HEPATITIS C AND G VIRUS INFECTION AND NON-HODGKIN LYMPHOMA IN A CASE-CONTROL STUDY FROM BRITISH COLUMBIA, CANADA

by

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Abstract

Background & Aims: Both Hepatitis C virus (HCV) and Hepatitis G virus (HGV) are single-stranded positive sense RNA viruses belonging to the Flaviviridae family. Epidemiological evidence has suggested an association between HCV infection and non Hodgkin lymphoma (NHL), but the association has mostly been seen in regions where the prevalence is high. Canadian studies have reported no significant association. The role of HGV infection in NHL has also been suggested, but there is little epidemiologic evidence. We investigated HCV, HGV and risk of NHL in a population-based case-control study in British Columbia, Canada.

Methods: Cases were aged 20-79, diagnosed between March 2000 and February 2004, and residents in Greater Vancouver or Victoria. Cases with HIV or a prior transplant were excluded. Controls were chosen from the Provincial Health Insurance Client Registry and were age/sex/region frequency matched to cases.

Results: Antibodies for HCV were measured in plasma of 795 cases and 697 control subjects. HCV seropositivity was 2.4% in cases and 0.7% in controls [odds ratio (OR)=3.4, (95% confidence interval (CI)=1.3-9.1)]. The highest risks were associated with diffuse large B-cell lymphoma (OR=8.3, 95%CI=2.9-23.9), marginal zone lymphoma (OR=4.5, 95%CI=1.1-19.2) and small lymphocytic lymphoma/chronic lymphocytic leukemia (OR=6.9, 95%CI=1.3-36.8).

HGV viremia was determined in plasma by the RT-PCR technique in 553 cases and 438 control subjects. The prevalence of HGV viremia was 4.5% in cases and

1.8% in controls (OR=3.2, 95%CI=1.4-7.3). The associations were strongest for cases with diffuse large B-cell lymphoma (OR=5.7, 95%CI=2.3-14.6), marginal zone lymphoma (OR=3.5, 95%CI=1.2-10.4) and other/unknown B-cell lymphoma (OR=4.9, 95%CI=1.3-17.6).

Interpretation: Our results provide further evidence that exposure to HCV and HGV contribute to NHL risk. The associations were strongest for cases with diffuse large B-cell lymphoma and marginal zone lymphoma.

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Abbreviations

EIA	Enzyme-immunoassay
ELISA:	Enzyme-linked immunosorbent assay
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGV	Hepatitis G virus
HHV8	Human herpesvirus 8
HIV	Human immunodeficiency virus
HTLV	Human T-cell leukemia virus
IDU	Injection drug use
MC	Mixed cryoglobulinemia
NHL	Non-Hodgkin lymphoma
PRC	Polymerase chain reaction
RIBA	Recombinant immunoblot assay
RNA	Ribonucleic acid

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1 Introduction

1.1 Purpose

The incidence of non-Hodgkin lymphoma (NHL) has been rising steadily for the past 30 years worldwide [1, 2]. In Canada, the incidence rates have doubled since 1970s to 9.0 per 100,000 and 13.3 per 100,000 in 1996, respectively, among males and females, and leveled off in recent years [3, 4]. The rates have also increased in Europe and Asia, but Canada and the United States are among the highest in the world [5-8]. The causes of the increasing incidence of NHL are largely unknown. The strongest risk factor identified is immunodeficiency; rates of NHL are greatly increased with relative risks of 10-100 or more in people with immune deficiency after transplantation who undergoing immunosuppressive therapy [9, 10]. In addition, autoimmune diseases such as rheumatoid arthritis, systematic lupus erythematosis, and Sjogren's syndrome have showed to be associated with a 2-5 fold increase in risk of NHL [11-15].

Viruses are considered the second most important cause of cancer in humans and contribute to 10 to 20% of all cancer cases in the world [16, 17]. Epidemiologic, clinical, and laboratory features also suggest that viruses play a pathogenic role in the development of NHL, however, the mechanisms are not completely understood.

There are two known biophysical activities that may promote the oncogenic role of viral agents in humans. First, oncogenesis is a multistep process that requires numerous genetic changes by the cell [17]. This is the result of two phenomena. The activation of oncogenes promoting cellular growth such as the ras family, and the inhibition of tumor suppressor genes leading to growth deregulation of neoplastic tissues and the p53 is the most well known tumor suppressor gene [18, 19]. Second, an over-expression of proteins or enzymes regulating angiogenesis may contribute to cancer expansion by facilitating the dissemination of neoplastic cells [20]. Both herpes viruses and retroviruses are frequently responsible for cancer in humans. Herpes viruses such as the Epstein-Barr virus are characterized by marked lymphotropism associated with lymphoproliferative disorders [21, 22]. In vitro, the Epstein-Barr virus can infect human and B lymphocytes and can immortalize these cell lines; whereas in vivo, lymphoid and epithelial cells are its preferential target tissues. These experimental observations can explain the role of the Epstein-Barr virus in Burkitt's lymphoma and immunoblastic lymphoma [21]. Two retroviruses are responsible for cancer in humans and they are the human T-cell leukemia virus (HTLV) and the human immunodeficiency virus (HIV). Patients with HIV infection may develop cancer due to the chronic immunosuppressive activity of this virus [23]. HIV virus-infected individuals may also develop human herpesvirus 8 (HHV8) related lymphomas such as Kaposi's sarcoma.

1.2 Research Objectives

- To determine whether HCV infection is related to the risk of non-Hodgkin Lymphoma, and to examine the relationship by subtype of NHL;
- To determine whether HGV infection is related to the risk of non-Hodgkin Lymphoma, and to examine the relationship by subtype of NHL;

1.3 The Following Chapters

Chapter 2 reviews the literature and summarizes the evidence for the role of hepatitis C virus and hepatitis G virus infection in non-Hodgkin lymphoma. The chapter begins with an overview of hepatitis C virus. The epidemiology of HCV will be reviewed with information on prevalence, modes of transmissions, biology of HCV, diagnostic testing and therapy, and then, the possible roles of HCV in NHL will be discussed. Second, the prevalence, risk factors, and biology of HGV, including the clinical manifestations and diagnostic testing will be presented, followed with the natural history and a discussion of the possible roles of HGV in NHL. Third, the epidemiology of NHL including known risk factors, molecular pathology, histopathological classification and therapy will be introduced. Chapter 3 highlights the methodology of the study. This section introduces the study design including the selection of cases and controls, data collection, blood collection of the study, measurement of HCV and HGV, and methods of analysis. Chapter 4 presents the study results. The risks of HCV and/or HGV infection for NHL are presented, including the association between HCV and /or HGV

infection and histological subtypes of NHL. Chapter 5 discusses the study results, some of the implications and limitations of the study, and links the results with the existing body of published literature. Chapter 6 provides a summary, and suggests future research to answer the remaining questions.

2 Background

2.1 Overview

Non-Hodgkin lymphoma is the fifth most common cancer in North America [3, 24, 25]. The incidence of NHL has increased 80% since the 1970s and stabilized recently. In the United States, the incidence of NHL began to decrease between 1996 and 2000 [24]. In Canada, the incidence rate has continued to increase but modestly since the mid-1990s [26]. Improved diagnostic techniques and classification of NHL, the HIV endemic, and immunosuppressive therapies may account for one third of the increase. Various infections, other lifestyle, environmental, and occupational exposures may contribute to the remaining increase in incidence.

The possible association between HCV and NHL was first suggested in 1993 [27]. The epidemiological association between HCV infection and NHL has been explored in many countries; results from Canada in the past did not support the association, but that could be due to limitations of the study design and small numbers [28, 29]. The first report to evaluate the risk of HGV infection in lymphoproliferative disorders including non Hodgkin-lymphoma was published in 1997 [30]. Studies in this area have been limited and the reported results were controversial. The following sections will introduce HCV, HGV and NHL respectively, and their possible roles in NHL.

2.2 Hepatitis C Virus

The Hepatitis C virus was first identified in 1989, and was known as "Non-A, Non-B Hepatitis" (NANB) to describe inflammatory liver disease not attributable to infection with hepatitis A virus (HAV) or hepatitis B virus (HBV) [31]. Chronic hepatitis C infection is one of the most common causes of chronic liver disease, cirrhosis and hepatocellular carcinoma. Chronic hepatitis C infection accounts for one-third of all cirrhosis cases. Between 20% and 30% of people with chronic hepatitis C develop cirrhosis after 20 years, and that may lead to liver failure [32]. HCV infection is believed to precede the development of hepatocellular carcinoma by a long time lag. It is an important risk factor for liver cancer, independent of HBV co-infection, alcohol abuse, age or gender [33]. It is also the most common indication for liver transplantation [34]. In addition, a substantial amount of clinical investigations suggested that HCV infection is associated with benign and malignant lymphoproliferative disorders such as mixed cryoglobulinemia (MC), and B-cell non-Hodgkin lymphoma (NHL) [35-37].

2.2.1 Epidemiology & Transmission

The World Health Organization estimates there are 170 million people, about 3% of the world's population, infected with hepatitis C, with 3-4 million new infections per year [38]. In Canada, it is estimated that there are currently between 210,000 and 275,000 people (0.8%) who are infected with hepatitis C and that as few as 30% of those who have hepatitis C know they have been infected [39]. The infection prevalence is higher in males (0.96%) than in females

(0.53%) [40]. About 5,000 persons are newly infected each year, and the number is expected to rise until 2022 [41]. According to seropositivity rates for first time blood donors in 1997 (Canadian Red Cross, unpublished data), British Columbia has the highest seropositivity rate (0.27%) and Newfoundland has the lowest (0.0%) prevalence. In 2000, 4302 HCV cases were reported in BC, responsible for 59% of viral hepatitis cases [42]. People in age groups 20-39 years and 40-64 years were estimated to have a highest prevalence rate, 1.51% and 0.75%, respectively, compared with other 5 years age groups [43]. This phenomenon may represent infection acquired in the 1960s and 1970s. From 1979 to 1997, there was an increasing trend in mortality from hepatitis C infection. Age-standardized mortality rates increased respectively, from 0.03 and 0.12 deaths per 100,000 population in 1979 to 0.26 and 0.41 deaths per 100,000 in 1997 [44].

Hepatitis C is primarily transmitted through blood or body fluids contaminated with the virus [45]. The most frequent method of transmission is injection drug use (IDU) accounts for 40% -70% of all prevalent HCV infections. There is a potential to become infected through the use of contaminated drug equipment, including straws, spoons, and other devices employed in drug use even without injection. An retrospective study in Ottawa suggested that 43% of infection was attributed to injection drug use and 33% to transfusion of blood products [46]. A cohort study reported that before the introduction of HCV screening in 1990, the post-transfusion hepatitis rate per 1000 transfusion recipients was 20.2. After the introducing of HCV screening, the post-transfusion

hepatitis rate dropped to per 1000 [47]. In 2000, Zou reported that the risk associated with blood, blood components and blood products is less than 1/100,000 [43]. It is thought that current transmission rate drops even further. Other common ways of being infected with the HCV virus are by sharing articles such as toothbrushes or razors, sexual transmissions, getting a tattoo, and body piercing or acupuncture from an operator who does not use sterilized equipment. Rate in males are two times higher than females [39].

