risk of infection in SLE. In one such study, immunosuppressive use was examined in a moderate size academic cohort of adult patients with SLE. Immunosuppressive use was associated with major infections in univariate analysis but not in a logistic regression model – after accounting for all possible confounders (Ruiz-Irastorza et al. 2009). In contrast, two other large academic cohorts that examined the risk of infection in adult patients with SLE found that immunosuppressive use was associated with increased odds of infection even after adjustment for other potential confounders (Gladman et al. 2002; Petri and Genovese 1992). This finding was further confirmed in a larger population study (Feldman et al. 2015). What further complicates the estimation of infection risk in patients receiving all of these medications is the confounding effect from all other medications, as well as the disease state which indicated the use of such medications in the first place (channelling bias) (Hudson and Suissa 2010). These factors, along with differences in study designs and methods of analysis, may explain why the association of any of these immunosuppressive medications and infection risk in SLE patients may not persist after adjusting for other variables in regression models (Duffy, Duffy, and Gladman 1991).

In order to dissect this issue further, a recent network meta-analysis study, conducted by Tian et al., examined reported adverse events – including serious infection – in clinical trials of immunosuppressive medications used to treat disease activity in SLE. The authors conducted both regular paired meta-analysis and network meta-analysis. In the paired metanalysis, most of the medications were compared to cyclophosphamide in terms of serious infection. In particular,
prednisone, MMF and Azathioprine had the highest number of studies. Estimated pooled odds of infection did not differ between cyclophosphamide and any of the other immunosuppressive medications, with the exception of Tacrolimus, which was associated with reduced odds of infection. However, this was based on two trials with a small number of total patients contributing to the results (113 patients, with wide confidence interval: 1.10, 17.51). In the network metaanalysis, Tacrolimus also had the best safety profile from an infection risk perspective, as it was found to be superior to cyclophosphamide, azathioprine or MMF combined with tacrolimus. Unfortunately, several other comparisons were imprecise to draw any other conclusions (Tian et al. 2018). Similar safety profile, in terms of infection risk among SLE patients, was also observed in a large population-based study using a Medicare database. In this study, MMF was compared to Azathioprine and to Cyclophosphamide (using 1350 propensity score matched pairs of MMF and Azathioprine and 674 pairs of MMF and cyclophosphamide) in an intention to treat analysis. Infection rate was lower in the first pair compared to the latter, which might reflect the different dosages and indications of use. However, there was no difference in the hazard of infection among the different pairs (Feldman et al. 2017).

The findings of these studies are somewhat reassuring in terms of using immunosuppressive medications collectively rather than individually, since the impact of most of the medications is similar on infection risk – with perhaps some exception for tacrolimus, which is not frequently used as a single agent in SLE management.

Accrued damage

Accrued damage has been regarded as an important risk factor for mortality and a major risk of accumulating new damage. This was clearly shown in a study by Bruce et al. using cohort data from Systemic Lupus International Collaborating Clinics (SLICC). In this study, accrued
damage, which was measured by the SLICC/ACR damage index (SDI), was assessed using the multi-state model for factors associated with transition from one state of damage to another. The authors found that age and male gender were associated with an increased rate of damage progression. They also identified hypertension, disease activity (measured by SLEDAI-2K), and prednisone to be important risk factors for damage progression. Anti-malarial use, meanwhile, was associated with reduced progression – from no damage to damage state and from damage state to higher state (Bruce et al. 2015). What is interesting about this study is that many of these factors were associated with risk of infection. Hence, in theory, damage could be a risk factor for infection development, and infection might be a potential risk for damage development and progression. While literature evidence is lacking for the first theory, a study from Johns Hopkins was presented at the American College of Rheumatology’s annual meeting in 2011 addressing this very question. In that study, patients with no previous damage were assessed for development of damage measured as SDI > 0 after one year. The analysis was time to damage accumulation modelled using cox-regression model. Any infection was found to increase the instant risk of damage – after adjustment for age, ethnicity, gender, disease duration at cohort entry, adjusted mean SLEDAI and prednisone use. Although it was unclear if immunosuppressive use was adjusted or not, the results shed some light about the relationship between damage accrual in SLE and infection (Amanda Eudy 2011).

**Outcomes of infection in SLE**

To appreciate the importance of infection risk among SLE patients, it is helpful to first understand the potential outcomes of this risk and its impact on patients. Infection outcomes among the SLE population range from increased risk of hospitalization, accumulated damage, or even mortality. In a large U.S population-based study, patients with SLE tended to have a higher risk of
hospitalization with infection. They also had an increased risk of age-adjusted inpatient mortality compared to the general population – due to opportunistic infections and infections requiring mechanical ventilation (Tektonidou et al. 2015). These outcomes depend on the type of infection, and the requirement of hospitalization likely reflects the severity of the infection (Gladman et al. 2002; Tektonidou et al. 2015). Realizing that infection is a major cause of mortality is crucial in order to improve survival among SLE patients. In general, mortality rates for lupus patients are three times higher than the general population (Abu-Shakra and Novack 2012). Infections, cardiovascular disease (CVD), and end organ damage are the most common causes of mortality (Abu-Shakra and Novack 2012; Bernatsky et al. 2006). This fact was evident in a large, multicentric study conducted to assess mortality in SLE patients. In this study 9,547 patients were examined to estimate the SMR. Circulatory causes, infections, malignancies (in particular, hematologic) and renal diseases were the dominant causes of mortality, with an overall SMR of 2.4. Stratifying SMR by cause revealed infections and renal diseases to have the highest SMRs compared to all other causes. Though this risk has fortunately been trending down over the years, these issues continue to lead the risk of death among SLE patients (Bernatsky et al. 2006).

Unfortunately, infection risk persists throughout the SLE disease course, regardless of the disease activity or the doses of immunosuppressive therapy (Rubin, Urowitz, and Gladman 1985; Urowitz et al. 1976). This may suggest additional disease inherent risk factors rather than solely attributing the risk to prednisone and immunosuppressive medications (Danza and Ruiz-Irastorza 2013).

**Immunoglobulins in SLE**

*Overview of structure and function*
Immunoglobulins are plasma cell-secreted proteins that bind to various pathogens and facilitate their elimination (Horton and Vidarsson 2013). These proteins are composed of heavy chains and light chains. Together, these chains form two functional domains in the immunoglobulins: constant regions and variable regions (Schroeder and Cavacini 2010). Variable regions are designed to recognize pathogens. This is achieved through a complex gene rearrangement process and somatic hypermutation that takes place on the variable region of both heavy and light chains. As a result, immunoglobulins are capable of efficiently recognizing a large repertoire of pathogens (Dudley et al. 2005). The constant region of immunoglobulins, on the other hand, is responsible for the effector function of immunoglobulins, which includes both binding complement and FC receptor on immune cells to facilitate pathogens elimination. Heavy chains of constant region also determine the type of immunoglobulin (Normansell 1987). Immunoglobulins have five main isotypes, including IgM, IgD, IgG, IgA, and IgE. Switching from one isotype to another occurs to change effector function without changing the recognition domain (namely, the variable region) (Schroeder and Cavacini 2010; Horton and Vidarsson 2013). The biological function of each isotype varies.

IgM is usually the first immunoglobulin produced, and it serves in monomeric shape as a receptor on naïve B-cells and as pentamer in the secreted form. IgM dominates primary immunoglobulin response to pathogens. Its pentamer secreted form provides efficient opsonization function. IgM is particularly very powerful in activating complement pathways. The relative low affinity of IgM to antigens also allows the isotype to function as natural antibodies (Boes 2000).

IgD is co-expressed early on naïve B-cells along with IgM (Geisberger, Lamers, and Achatz 2006). The secretory form is short-lived and its function is not very clear (Schroeder and Cavacini 2010). In contrast, IgG is the most abundant and long-lived immunoglobulin isotype
IgG is secreted as a monomeric protein with strong opsonizing function. IgG antibodies are capable of crossing the placenta to provide fetal protection against pathogens. They also go through extensive somatic hypermutation which renders them with a high affinity to antigens (Kitaura et al. 2017; Schroeder and Cavacini 2010). Four subclasses of the IgG isotype have been identified, with some variation in effector function: IgG1, IgG2, IgG3 and IgG4 (Vidarsson, Dekkers, and Rispens 2014).

IgA is the most abundantly secreted antibody in mucosal areas. Serum level of this isotype is higher than IgM but less than IgG. IgA has a very important role for mucosal protection against various pathogens (Woof and Mestecky 2005). Complement activation does not seem to be the principle effector function (Schroeder and Cavacini 2010). Activation of innate cells, like neutrophils, plays an important role in the protective function of IgA (Corthesy 2007). IgE, on the other hand, has the shortest half-life of all immunoglobulins and has an important role in hypersensitivity reaction and protection against parasitic infections (Burton and Oettgen 2011).

**Measurement of immunoglobulins and reference ranges**

The levels of immunoglobulin in the plasma depend on the isotype and also vary depending on the age and sex of the individual (Nordby and Cassidy 1983). Immunoglobulin levels usually drop after birth over the first six months of life and then increase back over time. The variation in levels is also affected by the ethnicity of an individual, which is why levels should be referenced according to the distribution of immunoglobulins in the population of interest (Tollerud et al. 1995). Interestingly, immunoglobulins do not follow a parametric distribution. Laboratories should always take this into account when reporting the levels and corresponding reference range (Bonilla et al. 2016). Furthermore, immunoglobulin levels in the serum of patients are dynamic. This means that levels change with changes in body fluids and inflammation, and they can also be
affected in so many other ways that may make it hard to capture in one reading (Gonzalez-Quintela et al. 2008). Hence, it is always important to have multiple readings to ensure the persistent nature of immunoglobulins. In addition, adjusting for both internal and external variables that may influence immunoglobulin levels is important to reduce any potential bias (Gonzalez-Quintela et al. 2008). It is also important to ensure that all readings are done in the same laboratory, given the potential variations in laboratory techniques in measuring immunoglobulins and, consequently, the results of the immunoglobulin levels (Bonilla et al. 2016).

**Hypogammaglobulinemia**

Hypogammaglobulinemia is a condition characterized by low immunoglobulins (quantitatively) or impaired immunoglobulin function (qualitatively) (Compagno et al. 2014). This condition can occur in primary or secondary fashion. Definitions of hypogammaglobulinemia vary significantly in different studies, partially because of measurement methods, as mentioned above. In addition, some authors use the reference range of a local laboratory, while others rely on a strict definition (van VOLLENHOVEN et al. 2010). For example, the lowest level of the normal range in most laboratories for IgG is between 6–8 g/l. However, other studies used levels below 4.5g/l to define hypogammaglobulinemia or categorize the levels into mild, moderate and severe deficiency (Ameratunga et al. 2013). On clinical grounds, physicians question the significance of both abnormal low level and the severity of the low level. Hence, it may be best to use both approaches mentioned above when studying hypogammaglobulinemia in order to produce a more comprehensive answer to our clinical inquiry.

**Primary hypogammaglobulinemia**

Immunoglobulin dysfunction might develop primarily. The exact nature of the defect varies – from complete absence of immunoglobulin due to the lack of B-cells (a condition known
as agammaglobulinemia) to normal immunoglobulin levels with aberrant immunoglobulin function (Perez et al. 2017). Other forms, such as subclass deficiencies, have also been described in the literature. What unifies all forms of primary immune deficiency is the consequence – namely, infection (Ballow 2002). In general, patients with hypogammaglobulinemia tend to have recurrent or severe infections that require hospitalization. In addition, many of these patients demonstrate a reduction of antibody titers for childhood vaccines or impairment of vaccine response, a condition referred to as common variable immune deficiency (CVID) (Resnick et al. 2012). This condition is defined based on a set of criteria that includes at least one clinical feature in the form of infection, autoimmunity or lymphoproliferation. CVID patients usually have low IgG levels, in addition to low levels of either IgM or IgA. The International Consensus Document (ICON) criteria for CVID uses a definition of low level that is based on age-specific laboratory ranges with no strict level; however, about 90 percent of patients will have levels below 4.5 g/l (Chapel et al. 2008; Quinti et al. 2007). Interestingly, low IgA seems to be more specific to CVID and is usually at a very low level (70 percent of patients tend to have levels below 0.1 g/l) (Bonilla et al. 2016). In addition, some patients with low IgA may present with isolated low IgA before they start to develop low IgG level (Aghamohammadi et al. 2008).

The definition of low immunoglobulin according to the ICON criteria requires two measurements at least three weeks apart to ensure the persistent nature of the low immunoglobulins. Finally, the exclusion of other conditions causing hypogammaglobulinemia is required. Patients meeting at least two of the five criteria are classified as common variable immune deficiency patients (Bonilla et al. 2016). Interestingly, many of these patients may not have all of the features at presentation and fulfill most of the features over time. It is important to note that once a patient acquires low immunoglobulins, they tend to remain low over time. This
persistent nature of hypogammaglobulinemia might help differentiate primary form from hypogammaglobulinemia due to secondary causes (Quinti et al. 2007).

Replacing immunoglobulins in CVID patients results in a substantial reduction in infection risk and hospitalizations (Busse, Razvi, and Cunningham-Rundles 2002). This effect of immunoglobulin replacement has been demonstrated across six general clinical categorizes within primary immune deficiencies, including: agammaglobulinemia; hypogammaglobulinemia with/without impaired antibody production; normal immunoglobulins with impaired antibody function; isolated IgG subclass deficiency with recurrent infections; and recurrent infections due to a complex immune mechanism involving genetically ascertained primary immune deficiency (Perez et al. 2017). As can be appreciated from these categories of primary immune deficiency, immunoglobulin levels may not always be low. This might be because of selective subclass deficiency, overcompensation of B-cells for the deficiency by increased production, or even abnormality in the function of the immunoglobulin. Hence, reliance on the clinical features of immunodeficiency is paramount in order to recognize primary immune deficient patients. This also emphasizes the importance of careful interpretation of the relationship between immunoglobulin levels and their protective function against infection.

Secondary hypogammaglobulinemia

Several conditions have been associated with impaired immune function and a reduction in immunoglobulin levels. The pathogenesis of hypogammaglobulinemia varies according to the secondary cause. The mechanism in some conditions, like nephrotic range proteinuria, burns, or protein losing enteropathy, is less complicated and usually mediated through excessive loss of protein (Compagno et al. 2014). However, the loss process does not affect all types and subclasses in the same way. This was found in a study of pediatric patients with membranous nephropathy in
which immunoglobulin isotype and subclasses of IgG isotype were compared during disease activity and remission. In this study, total IgG levels were low – in both active and remission state. Specifically, IgG1 subclass deficiency occurred in the early phase of active state, while IgG1, IgG2 and IgG3 subclasses were low later in the course of active disease. In contrast, only IgG2 remained low during long disease remission states (Kemper et al. 2002). What is important about IgG2 is that this major subclass is responsible for protection against polysaccharide capsular organisms, such as pneumococcus, haemophilus influenza, and meningococcal bacteria (Siber et al. 1980). In addition, this subclass deficiency tends to be found frequently with IgA deficiency (Oxelius et al. 1981). Interestingly, IgG2 subclass deficiency has also been reported in SLE (Oxelius 1984). These findings suggest that immunoglobulin loss and consequences of such deficiency is a much more complex process than a simple drop in immunoglobulin level.