2.2.2 Biology/ Viral pathogenesis

HCV is classified as a member of hepacivirus genus within the flaviviridea family with a positive sense single-stranded genome. Like other RNA viruses, HCV has a high mutation rate. It is characterized genetic heterogeneity, has at least 6 major genotypes and more than 100 different subtypes of the virus have been identified. In Canada, the major genotypes are 1, 2, and 3. Genotypes 4 and 5 are common in Africa, and genotype 6 primarily in Asia [48].

The hepatitis C virus is a small, envelope virus consisting of core protein and viral genomic RNA. HCV-RNA does not integrate into the cellular genome and little is known about the biologic activities of HCV proteins. Translocation of HCV core protein into the nucleus of cells has been suggested to play an important role in the development of hepatocellular carcinoma [49]. Due to the lack of reliable HCV culture system, the exact mechanism by which HCV enters host cells to initiate infection is not well understood. However, it is known that E1 and E2 envelope proteins exhibit significant genetic heterogeneity. These

proteins play a role in cellular receptor binding, and subsequent fusion of the virus to a host cell [50]. Recently, CD81 has been identified as one of the HCV-receptor candidates on B-lymphocytes [51]. Binding of HCV particles to a CD81-containing complex might facilitate B-cell activation [52]. This may partially explain the association between HCV, B-cell activation and lymphoproliferative diseases.

HCV chronic infection is becoming an increasingly important clinical disorder worldwide as the majority of HCV positive individuals develop a chronic infection leading to chronic hepatitis and cirrhosis [53]. HCV chronic infection has also been suspected to be associated with other exprahepatic manifestations such as B-cell neoplasias either alone or in combination with other factors (infectious, environmental and/or genetic). HCV may be one of many agents involved in the multistep mechanism of lymphomagenesis by inducing clonal proliferation of B cells and inhibition of apoptosis [54]. Or Hepatitis C antigens may represent a chronic stimulus for the immune system which leads to a mono- or polyclonal B-cell expansion [27, 55-57].

Clinical Manifestations & Testings

Seventy to eighty percent of HCV infections are asymptomatic [58]. Symptoms may include weakness, fever, and jaundice, very much like the symptoms of the influenza virus [59]. Very often patients are found incidentally to be infected when the alanine aminotransferase levels are reported elevated on

the ALT test or on a test for antibodies to HCV at the time of blood donation. About 15 to 20 percent of infected people resolve from the infection, however, the majority progress to chronic infection slowly. About 10%-15% of the people with chronic infection will advance to cirrhosis within 20 years, and 1 in 8 will die of liver disease [60]. Mechanisms of hepatocellular injury in HCV infection are not completely understood. Some evidence suggests that the host immune response plays the major role in controlling HCV infection and causing hepatocellular damage [61]. Over 50% of patients with chronic HCV infection will develop extrahepatic manifestations such as mixed cryoglobulinemia (MC), Sjogren syndrome and membranoproliferative glomerulonephritis [14, 27, 62].

Anti-HCV testing using Enzyme immunoassay (EIA) is usually done first to evaluate the HCV status. A supplemental recombinant immunoblot assay (RIBA) may be used to confirm a positive EIA test. Alternatively, if the results of the anti-body testing are indeterminate and the suspicion of disease is strong, confirmatory testing to detect HCV-RNA by PCR is recommended.

Four tests to detect HCV infection are commonly used. The initial diagnostic test for HCV infection is an enzyme immunoassay, a test that detects antibodies to multiple HCV antigens.

Anti-HCV tests detect the presence of antibodies to the virus, indicating exposure to HCV. These tests cannot tell if there is an active viral infection, only

exposure to HCV. These tests cannot tell if there is an active viral infection, only show there was virus exposure in the past. The current standard test is a 3rd generation enzyme immunoassay (ELISA). This assay can detect HCV infection on average 70 days after contact with the virus. In populations with a high risk for acquiring HCV, the accuracy of the HCV antibody tests is about 99%. In populations that are not at risk for hepatitis C the accuracy rate of a positive result is lower [64].

HCV RIBA test is an additional test to confirm the presence of antibodies to the virus. In most cases, it can tell if the positive anti-HCV test was due to exposure to HCV (positive RIBA) or represents a false signal (negative RIBA). In a few cases, the results cannot answer this question (indeterminate RIBA). Like the anti-HCV test, the RIBA test cannot tell if you are currently infected, only that you have been exposed to the virus.

HCV-RNA test detects whether the virus is in the blood by polymerase chain reaction. The qualitative test detects serum HCV at low levels and confirms the presence of active infection. This is particularly important before performing diagnostic liver biopsy or commencing medical therapy. The test may also be used after treatment to see if the virus has been eliminated from the body.

Viral Load or Quantitative HCV tests determine the number of viral RNA particles in the blood. Viral load tests are often used before and during treatment

to help determine response to treatment by comparing the amount of virus before and after treatment (usually after 3 months); successful treatment causes a decrease of 99% or more (2 logs) in viral load soon after starting treatment (as early as 4-12 weeks).

In addition, viral genotyping is used to determine the kind, or genotype, of the virus present. There are 6 major types of HCV; the most common (genotype 1) is less likely to respond to treatment than genotypes 2 or 3 and usually requires longer therapy (48 weeks, versus 24 weeks for genotype 2 or 3). Genotyping is often ordered before treatment is started to provide prognostic information to determine the likelihood of response and help delineate the length of medical therapy.

Treatment

Hepatitis C is one of the most common blood-borne infections in the world, and the persistence of the infection is high [38]. Only about 15% can clear the hepatitis C virus by their own immune system. In the remaining patients, the infection becomes chronic, meaning that it persists and is documented in your bloodstream for six months or longer. Antiviral treatment is determined on an individual basis, is generally based on genotype and severity of liver disease. A complete end-treatment response occurs if HCV RNA is undetectable at the end of medical therapy. A sustained response occurs if HCV RNA is undetectable 24 weeks after medication discontinuation.

The standard anti-viral therapy currently available is the combination of interferon-alpha and ribavirin. The duration of therapy is typically six to twelve months, depending on genotype of virus. Interferon combined with ribavirin is effective in about 55% of patients [38]. Patients with genotype 1 have sustained response rates of 42% to 46% [65]. Patients with genotype 2 or 3 have sustained response rates of 78% to 82% [65]. In North America types 1a and 1b are the most common in non-migrant people, but there are regional variations. Immigrants to Canada may have acquired different genotypes in their country of origin [66]. The subtypes are also geographically distributed. No vaccine is yet available.

2.2.3 Role of HCV in NHL

The possible association between HCV infection and NHL was first suggested in 1993 [27]. This association was proposed based on epidemiologic, clinical and laboratory observations that the hepatitis C virus is strongly associated with mixed cryoglobulinemia [35, 53, 67, 68]. and that mixed cryoglobulinemia is considered a variant of low-grade B-cell non-Hodgkin lymphoma which often progresses to B-cell NHL [69]. The identification of HCV as the triggering factor of the mixed cryoglobulinemia [14, 35, 69, 70] also suggested possible involvement of this virus in other hematological malignancies [71-73]. Although HCV is known to be lymphotropic [74, 75], the exact mechanism responsible for HCV-related benign lymphoproliferation and its

possible evolution to B-cell malignancies are still unclear.

The first case-control study reported a possible association between HCV and NHL [76] found that the prevalence of HCV seropositivity in NHL patients was 32% while in the healthy controls the rate was 1.3%. Other Italian studies reported a 9% to 32% prevalence of chronic HCV infection in patients with B-cell NHL, compared with a 1% to 5.4% prevalence in healthy controls [77-80]. Subsequent epidemiological studies suggesting a causal relation between HCV and NHL were originated from high of HCV prevalence areas such as Japan, Southern Italy, Egypt, where the prevalence of HCV might reach up to 12% [79, 81-87]. By contrast, several studies in low prevalence countries including the United Kingdom, Germany, France and the United States [28, 29, 88-94] did not confirm such an association. Two the studies from the United States reported an association in low grade indolent NHL [95, 96]. Prevalence of HCV in Canada is about 0.8%-1.5%, similar to that in Western Europe. Two Canadian studies reported in the late 1990s did not find association between HCV and NHL, but the studies were not population-based and contained very few cases [28, 29]. The reason for these conflicting reports is unclear but has been suggested that it might be attributed to geographic variation in other environmental and genetic factors [70, 90, 97].

Between 2003 and 2006, four systematic reviews and meta-analyses of the association between HCV and NHL were published [98-101]. Three of the

publications reported quantitative meta-analyses of case-control studies examining the risk of HCV in NHL and in B-cell NHL. Dal Maso reported the pooled RR from case-control studies of 2.5 NHL and 2.0 from cohort studies. Matsuo concluded the OR for NHL was 5.7. Similar trends were observed in the subgroups B-NHL (OR=5.04, 95%CI=3.59-7.06) and T-NHL (OR=2.51, 95%CI=1.39-4.56). Gisbert reported the highest OR of 10.8 (95%CI=7.4-16) in B-NHL; the OR increased up to 14.1 when only Italy studies were considered. The authors suggested that genetic and/or environmental factors possibly involved in the pathogenesis of B-cell lymphomas. Negri, author of the systematic review, concluded that although an association with an approximately 2-4 fold increased risk of NHL was found in different studies, different potential biases including publication bias and misclassifications could not be ruled out. The authors also pointed out that the proportion of B-NHL attributable to the HCV exposure would be high in only in high endemic areas.

A detailed review of the recent studies is provided. Results from a cohort study and several additional population-based and clinic-based studies using well selected control groups have been reported. A multicenter case-control study performed in 10 cities throughout Italy reported the prevalence of HCV significantly higher in NHL cases than controls (OR=3.1, 95%CI=1.8-5.2) ([102]. In a cohort study of 27,150 HCV-infected Swedish individuals, Duberg et al reported a significantly increased risk for HCV patients to develop B-cell NHL (SIR=1.89, 95%CI=1.1-3.0) [103]. In an Italian clinic-based study, Talamini et al

found a significantly increased risk of all NHL (OR=2.6, 95%CI=1.6-4.3) [104]. In a population based case-control study in four US centres, Engels et al reported a significantly increased risk of NHL among HCV seropositive subjects (OR=2.0, 95%CI=1.1-4.0) [95]. Three other studies found non-significant increased risks. These were a population-based case-control study among women in Connecticut by Morton et al (OR=2.0, 95%CI=0.6-8.2), a clinic-based study from Spain by de Sanjose et al (OR=1.6, 95%CI=0.9-2.8), and a population-based case-control study from Australia by Vajdic et al (OR=1.3, 95%CI=0.2-8.0) [96, 105, 106]. Although the results of these studies were not entirely consistent, they provide substantial epidemiological evidence that HCV is a possible causal factor in the etiology of NHL.