On the other hand, other conditions, like malignancy or medication-related hypogammaglobulinemia, are much more complex. Several malignancies have been associated with hypogammaglobulinemia, including hematological malignancies like chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) (Compagno et al. 2014). In the former, hypogammaglobulinemia has been related to the severity and progression of the disease (Morrison 2010). There is usually an inverse relationship between the levels of immunoglobulin and the frequency of infection in these patients. More importantly, the acquired nature of hypogammaglobulinemia in CLL patients seems to be persistent even after disease treatment and remission. The etiology of hypogammaglobulinemia is not very clear, and it may include the aberrant malignant lymphocyte, chemotherapeutic agents used in treatment, and co-morbidities associated with CLL patients (Morrison 2010). Furthermore, many of these factors have also been implicated in the pathogenesis of infection in the CLL population, which may confound the
relationship between hypogammaglobulinemia and infection. Yet, immunoglobulin replacement in this population seems to reduce the risk of infection, which supports its important predictive role of infection (Compagno et al. 2014).

Meanwhile, patients with MM tend to commonly present with hypogammaglobulinemia. The mechanism of hypogammaglobulinemia in MM is not very well defined. Several factors have been identified in the pathogenesis of such abnormality. These include: suppression of B-cell by aberrant macrophages; excessive inhibitory cytokines; and altered B- and T-cell functions (Kyrtsonis, Mouzaki, and Maniatis 1999). The early presentation of hypogammaglobulinemia in MM patients tends to be temporally correlating with infections which occur earlier in the disease course (Compagno et al. 2014; Ludwig and Zojer 2007), though this time period is also confounded by the initiation of chemotherapy and its associated complications.

The other major cause of secondary hypogammaglobulinemia is medications side effects. Many medications have been linked with hypogammaglobulinemia. These include immunosuppressive medications, such as cyclophosphamide, prednisone and azathioprine, as well as selective B-cell depleting agents like Rituximab and Belimumab. Other commonly reported medications, associated with secondary hypogammaglobulinemia, include anticonvulsants (Compagno et al. 2014). The acquisition of hypogammaglobulinemia in these situations can be transient or persistent.

Most commonly reported studies on secondary hypogammaglobulinemia are on B-cell depleting agents, and in particular rituximab. This biological agent is a monoclonal antibody against CD20 marker on mature B-cells (Schioppo and Ingegnoli 2017). Targeting this marker results in the elimination of B-cells and consequently reduces their pathogenic role in various diseases where it is implicated. The depletion of B-cells using this agent usually lasts between
three to six months until B-cells repopulate again. However, persistent B-cell depletion has also been reported (Cambridge et al. 2006). Interestingly, immunoglobulin levels are not always affected by this depletion because of the inability of this agent to deplete long-lasting memory plasma cells (Cooper and Arnold 2010). However, the impact of rituximab is complicated by the underlying disease for which it is used. Many reports about rituximab initially came from hematological malignancies literature, in which underlying malignancies can, by themselves, contribute to hypogammaglobulinemia and an associated risk of infection (Cooper and Arnold 2010). On the contrary, reports from autoimmune conditions are much more controversial. For example, the use of rituximab in anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis has been complicated by the development of hypogammaglobulinemia, although this is more common in patients who had low immunoglobulin levels at the time of treatment due to prior use of cyclophosphamide (Besada, Koldingsnes, and Nossent 2013; Cooper and Arnold 2010). This sequential therapy complicates the interpretation of the effect of rituximab on hypogammaglobulinemia and the risk of infection in those patients studied. On the other hand, the use of rituximab in other autoimmune conditions, such as rheumatoid arthritis or idiopathic thrombocytopenia, is very well-tolerated and is associated with a low prevalence of hypogammaglobulinemia and infection (Khellaf et al. 2014). More recently, Boleto et al demonstrated higher prevalence of hypogammaglobulinemia in a relatively small longterm multicenter study of rheumatoid arthritis patients treated with rituximab, in which hypogammaglobulinemia occurred at rate of 2.7 per 100 person-year. The study also found an association between patients with hypogammaglobulinemia and infection risk. However, this finding was based on univariate analysis that did not account for several confounders including comorbidities (Boleto et al. 2018).
Fortunately, many of these secondary conditions can be modified in order to restore the protective effect of the immune system or patients may benefit from immunoglobulin therapy in ameliorating the risk of infection. These secondary conditions include: malignancies, such as CLL and MM; drug-related, such as rituximab and cyclophosphamide; or even infection-related, including HIV or parvovirus B19 (Duraisingham et al. 2014). However, the requirement of immunoglobulin replacement is primarily dictated by the occurrence of infection in patients or the severity of immunoglobulin deficiency – in addition to the nature of the secondary cause (Duraisingham et al. 2014). This was demonstrated in CLL patients at risk of infection (those who had 50 percent or lower levels of immunoglobulins or a history of serious infection since their diagnosis) in a multicentric, double-blind randomized clinical trial in which immunoglobulin replacement was shown to reduce the risk of infection compared with placebo (Gale et al. 1988). Reports from patients with hypogammaglobulinemia secondary to the use of immunosuppressive medication in organ transplant patients have also shown potential benefits from immunoglobulin replacement in patients with recurrent infections (Jordan et al. 2011). All of this data from various conditions with hypogammaglobulinemia demonstrate the protective effect of immunoglobulins when used in the appropriate setting (Perez et al. 2017).

**Relationship between immunoglobulin and SLE**

SLE patients have the tendency to develop various immunological abnormalities. This ranges from a transient drop in white blood cells (leukopenia), including different subsets of the leukocytes, such as lymphopenia or neutropenia. However, the impact of such abnormalities on infection risk is not always significant (Lertchaisataporn et al. 2013a). Several factors can contribute to infection risk, as mentioned above, including the immunoglobulin level. Patients with SLE tend to have normal immunoglobulin levels (or hypergammaglobulinemia), especially during
disease flare-up. This is thought to be related to activated aberrant humoral immunity. The significance of hypergammaglobulinemia clinically reflects an activated immune system (Draborg et al. 2015; Bakri Hassan et al. 1998).

Interestingly, acquired immunoglobulin deficiency has been seen in lupus patients (Jesus et al. 2011; Yong et al. 2008). Unfortunately, most of these observations have come from a few small descriptive reports. The case reports and case series included various degrees of immunoglobulin deficiencies, ranging from selective isotype deficiency (including IgA, IgM or IgG) to CVID overlapping with SLE. In addition, some studies focused on overlapping primary hypogammaglobulinemia and SLE, while others examined secondary hypogammaglobulinemia in SLE patients.

**SLE and primary hypogammaglobulinemia**

The link between primary immunodeficiency and autoimmunity has been shown in several studies – and in various forms of primary immunodeficiencies. Autoimmunity is one of the clinical features in patients with CVID (Bonilla et al. 2016), and it might be the first presentation of such disease (Seve et al. 2008). Several types of autoimmune diseases have been reported in CVID, including SLE (Fernandez-Castro et al. 2007). Other selective types of primary hypogammaglobulinemia have been linked to autoimmunity and development of SLE, including IgA deficiency. This link was supported by a case-control study that found an association between a history of IgA deficiency and a later diagnosis of SLE (Yewdall et al. 1983). Following this, several international cohorts of SLE patients examined the prevalence of IgA deficiencies in SLE populations. Most of these cohorts noticed an increased prevalence of IgA deficiency in SLE (Wang et al. 2011). Unfortunately, most of these cohorts lack information about the IgA levels prior to SLE diagnosis, and thus the nature of this deficiency is unresolved. Interestingly, some
studies examined the presence of autoantibodies in the sera of patients with primary IgA deficiency and noted an increased prevalence of anti-nuclear antibodies (Gulez et al. 2009). This indeed supports the notion of increased tendency of those patients to develop several forms of autoimmunity. The mechanism of SLE development in patients with primary immune deficiencies is unclear. However, genetic studies in these conditions suggest a common genetic predisposition between these immune deficiencies and SLE. These include both MHC- and non-MHC-associated SNPs located in genes involved in pathogenies of SLE, such as Interferon-induced helicase 1 gene (Wang et al. 2011). Some data suggest that a lack of immunoglobulin complex may reduce the inhibitory signal through negative feedback receptors like FcγRIIB (Warnatz and Voll 2012). This inhibitory signal is important for immune homeostasis and tolerance (Baerenwaldt et al. 2011). Furthermore, mutation in this receptor has been associated with SLE development (Bolland and Ravetch 2000). Other factors leading to hypogammaglobulinemia include defects in B-cell selection and elevated B cell-activating factors (BAFF), which are also involved in the SLE pathogenesis and a target of recently approved biological therapy in SLE (Kreuzaler et al. 2012; Furie et al. 2011).

**SLE and secondary hypogammaglobulinemia**

SLE and immunoglobulins have a very unique relationship. The beginning of this relationship starts in the contribution of immunoglobulins to the pathogenesis of SLE. Autoantibodies are the hallmark of the disease. As a result, immunoglobulin levels are usually elevated – especially during the active phases of the disease. However, this unique relationship can be affected by other forces that may shift the levels of the immunoglobulins during the course of the disease.
In SLE, the development of hypogammaglobulinemia can be attributed to the presence of proteinuria, prednisone, immunosuppressive medications, B-cell depleting agents (such as Rituximab), and perhaps other non-defined factors (Reddy et al. 2017). In theory, the mechanism mediating the reduction in immunoglobulin levels could involve any step from immunoglobulin synthesis, class switch, transfer from serum to mucosal areas, excessive loss or even elimination. Studies in pediatric patients with SLE and IgA deficiency have demonstrated the presence of anti-IgA antibodies in all patients compared to only 25 percent of patients without SLE. A study of lupus nephritis and associated hypogammaglobulinemia was conducted to examine the association with infection. In this study, patients with hypogammaglobulinemia were compared to patients without such abnormality. Interestingly, the degree of hypogammaglobulinemia correlated with the severity of proteinuria. In addition, improvement in hypogammaglobulinemia correlated with improvement in proteinuria.

The extent and significance of secondary hypogammaglobulinemia, especially in relation to infection risk, has not been well-studied (Jesus et al. 2011; Saiki et al. 1987; Senaldi et al. 1988). The best descriptions come from a literature review of published cases by Yong et al. (Yong et al. 2008). In this paper, several cases with various forms of hypogammaglobulinemia were summarized. The first case was a patient with active SLE who presented with CNS lupus and later lupus nephritis. In this patient, hypogammaglobulinemia – affecting all immunoglobulin isotypes – developed several years after aggressive treatment of lupus nephritis and cerebritis with cyclophosphamide, MMF and methylprednisolone. The patient improved significantly with no residual proteinuria. Interestingly, the patient’s hypogammaglobulinemia gradually improved over three years with no infectious sequelae. In the same paper, infection occurred in one out of every five cases of hypogammaglobulinemia. The significance of this infection came in the form of
recurrent respiratory tract infection. The levels of immunoglobulins were: IgA < 0.06 g/l, IgM < 0.05 g/l and IgG < 1.7 g/l. The patient did not tolerate IVIG replacement due to side effects and eventually died from, presumably, an unrelated cause (Yong et al. 2008).

Most recent papers on hypogammaglobulinemia in SLE are focused on new B-cell depleting biologics, such as rituximab and belimumab. For example, Reddy et al. (Reddy et al. 2017) specifically looked at the development of hypogammaglobulinemia in SLE after rituximab treatment. In this cross-sectional study, 57 patients with SLE were treated with rituximab for refractory SLE disease, and their baseline immunoglobulin levels were analyzed before and after infusion of rituximab. Interestingly, the occurrence of low IgG was rare compared to IgM (which occurred in 25 percent) of the patients. Furthermore, this study showed no increased risk of infection in those patients who acquired hypogammaglobulinemia compared to those who did not post-rituximab treatment (Reddy et al. 2017). More recently, Belimumab, which is a humanized monoclonal antibody that targets B-cell activating factor (BAFF), was approved for SLE treatment. The medication works by inhibiting the survival of autoreactive B-cell. The safety of this medication, including the risk of developing hypogammaglobulinemia, was studied by Wallace et al. (Wallace et al. 2012). In their study, pooled data from phase 2 and 3 clinical trial of belimumab efficacy in SLE were examined. It is interesting to note that the frequency of hypogammaglobulinemia is variable according to isotype. While patients receiving belimumab had a higher frequency of hypogammaglobulinemia (≈ 5 percent for IgG, 2.5 percent for IgA, and 19 percent for IgM), the frequency of infection was not significantly different from the placebo group (Wallace et al. 2012). This effect of belimumab is unlikely to be related to common confounders, such as proteinuria, because of the similarity across all groups at baseline.
This diversity in presentation, effects of confounders, and potentially significant consequences of hypogammaglobulinemia warrant a larger, well-designed study to evaluate the significance of acquiring such abnormality among adult patients with SLE.
Chapter 2

Study Question, Objective, Hypothesis, Rationale and Methods

**Hypothesis:**

Acquiring any low immunoglobulin levels increases the risk of clinically relevant infection in adult patients with SLE within two years.

**Objective:**

To examine the association between any low immunoglobulin levels and the risk of clinically relevant infection in adult patients with SLE.

**Rationale:**

Infection remains a leading cause of death in SLE patients throughout the course of the disease. Understanding various factors contributing to this risk may lead to interventions that could modify this risk and hopefully improve overall mortality.

**Methods:**

**Data source**

This is a retrospective analysis of prospectively-collected data. The study population was identified from Toronto Lupus cohort, which was established in 1970 (Urowitz and Gladman 2005). Patients are followed prospectively at two- to six-month intervals, according to a standard protocol which includes demographics and detailed clinical and laboratory assessments. Data are collected by trained physicians. Infections are recorded within one month of a visit, according to the patient, along with the infectious agent where available, and the treatment prescribed. Immunoglobulin levels are performed annually. Overall disease activity is scored according to the
SLE Disease Activity Index (SLEDAI)-2K (Gladman, Ibanez, and Urowitz 2002), and accumulated damage is recorded according to the SLICC/ACR damage index (SDI) (Gladman et al. 1996). Immunoglobulins are measured in LifeLab according to standardized turbidimetric method (von Eckardstein et al. 2013).

The choice of this cohort as the data source was based on the following factors:

- Availability of data. The fact that all data are collected, cleaned, stored and ethically approved by local IRB (Urowitz and Gladman 2005).
- Consistency in data collection, which is collected prospectively according to the standardized protocol by trained physicians to minimize several forms of information bias, including observer/interviewer bias, measurement bias, and even recall bias (Fletcher, Fletcher, and Fletcher 2014; Grimes and Schulz 2002; Delgado-Rodriguez and Llorca 2004; Hartman et al. 2002).
- Finally, having data on uncommon disease like SLE is not easy. In fact, few cohorts in the world have such data collected prospectively, and Toronto Lupus Cohort is one of the largest (Urowitz and Gladman 2005). Hence, using this cohort may enhance the power of study and allow us to conduct designs that may not be feasible otherwise.

**Identification of the study cohort**

**Inclusion criteria:**

Patients who are 18 or above fulfilling the ACR classification criteria for SLE or had three criteria and evidence of lupus documented on biopsy (SLICC criteria) (Hochberg 1997; Petri et al. 2012). Mandating multiple measurements is essential to handling issues related to variation in values within individuals and false values related to possible lab error. These problems can lead to
an important source of biases, including regression-dilution bias and misclassification bias (Tripepi et al. 2008).

**Exclusion criteria:**

Patients with proceeding diagnosis of immunodeficiency, concurrent HIV infection, or those with low immunoglobulins at their first measured sample were excluded. To ascertain the acquired nature of low immunoglobulins (people who develop low immunoglobulin levels during their disease as opposed to those who might have persistent congenital low immunoglobulin levels, or those with existing diagnosis of CVID), we excluded patients with low immunoglobulins at first measurement, as mentioned above, and required the availability of at least two measurements for cohort inclusion (Conley, Notarangelo, and Etzioni 1999).