The first systematic review with regard to the HCV infection and risk for NHL which also looked at the subtype analysis [107] indicated that HCV infection was not associated with T-cell NHL, but increased risk for all subtypes of B-cell NHL. Generally, the association was found to be stronger for follicular and marginal zone lymphomas [77, 78, 108]. This observation was confirmed by another two recent systematic reviews and meta-analyses [98, 100]. In fact, the majority of individual studies reported a higher risk for low-grade indolent lymphoma such as follicular lymphoma, marginal zone and mantle cell lymphomas [87, 95, 96, 102, 109-112] despite the differences in HCV prevalence in the different study countries. Recent meta-analysis reported that the relative risks for follicular lymphoma across all selected studies were 2.73 (95%CI=2.20-

3.38, p=0.74) and 3.41 (95%CI=2.39-4.87, p=0.13) for marginal zone lymphoma, respectively. The association with diffuse large B-NHL was mainly observed in a few Italian studies where the prevalence of HCV was high. RRs for diffuse large B-NHL were 5.78 (95%CI=3.28-10.20), 3.77 (95%CI=2.17-6.55) and 2.49 (95%CI=1.43-4.36) in the studies of Vallisa, Mele and Talamini, respectively [102, 104, 112]. Studies in mid to low endemic countries such as Spain and the United States reported an increased risk for diffuse large B-NHL but the results were not statistically significant. The RRs were 2.28 (95%CI=0.87-6.01), 1.60 (95%CI=0.30-9.80) and 1.28 (95%CI=0.49-3.38) in the studies of de Sanjose, Morton and Engels, respectively [95, 96, 105]. However, the recent meta-analysis showed an overall excess risk for diffuse large B-NHL [RR=2.65 (95%CI=1.88-3.74, p<0.01)] [98].

A laboratory model to explain the role of HCV infection in the genesis of lymphoproliferative diseases has been developed. In this model, HCV antigen-driven chronic stimulation of B-cells lead to the polyclonal and subsequent monoclonal expansion of these cells which contribute to lymphocyte proliferation [37, 113]. In other words, HCV may play a role in the multistep mechanism of lymphomagenesis by inducing clonal proliferation of B cells and inhibition of apoptosis [54]. This hypothesis is supported by the evidence that monoclonal B cells are found in lymphoproliferative disorders and malignant lymphoma [55, 110, 114]. Evidence has supported an HCV antigen-driven process in the genesis of B cell lymphoproliferative disorders; however, the identity of the HCV

antigen(s) responsible for this process is still to be determined. Well designed clinical studies on the effect of anti-HCV treatment on B cell lymphoma are needed to confirm these findings.

The exact mechanisms responsible for HCV related B-cell malignancies are uncertain. Experimental investigations illustrated that the persistence of HCV in the peripheral blood mononuclear cells of patients with chronic hepatitis C may chronically stimulate B lymphocytes. And that chronic HCV infection, alone or in combination with other factors, may lead to the development of B-cell lymphoma [35]. It is reasonable to assume that several different pathogenetic mechanisms operate in the wide spectrum of HCV-related lymphomas [36]. One possibility is that HCV stimulates the proliferation of monoclonal B cell via their HCV specific B cell receptor on the cell surface. Quinn et al 2001 [115] found that B cell receptor (BCR) of a pre-malignant B cell bound to the HCV-E2 envelope glycoprotein. Further stimulation of the pre-malignant cells by the viral antigen could expand these cells, subsequently leading to the malignant phenotype. Although the identification of a pre-malignant B cell clone that subsequently converted to an overt B cell lymphoma can only be seen in some patients, this finding implicated that the same B cell clone present in an HCV-infected MC patient early in the course of the disease is later detected as a NHL.

A causal relation between HCV infection and NHL is further supported by the fact that splenic marginal zone lymphomas regressed in HCV positive patients treated with interferon-α ± ribavirin, but interferon treatment did not have

a similar anti-lymphoma effect on patients with marginal zone lymphoma who were not infected with HCV [116, 117]. Taken together, this biological evidence strongly supports the causal role HCV in the etiology of NHL. Understanding the molecular basis of HCV induced lymphomagenesis will potentially help in the design of novel ways to manage lymphoma.

2.3 Hepatitis G Virus

2.3.1 Epidemiology & Transmission

The prevalence of HGV-RNA in blood donors indicating indicates active viremia has been relatively consistent universally, ranging from approximately 1% to 4% (Germany (1.3%-1.9%), France (4.2%), the US (1.7%) and Japan (0.5%01.2%)) [118-120]. Rates of anti-E2, indicating resolved HGV infection, range from 3% to 14% [121, 122]. A Canadian study examining the prevalence of HGV in blood donors published in 2000 found HGV viremia in blood donors of 1.1% and anti-E2 of 7.3% [123]. Many studies have suggested that HGV does not cause any form of liver disease or any other clinical diseases. Investigations of viral RNA in sera of patients with hepatocellular carcinoma (HCC) and chronic liver diseases of different etiology, as well as detection of its nucleid acids in the liver tissue, did not find any association between HGV and HCC, and that suggests that HGV may not have a role attribute to chronic liver diseases and hepatocellular carcinoma like HCV [124].

The transmission routes of HGV are not well known, but it is suggested that the most common routes are through infected blood products [125]. This may include drug injections, blood transfusions, haemodialysis, tissue and organ transplants or unsafe tattooing and/or piercing [126]. Other common possible routes of transmission include sexual [127] and perinatal exposure [128]. The majority of those who are infected clear the virus and subsequently develop antibodies to the envelope glycoprotein (E2); only a small number of infections are chronic and those may persist for decades. A prospective study of 31 individuals [129] to evaluate the persistence of HGV viremia and duration of antibody response of 31 patients over a period of three years reported that infection with HGV can be fairly long lived in infected individuals. 11 individuals who were RNA positive on the first bleed date remained viremic throughout the course of the study. Similarly, all 20 individuals who were antibody positive on the first bleed date remained so throughout the course of study.

HGV is reported has ability to replicate in the host for many years producing chronic viral infection. The virus persists in approximately 15%-30% for up to nine years [130]. Since the prevalence of HGV infection is quite high in blood donors and is present in the blood products for many years, it is likely that hundreds of thousands of people worldwide have acquired HGV infection by transfusion. Whether HGV screening of donated blood and blood products should be administrated has been a highly debated topic, but currently there are no countries that screen the blood supply for HGV [121].

2.3.2 Biology & Viral Pathogenesis

The hepatitis G virus (HGV) was discovered in 1996 from the plasma of a patient with chronic non-A-E hepatitis [118]. HGV also called GB virus C. It has been suggested to be a new member of the hepacivirus genus within the Flaviviridea Family [131]. Both HCV and HGV are positive-strand enveloped viruses. Genomes of both viruses contain 5' and 3' untranslated regions, and encode a single, continuous open reading frame of about 3,000 amino acids. Sequence analysis of the main terminal 5' untranslated region (5' UTR) showed a certain degree of heterogeneity among different isolates. This domain of HGV is relatively lengthy and does not share a significant primary or secondary structure with the same domain of HCV [132]. Unlike HCV, the mutation rate of HGV was not high, suggesting that HGV may evolve with a pattern different from HCV [133-135]. Thus, these data enabled speculations that the biophysical structure of HGV could be different from HCV or other flaviviruses.

Current data about HGV genomic structure indicate that its genome, besides coding for structural proteins of the viral core and envelope (core, E1, E2), also codes for a number of nonstructural proteins that are important during viral replication (NS2-NS5) [136]. In addition, the E2 coding region, unlike HCV, does not contain a hypervariable region, thus immune escape may not be the mechanism involved in virus persistence [133]. The analysis of sequences derived from the 5' UTR region has demonstrated the existence of at least three

genotypes [136] which are correlated with geographic origin. Type 1 is found in West Africa, type 2 in the North America and Europe and type 3 in the Far East [131].

Both HGV and HCV are capable of establishing persistent, frequently lifelong infections characterized by high levels of continuous replication [137]. Cuceanu et al 2001 [138] suggested that the RNA molecule may be extensively folded through local and possible longer-range interactions to form a tertiary RNA structure, however, the mode of viral replication of HGV has not been yet elucidated. The envelope proteins (E) of flaviviruses have been described as class II fusion proteins [139, 140]. Experimental studies in the E hepatitis G virus protein has been proposed that E protein may serve as an internal fusion peptide [115, 139, 141]. Fusion events are associated with the entry of enveloped viruses into host cells. The fusion peptides operate at the interface between the extracellular medium and the membrane surface of the host cell. Through this process the virus can insert its genome into the cellular cytoplasm and carry out subsequent replication.

Other experimental data have suggested that the internal region of the E2 (279-298) structural peptide could be involved in an internal fusion process of the HGV [142]. The E2 (279-298) sequence was able to bind with high affinity to negatively charged membranes, and to promote inter-vesicle fusion. This fusogenic activity could be related to the induced peptide conformation upon

interaction with the target membrane [139].

The site of virus replication remains unclear, the issue about the viral capability to replicate in hepatocytes is very contradictory. Madejon et al [143] detected its negative sense (minus-RNA) in all seven livers that they examined, while in another study, none of the ten livers examined contained HGV-RNA [144]. A few recent studies [145, 146] failed to prove the causal relation between infection of the HGV and presence of liver pathology. Recent studies have found evidences of negative sense of viral RNA in spleen and bone marrow. These findings were so convincing that HGV was primarily lymphotropic virus. Its mechanism might be similar to EBV, in that it is able to activate cellular genes-oncogenes (c-myc, bcl-2) and induce cell transformation. Another possible mechanism might be that HGV, as chronic antigenic stimuli, causes lymphoid hyperplasia, and in the last stage of the process, leads to the clonal expansion of lymphocytes and to the development of malignant lymphomas [147].

2.3.3 Clinical Manifestation & Testing

The clinical manifestations of HGV are unknown. The majority of HGV infections (70.2%) are self-limiting and not persistent in patients [120]. However, the virus can establish both acute and chronic infection. Usually viruses that establish persistent infection in humans produce chronic diseases in their hosts, however, patients with the virus do not seem to have any medical complications [119, 145].

Approximately 50% to 75% of HGV infections are spontaneously cleared by the host immune system [148]. In contrast, an estimated 25% of HCV infections are cleared [149]. HCV infection frequently results in chronic, progressive liver disease; but HGV infection has not been convincingly associated with any disease [45]. It was suggested that HGV did not affect the clinical course in patients with hepatitis A, B, or C. However, long term clinical studies are still required to clarify the actual impact of HGV infection. It may be possible that HGV may be responsible for other extrahepatic diseases ranges from hematological and lymphomagenesis diseases such as cryoglobunemia and B-cell lymphomas [150-152].

Studies have shown that clinical manifestations of HGV infection are very mild or even absent. Most of patients with HGV have no any sign of acute or chronic hepatitis, although they are infected for years [148, 153]. The prevalence of HGV-RNA in patients with hepatitis B and C was found to be 16% and 34% [153, 154]. It appears that HGV/HCV co-infection does not seem to aggravate the course of chronic hepatitis B or C [155], or increase the severity of liver disease [146, 156, 157]. Molecular studies also show that there was no interaction between HCV and HGV in terms of viral replication [158, 159]. Studies that evaluated IFN therapy concluded that HGV was sensitive to this antiviral treatment [160]. However, co-infection with HGV may delay the spontaneous elimination of HCV-RNA from the blood [161].

HGV infection is widespread in the general population, causes persistent infection, and is transfusion-transmissible; so its potential risks should not be undermined. Recent studies suggest that HGV infection in HIV-positive individuals is associated with prolonged survival. In vitro co-infection of human lymphocytes with HGV and HIV lead to decreased HIV replication [162, 163]. The beneficial effect of HGV on HIV infected patients was confirmed in eight of ten studies [164]. Further understanding of the mechanism(s) responsible for this interaction with HIV may provide novel approaches for treating HIV and AIDS.

Testing

The two most popular methods to detect HGV infection are by HGV-RNA or antibodies directed against the HGV envelope protein E2 (anti-E2). The detection of HGV-RNA using reverse transcriptase polymerase chain reaction (RT-PCR) is the most popular diagnostic method to identify an ongoing HGV infection. Anti-HGV testing to detect antibodies to the envelope protein E2 of HGV is aimed to assess past HGV infection. This serological marker is considered as an indicator of the virus clearance. HGV-RNA appears shortly after the infection with HGV, becoming detectable in 2 to 3 weeks after exposure. Once the infected person successfully clears HGV infection, HGV-RNA disappears as anti-E2 becomes detectable over an interval of several months. Most individuals exposed to HGV are either HGV-RNA positive/HGV E2 negative or HGV E2 antibody positive/HGV-RNA negative [165, 166] Because anti-E2 and

HGV-RNA are mutually exclusive, testing for both antibody to HGV E2 and HGV-RNA is necessary to determine exposure to HGV. In a small proportion of cases, anti-E2 response does not develop, HGV may become persistent for many years [167]. Consequently, HGV-RNA testing correlates of persistence. RNA is a marker of the onset of infection, therefore RNA based study of HGV prevalence significantly underestimated the occurrence of HGV infection.

2.3.4 Roles of HGV in NHL

HGV infection is common in humans and may persist for decades. The majority of infected people clear the virus and subsequently develop antibodies to the envelope glycoprotein [147]. Since both HGV and HCV belong to the same family of Flaviviridea, and share similar genetic and biological features, HGV may also play a role in pathogenesis of NHL. This hypothesis is supported by epidemiologic studies and molecular evidence. The Hepatitis G virus has been found in peripheral blood [143, 168, 169]. HGV-RNA and its protein products have also been detected in lymphocytes [168, 170, 171]. It may be possible that the virus associates with B-cell lymphoproliferative disorders and B-cell lymphoma. In addition, molecular analysis showed that HGV replicated in PBMC in vitro suggesting that HGV is a panlymphotropic virus [122].

Whether HGV infection is a causative or contributing factor in the pathogenesis of NHL is not clear. A meta-analysis reported that HGV-positive rates in B-cell non-Hodgkin lymphoma patients and healthy subjects were 8.4%

and 0.8%, respectively, with an odds ratio of 10.8 [172]. The systematic review by Wiwanitkit et al only found three reports investigating the prevalence of HGV and the risk for B-cell NHL with HGV infection [88, 173, 174] . Of the three reports, only two were used [173, 174] for further meta-analysis because one [88] of them did not have complete data on the prevalence in both patients with B-cell NHL and healthy control subjects. As a result, 178 cases and 355 healthy were included in the meta-analysis, and an odds ratio of 10.8 determined.

Table 2.1 summarizes the previous results of the link between HGV infection and NHL. The prevalence of HGV was determined by detecting HGV-RNA in the majority of the studies except the study of Pavlova et al [150] that serum samples from all patients were examined for HGV-RNA and antibodies to E2 envelope protein of HGV. So far, only four case-control studies on HGV in NHL have been reported. One was a population-based case-control study [173] reporting a non-significant elevated risk of HGV with the odds ratio of 5.6 (95%CI, 0.61, 48.39). The second was a hospital based case-control study [29] that did not find significant difference in the incidence of HGV infection between patients with NHL and controls and suggested that HGV infection did not play an important role in the pathogenesis of NHL (OR=5.31, 95%CI=0.60-46.66). HGV viremia positive was found in 5 out of 100 NHL patients, and 3 out of 100 non-hematological malignancy controls (OR=1.7, 95%CI=0.40-7.32). As this was a hospital based study, it is unclear whether the cases or the controls were drawn from the same population. As a result, the study results may be biased. Another

hospital based case-control study comprised a total of 47 hematological patients,19 control patients with clonal stem cell disease and 28 cases with malignant hematological diseases. Among the 28 cases, 13 patients were diagnosed with NHL [150]. Serum samples of 29 of 47 (62%) hematological patients were RT-PCR positive. The serological analysis showed that antibodies to E2 were detected in 5 of 29 HGV-RNA positive hematological patients. Among the 28 hematological patients, 21 were HGV-RNA positive. Among the 13 NHL patients, 13 (76.9%) were HGV-RNA positive, and 3 negative (13%). Among the 19 clonal stem cell disease control patients, 8 were HGV-RNA positive (42%), 11 were negative (61%) respectively. Among the 29 HGV-RNA positive patients, HCV-RNA and HCV antibody were detected in 1, the co-infection rate was 3.45%. In conclusion, the prevalence of HGV-RNA in the group of malignant hematological diseases patients (72%) was significantly higher (p=0.02) than in the patients with clonal stem cell diseases (28%), the prevalence of HGV-RNA positive was also higher (76.9%) among patients with NHL compare with patients with other malignant homological diseases. The forth study, by Giannoulis et al, reported a high prevalence of HGV viremia in patients with NHL (9.3%) whilst the prevalence of HGV in blood donors was 0.7% (OR=14.4, 95%CI=3.11-67.05) [174]. This study may also have been biased as it used population-based cases and blood donors as the control subjects. Two other epidemiologic studies observed the prevalence of HGV in NHL cases, no control sample was recruited. Ellenrieder et al 1998 [88] was one of the very first studies reported an increased prevalence of HGV infection in patients with NHL, especially with low grade NHL

(16.3%). The other case only study reported a contradictory result, as no HGV infection was found in the study subjects [175].

There have also been several epidemiological studies of lymphoproliferative disorders. One UK study showed that patients with lymphoma (NHL and HD) had an increased incidence of HGV viremia compared with normal blood donors, another UK study only found one HGV viremia in 75 lymphoproliferative disorders cases, therefore concluded that HGV viremia was not associated with the lymphoproliferative disorders [30, 169]. The Italian study [151], however, reported statistically significant difference of HGV in B-cell lymphoproliferative disorders (7.8% vs. 0.9%, $p<0.03$) than in controls. Among the various B-LPD neoplasms, HGV infection was more frequent in B-NHL (11.5%).

Experimental studies further support the lymphogenesis role of HGV. HGV- RNA replicates in peripheral blood mononuclear cells, CD4(+) and CD8(+) T lymphocytes, and CD19(+) B lymphocytes [122], further promoting the causative role in the pathogeneses of B- cell NHL. HCV and HGV were shown to replicate in bone marrow progenitors and hematopoietic cell lines which further confer that HCV and HGV may play a role in the lymphoproliferative diseases or non-Hodgkin lymphoma [176]. Another possible mechanism may be that HGV as a chronic antigenic stimuli, may cause lymphoid hyperplasia and lead to the clonal expansion of lymphocytes, and to the development of malignant

lymphomas. The process of HGV lymphomagenesis and malignant transformation may be heterogeneous; and the evolution of the HGV may not only involve multiple steps but may also engage with extremely slow long term evolution [134].

The role of HGV in lymphotropism and its potential oncogenic role in NHL has been suggested [30, 177], but the reports have been inconsistent [136, 170]. Experimental data has illustrated that HGV replicates in both T and B lymphocytes in vitro [122], and demonstrated that HGV replication in human lymphoid cells [178, 179]. Ellenrieder et al have demonstrated an increased prevalence of HGV (16.3%) in patients with low-grade NHL [88]. This fact, together with the reported association of HGV with mixed cryoglobulinemia, may be indicative of a relationship between HGV infection and lymphoid disorders.

Overall, the causal role of HGV with B-cell lymphoproliferative disorders, NHL in particular, is primarily supported by epidemiologic and serologic studies. Molecular evidence has not been proven beyond doubt, as the virus has been found in peripheral blood mononuclear cells (PBMC). Whether this implied an etiological role for the viral agent in the development of NHL cannot be conclusively answered. In conclusion, it is thought that the process of lymphomagenesis and malignant transformation is likely heterogeneous, and the evolution of this RNA virus involves multiple steps over a long period.

2.4 Non-Hodgkin Lymphoma

The incidence of non-Hodgkin lymphoma has increased steadily in the last three decades in both males and females, and is now the fifth most common cancer in Canada. The number of deaths each year from NHL has also increased over the last thirty years. These increasing rates are likely due to both a true increase and improvements in the detection and classification of NHL. The introduction of immunological and genetic techniques has improved our ability to preferably diagnose and categorize the condition. The etiology of NHL is not well understood. The strongest association is with both primary and secondary immunodeficiency [180]. Recent studies also suggested that the use of highly active antiretroviral therapy (HAART) decreased the risk of NHL [181], whereas HIV DNA load increased risk of NHL. Despite the known risk factors, it is estimated that 40% of the NHL cases remained unexplained [182].

2.4.1 Epidemiology & Etiology

Incidence rates of NHL in Canada and the United States are among the highest in the world, intermediate in Africa and low in east Asia [5]. Liu et al 2003 reported that incidence rates of NHL has increased from 7.3 and 5.2 per 100,000 in 1970-1971 to 14.0 and 10.0 per 100,000 in 1995-1996 in males and females, respectively, in the United States [4]. Improvements in diagnosis, changes in NHL classification and the increase in AIDS-associated NHL contribute to the marked increase in NHL, but changes in risk factors may also have contributed to the increase of NHL. The rate of NHL increases exponentially with increasing

age (median age is 50). In America; incidence rates are higher in whites than blacks. Hispanic women have the second highest incidences rates after whites. In Canada, an estimated 3,600 men and 3,000 women will develop NHL (500 men and 400 women in BC), constituting 5% of all new cases. 1,650 men and 1,350 women might have died of NHL in 2006 [3]

Medical Conditions

We do not yet fully understand what causes NHL, although certain risk factors make individuals more prone to the development of NHL. Among these many factors, some of them such as age and genetics are beyond our control. Other factors such as environmental or lifestyle related variables are modifiable. People with a weakened immune system or autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosis have been observed to have increased risks. A study in Sweden found a two-fold increase in people diagnosed with celiac disease, and a fifty-fold increase in some types of T-cell NHL [183]. Human immunodeficiency virus (HIV) is characterized by a deficiency of CD4-positive T cells. HIV infection markedly increases risk of NHL. A study of the US veterans found that HIV-positive subjects had 9.71 times (95% CI, 6.99-13.49) greater risk of NHL than HIV-negative veterans [184]. Evidence showed that NHL risk is increased in patients undergoing immunosuppressive therapy to prevent rejection after transplantation with donor organs or tissues. During the first year after transplantation, the risk of NHL increases about twenty-fold, and drops when immunosuppressive medication is reduced or stopped [185].