**Time of entry:**

Time of entry to the cohort was the index date for both the low immunoglobulin or non-low immunoglobulin groups. Follow-up time is two years from the index date for the occurrence of the outcome (infection). This duration was chosen based on preliminary data on the normalization of low immunoglobulins in our cohort.

**Low immunoglobulin (Exposure)**

Low immunoglobulins was defined based on the presence of two recorded (consecutive or non-consecutive) low immunoglobulin levels below the normal reference range. The index date was the first measurement of low immunoglobulins. Overlap of other low immunoglobulin types was allowed. Restricting exposure to two consecutive measures aimed to minimize misclassification of lab error as exposure (Fletcher, Fletcher, and Fletcher 2014). However, sensitivity analysis was conducted for those patients with only one measurement of low immunoglobulins to determine whether their exclusion affected the outcome.
Non-low immunoglobulins (Control)

The non-low immunoglobulins group was defined based on the presence of two non-low immunoglobulin levels in those who never experienced any low immunoglobulin levels in their follow-up. The index date was first measurement of non-low immunoglobulins. Immunoglobulins (IgA, IgM and IgG) in this study were categorized as low or normal and then as continuous variables.

Outcome

Clinically-relevant infection was defined as infections requiring the use of any antibiotics (oral or parenteral) within two years of index date (Costa-Carvalho et al. 2014). Multiple sensitivity analyses were carried out to assess other infection definitions, including clinically relevant infection (as defined above) within one year of index date, severe infection (requiring parenteral antibiotic), or any infection (viral, fungal or parasitic) within two years of index date, regardless of treatment (Fletcher, Fletcher, and Fletcher 2014; Grimes and Schulz 2002; Rothman, Greenland, and Lash 2015).

Statistical analysis

This is a retrospective analysis of prospectively collected data, in which low immunoglobulin and non-low immunoglobulin groups are compared in terms of clinically relevant infection. The primary analysis was time-to-event using a Cox regression model (Harrell 2001). Missing values were addressed using multiple imputation. Both study groups were matched according to disease decade. Further group balance was achieved using propensity score methods in the form of covariate adjustment in the regression model and inverse probability weighted treatment (IPTW) (Austin 2009b; Johnson, Tomlinson, et al. 2018). Multiple sensitivity analyses, including exact matching on the most important confounders to reduce confounding bias, as well
as examining several exposure and outcome definitions, were performed to ensure result consistency (Rothman, Greenland, and Lash 2015).

This study followed the guidelines and recommendations from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. STROBE was developed in 2004 by a group of methodologists, major medical journal editors, and researchers. It consists of a set of 22 items that are considered essential in reporting observational studies (coHORTS, case-control and cross-sectional designs). These 22 items cover issues related to title, abstract, introduction, methodology, results, discussion and funding. The aim of using such recommendations is to improve quality and transparency of reporting observational studies (von Elm et al. 2007). However, although STROBE provides a nice framework of items that need to be addressed in observational study, it is not a tool for assessing quality of study. Most of the observational study quality checklists are usually designed for non-randomized interventions. For example, ROBINS-I is a tool designed to assess quality of observational studies when the assignment of intervention is not by chance. The checklist tool may not be easily translated when the exposure is not an intervention, but rather the prognostic is variable. However, the tool covers common issues that threaten internal validity of observational study – particularly selection bias, information biases, and confounding bias. In addition, it stresses the methods used to account for such biases in design and analysis (Sterne et al. 2016).

**Missing values**

Missing values pose a significant threat to the internal validity of any observational study. Missing variables can be classified into three general categories: missing completely at random (MCAR); missing at random (MAR); and missing not at random (MNAR) (Haukoos and Newgard 2007; Sterne et al. 2009; Stephens et al. 2018). Understanding the distinction between these
categories is crucial to properly handling missing values and applying the right method. For example, MCAR can be handled by complete case analysis (dropping observations with missing values from analysis) (Sterne et al. 2009). Other forms require more sophisticated methods to handle the missing values (Haukoos and Newgard 2007).

In our study, we assumed that missing values were MAR. Based on that, we used multiple imputation to address this problem (choosing MAR for its strength in reducing bias and its ease of application) (Newgard and Haukoos 2007). Multiple imputation is a technique based on Bayesian theory (Sterne et al. 2009). In this method, we use all the observed dataset to estimate a distribution of variables that has missing values and then generate multiple datasets with different imputed values based on estimated distribution. As a result, variations due to uncertainty about imputed values can be incorporated into analysis (Newgard and Haukoos 2007). Following that, datasets are used with the statistical model of choice to study the association between a variable with missing values and the outcome. Finally, regression coefficients obtained from the statistical model – using multiple datasets – will be averaged in order to get the final regression coefficient estimate. The Rubin method will typically be used to have standard error for this regression coefficient. The major advantage of this technique is that it takes into account all variation between data sets (Sterne et al. 2009; Newgard and Haukoos 2007).

In our study, multiple imputation was carried out to address missing values on Antiphospholipid Antibodies (APA) (42 patients had missing values in categorical APA variable at index)(Sterne et al. 2009). Five data sets were created, each of which had the same mean and standard deviation (0.23 ± 0.41), thus suggesting no impact of imputation on mean APA (Sterne et al. 2009).
**Propensity score methods**

*Overview*

Propensity score is a balancing score, as described by Rosenbaum and Rubin in 1983, in order to account for the systematic difference between exposure and control groups (Rosenbaum and Rubin 1983). This potential difference can distort the estimation of the exposure effect if not accounted for (Johnson, Tomlinson, et al. 2018; Rosenbaum and Rubin 1983). The score is constructed by estimating the probability of exposure for each study cohort subject. This is achieved by conditioning probability of exposure on available observed variables. Following that, propensity score can then be used in one of four methods: matching, stratification, adjustment, and inverse-weighted treatment.

*Matching:*

In this method, patients are matched based on their propensity score using a proximity method with predefined caliper width. This caliper width is based on the standard deviation of the logit of propensity score (Austin 2011; Johnson, Tomlinson, et al. 2018). Following that, adequacy of matching is assessed using either statistical testing or standardized difference between baseline covariates in the exposure and control groups (Austin 2009a; Austin 2011). There is some controversy on what constitute optimal standardized difference. Some authors use a standardized difference of 0.1 as upper limit of acceptable imbalance in baseline covariates, while others uses divide standardized difference into several cutoffs in which a difference less than 0.2 indicates low imbalance between matching groups, while 0.5 is moderate and 0.8 is considered large imbalance (Austin 2009a).

The matching method is usually used when having a large pool of subjects in a control group. Otherwise, a significant loss of sample size may occur due to the lack of a match. As a
result, this method may lead to significant sample size loss. In addition, this method will only account for variables that we match, which may leave some confounding effects unaccounted for (Austin 2009a; Austin 2011; Johnson, Tomlinson, et al. 2018).

**Stratification:**

In this method, patients are stratified based on their propensity score and exposure, and control groups are compared within each stratum. As a consequence, significant bias elimination can be achieved. However, sample size can be small within each stratum, which may in turn affect the power of the study (Austin 2009b; Johnson, Tomlinson, et al. 2018).

**Adjustment:**

In this method, estimated propensity score is included in a regression model of choice along with indicator of exposure assignment. By doing this, a limited sample size with many confounding variables can still be used in a stable regression method. Unfortunately, this means that both dependent outcomes and independent variables (propensity score and exposure assignment indicator) are all in one model, thus potentially rendering the results subject to bias estimates (Johnson, Tomlinson, et al. 2018; Rosenbaum and Rubin 1983).

**Inverse-weighted treatment:**

This unique method uses propensity score to create a state of pseudo-randomization. This is achieved by weighting the exposure group based on the inverse of their estimated propensity score, while weighting the control group based on the inverse of 1-estimated propensity score. As a result, all subjects can be used in the study while reducing bias related to the systemic difference between exposure and control subjects (by giving appropriate weight based on estimated propensity score) (Rosenbaum and Rubin 1983). One of the caveats of this method is that it may lead to imprecise estimates if subjects have an extreme estimated propensity score (i.e.
approximate to 0 or 1) (Williamson et al. 2012). However, there are several proposed mechanisms to account for this occurrence, such as using stabilized weight (Austin and Stuart 2015). Finally, the adequacy of balancing groups using this technique can be assessed by comparing the weighted average of the subjects’ baseline covariates in both groups (Austin 2009b, 2009a; Austin 2011; Johnson, Tomlinson, et al. 2018; Rosenbaum and Rubin 1983).

**Propensity score methods in our study**

Propensity score in our study was completed using three methods to evaluate the consistency of our results. The main reason of using three methods was the low event rate with respect to our outcome and a relatively small sample size contributing to these events. As a result, we wanted to minimize sample loss while maintaining rigorous methods to account for different types of biases. We first used propensity score as an adjustment in the Cox-regression model. We then confirmed the results by repeating the analysis using the inverse probability weighted treatment method. Finally, we compared the results with the propensity score match, knowing that a loss of sample size may reduce the statistical power. We also accounted for matching on PS using cluster robust standard error as well as stratified cox-regression model (Austin 2011, 2014).

**Construction of propensity score in our study**

Propensity score was derived using the logistic regression model (Newgard et al. 2004). In that model, low immunoglobulins was the dependent variable and the following variables were independent variables: age at index (as a continuous variable), sex, disease duration at index, disease activity (measured by SLEDAI-2K score), nephrotic range proteinuria, protein-losing enteropathy, antiphospholipid antibodies (APA), prednisone use and dose, immunosuppressant use, and use of biologics (Danza and Ruiz-Irastorza 2013). The choice of these covariates was
based on the available knowledge in the literature regarding associated or predisposing factors to low immunoglobulin states.

To construct a logistic regression-based propensity score model, we first estimated it by calculating the probability of having any low immunoglobulin levels using the whole study cohort (Austin 2009b; Austin 2011). Because of the missing values for the APA variable, we used the five datasets created through the multiple imputation step. Following that, five propensity scores were estimated using these datasets. We then used the propensity score from each dataset as a covariate in the Cox-regression model to adjust for confounders and then used the MIANALYZE Procedure (in SAS) to combine regression coefficients and reach our final results (Azur et al. 2011).

**Exact matching**

One of the methods to address selection (and, more precisely, confounding bias) in an observational study is to match the groups on variables that are thought to be responsible for this difference (Fletcher, Fletcher, and Fletcher 2014; Grimes and Schulz 2002). Unfortunately, this method is limited by several factors, including the number of variables to match, which consequently will limit the sample size (Fletcher, Fletcher, and Fletcher 2014). Hence, we decided to use exact matching here as sensitivity analysis, aware that the results will be limited by a smaller number of events.

We conducted 1:1 matching based on known risk factors for infection in SLE patients as reported in the literature (Grimes and Schulz 2002). These included disease activity assessed by SLEDAI-2K (within 1 point) and prednisone and immunosuppressant use (Danza and Ruiz-Irastorza 2013), as well as major demographic factors, including age (within 5 years), sex, time of diagnosis (by decade), and duration from diagnosis to index date (within a year). Cox-regression
model was used to model time to event analysis where IgG, IgM and IgA were independent variables and clinically relevant infection was dependent variable. Cluster robust standard error was used to account for matching.

**Time-to-event analysis**

The choice to use time-to-event analysis here was based on the various entry points and follow-up periods of patients entering the cohort. As a result, time contributed from each subject in the study cohort may vary from one another. In addition, infection as an event may happen early or late in the follow-up time, which might lead to skewed distribution and necessitate time-to-event based analysis (Clark et al. 2003). We used Cox-regression hazard to model our survival analysis for its flexibility in terms of assumption of outcome distribution (Bradburn et al. 2003). Several questions needed to be addressed when we chose such an analysis. These questions included the following:

*Are censored events due to loss of follow-up informative?*

One of the major issues that can arise due to loss of follow-up is whether this loss has a prognostic effect on survival. This simply means that the probability of developing the event is systematically different in those with loss of follow-up compared to those who continued to be followed in the cohort. Fortunately, we did not have any significant loss of follow-up in our study.

*Is there any competing risk?*

Death is an important cause of loss of follow-up. Failure to account for that can result in an important bias known as competing risk bias (Austin, Lee, and Fine 2016). This issue concerns time-to-event-based analysis. It occurs when death competes with the ability of an individual to develop the outcome of interest (infection, in the case of our study). Hence, in our study, mortality was assessed in both groups to ensure no competing risk of death (Austin, Lee, and Fine 2016).
Is there any effect of cohort on survival?

This issue concerns long-term open observational studies, such as our cohort which was established back in 1970. In simple survival analysis, there is an assumption of homogeneity across all cohort members. This simply means that survival probability for those who entered the cohort early should be similar to those who entered later (apart from exposure of interest). However, things change over time, including treatment strategy, general care, or even disease severity. As a result, the survival probability of individuals in such a cohort may not be the same (Clark et al. 2003). To address this further, we chose to match the exposure and control groups at the beginning for age and disease decade. This aims to minimize the effect of such bias and improve homogeneity across our study subjects.

Final modelling

Comparisons were conducted using the Student’s t-test for continuous variables and the Fisher’s exact test and chi-square test for categorical variables (Daniel and Cross 2013). Four Cox proportional-hazards regression models were constructed conceptually. The first model consisted of each immunoglobulin (IgA, IgM, IgG) and PS as independent variables, while clinically relevant infection was the dependent variable. The second model consisted of each immunoglobulin as independent variables (IgA, IgM, IgG), weighted according to their 1/PS for low immunoglobulins group and 1/(1-PS) for normal immunoglobulins group. In that model, clinically relevant infection was the dependent variable. The third model used PS to match exposure and control groups with caliper of 0.2. Following that, three immunoglobulins were independent variables and clinically relevant infection was the dependent variable. The fourth model was for exact match, and the independent variables consisted of IgA, IgM, IgG, age (within 5 years), disease decade (within a decade), disease duration (within 5 years), disease activity using
SLEDAI (more than 6 or less than 6), prednisone dose (more than 10 or less than 10), and the use of immunosuppressive medication. Clinically relevant infection was the dependent variable.

Model fitness was assessed using martingale residual against our independent variables. The absence of a slope for the residual is considered an indication of adequacy of fitness (Bradburn et al. 2003).

We did not conduct any optimization steps for our four models, given that these models are conceptual in construction and aim to be explanatory rather than predictive (Shmueli 2010). Because of the fluctuating nature of immunoglobulins, several assumptions were tested to ensure that statistical modelling was well-constructed. These included the following:

*Nonlinearity*

The first assumption was nonlinearity. We checked nonlinearity of immunoglobulins by plotting martingale residual vs. independent variable values. We also confirmed this by plotting beta estimates against independent variable category midpoints (here, we used the quantiles to categorize independent variables) (Fang, Austin, and Tu 2009). Failure to account for non-linearity will result in reduction of the power of the statistical modelling and may compromise the results (Kahan et al. 2016). To address this, we used restricted cubic splines function in our regression model to account for the non-linearity of our independent variables (Andersen 2003). In this method, continuous variable range is divided into smaller windows using knots. This results in smaller polynomial pieces that are forced to join at the knots, allowing it to model curvy shapes while adding smoothness to it. In addition, the restricted cubic spline method constrains the tails of continuous variable figure tails to be linear. Together, the final model will use fewer degrees of freedom and perform better (before and after first and last knots, respectively) (Harrell 2001). We decided to use this method because of the above-mentioned properties and its superior performance.
compared to other methods, such as categorization, linear assumption or fractional polynomial using one polynomial term (Kahan et al. 2016).