Infectious Diseases

Several infectious agents are known to increase risks of NHL. Helicobacter pylori, a bacterium that may infect gastrointestinal tract, is associated with the development of gastric NHL. Burkitt's lymphoma in Africa is associated with prior infection with Epstein-Barr (EBV) virus. The virus may play a role in the development of other subtypes of NHL as well. Infection with human T-lymphotrophic virus type I (HTLV-1), especially in early childhood, is associated with increased risk of adult T-cell leukemia/lymphoma (ATL) [186]. ATL is rare in Canada, but more common in southern Japan and the Caribbean. The possibility that some low grade NHL and other B-cell disorder are associated with hepatitis C virus (HCV) has been suggested [86, 187-191]. E2 protein is speculated to be the chronic antigenic stimuli involved in the lymphomagenetic process [115, 192]. In Canada, 88% of HCV infection occurs in injection drug users [193]. HCV infection is shown to be strongly associated with mixed cryoglobulinemia, a benign lymphoproliferation that can evolve into B-cell NHL [80]. Thus, Hepatitis G virus (HGV) may be associated with lymphoproliferative disorders [30] since both HGV and HCV belong to the Flaviviridae family and have similar genome sequences and structure. However, studies in this area have been limited, and the reported results are controversial. These findings suggest that exposure to infectious agents causes the proliferation of lymphoid cells as an aberrant, uncontrolled immune response; chronic antigenic stimulation and or inflammation may underlie these entities.

Occupational & Environmental Exposure

Reports on the association of NHL with occupational exposures are inconsistent. Since most studies of occupational exposures were based on job titles, it is hard to interpret specific exposure. Among different occupational groups, farmers and people living in rural communities have been extensively studied. The main hypothesis for the occupational risk of NHL focuses on exposure to pesticides, herbicides, fungicides, paints, fuel, oils and other organic solvents [194, 195]. Work involved with animals, such as meat workers, meat inspectors, and veterinarians, has been inconsistently associated with increased risk pf NHL. Exposure to solvents and NHL has been extensively investigated, but there is no consensus of the relationship. A recent Australian study found a 30% increase in NHL risk in those occupationally exposed to benzene or other benzene containing petroleum products in their work [196, 197].

Lifestyle

The association between various lifestyle factors (physical activity, nutrition, reproductive and hormonal factors) and NHL have been extensively studied, although most of the results appear negative or not statistically significant. Several studies have investigated the relationship between smoking and NHL. A pooled analyses comprising studies in the US, Europe and Australia found significantly increased risk for the association between follicular lymphoma and smoking history, but alcohol consumption in particular wine has been

reported to have a protective effect on risk of NHL. Nevertheless, dose-response relationship between type of alcohol and subtype of NHL is undetermined [198]. Sunlight exposure has also been found to reduce risk of NHL in at least two case-control studies in Australia and Sweden [199, 200]. The Australia study reported that the reduction in risk was strongest for exposure on non-working days, with an odds ratio in the highest quarter of exposure of 0.47 relative to those with the lowest quarter of exposure [200].

Genetic Susceptibility

The role of genetic susceptibility in NHL is supported by the evidence of common genetic variations altering NHL risk. A pooled analysis of over 10,000 cases and 11,000 controls from the InterLymph Consortium confirmed that risk of NHL was elevated among those with hematopoietic malignancies in first degree relatives [201]. Genetic polymorphisms controlling immune function are suggested to influence NHL risk. This area is being studied intensively, and new findings may be reported in the near future.

2.4.2 Biology & Histopathological Classification

It is now known that all cancers, including lymphoma, begin as a mutation in the genetic material - the DNA (deoxyribonucleic acid) - within certain cells. The external or internal causes of such changes probably add up over a lifetime. DNA errors may occur in the form of translocations - damage produced when part of one chromosome becomes displaced and attached to another

chromosome. Translocations disrupt the normal sequencing of the genes. As a result, oncogenes on the chromosomes may be switched on, whereas tumor suppressors may be switched off. These changes often occur in lymphoma. Numerous risk factors may be responsible for DNA damage within the body's lymphocytes.

Lymphoma is a general term for cancers that develop in the lymphatic system. Lymphocytes are cells that originate in the bone marrow and circulate in the blood vessels, and are part of our immune system designed to fight infection. Lymph nodes are present in the underarms, groin, neck, chest, and abdomen. Lymphocytes are also found in many body organs such as the spleen, liver, bone skin marrow and intestine. There are two types of lymphoma: Hodgkin disease and non-Hodgkin lymphoma. NHL is classified according to the type of lymphocytes from which they arise: B-cell lymphoma originate from lymphocytes which develop in the bone marrow and T-cell lymphomas from the lymphocytes which develop in the thymus.

Over 85% of the NHL cases in Western countries are B-cell lymphoma; T-cell lymphoma is more common in Asia. B-cells help protect the body against bacteria by maturing into plasma cells and producing immunoglobulin (antibodies). Antibodies are part of the humoral immune system response to bind to surface antigens. This results in the killing of bacteria. T cells help protect the body against viruses, fungi and certain bacteria. They recognize specific

substances found in virus-infected cells and destroy these cells. T cells also release cytokines to attract other types of white blood cells, which then digest the infected cells.

Non-Hodgkin lymphoma is a group of malignancies of immune system with diverse molecular features. Because it is heterogeneous, NHL can start almost everywhere and can spread to almost any organ in the body. There are over 28 different types of NHL. Each type has its particular appearance when examined under the microscope, and has different types of proteins on the surface of the cells with different rates of growth. The most typical B-cell lymphomas are of diffuse large B-cell (33%) followed by follicular lymphoma (22%). All other types of lymphoma are less than 10% [202]. Different types of lymphoma response to different treatments; therefore, it is important to determine the type of lymphoma in considering treatment options.

NHL is categorized into B-cell and T-cell neoplasms based on their immunophenotypic characteristics. Due to the wide variety of disease entities, classification of NHL has been difficult. Before the introduction of the World Health Organization (WHO) system, the Working Formulation (WF) was used to categorize NHL according to histologic type (diffuse, follicular), tumor grade (low, intermediate, high), and immunologic type (B-cell, T-cell). Supported by immunophenotype and genetic examination techniques, lymphomas were identified as specific entities on the basis of morphological appearances. This

new classification was subsequently published in the Revised European-American Classification of Lymphoid Neoplasms (REAL). In 2000, WHO updated the classification system based on the REAL, thereafter, the WHO system became has become the standard classification system worldwide [202].

2.4.3 Clinical Manifestations & Treatment

The most common sign of indolent NHL is a painless swelling in one or more of the lymph nodes of the neck, collarbone region, armpits, or groin, patients with indolent NHL do not present with B symptoms (weight loss greater than 10%, night sweats and fever), but the B symptoms usually occur to patients with intermediate and high grade NHL.

The diagnosis of lymphoma is made by tissue biopsy. Lymph node biopsy is required for diagnosis and staging. Biopsy samples usually are sent to a laboratory for a number of additional tests, such as immunocytochemistry, flow cytometry, and cytogenetic studies. These tests help to identify specific types of lymphoma. Because NHL is a heterogeneous malignancy, prognosis may vary from an aggressive to an indolent course.

Treatment for NHL depends on the type, location, grade, and stage of disease, as well as the patient's age and overall health. If NHL has spread to the lymph nodes and other organs beyond the skin, other treatments will be required, such as systemic chemotherapy or biological therapies with substances such as

interferon, monoclonal antibodies, cis-retinoic acid (a chemical relative of vitamin A), or other new compounds: cytotoxic fusion protein. Cytotoxic fusion protein binds to cancers cells and causes them to die. The role of radiotherapy for the treatment of non-Hodgkin lymphoma depends on the type and stage of disease, as well as the health status of the patient. The exceptions are early-stage low-grade lymphomas, which often can be treated by radiotherapy alone, as well as lymphomas of certain organs, such as the eye. In select cases, a stem cell transplant may be needed.

2.5 Summary

For several decades the incidence of NHL has increased more rapidly than that of any other cancer. Reasons for the increase of some NHL subtypes are well established such as immunosuppression, autoimmunity, and HIV infection, but these only explain a fraction of the cases. Since the early 1990s, increasing evidence suggests a role of HCV infection in the etiology of malignant lymphoma. Recent multi-centre case-control in five European countries supports that chronic HCV infection contributes to the etiology of NHL [203] because it shares similar genome structure and organization to HCV, it is suggested that HGV may also lead to the development of NHL. Several studies identified an increase prevalence of HGV infection in patients with low grade NHL. Whether this feature represents merely epiphenomenon or implies an etiological role for the viral agent in the development of NHL is not yet conclusively answered.

Table 2.1 Studies on HGV in non-Hodgkin lymphoma

Study types	Year	Authors	Country	# Cases	HGV Prevalence in cases (%)	Type of Controls	# Controls	HGV Prevalence in controls (%)
1	1999	Collier	Canada	100	5.0	Non NHL malignancy	100	3.0
1	2002	Kaya	Turkey	70	7.1	Healthy subjects	70	1.4
1	2004	Giannoulis	Greece	108	9.3	Blood donors	285	0.7
2	1998	Ellenrieder	Germany	69	4.3	NA	NA	NA
2	1999	Pavlova	Austria	13	76.9	Clonal stem cell disease	19	42.1
2	2000	Arican	Turkey	44	0	NA	NA	NA
3	1998	Minton	UK	76*	9.2	Blood donors	100	1.0
4	1997	Keenan	UK	75**	1	NA	NA	NA
5	2002	De Renzo	Italy	127**	7.8	Healthy subjects	110	0.9

Study types:
1: NHL, case-control, 2: NHL, cases only, 3: NHL & HD, case-control, 4: LPD, cases only, 5: LPD, case-control
NA: not available
* NHL plus HD
** Lymphoproliferative disorders (LPD)

3 Methods

3.1 Study Design

The main objectives of this study were to determine:

1. whether prior medical history, particularly with respect to factors related to immune stimulation and suppression, is related to the risk of NHL.
2. whether plasma levels of a number of organochlorine compounds including DDT, PCB congeners, and selected other organochlorines is related to risk of NHL, and whether the risk is associated with increasing levels of combined or specific organochlorine compounds; and
3. whether ultraviolet radiation (UVR) exposure (artificial or natural solar) is related to the risk of NHL, whether there is an increasing risk of NHL associated with increasing cumulative UVR, and whether the character and timing of exposure to UVR is related to the risk of NHL.

Between March 2000 and February 2004, subjects from the Greater Vancouver Regional District (GVRD) and the Capital Regional District (CRD), which includes the city of Victoria, were enrolled from the British Columbia Cancer Registry. Together, these 2 large districts represent about 59% of the population of the province. Cases included subjects with newly diagnosed NHL aged 20 to 79 without evidence of HIV infection or prior transplantation. Subjects unable to give informed consent, were deceased prior to contact, too ill or unable to complete the questionnaire due to language were excluded to the study. A

package including an introduction letter, patient information and consent form were sent to the subjects to invite them to participate in the study. Study material was made available in the four most commonly spoken languages in the catchment area: English, Chinese, Punjabi and Tagalog. Follow- up calls were made to subjects who did not respond after two weeks. Subjects who agreed to participate were asked to provide a written informed consent, complete a self-reported questionnaire on family history, and complete a computer-assisted telephone interview (CAIT). In addition, subjects were asked to provide a blood sample. For those did not want to provide a blood sample, they were asked to provide a saliva sample. By the end of the data collection, a total of 828 and 848 eligible cases and controls, respectively consented to the study.

This study was approved by the BC Cancer Agency-University of British Columbia Research Ethics Board. Written informed consent was obtained from each participant. Subjects unable to informed consent, deceased prior to contact, too ill or unable to complete the questionnaire due to language were excluded to the study.

3.1 1 Cases

Eligible cases were NHL patients age 20-79 diagnosed between 2000- and 2004 residing with the GVRD or CRD. Cases were ascertained from the BC Cancer Registry. All cases were reviewed by one of two pathologists (Randy D. Gascoyne or Brian Berry) and classified using the World Health Organization

classification [204].

The total number of eligible cases available in the parent study was 1263. Of those eligible cases, 133 (10.5%) died before being contacted with a median time between diagnosis and death of 32 days (interquartile range 6-78 days), 62 (4.9%) were unable to be located, and 1068 (84.6%) were contacted. Of those contacted, 73 (6.8%) could not participate due to poor health, 8 (0.7%) could not participate due to language, 147 (13.8%) refused, and 840 (78.7%) consented. After pathological review, 12 cases with insufficient material to classify or with prior transplantation were eliminated leaving 828 cases for analysis. All 828 cases completed at least part of the questionnaire, 769 (92.9%) provided a blood sample, 28 (3.4%) provided only a saliva sample, and 31 (3.7%) did not provide a blood or saliva sample (Table 3.1).

3.1 2 Controls

Population controls were frequency matched to cases by sex, age (within 5-year age group), and residential location (GVRD or CRD) in an approximately 1:1 ratio. Controls were selected from the Client Registry of the BC Ministry of Health. There were 2373 controls selected in the parent NHL study. Of those selected, 13 (0.5%) were deceased, 504 (21.2%) could not be contacted, and 1856 (78.2%) were contacted. For those contacted, 103 (5.5%) could not participate due to poor health, 49 (2.6%) could not participate due to language, 856 (46.1%) refused, and 848 (45.7%) consented. All consenting controls

completed at least part of the questionnaire, 680 (80.2%) gave a blood sample, 113 (13.3%) gave only a saliva sample, and 55 (6.5%) did not provide either sample.

3.2 Questionnaire

Questionnaires comprised two parts: self-reported and telephone interview. A questionnaire was enclosed in the initial package sending to the subjects in order to solicit family medical history, personal residence and work history. The full interview was administrated by trained interviewers using a computer-aided telephone interview (CATI). The interviewer team was made up of interviewers fluent in four most common foreign languages in BC, i.e. Cantonese, Mandarin, Punjabi and Tagalog in addition to English. The telephone interview covered detailed medical history, physical activities, sunlight exposure, tattoo and body piercing, pet exposure, hair dye, and demographic information.

3.3 Blood Samples & Lab Methods

A blood sample of 40mL was drawn into four ACD-and two EDTA tubes. All blood was processed at the BC Cancer Agency. Each sample was assigned a unique random identification number to blind the laboratory with respect to the disease status of the sample. For serology testing, plasma was separated from the whole blood in the EDTA tubes within 24 hours after vein puncture, and then transferred to 1ml vials stored at -80°C for 1-4 years before testing.

3.3 1 HCV

This study of HCV risk included cases and controls that had an HCV test in British Columbia (BC) or had sufficient plasma to ascertain HCV serology. A database containing the results of all HCV tests in BC since 1992 is maintained by the British Columbia Centre for Disease Control. HCV testing is recommended for all newly diagnosed non-Hodgkin's lymphoma cases [205]. HCV serology results were obtained from the HCV Registry for 458 (55.3%) cases and 93 (11.0%) controls. Cases and controls with sufficient plasma and either without a linkage to the HCV database or with an equivocal result from the registry (3 cases) were tested for HCV serology. In addition, 101 subjects with a result in the HCV Registry were also tested for HCV serology in plasma. From all sources, HCV serology was determined for a total of 795 (96.0%) cases and 697 (82.2%) controls.

Serology

The HCV serology was performed at either the National Microbiology Lab (NML) in Winnipeg, MB (n=467) or the BC Centre for Disease Control (BCCDC) in Vancouver, BC (n=578). Plasma samples were tested for HCV antibodies at NML by a second generation enzyme immunoassay (EIA), whereas those tested at BCCDC were tested using a third generation dual EIA procedure. At NML, primary screening was performed by Abbott HCV EIA v.2.0, and reactive samples were retested by recombinant immunoblot assay (RIBA). At BCCDC,

primary screening was performed by Abbott AxSYM HCV 3.0, and reactive samples were retested by Ortho Vitros Eci HCV 3.0 (to February 2005). From March 2005, primary screening was performed by Bayer ADVIA Centaur HCV and reactive samples were retested with Abbott Architect anti-HCV. Only samples reactive by both manufacturers' tests were considered to be anti-HCV reactive. Samples were considered anti-HCV equivocal when only one EIA was reactive.

3.3 2 HGV

This study of HGV risk included cases that had sufficient plasma to ascertain HGV-RNA. HGV viremia was determined for a total of 553 (66.8%) cases and 438 (51.7%) controls.

Nucleic acid extraction

Nucleic acid was purified from 250 µL of plasma on a Qiagen BioRobot 9604 using the QIAamp Virus BioRobot 9604 Kit (Qiagen, Mississauga, ON) according to manufacturer's instructions. Nucleic acid was eluted in 70 µL RNase-free buffer and stored at –80 °C until amplified.

PCR primers, probes and conditions

HGV primers and probe (Table 3.2) were directed toward the conserved 5' non-coding region of the viral genome.[206] Amplification of extracted nucleic acid was performed on an ABI 7900HT Fast Real-Time PCR system cycler

(Applied Biosystems, Streetsville, ON). Cycling conditions included a 30 minute incubation at 50°C for reverse transcription, a 15 minute incubation at 95°C to activate HotStarTaq DNA polymerase, and 50 two-step cycles of 15 seconds at 95°C and 1 minute at 60°C for amplification of DNA.

Successful nucleic acid extraction, functional reactivity of amplification reagents and absence of inhibitors were verified by detection of endogenous ß-globin DNA by PCR in cases and controls. Primers and probe were based on a Hydrolysis Probe Assay (Roche Applied Science, Laval, QC). Cycling conditions included a 15 minute incubation at 95°C followed by 45 two-step cycles of 94°C for 15 seconds and 60°C for 30 seconds. Specimen quality successful nucleic acid amplification and absence of inhibitors was demonstrated by detection of endogenous ß-globin. HGV Armored RNA (Ambion Diagnostics, Austin, TX) also served as a positive extraction and amplification (including reverse transcription) control for all runs.

Table 3.1 Study design and ascertainment flow chart of NHL study

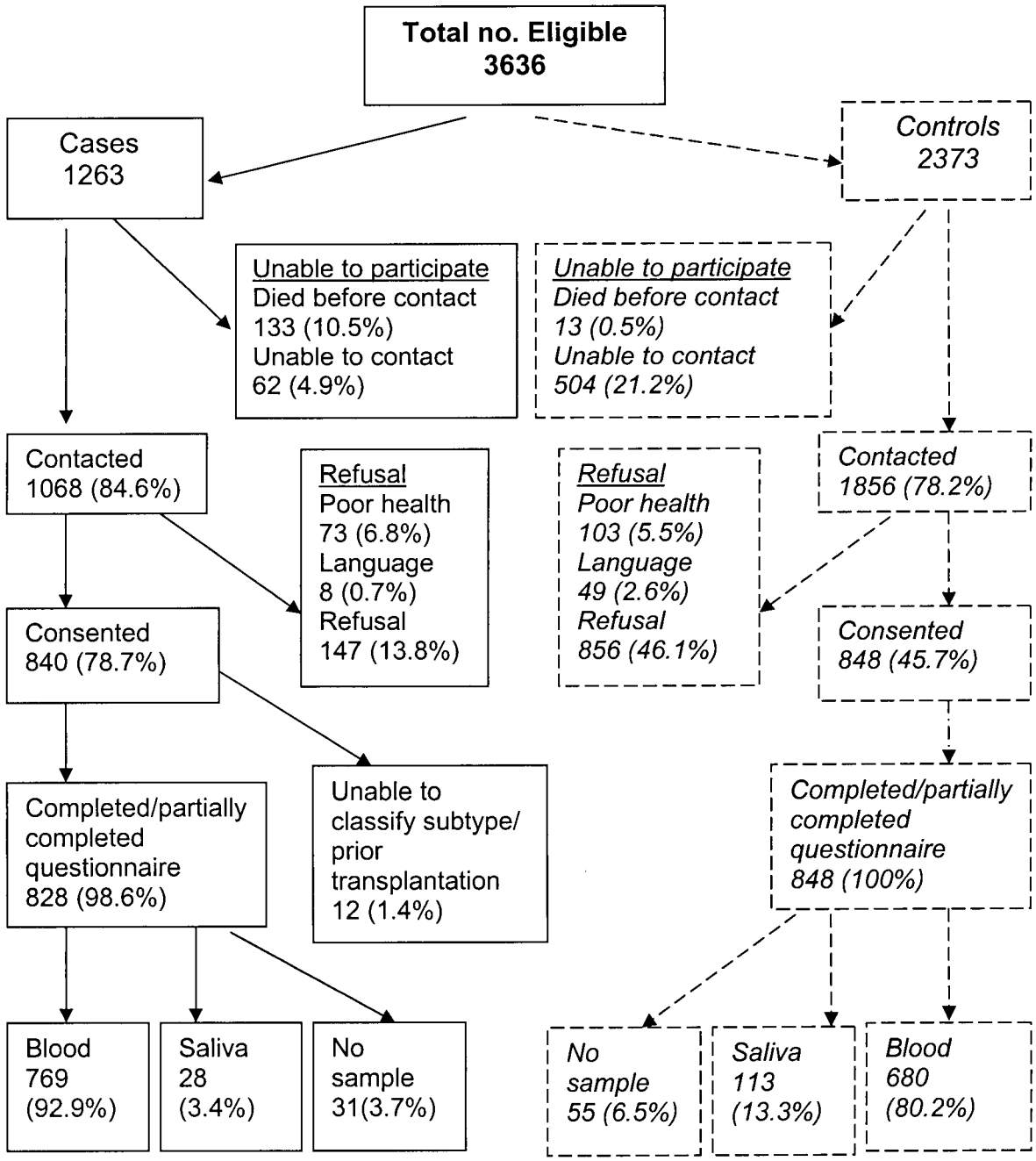

Table 3.2 Primers and probes used in the RT-PCR-based detection of HGV-RNA, PCR-based detection of ß-globin DNA and HGV genotyping

Name	Sequence	Amplicon size
HGV		
Forward	5'-AAGGTGGTGGATGGGTGATG-3'	64 bp
Reverse	5'-AGTGGCTACCARGRTGACCGG-3'	
Probe	5'-FAM-CAGGGTTGGTAGGTCGT-MGB-3'	
ß-globin		
Forward	5'-ACCCAGAGGTTCTTTGAGTCC TTT-3'	82 bp
Reverse	5'-TGCCATGAGCCTTCACCTTAG-3'	
Probe	5'-FAM-ATCTGTCCACTCCTGATGCTGTTATGGGC-TAMRA-3'	

3.4 Data Coding

A common protocol was used for questionnaire coding. Variables used from the parent study data include: age (4 groups, 1=20-49, 2=50-59, 3=60-69, 4=70+), sex (1=male, 2=female), region (1=GVRD, 2=CRD), education (1=less than high school, 2=high school graduate, 3=university graduate), ethnicity (1=European, 2=Asian, 3=South Asian, 4=Other/Mixed), self reported HCV (yes/no), ever use IV drug (yes/no), ever had blood transfusion (yes/no), family history of NHL (yes/no), ever tattoo (yes/no), ever piercing (yes/no). All variables described above are categorical variables Age was also examined is less five groupings (10 year age groups, 4 groups, 1=20-49, 2=50-59, 3=60-69, 4=70+). Age was also examined as a continuous covariate, including the examination of a quadratic term. Two variables, income and education were ascertained to represent socio-economic status. Because the variable for income level had more missing data compared to that for education level (8 cases and 18 controls, total 1.7% missing data) and both variables were highly correlated [207, 208], therefore only education was selected to represent socio-economic.