Collinearity

One of the issues that can arise while building models is collinearity. This refers to a strong correlation between one or more independent variables which renders effect estimation of these covariates difficult to separate and, consequently, less reliable to use in one’s inference (‘Encyclopedia of Research Design’ 2010; Rothman, Greenland, and Lash 2015). We assessed collinearity using the variance inflation factor (VIF). VIF is a factor that measures the degree of correlation by showing how much variance for an independent variable is explained by collinearity in the model being studied (Johnston, Jones, and Manley 2018). Values above 10 are generally considered to be a strong indicator of collinearity, although the threshold value remains a subject of debate among statisticians (Johnston, Jones, and Manley 2018).

Time-varying effect

One of the important assumptions in the Cox-regression proportional hazard model is that independent variables’ contribution to overall hazard is constant. That is, their regression coefficient is the same over time and, consequently, the slope of these coefficients over time should be zero. This is referred to as proportional hazard assumption (Bradburn et al. 2003; Harrell 2001). Assessing this is essential in order to produce valid estimates. Several methods have been proposed to assess proportional hazard, including numerical tests (like the supremum test) and graphical tests (like the Schoenfeld residual test) (Harrell 2001; Hiller, Marshall, and Dunn 2015; Huque 2013). We chose to conduct both tests to ensure that proportional hazard is consistently satisfied.
Chapter 3

Results

Cohort creation

Out of 1935 patients in Toronto lupus cohort, we identified 448 patients in the low immunoglobulin group (221 had consecutive low immunoglobulin levels, and 227 had non-consecutive low immunoglobulins) and 656 disease decade-matched non-low immunoglobulin patients (Baseline characteristics of overall Toronto lupus cohort can be seen in Table a in supplementary appendix). Of the low immunoglobulin group, 94 patients had low IgG, 123 patients had low IgM, and 54 patients had low IgA.

We examined the characteristics of non-eligible patients due to lack of two measurements of immunoglobulins compared to the rest of eligible patients and found no significant difference between both groups (Table b in supplementary appendix).

The number of overlaps was small (11 patients had both low IgG and IgM, 15 patients had both low IgG and IgA, and only three patients had all three isotype levels low at index). The average time between two low IgG measurements in the consecutives group was 6.8 months, while in the non-consecutive group it was two years.

The following flow diagram represents assembly of the cohort:
Baseline characteristics

Baseline characteristics of low immunoglobulin and non-low immunoglobulin groups are presented in Table 1:

Table 1: Baseline characteristics of the study cohort

<table>
<thead>
<tr>
<th>Variables at index</th>
<th>Unit</th>
<th>Non-low immunoglobulins</th>
<th>Low immunoglobulins</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease duration</td>
<td>Mean ± SD</td>
<td>7.6 ± 8.0</td>
<td>11.2 ± 9.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td>90 (13.7%)</td>
<td>58 (13.3%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td>436 (66.5%)</td>
<td>292 (66.8%)</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td></td>
<td>57 (8.7%)</td>
<td>50 (11.4%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>73 (11.1%)</td>
<td>37 (8.5%)</td>
<td></td>
</tr>
<tr>
<td>SLEDAI-2K score</td>
<td>Mean ± SD</td>
<td>5.9 ± 5.9</td>
<td>6.2 ± 6.3</td>
<td>0.02</td>
</tr>
<tr>
<td>SDI Score</td>
<td>Mean ± SD</td>
<td>0.5 ± 1.0</td>
<td>1.2 ± 1.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Had lupus nephritis before the index</td>
<td>N (%)</td>
<td>117 (17.8%)</td>
<td>196 (44.9%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>N (%)</td>
<td>74 (11.3%)</td>
<td>112 (25.6%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Any low complement</td>
<td>N (%)</td>
<td>274 (41.8%)</td>
<td>162 (37.1%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Increase DNA binding</td>
<td>N (%)</td>
<td>315 (48.0%)</td>
<td>187 (42.8%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>N (%)</td>
<td>21 (3.2%)</td>
<td>18 (4.1%)</td>
<td>0.36</td>
</tr>
<tr>
<td>APA</td>
<td>N (%)</td>
<td>168 (26.2%)</td>
<td>62 (15.2%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Prednisone use</td>
<td>N (%)</td>
<td>349 (53.2%)</td>
<td>332 (76.0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Oral prednisone dose (mg/day)</td>
<td>Mean ± SD</td>
<td>15.3 ± 14.6</td>
<td>16.8 ± 16.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Immunosuppressive use</td>
<td>N (%)</td>
<td>152 (23.2%)</td>
<td>201 (46.0%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Biologics treatment</td>
<td>N (%)</td>
<td>1 (0.2%)</td>
<td>5 (1.1%)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Propensity score derivation

Propensity score derived from the different datasets created by multiple imputation is shown in the following figures (1a, b, c, d):

Figure 1: Propensity score distribution in imputed datasets. The figures (a-d) provide visual check of PS distribution in all generated datasets by multiple imputation.

Figure 1a. Propensity score distribution in imputed dataset 1.
Figure 1b. Propensity score distribution in imputed dataset 2.

Figure 1c. Propensity score distribution in imputed dataset 3.
Propensity score distribution is appropriately reflected by the percentage of patients in exposure and control groups, with higher and lower propensity scores, respectively. Adequacy of balance was assessed using standardized difference before and after using propensity score in matching and inverse probability weighted treatment (Table 3).
Table 2: Adequacy of balancing between low immunoglobulins and Normal immunoglobulins groups after using PS in matching and IPWT

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>VALUE</th>
<th>Normal Ig</th>
<th>Low Ig</th>
<th>STD. Diff before PS methods</th>
<th>STD. Diff after PS matching</th>
<th>STD. Diff after IPW weighting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N = 656</td>
<td>N = 437</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>37.69 ± 16.01</td>
<td>42.37 ± 14.10</td>
<td>0.19</td>
<td>0.14</td>
<td>0.176</td>
</tr>
<tr>
<td>Female</td>
<td>N(%)</td>
<td>388 (89)</td>
<td>570 (87)</td>
<td>0.06</td>
<td>0.04</td>
<td>0.004</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>Mean ± SD</td>
<td>7.6 ± 8.0</td>
<td>11.2 ± 9.1</td>
<td>0.43</td>
<td>0.21</td>
<td>0.334</td>
</tr>
<tr>
<td>SLEDAI_2K</td>
<td>Mean ± SD</td>
<td>5.9 ± 5.9</td>
<td>6.2 ± 6.3</td>
<td>0.05</td>
<td>0.04</td>
<td>0.024</td>
</tr>
<tr>
<td>SDI</td>
<td>Mean ± SD</td>
<td>0.5 ± 1.0</td>
<td>1.2 ± 1.6</td>
<td>0.59</td>
<td>0.32</td>
<td>0.46</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>N (%)</td>
<td>74 (11.3%)</td>
<td>112 (25.6%)</td>
<td>0.39</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
<td>APA</td>
<td>N(%)</td>
<td>168 (26.2%)</td>
<td>62 (15.2%)</td>
<td>0.25</td>
<td>0.26</td>
<td>0.17</td>
</tr>
<tr>
<td>Steroid use</td>
<td>N (%)</td>
<td>349 (53.2%)</td>
<td>332 (76.0%)</td>
<td>0.48</td>
<td>0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>Steroid dose (mg/day)</td>
<td>Mean ± SD</td>
<td>15.3 ± 14.6</td>
<td>16.8 ± 16.8</td>
<td>0.32</td>
<td>0.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Immuno-suppressive Biologics</td>
<td>N (%)</td>
<td>152 (23.2%)</td>
<td>201 (46.0%)</td>
<td>0.5</td>
<td>0.17</td>
<td>0.36</td>
</tr>
<tr>
<td>Biologics</td>
<td>N (%)</td>
<td>1 (0.2%)</td>
<td>5 (1.1%)</td>
<td>0.13</td>
<td>0.01</td>
<td>0.09</td>
</tr>
</tbody>
</table>

N= represents the total number of patients in the study cohort after matching on enrolment decade followed by application of each PS method. Both PS methods reduced groups imbalance. PS match improved the groups’ balance more than IPW but resulted in smaller sample size.

Assessment of linearity of independent variables

All immunoglobulins showed evidence of non-linearity as well as PS (Figure 2a, b, c, d).
Figure 2: Martingale residual of independent variables in study models. Visualization of the figures suggests non-linearity.

Figure 2a. Martingale residual of PS
Figure 2b. Martingale residual of IgG

Figure 2c. Martingale residual of IgM
Figure 2d. Martingale residual of IgA
Similar findings were seen using beta coefficient against mid-points of independent variables in the study model quantiles (Figure 3a, b, c).

Figure 3: Beta coefficient against mid-points of independent variables quantiles. Significant variation in beta coefficient indicates non-linearity.

Figure 3a: Beta coefficient against mid-points of propensity score quantiles.
Figure 3b: beta coefficient against mid-points of IgG levels quantiles.

Figure 3c: beta coefficient against mid-points of IgM levels quantiles.
Likelihood ratio test was statistically significant when used to compare goodness of fit between cox-regression models with restricted cubic spline function and without it. This was only conducted in models that used PS as adjustment covariate or weighting in IPWT method.

**Proportional hazard assumption test**

Proportional hazard assumption was satisfied for immunoglobulins and propensity score.

**Main findings**

The mean levels at index among the low immunoglobulin group were IgG $11.5 \pm 6.1$ g/l, IgM $0.8 \pm 1.1$ g/l, IgA $2.4 \pm 1.6$ g/l, while the non-low immunoglobulin group had mean of IgG $16.3 \pm 6.4$ g/l, IgM $1.8 \pm 1.2$ g/l and IgA $3.2 \pm 1.5$ g/l, respectively.

There were 97 events (47 in low immunoglobulin group and 50 in non-low immunoglobulin group) when propensity score was used as covariate adjustment and inverse
weighting (IPWT) was applied. When we used propensity score to match, we ended up with a total of 85 events (45 in low immunoglobulin and 40 in non-low immunoglobulin).

**Using immunoglobulin levels as a categorical variable**

Only low IgA level was associated with increased hazard of infection. This was evident using the propensity score matched method as seen in the Kaplan-Meier curve (Figure 4). Low IgG and IgM levels were not associated with increased hazard of infection (Figures 5, 6).

![Graph showing Kaplan-Meier curve of low IgA versus non-low IgA group.](image)

**Figure 4**: Kaplan-Meier curve of low IgA versus non-low IgA group.
Figure 5: Kaplan-Meier curve of low IgG versus non-low IgG.

Figure 6: Kaplan-Meier curve of low IgM versus non-low IgM.
The results of the Cox-regression model were consistent with the above results after adjustment of other immunoglobulins isotypes using cluster-robust standard error. Using stratified cox-regression model to account for matching on propensity score, similar estimates were found but with loss statistical significance when test at alpha level 0.05 (Table 3)

Table 3: Hazard ratio of low immunoglobulins treated categorically after PS match

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th></th>
<th>IgG</th>
<th></th>
<th>IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>P-Value</td>
<td>Hazard Ratio</td>
<td>P-Value</td>
<td>Hazard Ratio</td>
<td>P-Value</td>
</tr>
<tr>
<td>Low Ig</td>
<td>2.24</td>
<td>0.01</td>
<td>1.15</td>
<td>0.63</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>(1.61- 3.12)</td>
<td></td>
<td>(0.84- 1.59)</td>
<td></td>
<td>(0.73- 1.23)</td>
<td></td>
</tr>
<tr>
<td>Low Ig stratified</td>
<td>2.35</td>
<td>0.06</td>
<td>1.20</td>
<td>0.66</td>
<td>0.90</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>(0.94- 5.88)</td>
<td></td>
<td>(0.52- 2.72)</td>
<td></td>
<td>(0.49- 1.64)</td>
<td></td>
</tr>
</tbody>
</table>
Similar results were found when propensity score was used as a covariate in the Cox-regression model (Table 4):

*Table 4: Hazard ratio of low immunoglobulins treated categorically after PS adjustment*

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>P-Value</th>
<th>IgG</th>
<th>P-Value</th>
<th>IgM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low Ig</strong></td>
<td>3.19</td>
<td>0.02</td>
<td>1.87</td>
<td>0.16</td>
<td>0.63</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(1.17-8.71)</td>
<td></td>
<td>(0.77-4.54)</td>
<td></td>
<td>(0.34-1.17)</td>
<td></td>
</tr>
<tr>
<td><strong>PS score</strong></td>
<td>10.18</td>
<td>0.001</td>
<td>7.06</td>
<td>0.01</td>
<td>20.35</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>(2.69-38.52)</td>
<td></td>
<td>(1.63-30.64)</td>
<td></td>
<td>(4.42-93.77)</td>
<td></td>
</tr>
</tbody>
</table>

In the above table, PS was used as covariate in regression model along with immunoglobulins. The effect of the PS on risk of infection is about three times the effect of low IgA, which highlights the magnitude of PS (particularly variables used in building the PS, including disease activity, prednisone and immunosuppressive medications) on the risk of clinically relevant infection.
Results of low IgA were also consistent in all three propensity score methods (matching, adjustment and inverse probability weighted treatment). The following table shows hazard ratio across various propensity score methods (Table 5).

*Table 5: Hazard ratio of low immunoglobulins treated categorically after using various PS methods*

Using immunoglobulin levels as a continuous variable

Only IgA showed increased hazard at low levels (0.75 g/l) after adjustment for propensity score or weighted using IPWT (Figure 8). There was a trend in IgG to increase hazard at very low levels, but there were not enough events to make any inference (only two events below 1.3 g/l) (Figure 7a). All three immunoglobulins were associated with decreased hazard of infection in the specified knots after adjustment of propensity score, as shown in Figures 7 and 8:

<table>
<thead>
<tr>
<th></th>
<th>Low IgA</th>
<th></th>
<th>Low IgG</th>
<th></th>
<th>Low IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>P-Value</td>
<td>Hazard Ratio</td>
<td>P-Value</td>
<td>Hazard Ratio</td>
<td>P-Value</td>
</tr>
<tr>
<td><strong>PS matching</strong></td>
<td>2.24 (1.61-3.12)</td>
<td>0.01</td>
<td>1.15 (0.84-1.59)</td>
<td>0.63</td>
<td>0.95 (0.73-1.23)</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>PS IPWT</strong></td>
<td>1.75 (1.01-3.02)</td>
<td>0.04</td>
<td>1.37 (0.82-2.30)</td>
<td>0.22</td>
<td>0.54 (0.35-0.82)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>PS adjustment</strong></td>
<td>3.19 (1.17-8.71)</td>
<td>0.02</td>
<td>1.87 (0.77-4.54)</td>
<td>0.16</td>
<td>0.63 (0.34-1.17)</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Figure 7: Hazard of infection for immunoglobulins treated as continuous variables after adjustment for PS. Dots correspond to knots in the cubic spline function. Red line represents hazard of infection, while gray lines represent confidence intervals of the hazard.

Figure 7a: hazard risk at different IgG levels after PS adjustment.
Figure 7b: hazard risk at different IgM levels after PS adjustment.

Figure 7c: hazard risk at different IgA levels after PS adjustment.
Findings of inverse probability weighted treatment are shown below (Figures 8a, b, c).

Figure 8: Hazard of infection for immunoglobulins treated as continuous variables after using PS in IPWT. Dots correspond to knots in the cubic spline function. Red line represents hazard of infection, while gray lines represent confidence intervals of the hazard.

Figure 8a: Hazard risk at different IgG levels after using PS in IPWT.
Figure 8b: Hazard risk at different IgM levels after using PS in IPWT.

Figure 8c: Hazard risk at different IgA levels after using PS in IPWT.
Propensity score match, using immunoglobulin levels as continuous variables, shows similar shape between immunoglobulin levels and hazard of infection. Precision of estimate drops with wider confidence interval (Figure 9).