HCV seropositivity is defined as a collective variable of the testing results from the serology testings in NML or BCCDC, or database linkage from the BCCDC laboratory database. Virus state of HCV and HGV is a binary variable (0=non-reactive, 1=reactive). NHL is categorized into two

groups, B-cell and T-cell neoplasms based on their immunophenotypic, and then categorized into four large B-cell sub-groups (1=diffuse large cell, 2=follicular, 3= marginal zone, 4=other/unclassified B-cell) and seven smaller B-cell subgroups (1=diffuse large cell, 2=follicular 3= marginal zone/MALT, 4=mantle cell, 5=small lymphocytic lymphoma/chronic lymphocytic lymphoma , 6= lymphoplasmocytoid lymphoma, 7=other/unknown B-cell lymphoma) based on their histologic manifestations. Disease state of NHL is a binary variable (0=controls, 1=cases).

3.5 Data Analyses

3.5.1 The Logistic Regression Model

Data analyses were preformed using the SPSS Version 11.0. Statistical analyses for the risk of NHL and exposure to HCV or HGV were done independently. The odds ratio (OR) and 95 percent confidence interval (CI) for the risk of NHL for HCV, or HGV were estimated using logistic regression.

3.5.2 Selection of Confounders

The change-in-estimate method [209] was used to select confounders whose exclusion changed the NHL parameter estimate by 5% or more, with backward elimination of predictors that changed the

effect of NHL coefficients least. When no further variables could be eliminated without changing the effect of NHL coefficients by less than 5%, the main-effects model (final logistic regression model) was completed. In short, by using the change-in-estimate method, we selected only those predictors that actively confounded the effect of NHL, while variables not confounding the effect of NHL were eliminated from the model.

3.5.3 Final Model, Interactions & Additional Analyses

For the HCV analysis, the possible cofounder included age, sex, region, ethnicity, education level, family history of NHL, prior history of tattoo, piercing and blood transfusion. Only the removal of prior history of injection drug use (IDU) changed the OR for HCV seropositivity more than 5%, therefore IDU was kept in the final model for HCV analyses

For the HGV analysis, the same cofounding variables were put in the full model. The OR for HGV-RNA positivity changed more than 5% when prior history of blood transfusion, region and age were removed. The final model for HGV comprised prior history of blood transfusion, region and age. To allow comparisons between previous results, unadjusted odds ratios and odds ratios adjusted for IDU were presented for the HCV analysis. Unadjusted odds ratios and odds ratios adjusted for blood transfusion, region and age were presented for the HGV analysis.

Interactions between HCV seropositivity and potential confounding variables: sex, age (<60 vs. 60+), region (Vancouver vs. Victoria), ethnicity (European vs. non European), education (high school graduate vs. no high school diploma) were examined by entering the interaction term into the logistic regression model. Significance was based on the likelihood ratio p-value for the interaction term. The same protocol was applied to the logistic regression model for HGV interaction analyses.

Sub-group analyses of the association between HCV and HGV seropositivity and NHL were also performed for the histological subtypes. Both unadjusted odds ratios and odds ratios adjusted for confounding variables are presented for comparison. Finally, a descriptive analysis of HCV and HGV co-infection analysis was performed by reviewing the demographic and personal habit exposure data of subjects positive for both HCV and HGV.

4 Results

4.1 Overview

Selected characteristics of cases and controls of NHL study were compared (Table 4.1). The frequencies with regard to sex, age, region, education, and family history of NHL were significantly different between cases and controls. Compared to controls, cases were more likely to be older (mean age 57.6 vs 60.4), male (58% vs 53%), from Vancouver (83% vs 78%), not high school graduate (20% vs 14%), and have a family history of non-Hodgkin lymphoma (4.3% vs 2.5%). In addition, cases and controls were significantly different with respect to self-reported HCV status, injection drug use and blood transfusion, factors which relate to viral exposure. Compared to controls, cases had a higher rate of self-reported HCV (7.0% vs 6.1%), injection drug use (1.8% vs 0.5%) and blood transfusion (21.4% vs 13.9%). Cases and controls were not significantly different with respect to ethnicity, piercing or tattooing.

4.2 HCV Study Results

The frequencies of the study sample with respect to sex, age, region, ethnicity and education between the parent NHL study and HCV the HCV study were similar (Table 4.2).

HCV seropositivity was either confirmed through database linkage to the BCCDC database or determined by serology testing. Of the 101 subjects with a non-equivocal HCV determination in both the database and by serology, 96 were negative on both, 4 were positive on both and 1 was positive on serology, but negative in the database. The concordance rate is 99.0% [95%CI (94.6%-100.0%)]. All three cases recorded as "equivocal" in the HCV BCCDC database were negative by serology were considered negative for all analyses.

Table 4.3 summarizes the prevalence of HCV infection by study design variables, sex, age, region, and other socio-demographic variables for cases and controls. HCV seropositivity rates were similar with regard to region, ethnicity, education, blood transfusion, piercing and family history of NHL. A higher prevalence of HCV seropositivity was observed for males compared to females (20/837, 2.4% vs. 4/655, 0.6%, p=0.007), and in younger individuals compared to older people (19/671, 2.8% vs. 5/821, 0.6%, p=0.001). The mean age of the seropositive subjects was 53 years old compared to 60 years old for the seronegative individuals, The proportion of HCV seropositivity in injection drug users was much higher than the non injection drug users (9/18, 50% vs. 14/1428, 0.98%, p<0.001). There was also a higher rate of HCV seropositivity in individuals who had tattoos (7/88, 8.0% vs. 16/1365, 1.17%).

Four seropositive subjects reported a blood transfusion in the past. Nine subjects who reported no history of IV drug use or blood transfusion were HCV positive. Of the 24 subjects with a positive HCV test result, only 9 self-reported an HCV diagnosis. An additional 3 subjects reported Hepatitis but unknown type. One individual did not answer the question. Four of the 11 remaining seropositive subjects had a record of the HCV seropositivity in the BCCDC database but claimed that they had no HCV infection. It is possible that the subjects did not know the results of the test when they completed the questionnaire. The remaining seven subjects not reporting an HCV diagnosis were identified from serology alone. Two of the non self-reported HCV seropositive subjects reported a previous blood transfusion and none reported IV drug use. Three of these subjects reported previous injection drug use. One subject reported an HCV diagnosis, but tested negative for HCV. In addition, out of these 24 HCV seropositive subjects, one subject reported duel exposure to IV drug and blood transfusion. This subject also co-infected with HBV. Three post-NHL diagnosed HCV positive subjects did not have the anti-HCV testing four months after the diagnosis of lymphoma.

Table 4.4 shows the analysis of HCV infection for all cases and for the different NHL subtypes. Overall, there was a significant association between HCV infection and NHL, with HCV seropositive subjects having an unadjusted OR of 3.39 (95%CI=1.26-9.12) compared to subjects with negative serology. A larger risk was associated with B-cell neoplasm

(OR=3.56, 95%CI=1.13-9.65) than T-cell neoplasm (OR=1.80, 95%CI=0.21-15.6). Significantly increased risks associated with HCV were observed for diffuse large cell lymphoma, an aggressive B-NHL (OR=8.30, 95%CI=2.89-23.85) and marginal zone lymphomas, an indolent B-NHL (OR=4.51, 95%CI=1.06-19.2). No excess risk was observed for follicular lymphoma. Other B-cell lymphomas comprised marginal cell lymphoma (MCL), small lymphocytic lymphoma/chronic lymphocytic lymphoma (SLL/CLL), lymphoplasmacytic lymphomain (LPL) and miscellaneous B-cell lymphoma (misc. BCL). The risk of HCV for SLL/CLL was statistically significant (OR=6.92, 95%CI=1.30-36.78), a non-statistically significant was observed for LPL (OR=3.64, 95%CI=0.42-31.95).

Table 4.5 shows the results adjusted for IDU for those subjects with known IDU status (758 cases and 688 controls). All odds ratios were attenuated. After adjustment for IDU, the OR for risk of NHL was elevated, but no longer statistically significant (OR=2.57, 95%CI=0.89-7.44). The risk of HCV for B-cell neoplasm was also reduced, but remained statistically significant (OR=2.94, 95%CI=1.00-8.58). Attenuated risks were observed after adjustment for IDU included diffuse large cell (OR=7.31, 95%CI=2.14-25.0) and marginal zone (OR=6.08, 95%CI=1.09-33.9) lymphoma. Similar to the unadjusted analysis, no excess risks were observed for other B-cell or T-cell lymphoma.

Ten study characteristics including sex, age, region, education, self-reported hepatitis status, injection drug use, blood transfusion, piercing, tattoo and family history of NHL were examined for interactions with HCV (Table 4.6). No significant interactions were observed between HCV seropositivity and sex, age, region, education, family history of NHL or prior history of injection drug use, piercing, or tattoo. There was a significant interaction for ethnicity (p interaction=0.041). After adjustment for IDU, the odds ratio for European ethnicity was 4.21 (95%CI=1.16-15.3) and for non-European ethnicity was 0.23 (95%CI=0.01-4.48).

4.3 HGV Study Results

The frequencies of the study sample with respect to sex, age, region, ethnicity and education between the parent NHL study and HGV the HGV study were similar (Table 4.2). The prevalence of HGV viremia in the control subjects of our study was 1.8%, the frequency was similar to that in other countries and similar to that of the Canadian blood donors (1.1%).

Table 4.7 summarized the prevalence of HGV viremia by study design variables, sex, age, region, and other socio-demographic variables for cases and controls. HGV viremia rates were similar to all exposure variables except prior blood transfusion and injection drug use. . A higher prevalence of HGV viremia was observed for injection drug users

compared to non injection drug users (2/14, 14.3% vs. 30/943, 3.2%, p=0.022), and for individuals with previous blood transfusion compared to those without previous blood transfusion (10/175; 5.7% vs. 21/773, 2.7%, p=0.044).