![Figure 9: Hazard risk at different IgA levels treated as continuous variable after PS matching. The blue line corresponds to a hazard of 1.](image)

Exact match analysis resulted in 300 pairs. There was no change with respect to increased hazard ratio at low immunoglobulins, but the reduction in hazard at normal ranges of immunoglobulins lost significance (Figure 10).
Figure 10: Hazard risk at different IgA level treated as continuous variable after exact match. Blue line corresponds to hazard of 1.

Competing risk

Death was rare (2 out of 97 in infection group and 8 out of 996 in non-infection group). Hence, no competing Cox-model was conducted.

Sensitivity analyses

Multiple sensitivity analyses were conducted. I examined several definitions of low immunoglobulins and infection. The overall findings of these sensitivity analyses support the findings of the main results with preservation of the shape of the figures. However, they also resulted in a significant drop in the number of patients and vents, which led to wider confidence intervals and a loss of statistical significance tested at alpha level of 0.05.
The results of the sensitivity analyses:

1- Defining low immunoglobulins as two consecutive low immunoglobulins, infection outcome was defined as clinically relevant infection:

A total of 214 low immunoglobulin patients and 303 non-low immunoglobulins patients were found using the above definition. Results of the model are presented in Figure 11:

![Figure 11: Hazard risk at different immunoglobulin levels treated as continuous variable after PS adjustment in first sensitivity analysis. Restricting the definition of low immunoglobulin to two consecutive measurements resulted in similar shape to the main analysis, but with wider confidence interval and weaker association with clinically relevant infection.](image)

Figure 11a: Hazard risk at different IgG levels in first sensitivity analysis.
Figure 11b: Hazard risk at different IgM levels in first sensitivity analysis.

Figure 11c: Hazard risk at different IgA levels in first sensitivity analysis.
2- Defining outcome as infections requiring IV antibiotics within 2 years: Low immunoglobulin is defined here similar to the main analysis as two low measurements (consecutive and non-consecutive).

This definition yielded 44 infections. No association between low immunoglobulins of any type and infection risk was noted (Figures 12a, b, c)

Figure 12: Hazard risk at different immunoglobulin levels treated as continuous variable after PS adjustment in second sensitivity analysis.

Figure 12a: Hazard risk at different IgG levels in second sensitivity analysis.
Figure 12b: Hazard risk at different IgM levels in second sensitivity analysis.

Figure 12c: Hazard risk at different IgA levels in second sensitivity analysis.
3- Defining outcome as any infections within 2 years irrespective of use of antibiotics: Low immunoglobulin is defined here similar to the main analysis as two low measurements (consecutive and non-consecutive).

A total of 102 events were found based on this definition. The results of the regression models are similar to primary analysis as shown below (Figures 13a, b, c):

Figure 13: Hazard risk at different immunoglobulins levels treated as continuous variable after PS adjustment in third sensitivity analysis.

Figure 13a: Hazard risk at different IgG levels in third sensitivity analysis.
Figure 13b: Hazard risk at different IgM levels in third sensitivity analysis.
Figure 13c: Hazard risk at different IgA levels in third sensitivity analysis.

4- Defining outcome as infections requiring any antibiotics within one year of index: Low immunoglobulin is defined here similar to the main analysis as two low measurements (consecutive and non-consecutive).

This results in a total of 25 events. The results of regression models based on these events are shown below (Figures 14a, b, c):

Figure 14a: Hazard risk at different IgG levels in fourth sensitivity analysis.

Figure 14b: Hazard risk at different immunoglobulin levels treated as continuous variable after PS adjustment in fourth sensitivity analysis.

Figure 14c: Hazard risk at different IgG levels in fourth sensitivity analysis.
Figure 14c: Hazard risk at different IgA levels in fourth sensitivity analysis.

Figure 14b: Hazard risk at different IgM levels in fourth sensitivity analysis.
Interaction between various isotypes of immunoglobulins and infection risk

We tested for interaction between IgA and IgG due to the previous literature suggesting common deficiency of IgG subclasses with IgA. This was done using immunoglobulins as categorical and as continuous variables. There was no evidence of interaction between two low immunoglobulins (Table 6, 7).

Table 6: Results of interaction term in Cox-regression model using immunoglobulins level categorical

<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>P-Value</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>Low Ig</td>
<td>2.89 (1.47-5.68)</td>
<td>0.001</td>
<td>1.45 (0.76-2.75)</td>
</tr>
<tr>
<td>IgA*IgG</td>
<td>HR:0.32, p-value: 0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7: Results of interaction term in Cox-regression model using immunoglobulins level as continuous variable. Levels correspond to the knots used in restricted cubic spline function.

<table>
<thead>
<tr>
<th>IGG*IGA VALUE</th>
<th>HAZARD RATIO</th>
<th>CONFIDENCE INTERVAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.56</td>
<td>0.70</td>
<td>0.39 - 1.23</td>
</tr>
<tr>
<td>24.32</td>
<td>0.46</td>
<td>0.19 - 1.10</td>
</tr>
<tr>
<td>35.53</td>
<td>0.26</td>
<td>0.11 - 0.58</td>
</tr>
<tr>
<td>49.06</td>
<td>0.44</td>
<td>0.22 - 0.85</td>
</tr>
<tr>
<td>72.86</td>
<td>0.65</td>
<td>0.31 - 1.36</td>
</tr>
</tbody>
</table>

We also tested other interactions, including low complements or leukopenia at index, which were also pre-specified in the study before the analysis. There was no evidence of effect modification of low complements on the effect of individual immunoglobulin isotypes (IgA, IgG or IgM).

**Immunoglobulin level recovery**

Finally, we assessed the recovery of hypogammaglobulinemia. We noticed that 263 out of 434 (60.6 percent) of the patients had overall recovery of low immunoglobulin levels over 4 or more years (Table 9).
Table 8: Recovery of immunoglobulin levels from abnormal low in exposure and abnormal high in control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Follow-up years available</th>
<th>&lt; 1 year</th>
<th>&lt; 2 year</th>
<th>&lt; 3 year</th>
<th>&lt; 4 year</th>
<th>4 or more</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low immuno cohort</td>
<td>Count</td>
<td>11</td>
<td>36</td>
<td>44</td>
<td>80</td>
<td>263</td>
<td>434</td>
</tr>
<tr>
<td></td>
<td>Row percent</td>
<td>2.53</td>
<td>8.29</td>
<td>10.14</td>
<td>18.43</td>
<td>60.6</td>
<td></td>
</tr>
<tr>
<td>Normal/high cohort</td>
<td>Count</td>
<td>12</td>
<td>65</td>
<td>71</td>
<td>117</td>
<td>376</td>
<td>641</td>
</tr>
<tr>
<td></td>
<td>Row percent</td>
<td>1.87</td>
<td>10.14</td>
<td>11.08</td>
<td>18.25</td>
<td>58.66</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>23</td>
<td>101</td>
<td>115</td>
<td>197</td>
<td>639</td>
<td>1075</td>
</tr>
</tbody>
</table>

We also compared the clinical variables of those who recovered and those who remained low at 4 years to ensure homogeneity of the low immunoglobulin group. No difference was observed between both groups (Table 9).

Table 9: Baseline characteristics of patients who had persistent low immunoglobulins versus those who recovered at 4 years of index date

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Persistent low group</th>
<th>Recovered group</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at index</td>
<td>N=306</td>
<td>N=128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>42.6 ± 13.9</td>
<td>40.1 ± 13.4</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>261 (85.3%)</td>
<td>116 (90.6%)</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>45 (14.7%)</td>
<td>12 (9.4%)</td>
<td></td>
</tr>
<tr>
<td>Disease duration at Index</td>
<td>Mean ± SD</td>
<td>11.6 ± 9.2</td>
<td>10.4 ± 9.0</td>
<td>0.208</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Black</td>
<td>35 (11.4%)</td>
<td>23 (18.0%)</td>
<td>0.301</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>208 (68.0%)</td>
<td>81 (63.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>35 (11.4%)</td>
<td>15 (11.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>28 (9.2%)</td>
<td>9 (7.0%)</td>
<td></td>
</tr>
<tr>
<td>SLEDAI-2k_index</td>
<td>Mean ± SD</td>
<td>6.2 ± 6.2</td>
<td>6.2 ± 6.6</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>4 (2-8)</td>
<td>4 (2-9)</td>
<td>0.952</td>
</tr>
<tr>
<td>SDI_index</td>
<td>Mean ± SD</td>
<td>1.3 ± 1.7</td>
<td>1.0 ± 1.3</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>1 (0-2)</td>
<td>1 (0-2)</td>
<td>0.163</td>
</tr>
<tr>
<td>Had lupus nephritis before the index</td>
<td>N (%)</td>
<td>133 (43.5%)</td>
<td>63 (49.2%)</td>
<td>0.272</td>
</tr>
<tr>
<td>Proteinuria positive at index</td>
<td>N (%)</td>
<td>79 (25.8%)</td>
<td>32 (25.0%)</td>
<td>0.859</td>
</tr>
<tr>
<td>Condition</td>
<td>N (%)</td>
<td>Median (IQR)</td>
<td>Mean ± SD</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Low complement (C3 or C4) at index</td>
<td>114 (37.3%)</td>
<td>11.6 ± 6.4</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Increase DNA binding at index</td>
<td>130 (42.5%)</td>
<td>11 (7-14)</td>
<td>0.529</td>
<td></td>
</tr>
<tr>
<td>Leukopenia at index</td>
<td>10 (3.3%)</td>
<td>2 (1-3)</td>
<td>0.355</td>
<td></td>
</tr>
<tr>
<td>Antiphospholipid antibodies positive at index</td>
<td>237 (83.5%)</td>
<td>0.9 ± 1.1</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>IgG at index date</td>
<td>11.6 ± 6.4</td>
<td>11.1 ± 5.4</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>IgA at index date</td>
<td>2.3 ± 1.6</td>
<td>2.4 ± 1.6</td>
<td>0.491</td>
<td></td>
</tr>
<tr>
<td>IgM at index date</td>
<td>0.9 ± 1.1</td>
<td>0.7 ± 0.9</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Prednisone use at index</td>
<td>235 (76.8%)</td>
<td>95 (74.2%)</td>
<td>0.566</td>
<td></td>
</tr>
<tr>
<td>Oral Prednisone dose (mg/day) at index</td>
<td>16.8 ± 17.6</td>
<td>16.8 ± 14.9</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressant use at index</td>
<td>142 (46.4%)</td>
<td>10 (8-20)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>biologics treatment at index</td>
<td>4 (1.3%)</td>
<td>1 (0.8%)</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Had Infections within two years after index</td>
<td>30 (9.8%)</td>
<td>17 (13.3%)</td>
<td>0.288</td>
<td></td>
</tr>
<tr>
<td>Index to earliest infection in months</td>
<td>11.9 ± 6.7</td>
<td>14.2 ± 5.9</td>
<td>0.251</td>
<td></td>
</tr>
</tbody>
</table>

*Note: N (%) represents the number of patients with the condition as a percentage of the total population.*
Chapter 5
Discussion

This study examined the relationship of acquired hypogammaglobulinemia and risk of clinically relevant infection in adult patients with SLE. Hypogammaglobulinemia was more likely to be present in patients with longer disease duration, a history of lupus nephritis, active proteinuria, and those treated with prednisone and immunosuppressive medications. Not all low immunoglobulins were associated with clinically relevant infection in this study. In fact, only low IgA increased the risk of infection after adjustment of confounding factors. Furthermore, this study shows that the majority of acquired hypogammaglobulinemia recover over time. In this chapter, I will review the main findings of our study in the context of reported studies in the literature.

The presence of hypogammaglobulinemia in SLE

The presence of hypogammaglobulinemia is not rare in SLE (as people might otherwise think). In our cohort, 448 out of 1,375 patients had at least two measurements of low immunoglobulins during their follow-up. Hypogammaglobulinemia has been reported previously in adult and juvenile onset SLE (Tektonidou et al. 2015; Jesus et al. 2011; Yong et al. 2008). The largest study in the literature was a retrospective chart review study of patients with juvenile SLE who had developed low IgG immunoglobulin levels. A total of 86 patients with juvenile SLE were included, six of whom had low IgG. This study focused on the factors associated with IgG hypogammaglobulinemia, which included white race and lupus nephritis at time of hypogammaglobulinemia. However, the significance of this finding with respect to SLE has not yet been elucidated (Lim et al. 2013).
Clinical characteristics of SLE patients with hypogammaglobulinemia

The relationship between immunoglobulins and infection in SLE patients can be confounded by many variables, including immunosuppressive medications, prednisone, or disease activity (and its complications). This can easily be seen in the baseline difference between exposure and control groups. In our study, patients with acquired hypogammaglobulinemia tended to have longer disease duration (with a mean of 11 years), active disease, and more proteinuria. In addition, the majority were on prednisone and used more immunosuppressive medications. This is consistent with clinical characteristics of previously reported cases in the literature (Yong et al. 2008). Many of these factors have been associated with hypogammaglobulinemia and also infection risk in the SLE population. For example, in juvenile patients with SLE, lupus nephritis with active proteinuria were found to be associated with hypogammaglobulinemia (Lim et al. 2013). This is not surprising, knowing that proteinuria could lead to protein loss, including immunoglobulins. This was specifically examined in a recent paper using the Abatacept and Cyclophosphamide Combination Efficacy and Safety Study (ACCESS) data. In this cohort study, patients with IgG hypogammaglobulinemia before treatment initiation were compared with those who did not have this abnormality. Patients with hypogammaglobulinemia had more active lupus nephritis and proteinuria. The prevalence of hypogammaglobulinemia correlated with the degree of proteinuria, and the level of IgG was inversely correlated with urinary protein/creatinine ration. Taken together, this strongly suggests an association between immunoglobulin levels and lupus nephritis-related proteinuria (Smilek et al. 2017).

Interestingly, lupus nephritis was also found to be associated with infection risk in lupus patients, making it a potential confounder in our study (Danza and Ruiz-Irastorza 2013). Immunosuppressive medications and prednisone were commonly found in case reports of
hypogammaglobulinemia in SLE, and they are well-known risk factors for infection (Gladman et al. 2002; Lim et al. 2013). To account for potential confounding bias, we carried out multiple analyses and measures to ensure groups balance (Hudson and Suissa 2010; Johnson, Tomlinson, et al. 2018; Mann 2003). In particular, we used propensity score in various ways. We favoured using it as covariate adjustment and IPWT because of the concern about a drop in patient numbers (and a consequent effect on study power). However, our balance diagnostics indicated that adjustment and IPWT in our study had modest improvements in group balance – likely due to the relatively small sample size. We then used propensity score to match both groups and achieve better group balance.

**Low IgA levels are associated with increased risk of clinically relevant infection in SLE**

Our data shows that acquiring low levels of IgA was associated with an increased risk of infection over two years. This is not surprising, given the important protective function of IgA in mucosal areas where most SLE patients have their primary site of infection (Perez et al. 2017). The distribution of infection in SLE patients was assessed in several cohorts, with respiratory infections being the most frequent and severe infections requiring hospitalization, and urinary infections being the most prevalent infection in outpatient settings (Gladman et al. 2002; Zonana-Nacach et al. 2001). This was further confirmed in a large population-based study in the U.S. using a national inpatient sample database. In this well-executed study, patients with SLE between the ages of 18 to 64 were examined (from 1996 to 2011) for infection-related hospitalization and risk of mortality. Several interesting observations were found. First, the most common infection requiring hospitalization was respiratory tract infection (pneumonia, in particular), which confirms previous cohort studies. The study also found that mortality was increased among patients in
hospital with SLE and respiratory infection requiring mechanical ventilation (Tektonidou et al. 2015). Several theories have been postulated about the differential importance of IgA in the protection against sinopulmonary and urinary tract infection compared to other mucosal areas like gastrointestinal tract. One possible explanation is that deficiency of IgA in mucosal area can be compensated by other isotypes like IgG and IgM (Macpherson et al. 2008; Brandtzaeg et al. 1987). This is supported by a murine study that looked at protection against rotavirus infection in IgA knockout mice. In this study, compensation was achieved through an increased level of IgG level specific to rotavirus (O'Neal, Harriman, and Conner 2000). Another suggested theory is the impact of low IgA on vaccination response – particularly those infections involving the respiratory tract. This was examined in a very interesting study of IgA-deficient patients who were followed prospectively. Pneumococcal vaccine response was noticed to be reduced in those patients, although there was no correlation between this finding and infection outcome in those patients compared to a control group (Edwards, Razvi, and Cunningham-Rundles 2004). In contrast, IgA deficiency reduces the response to influenza vaccine and increases the susceptibility to infection in murine model (Arulanandam et al. 2001). Unfortunately, data from human subjects are limited and require further investigation. This controversy about the mechanism by which IgA deficiency mediate protective function and redundancy in delivering this effect calls for more studies in this area.

Irrespective of the mechanism by which IgA mediate its function, the importance of IgA in defending the human body against mucosal infection is well-documented in clinical studies. This was shown in a large retrospective cohort of pediatric patients with selective IgA deficiency. In this cohort, 35 percent of patients had recurrent sinopulmonary infections involving ear, sinus, or pulmonary infections. Interestingly, 11.5 percent of the patients in this cohort also had
concomitant autoimmune condition (Dominguez et al. 2012). Unfortunately, the majority of these findings come from the pediatric population, with the majority being primary IgA deficient. Data on the significance of acquired IgA deficiency in adult populations is sparse. Several studies have been conducted to examine the prevalence of IgA deficiency in patients with various autoimmune diseases, including SLE. These studies report prevalence ranging from 1:19 to 1:130 (depending on the study origin and the methodology used). Higher prevalence among patients with Chinese origin was noticed, which was not found in our study. However, few studies have looked at the significance of this link, and they have been limited by sample size and rigor of study methods. For example, a cross-sectional study from the U.S. examined the prevalence of IgA among 229 patients with SLE (77 pediatric and 152 adults). The study looked at the IgA levels and possible correlation with clinical outcomes such as infection. The results showed only 12 patients with low IgA. And of those patients, only one patient died because of septicemia. Obviously, the study is far too limited (by number and study design) to make any inference about acquired IgA and risk of infection (Cassidy, Kitson, and Selby 2007). It is worth noting that the majority of studies that look at hypogammaglobulinemia in SLE and infection outcomes did not examine IgA or IgM, but rather focused on IgG. This is perhaps related to the inherent notion that IgG is the most important immunoglobulin for body defense against infections, which is primarily driven from primary immune deficiency disease literature.

**Low IgG or IgM were not associated with increased risk of clinically relevant infection in SLE**

In our study, low levels of IgG and IgM did not carry increased risk of infection in our cohort. This is true whether using a definition of any infection, clinically relevant infection, or severe infection. Interestingly, this reality was also noticed in several observational studies of
patients with acquired hypogammaglobulinemia. Smilek and his colleagues looked at the outcome of IgG hypogammaglobulinemia in SLE patients from ACCESS study. In this study, 102 patients had immunoglobulin levels analyzed prior to the initiation of study therapy. Of these patients, 16 had low IgG levels. Those patients were not different from the rest of patients in the study in terms of ethnicity, prednisone or MMF use, but they had more lupus nephritis and nephrotic range proteinuria. Interestingly, patients with low IgG did not have an increased risk of infection compared to the rest of the study population (Smilek et al. 2017). Although the study sample was relatively small, and the reported outcome was serious infection requiring hospitalization, such results are in keeping with our findings. Acquired low IgG level was also assessed in other studies as a potential risk of infection in the context of rituximab usage. This was highlighted in a French case-control study conducted on patients treated with rituximab for various conditions, including SLE. Patients who suffered serious infection in their follow-up were compared to those who did not experience any serious infection. In univariate regression analysis, patients who had concomitant treatment with prednisone (15mg or more) did have increased odds of infection. A higher IgG level was associated with a lower risk of infection, with odds ratio of 0.87 (95% CI = 0.77–0.99, p = 0.03) (Heusele et al. 2014). It was not very clear in this study whether IgG level was treated as a continuous or categorical variable, whether duration of follow-up of each patient contributed to infection, or if the results remained the same in multivariate analysis. As a result, this limits the ability to reliably relate the results to our study.

The best data perhaps comes from a study of long-term outcomes of rheumatoid arthritis patients treated with rituximab, conducted by Van Vollenhoven et al. (van VOLLENHOVEN et al. 2010). In this study, data from six multicentre international randomized trials were pooled to assess the long-term safety of rituximab. A total of 2,578 patients were analyzed, giving rise to
5,013.5 patient-years of observation. All three isotypes of immunoglobulin levels were assessed prior to the treatment and after, in addition to serious infections and malignancies. Infection was defined as infections requiring IV antibiotics. While this is a more severe form of infection with low event rate in SLE, the sample size is large enough to examine such outcomes. In addition, milder infection that can be managed with oral antibiotics were not captured by this definition. To account for that, the authors also looked at infections in general in their analysis. Meanwhile, low immunoglobulins were defined as below the normal reference range of the measuring laboratory. This is a practical definition, and it is similar to the exposure definition in our study. Multiple variable Cox-regression model was used to identify baseline predictors of infection and also to identify risk factors for low immunoglobulins. Based on the study findings, there was no association between the level of immunoglobulins at the initiation of the study and infection risk over time. The authors noted that the number of patients who developed low IgA was too small to conduct any analysis. Nevertheless, IgG levels did not predict infection in those patients. The Van Vollenhoven study also examined IgM levels, finding that about 23 percent of the patients had a drop in IgM during the study period (however, levels of IgM did not predict infection risk either in the study) (van VOLLENHOVEN et al. 2010).

More recently, a group from Harvard examined the IgG levels in patients before treatment with rituximab and identified infection. In this interesting study, a total of 3,824 patients were included, of whom 27.7 percent were patients treated with rituximab for rheumatic conditions including rheumatoid arthritis, SLE and ANCA-associated vasculitis. Immunoglobulins were categorized into mild, moderate or severe low IgG. Among those with IgG hypogammaglobulinemia, severe infection was not clearly defined, but the assumption is that the study refers to infections requiring hospitalization in accordance to the references that they used.
Although the study was not primarily targeting patients with rheumatic conditions, several findings resonate with our own study. In particular, patients with rheumatic conditions had similar frequencies of infection – before and after rituximab. In addition, patients with hypogammaglobulinemia prior to rituximab therapy tended to have more serious infection (33.5 percent) compared to those patients with normal IgG levels (15 percent). However, no formal subgroup analysis was done in patients with rheumatic conditions. This is important given that the majority of the patients had malignancies in which hypogammaglobulinemia is a known risk factor for infection complication (Barmettler et al. 2018). All together, these findings share many similarities with our study findings – particularly with respect to IgG in various populations with rheumatic disease. Although SLE is a distinct rheumatic entity, immunoglobulins seem to behave in a similar fashion across various rheumatological conditions.

**Contrasting acquired hypogammaglobulinemia effect with primary hypogammaglobulinemia**

The importance of IgG, and to a lesser extent IgM, has been demonstrated in multiple other diseases, such as CVID (Busse, Razvi, and Cunningham-Rundles 2002). Several differences should be drawn to attention here. First, the average level of low immunoglobulins in CVID is much lower than average transient-acquired low levels in SLE patients (Quinti et al. 2007). This fact was repeatedly shown in multiple observational studies in SLE and other secondary causes of hypogammaglobulinemia. For example, in a study by Smilek et al., only 16 patients (15 percent of the study population) with SLE and lupus nephritis had low levels below 450 mg/dl (4.5 g/l) (Smilek et al. 2017). This can also be noticed in the rheumatoid arthritis study by Van Vollenhoven et al. In this study, only 3 out of 2,578 patients had very low IgG levels (below 3 g/l) (van Vollenhoven et al. 2010). The IgG levels in both studies are indeed different than the mean
level of low IgG that is reported from CVID literature, which is around 250 mg/dl (2.5 g/l) as seen in the CVID cohorts (Quinti et al., 2007). To delineate this further, we analyzed the immunoglobulins as a continuous variable to examine multiple levels without specification of what is low, normal or high (Froslie et al., 2010). We also accounted for the non-linear relationship between these levels and infection outcome by using restricted spline function. The findings were both informative and confirmatory. While only IgA had an increased hazard of infection, this was noticed at a level of 0.75 g/l, which is just below the normal range. This is different than that seen in patients with CVID, who have much lower levels. In addition, the graph of IgG levels showed a trend to increase hazard at very low levels, although the number of events was too small to make any inference. Interestingly, this very low level is close to the mean level of IgG in patients with CVID. These findings highlight the importance of resisting the temptation of categorization of continuous variables and rather treating it as continuous – or at least combining both approaches – in order to capture the full spectrum of the relationship and produce more accurate inferences (Kahan et al., 2016). This finding is also very interesting since the majority of the studies that treated immunoglobulin levels as a continuous variable in rheumatic conditions did not examine IgA as a risk of infection (or had very small numbers of patients with such an abnormality) (Van Vollenhoven et al., 2010; Smilek et al., 2018). Furthermore, patients with primary hypogammaglobulinemia tend to have many other features that differentiate them from secondary hypogammaglobulinemia. One of these features is poor response to vaccines. This is an important feature of the disease, though it is not always present in all patients (Orange et al., 2012). It is also not clear in the literature whether this feature is present in patients with secondary hypogammaglobulinemia. Patients with primary hypogammaglobulinemia tend to have multiple immunological alteration, including defects in T-cell, plasma cell deletion in bone marrow, and
low switching memory B-cells (Bonilla et al., 2016). Interestingly, some of these abnormalities, such as low switching memory B-cells, might be present in patients with secondary hypogammaglobulinemia, though to a lesser extent (Duraisingham et al., 2014). In addition, these patients tend to have recurrent infections (mostly upper respiratory tract infections) which might be complicated by bronchiectasis if left untreated. Secondary humoral immune deficiencies have been found to have relatively high prevalence of bronchiectasis. Whether this is mostly related to complications of hypogammaglobulinemia, or underlying diseases, is difficult to separate. However, in both populations, the most important factor is the presence of recurrent infection.

**The association between higher immunoglobulin level and risk of infection**

In our study, all immunoglobulins were associated with a lower hazard of infection at various levels. This association clustered around values within normal range. Precisely, all isotypes of immunoglobulins reduced the risk of infection at each knot we placed in graphs of immunoglobulin levels. These knots were chosen based on the quantile of immunoglobulin levels among both low immunoglobulin and non-low immunoglobulin groups. Similar findings on IgG levels were noted in the French study of 69 patients who were treated with rituximab for various autoimmune conditions (of which SLE represented one-third of the patients). In this case-control study, patients with severe infection were compared with those who did not have severe infection. Similar to our study results, IgG reduced the odds of getting infection “(OR = 0.87, 95%CI = 0.77–0.99, p = 0.03)” (Heusele et al., 2014). Unfortunately, it was not clear if immunoglobulins were treated as continuous variable or not, but their baseline characteristics reported IgG level as continuous variable. Nevertheless, our findings may potentially clear the confusion about the relationship between immunoglobulins and infection risk – particularly in SLE patients. While only low levels of IgA was associated with increased risk of infection, IgG, IgM and IgA may
continue to perform their biological function and reduce the risk of infection after adjusting for other factors that can influence infection risk in the SLE population, including disease activity, proteinuria, prednisone, immunosuppressive medications, and biological therapy. Supporting this theory of immunoglobulins, specifically involving IgG, is the data from Barmettler et al. (2018), which examined patients with hypogammaglobulinemia who were treated with rituximab for varied reasons (27.7 percent had autoimmune rheumatic conditions) for infection outcome. In this study, immunoglobulin replacement effect was examined among patients with hypogammaglobulinemia and among the whole patient cohort. The results showed that immunoglobulin replacement did reduce the risk of infection compared to those who did not receive it, and these results were consistent in both models (Barmettler et al., 2018) The effect of immunoglobulin infusion on infection risk has not been well-characterized in SLE patients. Based on the above findings, acquiring low immunoglobulins does not seem to carry an increased risk of infection (except for IgA), but all immunoglobulins remain important protectors against infection. However, further studies are needed to evaluate the use of IVIG in patients with SLE and severe and recurrent infection.

The effect of various analysis methods on our study results

The results of our study were consistent when using propensity score as adjustment, inverse probability weighting, matching, and even exact matching with respect to increase hazard. However, the matching methods (either exact matching or using propensity score match) led to a reduction in sample size and consequently the reduced hazard effect of immunoglobulins was not statistically significant. The use of multiple methods with propensity score allowed us to compare these methods in an observational study with a relatively small sample size (which is not commonly performed in observational studies). A paper from Johnson et al. (2018) described the
utilization of propensity score in observational studies as a way to improve group balance and reduce confounding bias. In her paper, the author highlights the rationale of using propensity score, the methods of propensity score application, and the common approaches used to examine the adequacy of group balance after the propensity score application by calculating standardized difference between study groups. She then uses data from a study of systemic sclerosis to simulate the use of propensity score in matching. In her example, matching on propensity score improved group balance between patients with systemic sclerosis with pulmonary hypertension who were treated with warfarin versus those who were not treated with the drug. There are two important points to notice in her simulation example: first, the improvement of balance was noticed in both groups, but it did not necessitate all study variables to have a standard difference of less than 0.2. This was explained by simulating a trial data for patients with systemic sclerosis with a sample size similar to her observational study and comparing standardized differences between the trial and observational study results. Interestingly, propensity score resulted in better overall standardized difference and consequently groups balance. The second important point to notice was the drop in the sample size using propensity score match to almost half the original sample (Johnson et al., 2018). In our study, we tried to compare three approaches using propensity score to ensure balance between adequacy of balance and maintenance of study power. We noticed that inverse probability weighting achieved a better balance than adjustment based on the standard difference, though both resulted in similar findings. Yet, the standardized difference was not optimal after inverse weighting using standardized difference of > 0.2 as indicator of significant imbalance. This might be related to the sample size of our study (compared to larger health administrative data, in which propensity score is commonly used). Hence, we used matching to achieve better balance. The results of PS match indeed improved the standardized difference.
overall with only one variable showing more residual imbalance (SDI). The data remained consistent with respect to the increased hazard of acquiring low IgA, but the reduction in hazard effect of immunoglobulin isotypes was lost with wider confidence intervals. The discrepancy between both results could be related to the advantage of inverse probability weighting in avoiding patient loss and consequently preservation of study power. Or it could be due to residual confounding bias that has been better accounted for by matching strategy. This will be further studied in future projects using trial data of SLE patients in simulation to examine the performance of both approaches using sample sizes similar to our study and comparing it with the results of our study. Interestingly, a study by Elze et al. (2017) examined the application of four methods of propensity scores using data from four cardiovascular studies. This paper uses relatively large sample sizes, which allowed propensity scores to be used in all four ways without significant loss of patient samples. One of the studies, called ADAPT-DES, compared patients on clopidogrel with high platelets reactivity (n=4,030) to those with normal platelets reactivity (n = 3,650) for time to stent thrombosis. The relevance lies in the number of events of the study (56 events in both groups), which is small and close to the number of events in our own study. After the application of four methods of propensity score, the authors noted that all methods led to similar hazard ratios (with the exception of stratification, which did not perform well with the small number of outcome events). Furthermore, when they compared matching to inverse probability weighting, the former had less precision as noticed by a wider confidence interval. Similar findings were noted with another cardiac observational study, which had a small number of outcome events (Elze et al., 2017). These findings perhaps support our choice of using inverse probability weighting and argue that perhaps when propensity score is used in observational studies with small number of outcome events, the IPWT method is warranted for its advantage of retaining study sample and perhaps
study power. The primary answer of our study question was consistent in that only acquired low IgA level increases the risk of clinically relevant infection in adult patients with SLE.

**Sensitivity analyses and its effect on primary findings**

Due to the nature of our exposure and outcome, several definitions of exposure and outcome can be used, and the changes in those definitions may change the studies’ findings. Hence, we conducted several sensitivity analyses to examine various definitions of exposures and outcomes. The first sensitivity analysis involved defining low immunoglobulins as those with two consecutive low immunoglobulins (instead of any two low immunoglobulin measurements). This was referred to in the other studies as ‘sustained low immunoglobulin’ (Van Vollenhoven et al., 2010). The advantage of restricting the definition to two consecutive measurements is a minimization of the risk of misclassification of laboratory error as low immunoglobulins. Unfortunately, this restriction resulted in a reduction in sample size to almost half of the original sample, which was reflected on the results of the analysis. The increased hazard of infection with low IgA lost its statistical significance, although the shape of the curve and direction of the hazard remained the same, suggesting that the result was limited by the reduced sample size and study power. Furthermore, we looked at our primary definition to ensure that the two measurements of low immunoglobulins were close enough to ensure that it fulfilled the reason behind choosing it (which was to reduce laboratory error misclassification as exposure). The results were indeed reassuring. The median time between the first and second measurements was 3.7 months, and the mean time was 6.8 months. In addition, the mean value of all three isotypes of immunoglobulins at first and second measurements was similar. These results suggest that those with two consecutive and two non-consecutive acquired low immunoglobulins are not systemically different.
We also looked at several definitions of outcome, including serious infection (which is referred to infections that require intravenous antibiotics and usually hospitalization). This definition is commonly used in clinical trials because it is regarded as a serious adverse event (Van Vollenhoven et al., 2010). It also tends to carry a higher mortality rate during hospitalization – especially if use of mechanical ventilation is required, as shown by Tektonidou et al. in their population-based study (Tektonidou et al., 2015). The caveat of choosing this definition is that total events with such a definition dropped to 44, which is less than half of the total events of primary definition (total events in primary analysis was 97). Interestingly, the shape of all the figures of three immunoglobulin isotypes remained the same, suggesting that these infections perhaps drove a significant proportion of the primary outcome definition. It is worth noticing that the choice of infection definition and spectrum of infections that it covers may explain the diversity in the reported literature about associated factors with infection in SLE patients. When severe infection definition is used, the results seem to be consistent – including prednisone and immunosuppressive medications (Gladman et al., 2002; Noël et al., 2002). In outpatient settings, disease activity might be more important (Zonana-Nacach et al., 2001). However, the caveat is that many other factors contribute to variation in results, including the cohort itself, methodology, and sample sizes of the different studies. Hence, it is important to consider in each study various definitions which may account to variations in results.

Because patient-reported infection within one month of the clinical visit are included in our data, there is a possibility of contaminating the outcome with viral infections that were treated with antibiotics by the treating physician. In order to investigate this possibility, we conducted a sensitivity analysis looking at a definition of any infection as the outcome. Using any infection did not change the primary results. In fact, all figures looked exactly the same, despite an increase in
the total events to 102. This suggests that the addition of other types of infections into our outcome definition did not have a great impact on the results. This should not be surprising, knowing that hypogammaglobulinemia is an impairment of humoral immunity that manifests mostly by bacterial infection, while viral infections are usually related to T-cell dysfunction (Dropulic and Cohen, 2011). This point is illustrated in a recent paper that examined characteristics of severe infection among patients with primary nephrotic syndrome, a condition commonly associated with hypogammaglobulinemia due to the leakage of immunoglobulins from the glomeruli of the kidney. In this study by Li et al., 138 patients with primary nephrotic syndrome were retrospectively analyzed for infection. They divided infections between mild infections (which required oral antibiotics) and moderate to severe infections (which required intravenous antibiotics). Patients had similar infection distribution to our study population. The majority of the infections were respiratory infections, and bacterial infections were the most common etiology. Interestingly, non-bacterial infections accounted for only 5 percent of the total number of infections, which is very similar to our study findings (total number of infection events was 102, while those requiring antibiotics numbered 97). Patients with severe infections had the highest cumulative dose of prednisone and more immunosuppressive use, both of which remained a risk factor of infection in multivariable regression model (Li et al., 2017).

We then examined clinically relevant infection defined as infection requiring the use of antibiotics within one year of the low immunoglobulin level. This was done because of the concern about the temporal relationship between the measurement of low immunoglobulin and the possibility of recovery of immunoglobulins within a period of two years. However, the caveat of such restriction is the same major limitation of study, which is power. When we applied this definition, only 25 events were found. Clearly, this is reflected in the figures of immunoglobulins
and hazard of infection, which does not indicate any association between immunoglobulins and risk of infection using such a definition.

**Recovery of acquired low immunoglobulin levels**

We also evaluated the recovery of immunoglobulins over time. This is an important part of understanding the relationship between immunoglobulins and infection risk. We noticed that the majority of low immunoglobulins did recover over four years or more. This is consistent with case reports and case series reported in the past (Yong et al., 2008). This was examined in depth by a Vollenhoven et al. study which examined hypogammaglobulinemia following rituximab treatment in patients with rheumatoid arthritis. In this study, low immunoglobulins were present in 1.7 percent of patients for IgG, 1.5 percent for IgM, and 0.7 percent for IgA prior to the initiation of rituximab. Following rituximab infusion, the percentages increased, as expected, to 5 percent for IgG and 23 percent for IgM (but the levels remained the same for IgA). In addition to that, the proportion of patients with low immunoglobulins continued to increase following several cycles of infusion for each isotype (except IgA, which remained low throughout). More importantly, recovery of these immunoglobulins seemed to vary according to the isotype. While most acquired low IgG levels were transient with quick recovery (only 1 percent had persistent low IgG), IgM tended to have a more persistent pattern after all infusion courses. However, over time, most of the immunoglobulins recovered. The authors also found that older age of the patient was a predictor of sustained low immunoglobulins. In addition, they did not find any association between those with persistently low immunoglobulins and risk of serious infection (Van Vollenhoven et al., 2010).

The recovery of hypogammaglobulinemia was also examined in patients with lupus nephritis. In a Smilek et al. study, patients with hypogammaglobulinemia from ACCESS trial data
were examined for recovery of hypogammaglobulinemia. The authors noted that 15.6 percent of the patients (total sample of 102 patients) had hypogammaglobulinemia at baseline. This percentage increased over two to four weeks from the initiation of induction treatment of lupus nephritis (including prednisone, cyclophosphamide and abatacept in treatment arm) to 30.4 percent. However, by week 24 almost all patients recovered from hypogammaglobulinemia (only one patient had persistent hypogammaglobulinemia by 24 weeks) (Smilek et al., 2017). It is worth mentioning that low immunoglobulin in this study was defined as IgG level below 4.5 g/l, which is lower than the usual laboratory definitions (usually below 8 or 7 g/l is the lower level of normal). This may explain the early complete recovery of the immunoglobulins compared to our study.

The pathogenesis of recovery of acquired hypogammaglobulinemia is complex. Some reports suggest a delay in memory B-cell recovery could explain such a process in hypogammaglobulinemia secondary to rituximab since it is a B-cell depleting agent (Nishio et al., 2006). This differs from hypogammaglobulinemia secondary to proteinuria, in which the degree of hypogammaglobulinemia correlates with the severity of proteinuria. In the latter situation, improvement of proteinuria is likely to improve hypogammaglobulinemia (Smilek et al., 2017). It seems that secondary hypogammaglobulinemia recovery depends on the underlying etiology, though the pathogenesis is complex and consequently so is the recovery. On the other hand, replacement of immunoglobulins in the case of lack of recovery is mostly dependent on the associated clinical features, specifically infection (Duraisingham et al., 2014).

In contrast, patients with CVID tend to have persistently severe low immunoglobulin levels over time (Resnick et al., 2012). Although transient normalization can take place in CVID, most of these patients observe a drop in their immunoglobulins over time. This was evident in the characteristics of patients with CVID based on several large registries. In these registries, a small
percentage of patients (5 to 15 percent) do not have evidence of hypogammaglobulinemia but may exhibit other features of the disease (Bonilla et al., 2016; Cunningham-Rundles et al., 1999). However, over time patients may start demonstrating more hypogammaglobulinemia (Aghamohammadi et al., 2008).

We also noticed in our study that characteristics of those patients who recovered versus those who did not are similar. In particular, the number of infections and other clinical features were the same. This supports the notion that acquired hypogammaglobulinemia patients in SLE are a homogenous group – whether they recover or not.

**Examining potential interactions between immunoglobulin isotypes and other variables**

Since the mechanism of infection is complex and involves multiple factors, we tried to explore several interactions between immunoglobulins and other potential immunological variables. One of our pre-specified factors was low complement. This is based on the physiological interaction between immunoglobulins and complement levels to execute some of immunoglobulins’ protective function. For example, lack of C1q component of the complement cascade significantly reduces antibody response (Sörman et al., 2014). Our results did not find any strong evidence of such an interaction with any immunoglobulin isotypes. Perhaps, low complement is part of a causal pathway of hypogammaglobulinemia rather than an effect modifier of low immunoglobulin levels on infection risk.

We then examined the interaction between immunoglobulin levels and the presence of leukopenia. The possibility of synergism was raised when we designed the study, and it is based on the inherent role of leukocytes in defending against infection. Our results did not find any significant interaction between immunoglobulin levels and leukopenia. This is perhaps due to
maintenance of the leukocyte protective function in SLE despite the presence of leukopenia, which is part of the disease process (Carli et al., 2015). It is important to note that we did not examine each specific subset of leukocytes (such as neutropenia or lymphopenia) for interaction, since this was not one of the study objectives. Hence, the results of such interactions should be interpreted with caution.

We also examined the interaction between different immunoglobulin isotypes. This was important since the presence of more than one isotype hypogammaglobulinemia may raise more concerns about clinical significance by a treating physician. Fortunately, there was no interaction between hypogammaglobulinemia from different isotypes. This provides another layer of reassurance regarding the effect of transient hypogammaglobulinemia in adult patients with SLE. In our study, only low IgA levels increased the risk of infection; however, all immunoglobulins showed a protective effect against infection. There is the question of whether immunoglobulin replacement in those who have a clinical presentation of recurrent infection would alter the risk of infection, or whether the use of vaccine response can stratify those at higher risk of infection from those with transient hypogammaglobulinemia. These questions require more studies to find proper answers. Overall, our findings encourage the incorporation of immunoglobulin tests in clinical care of patients with SLE.
Chapter 6

Conclusions

This is a cohort study using prospectively collected data to examine the relationship between acquired hypogammaglobulinemia and clinically relevant infection risk in adult patients with SLE. In our study, acquired hypogammaglobulinemia was more likely to occur among patients with longer disease duration and higher accumulated damage index, as measured by SDI. Hypogammaglobulinemia patients are more likely to have a history of lupus nephritis and active proteinuria at the time of acquiring low immunoglobulin levels. They are also more likely to be treated with prednisone and immunosuppressive agents. Our results suggest that in adult patients with SLE, acquiring low IgA levels increases the risk of infection within two years, with the majority of these infections occurring within a year from the hypogammaglobulinemia. Whether this directly relates to IgA itself or an unmeasured confounder(s) is yet to be determined. Our findings are reassuring, with respect to acquiring low levels of IgG or IgM, since both deficiencies did not increase the risk of infection. The majority of IgG hypogammaglobulinemia occurring in adult patients with SLE are mild, and they do not pose any increase risk of infection. We noted in our analysis that increases in the level of immunoglobulins isotypes (IgG, IgM and IgA) were associated with a lower risk of infection in adult patients with SLE. This effect was not persistent in all analysis methods, which cautions the interpretation of such findings. It is possible that this effect is small and requires more events to be able to detect it. The other possibility is that residual imbalance between group accounts for this finding, which was better addressed with matching strategies. Hence, further studies are required to examine whether increasing levels of immunoglobulins in adult patients with SLE would reduce the risk of infection. This is particularly important in SLE patients with recurrent or severe infections.
Finally, we noted that the majority of acquired hypogammaglobulinemia, regardless of the isotype, improve over four years. This supports the notion that acquired hypogammaglobulinemia in adult patients with SLE is transient. This may be due to recovery of precipitating factors, such as proteinuria, though this was not specifically examined in our study.

In light of our findings, we would recommend the incorporation of immunoglobulin measurement in the routine care of SLE patients aiming to identify patients at higher risk of infection. We also believe that further studies are required to investigate methods that might modify risk of infection in high risk groups such as those with low IgA using, for example, immunoglobulin replacement or prophylactic antibiotics.

**Strengths**

To our knowledge, this is the first dedicated study looking at the relationship between acquired low immunoglobulins and infection risk in a prospective cohort of adult patients with SLE. One of the strong features of this study is the use of Toronto Lupus cohort, which is one of the largest prospective cohorts in the world. The regular and standardized clinical visits reduced several potential biases, such as investigator lead and measurement bias. In addition, the number of patients with hypogammaglobulinemia is higher than most of the studies conducted in the past (which has been one of their major limitations).

This study aimed to clarify the question about hypogammaglobulinemia and risk of infection in adult SLE patients after accounting for all possible confounders. To ensure rigour of research reporting, we tried to follow the guidelines and recommendations from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement. Although STROBE is not designed to be a checklist for observational studies, it highlights important elements of reporting observational studies (von Elm et al., 2007). In our study, we ensured that
control selection was from the sample population that gave rise to our exposure group. In addition, we tried to ensure that measured variables that account for exposure were adjusted for in the analysis. In order to achieve that, we used propensity score in various methods – taking into account the effect on the number of outcome events. We also used matching to ensure consistency of the results. Although missing data was not a major issue in this study, we did apply multiple imputation to handle such issues. We also studied exposure variables in two approaches (categorization and continuous), which provides more insight into the relationship between hypogammaglobulinemia and infection risk. In addition, we accounted for non-parametric distribution of hypogammaglobulinemia using restricted cubic spline function. Finally, we examined various definitions of exposure and outcomes in multiple sensitivity analyses. Obviously, all these measures were taken to ensure appropriate inference and address issues related to previous studies on the same topic.

Besides study methodology, this study has a further advantage of examining all isotypes of immunoglobulins rather than IgG only. This was very fruitful, since only low IgA was associated with increased hazard of infection in this study.

**Limitations**

Despite all these strengths, our study is an observational one which faces several limitations. The first major limitation is low infection rate, which likely limited the power of the study. Although we have a relatively large number of patients, our primary analysis is time-to-event analysis, which is driven by the number of events. We tried to be conscious about that in our primary choices of exposure and outcome. We also tried to use analytic methods that do not lose significant sample size when they account for covariates. More specifically, we used propensity score as covariate adjustment and IPWT (Johnson et al., 2018). The main result was similar when
we applied more restricted methods, such as propensity score or exact matching. Yet, the effect of applying more restricted definitions of exposure and outcome on the number of events was seen on the multiple sensitivity analyses. As a result, the precision of exposure effect estimate was lower with a wider confidence interval. Hence, we should interpret the results cautiously, especially when there is discrepancy between the main result and sensitivity analysis.

Our second challenge was infrequent measurement of our exposure variable, which raised some concerns regarding potential misclassification of lab error as exposure or regression-dilution bias (Grimes and Schulz, 2002; Rothman et al., 2015; Mann, 2003). To account for this, we looked at the level of the second measurements in patients who had non-consecutive low immunoglobulins and we found that the levels were similar to the first measurements. In addition, the mean time to the second measurement was about two years. Furthermore, sensitivity analyses restricting the exposure to those who had consecutively low immunoglobulins were also conducted. (Figure I-K in supplementary appendix).

Another limitation was the lack of details on immunoglobulin isotype subclassification in our database. Information about immunoglobulins subclasses may improve our understanding of the association between low IgA levels and risk of infection in adult patients with SLE. In addition, our database also lacked details on surrogate markers of immunoglobulins function such as vaccine specific antibody’s titer. Absence of details on immunoglobulins function in our study limits causal inference of low immunoglobulins on the infection risk and limits the interpretation of the findings to an association between low IgA and infection risk in SLE patients that requires further investigation.

Our study presents issues with the way infection was recorded in the database. First issue relates to timing of infection which according to protocol occurred within one month of clinic visit.
Since patients are seen on average every three months, infections outside this one-month window would not be captured in our database. Failure to capture these infections might affect the strength of association between low immunoglobulins and risk of infection in our current study. The second issue relates lack of details regarding the nature of the infection in our study population. The infection variable recorded in the database is based on patient reported history of infection as interpreted by the recording physician. Despite such data limitation, we tried to examine various definitions of infection in our sensitivity analyses and found no major change in the direction of association between low immunoglobulins and risk of infection.

Another limitation of our study was the use of immunosuppressive agents collectively rather than individually with corresponding dose and duration. Since the number of patients on immunosuppressive was small (26.2% vs 46.0% in the non-low immunoglobulins vs low immunoglobulins respectively), and a number of different immunosuppressive were used in this patient population it would be difficult to analyze the impact of different immunosuppressive medications in this study.

A potential limitation may be the generalizability of these results. However, this Centre is an Academic Centre at a downtown area of a major Canadian City and functions as a referral centre for primary, secondary and tertiary care. Therefore patients in the cohort reflect the whole spectrum of lupus from mild to very severe disease. Toronto is a multi-ethnic city and this is reflected in the ethnicity of the lupus cohort (Table 1) The results are therefore generalizable to a similar population.

Finally, this study faces the same challenge of any other observation study when it comes to estimation of exposure or intervention effect estimation. Since the exposure assignment is not random, but rather a set of determined variables, failure to account for these variables may lead to
bias estimation of the exposure effect. We tried to account for this by using propensity score methods. However, propensity score will only account for known variables (Austin, 2009). Interpretation of the results should take into account the possible effect of unmeasured confounders.

**Future Directions**

This study opens doors for several important future approaches. Most of the previous studies focused on IgG as the key player in risk modification in adult patients with SLE. Little is known about the role of IgA in protecting SLE patients. In our study, low IgA increased the risk of clinically relevant infection in adult patients with SLE (but not low IgG or IgM). Hence, we aim to investigate the mechanism of such association further. One approach is to examine patients with low IgA for predisposing factors, including clinical and serological factors such as IgG subclass deficiency.

Another important finding that requires further study is the association between the increase in immunoglobulin levels and infection risk. In our primary analysis, the increase in immunoglobulin levels for all isotypes was associated with a reduction in the risk of infection. There have been several reports in the literature about the role of immunoglobulin replacement in reducing the risk of infection, as well as their potential immunomodulating effect. This might be important in patients at risk of clinically relevant infection, such as patients with low IgA, or in those who have already manifested recurrent or severe infection. This requires further study in order to confirm these findings.

Finally, it was clear (in this and previous studies) that many factors contribute to the risk of infection in adult patients with SLE, including immunological, clinical and medication-related factors. In our study, the effect of PS, which reflects many confounders for infection in SLE, was
large. One could incorporate all these confounders in a prediction model to stratify patients at risk of infection and then try to modify the infection outcome using low dose antibiotics, IVIG, or even by reinforcing vaccination.
### Supplementary Appendix

Table a: Baseline characteristics of whole Toronto lupus cohort at the study time.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>VALUE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VARIABLE</strong></td>
<td><strong>VALUE</strong></td>
<td><strong>TOTAL</strong></td>
</tr>
<tr>
<td>N=1,935</td>
<td></td>
<td></td>
</tr>
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<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inception Patients</td>
<td>Yes</td>
<td>826 (42.7%)</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>1,691 (87.4%)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>244 (12.6%)</td>
</tr>
<tr>
<td>ethnicity</td>
<td>Black</td>
<td>253 (13.1%)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1,290 (66.7%)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>191 (9.9%)</td>
</tr>
<tr>
<td></td>
<td>Filipino</td>
<td>63 (3.3%)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>138 (7.1%)</td>
</tr>
<tr>
<td>Post-secondary education</td>
<td>No</td>
<td>538 (34.9%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1,003 (65.1%)</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married/CL</td>
<td>799 (41.3%)</td>
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<tr>
<td></td>
<td>Other</td>
<td>504 (26.0%)</td>
</tr>
<tr>
<td></td>
<td>Single</td>
<td>632 (32.7%)</td>
</tr>
<tr>
<td>Alive</td>
<td>N (%)</td>
<td>1,631 (84.3%)</td>
</tr>
<tr>
<td>Disease duration at enrollment</td>
<td>Mean ± SD</td>
<td>4.5 ± 6.3</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>2 (0-6)</td>
</tr>
<tr>
<td>Disease duration at last visit in years</td>
<td>Mean ± SD</td>
<td>15.1 ± 11.2</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>13 (6-21)</td>
</tr>
<tr>
<td>Duration of follow up in the clinic in years</td>
<td>Mean ± SD</td>
<td>10.6 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>8 (3-15)</td>
</tr>
<tr>
<td>Age at SLE diagnosis</td>
<td>Mean ± SD</td>
<td>30.8 ± 14.5</td>
</tr>
<tr>
<td>Age at enrollment</td>
<td>Mean ± SD</td>
<td>35.3 ± 14.5</td>
</tr>
<tr>
<td><strong>Measurements</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline ACR criteria #</td>
<td>Mean ± SD</td>
<td>4.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>5 (4-6)</td>
</tr>
<tr>
<td>First_SLEDAI-2K</td>
<td>Mean ± SD</td>
<td>8.3 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>6 (2-12)</td>
</tr>
<tr>
<td>Last_SLEDAI-2K</td>
<td>Mean ± SD</td>
<td>4.3 ± 5.4</td>
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<td>Median (IQR)</td>
<td>2 (0-6)</td>
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<tr>
<td>First_SDI</td>
<td>Mean ± SD</td>
<td>0.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>0 (0-1)</td>
</tr>
<tr>
<td>Last_SDI</td>
<td>Mean ± SD</td>
<td>1.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td><strong>Treatments</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroid_ever</td>
<td>N (%)</td>
<td>1,593 (82.3%)</td>
</tr>
<tr>
<td>Antimalarial_ever</td>
<td>N (%)</td>
<td>1,484 (76.7%)</td>
</tr>
<tr>
<td>Immunosuppressive_ever</td>
<td>N (%)</td>
<td>1,180 (61.0%)</td>
</tr>
</tbody>
</table>
Non-participants in study cohort

A total of 560 SLE patients were non-eligible for the study cohort because of the absence of two measurements of immunoglobulins. The main reason for absence of two measurements of immunoglobulins was late introduction of regular measurements in the Toronto Lupus cohort. I examined the characteristics of patients lacking two measurements of immunoglobulins (non-eligible) versus patients who had two measurements of immunoglobulins (study participants) and found no major differences between the two groups.

Table b: Baseline characteristics difference between patients who had two measurements of Immunoglobulins (participants) and patients who did not have two measurements of immunoglobulins (Non-eligible).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>VALUE</th>
<th>Non-eligible</th>
<th>Participants</th>
<th>P-VALUE</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N=560</td>
<td>N=1,375</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline SLEDAI-2K score</td>
<td>Mean ± SD</td>
<td>8.4 ± 8.0</td>
<td>8.3 ± 7.5</td>
<td>0.727</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>6 (2-12)</td>
<td>6 (2-12)</td>
<td>0.761</td>
</tr>
<tr>
<td>Baseline SDI score</td>
<td>Mean ± SD</td>
<td>0.3 ± 0.8</td>
<td>0.3 ± 0.8</td>
<td>0.678</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.438</td>
</tr>
<tr>
<td>Baseline Treatment of oral steroids</td>
<td>N (%)</td>
<td>365 (65.2%)</td>
<td>839 (61.0%)</td>
<td>0.087</td>
</tr>
<tr>
<td>Baseline Treatment of Immunosuppressive</td>
<td>N (%)</td>
<td>156 (27.9%)</td>
<td>353 (25.7%)</td>
<td>0.322</td>
</tr>
<tr>
<td>Any low immunoglobulin measurement over follow up</td>
<td>N (%)</td>
<td>43 (7.7%)</td>
<td>607 (44.1%)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
Propensity score model (model information)

- Data Set: CURRENT_CASE_CONTROLS_IMPUTED
- Response Variable: low_immuno
- Number of Response Levels: 2
- Model: binary logit
- Optimization Technique: Fisher's scoring
- Number of Observations Read: 1,093
- Number of Observations Used: 1,093

<table>
<thead>
<tr>
<th>Value</th>
<th>immuno</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>437</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>656</td>
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Model Fit Statistics

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<th>Criterion</th>
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<th>Covariates</th>
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<tr>
<td>AIC</td>
<td>1473.041</td>
<td>1284.242</td>
</tr>
<tr>
<td>SC</td>
<td>1478.038</td>
<td>1344.202</td>
</tr>
<tr>
<td>-2 Log L</td>
<td>1471.041</td>
<td>1260.242</td>
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</tbody>
</table>

Testing Global Null Hypothesis: BETA=0

<table>
<thead>
<tr>
<th>Test</th>
<th>Chi-Square</th>
<th>DF</th>
<th>Pr &gt; ChiSq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likelihood Ratio</td>
<td>210.7995</td>
<td>11</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Score</td>
<td>194.2203</td>
<td>11</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Wald</td>
<td>161.0261</td>
<td>11</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Analysis of Maximum Likelihood Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DF</th>
<th>Estimate</th>
<th>Error</th>
<th>Chi-Square</th>
<th>Pr &gt; ChiSq</th>
</tr>
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<tbody>
<tr>
<td>Intercept</td>
<td>1</td>
<td>-1.8352</td>
<td>0.3291</td>
<td>31.0948</td>
<td>&lt;.0001</td>
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<tr>
<td>apla_index</td>
<td>1</td>
<td>-0.5198</td>
<td>0.1771</td>
<td>8.6155</td>
<td>0.0033</td>
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<tr>
<td>age_index</td>
<td>1</td>
<td>0.00812</td>
<td>0.00554</td>
<td>2.1497</td>
<td>0.1426</td>
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<tr>
<td>female</td>
<td>1</td>
<td>-0.0540</td>
<td>0.2116</td>
<td>0.0652</td>
<td>0.7985</td>
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<tr>
<td>disdur_index</td>
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<td>0.0359</td>
<td>0.00937</td>
<td>14.6298</td>
<td>0.0001</td>
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<tr>
<td>sledai_index</td>
<td>1</td>
<td>-0.0263</td>
<td>0.0138</td>
<td>3.6037</td>
<td>0.0577</td>
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<tr>
<td>SDI index</td>
<td>1</td>
<td>0.3219</td>
<td>0.0627</td>
<td>26.3759</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>proteinuria_index</td>
<td>1</td>
<td>0.8360</td>
<td>0.2123</td>
<td>15.5101</td>
<td>&lt;.0001</td>
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<tr>
<td>steroid index</td>
<td>1</td>
<td>0.5576</td>
<td>0.1820</td>
<td>9.3832</td>
<td>0.0022</td>
</tr>
<tr>
<td>sterdose_index</td>
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<td>0.01000</td>
<td>0.00617</td>
<td>2.6235</td>
<td>0.1053</td>
</tr>
<tr>
<td>immuno_index</td>
<td>1</td>
<td>0.6235</td>
<td>0.1560</td>
<td>15.9708</td>
<td>&lt;.0001</td>
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<tr>
<td>biologics_index</td>
<td>1</td>
<td>1.3797</td>
<td>1.1473</td>
<td>1.4462</td>
<td>0.2291</td>
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</table>
### Odds Ratio Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Confidence Limits</th>
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<tbody>
<tr>
<td>apla_index</td>
<td>0.595</td>
<td>0.420</td>
</tr>
<tr>
<td>age_index</td>
<td>1.008</td>
<td>0.997</td>
</tr>
<tr>
<td>female</td>
<td>0.947</td>
<td>0.626</td>
</tr>
<tr>
<td>disdur_index</td>
<td>1.037</td>
<td>1.018</td>
</tr>
<tr>
<td>sledai_index</td>
<td>0.974</td>
<td>0.948</td>
</tr>
<tr>
<td>SDI_index</td>
<td>1.380</td>
<td>1.220</td>
</tr>
<tr>
<td>proteinuria_index</td>
<td>2.307</td>
<td>1.522</td>
</tr>
<tr>
<td>steroid_index</td>
<td>1.746</td>
<td>1.222</td>
</tr>
<tr>
<td>steroidose_index</td>
<td>1.010</td>
<td>0.998</td>
</tr>
<tr>
<td>immuno_index</td>
<td>1.865</td>
<td>1.374</td>
</tr>
<tr>
<td>biologics_index</td>
<td>3.974</td>
<td>0.419</td>
</tr>
</tbody>
</table>

### Association of Predicted Probabilities and Observed Responses

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Concordant</td>
<td>75.4</td>
</tr>
<tr>
<td>Percent Discordant</td>
<td>24.4</td>
</tr>
<tr>
<td>Percent Tied</td>
<td>0.2</td>
</tr>
<tr>
<td>Pairs</td>
<td>286672</td>
</tr>
<tr>
<td>Somers' D</td>
<td>0.509</td>
</tr>
<tr>
<td>Gamma</td>
<td>0.511</td>
</tr>
<tr>
<td>Tau-a</td>
<td>0.245</td>
</tr>
<tr>
<td>c</td>
<td>0.755</td>
</tr>
</tbody>
</table>

### Missing values and multiple imputation

A total of 42 patients had missing only APA status at index. Mean and SD of APA status before imputation was 0.22±0.41. Multiple imputation was done only for APA missing values (yes or no at index), and was conducted with five datasets created as shown in the following Table b:
Table b: APA descriptive statistics in imputed datasets with respect to APA

<table>
<thead>
<tr>
<th>Data Set #</th>
<th>N</th>
<th>N Miss</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1093</td>
<td>0</td>
<td>0.23</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td>1093</td>
<td>0</td>
<td>0.23</td>
<td>0.41</td>
</tr>
<tr>
<td>3</td>
<td>1093</td>
<td>0</td>
<td>0.23</td>
<td>0.41</td>
</tr>
<tr>
<td>4</td>
<td>1093</td>
<td>0</td>
<td>0.22</td>
<td>0.41</td>
</tr>
<tr>
<td>5</td>
<td>1093</td>
<td>0</td>
<td>0.23</td>
<td>0.41</td>
</tr>
</tbody>
</table>

‘Mean’ represents the average of patients at index with positive APA in each imputed dataset.

Mean and standard deviation of APA in the five datasets did not change after imputation.
References

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