Table 4.8 shows the association of HGV viremia with NHL and the NHL subtypes with no adjustment for blood transfusion. There was a statistically significant association between HGV viremia and NHL, with an unadjusted OR of 3.16 (95%CI=1.38-7.27) for HGV viremia positive individuals compared to HGV viremia negative individuals. An increased risk was associated with B-cell neoplasm (OR=3.42, 95%CI=1.48-7.90). No excess risk was observed for T-cell lymphoma. The largest increased risks associated with HGV viremia was observed for diffuse large cell (OR=5.73, 95%CI=2.25-14.60), and other/unknown B-cell lymphoma (OR=4.86, 95%CI=1.34-17.64). Non statistically significant, but positive associations with HGV viremia were observed for all B-cell subtypes except LPL.

After adjustment for age, region and blood transfusion (Table 4.9), the observed ORs for all subtypes were slightly attenuated. The risk of HGV for NHL remained statistically elevated (OR=2.84, 95%CI=1.22-6.59). The odds for all B-cell (OR=3.04, 95%CI=1.30-7.09) and diffuse large cell lymphoma (OR=5.35, 95%CI=2.09-13.69) remained statistically

significant. The risk for other B-cell lymphoma was elevated, but not statistically significant (OR=2.98, 95%CI=0.95-9.32). All other B-cell subtypes were non-significant, HGV viremia did not increase risk for follicular and marginal zone lymphoma. Risk of HGV viremia was not observed for all T-cell and T-cell subtypes in this study.

Ten same study characteristics were examined for interactions with HGV (Table 4.10). No significant interactions were observed. However, a borderline significant interaction was observed for education status. Subjects with no high school diploma had an odds ratio of 0.468 (95%CI=0.097-2.169), whereas post high school graduates had an odds ratio of 6.282 (95%CI=1.693-23.309). after adjustment for sex, region and prior blood transfusion. Although not statistically significant different, a higher odds ratio was observed for males than females (OR=3.84, 95%CI=1.27-11.63; OR=2.05, 95%CI=0.48-18.74), and for younger compared to old individuals (OR=3.65, 95%CI=1.21-10.99; OR=2.23, 95%CI=0.60-8.33).

4.4 HCV Co-infection with HGV Viremia

Two NHL patients and one control subject were co-infected with both HCV seropositive and HGV viremia (Table 4.11). Our data shows that the prevalence of HGV and HCV co-infection was 10% lower than that reported by other studies [210, 211]. All three individuals were middle-

aged males. Both co-infected NHL patients were diagnosed with diffuse large cell lymphoma and reported having used injection drugs. One reported previous blood transfusion, tattoo and piercing. HCV and HGV may be similar in transmission with injection drug use the main transmitting factor for HCV and HGV infection. The control subject reported no injection drug use, blood transfusion or tattoo, but did report previous piercing. In conclusion, our data support the hypothesis that HCV and HGV infection are causative factors in the etiopathogenesis of NHL but their natural history, and role in pathogeneses may be different although they both belong to the Flaviviridae family.

Table 4.1 Characteristics of NHL study subjects [frequency (percentage)]

	Cases	Controls	P-value
Number	828	848	
Sex			0.038
Male	482 (58.2)	451 (53.2)	
Female	346 (41.8)	397 (46.8)	
Age			0
20-49	162 (19.6)	241 (28.4)	
50-59	197 (23.8)	174 (20.5)	
60-69	219 (26.4)	217 (25.6)	
70+	250 (30.2)	216 (25.5)	
Region			0.006
Vancouver	688 (83.1)	659 (77.7)	
Victoria	140 (16.9)	189 (22.3)	
Ethnicity			0.346
European	649 (81.2)	651 (78.6)	
Asian	83 (10.4)	97 (11.7)	
South Asian	29 (3.6)	43 (5.2)	
Other/Mixed	37 (4.8)	37 (4.5)	
Education			0.019
Less than high school	158 (19.5)	120 (14.3)	
High school graduate	425 (52.5)	469 (56.0)	
University graduate	226 (27.9)	249 (29.7)	
Self-reported HCV			0
Yes	55(7.0)	51 (6.1)	
No	736 (93.0)	785 (93.9)	
Injection Drug Use			0.012
Yes	14 (1.8)	4 (0.5)	
No	775 (98.2)	834 (99.5)	
Blood Transfusion			0.00
Yes	168 (21.4)	115 (13.9)	
No	617 (78.6)	712 (86.1)	
Piercing			0.124
Yes	313 (39.3)	363 (43.1)	
No	483 (60.7)	480 (56.9)	
Tattoo			0.997
Yes	46 (5.8)	49 (5.8)	
No	745 (94.2)	793 (94.2)	
Family History of NHL			0.05
Yes	34 (4.3)	21 (2.5)	
No	763 (95.7)	814 (97.5)	

Table 4.2 Characteristics of study subjects of NHL, HCV and HGV studies

	NHL			HCV			HGV		
NHL	**Cases**	**Controls**	**Pop Prop**	**Cases**	**Controls**	**Pop Prop**	**Cases**	**Controls**	**Pop Prop**
Number	828	848		795	697		553	438	
Sex									
Male	482	451	0.56	463	374	0.56	322	248	0.58
Female	346	397	0.44	332	323	0.44	231	190	0.42
Age									
<60	359	415	0.46	349	322	0.45	251	233	0.49
60+	469	433	0.54	446	375	0.55	302	205	0.51
Region									
Vancouver	688	659	0.80	661	525	0.79	475	329	0.81
Victoria	140	189	0.20	134	172	0.21	78	109	0.19
Ethnicity									
European	649	651	0.80	629	570	0.83	435	355	0.82
Non-European	150	177	0.20	138	108	0.17	102	69	0.18
Education									
< High school	158	120	0.17	146	98	0.17	94	62	0.16
≥ High school	651	718	0.83	631	591	0.83	446	369	0.84

Pop Prop- population probability

Table 4.3 Characteristics of study subjects and HCV seropositivity [frequency (percentage)]

			HCV Seropositivity	
	Cases	**Controls**	**Cases**	**Controls**
Number	795	697	19 (2.4)	5 (0.7)
Sex				
Male	463 (58.2)	374 (53.7)	16 (3.5)	4 (1.1)
Female	332 (41.8)	323 (46.3)	3 (0.9)	1 (0.3)
Age				
20-49	158 (19.9)	173 (24.8)	7 (4.4)	2 (1.2)
50-59	191 (24.0)	149 (21.4)	8 (4.2)	2 (1.3)
60-69	208 (26.2)	187 (26.8)	2 (1.0)	1 (0.5)
70+	238 (29.9)	188 (27.0)	2 (0.8)	0
Region				
Vancouver	661 (83.1)	525 (75.3)	15 (2.3)	5 (1.0)
Victoria	134 (16.9)	172 (24.7)	4 (3.0)	0
Ethnicity				
European	629 (79.1)	570 (81.8)	17 (2.7)	3 (0.5)
Asian	74 (9.3)	55 (7.9)	0	0
South Asian	27 (3.4)	27 (3.9)	0	1 (3.7)
Other/Mixed	37 (4.7)	26 (3.7)	1 (2.7)	1 (3.8)
Unknown	28 (2.7)	19 (2.7)	1 (3.6)	0
Education				
< high school	146 (18.4)	98 (14.1)	4 (2.7)	1 (1.0)
≥High school	414 (52.1)	386 (55.4)	9 (2.2)	4 (1.0)
University graduate	217 (27.3)	205 (29.4)	5 (2.3)	0
Unknown	18 (2.3)	8 (1.1)	1 (5.6)	0

Table 4.3 Characteristics of study subjects and HCV seropositivity [frequency (percentage)](continued)

			HCV Seropositivity	
	Cases	**Controls**	**Cases**	**Controls**
Self-reported HCV				
Yes	8 (1.0)	2 (0.3)	8 (100.0)	1 (50.0)
No	734 (92.3)	671 (96.3)	9 (1.2)	2 (0.3)
Unknown	53 (6.7)	24 (3.4)	2 (3.8)	2 (8.3)
Injection Drug Use				
Yes	14 (1.8)	4 (0.6)	7 (50.0)	2 (50.0)
No	744 (93.6)	684 (98.1)	11 (1.5)	3 (0.4)
Unknown	37 (4.7)	9 (1.3)	1 (2.7)	0
Blood Transfusion				
Yes	161 (20.3)	102 (14.6)	4 (2.5)	0
No	594 (594)	576 (82.6)	13 (2.2)	4 (0.7)
Unknown	40 (5.0)	19 (2.7)	2 (5.0)	1 (5.3)
Piercing				
Yes	303 (58.1)	304 (43.6)	6 (2.0)	2 (0.7)
No	462 (38.1)	389 (55.8)	12 (2.6)	3 (0.8)
Unknown	30 (3.8)	4 (0.6)	1 (3.3)	0
Tattoo				
Yes	46 (5.8)	42 (6.0)	5 (10.8)	2 (4.8)
No	715 (89.9)	650 (93.3)	13 (1.8)	3 (0.5)
Unknown	34 (4.3)	5 (0.7)	1 (2.9)	0
Family History of NHL				
Yes	34 (4.3)	19 (2.7)	0	0
No	732 (92.1)	676 (97.0)	18 (2.5)	5 (0.7)
Unknown	29 (3.6)	2 (0.2)	0	0

Table 4.4 HCV seropositivity association with NHL

Histologic Subtype	Cases HCV+/HCV-	Controls HCV+/HCV-	OR	95%CI
All NHL	19/776	5/692	3.39	1.26-9.12
All B-cell	18/699		3.56	1.13-9.65
Diffuse large cell	12/200		8.30	2.89-23.85
Follicular	0/212		-	-
Marginal zone	3/92		4.51	1.06-19.20
Other B-cell	3/195		2.13	0.50-8.99
MCL	0/48		-	-
SLL/CLL	2/40		6.92	1.30-36.78
LPL	1/38		3.64	0.42-31.95
MISC BCL	0/69		-	-
All T cell	1/77		1.80	0.21-15.60
MF	0/42		-	-
PTCL/MISC TCL	1/35		3.95	0.45-34.76

Table 4.5 HCV seropositivity association with NHL adjusted for injection drug use

Histologic Subtype	Cases HCV+/HCV-	Controls HCV+/HCV-	OR	95%CI
All NHL	18/740	5/683	2.57	0.89-7.44
All B-cell	17/665		2.94	1.00-8.58
Diffuse large cell	11/194		7.31	2.14-25.0
Follicular	0/199		-	-
Marginal zone	3/83		6.08	1.09-33.9
Other B-cell	3/189		1.59	0.32-8.00
MCL	0/47		-	-
SLL/CLL	2/40		6.627	0.82-53.35
LPL	1/37		6.135	0.62-60.38
MISC BCL	0/65		-	-
All T cell	1/75		0.37	0.02-6.06
MF	0/40		-	
PTCL/MISC TCL	1/35		0.773	0.04-16.23

Table 4.6 HCV interaction adjusted for Injection drug use

	OR	95%CI	Interaction P- value
Age			0.758
<60	2.369	0.696-8.067	
60+	3.353	0.375-29.946	
Sex			0.961
Male	2.524	0.771-8.265	
Female	2.038	0.184-22.59	
Region			0.477
Vancouver	1.762	0.579-5.357	
Victoria	668.521	0-5.7E+13	
Ethnicity			0.041
European	4.212	1.158-15.335	
Non European	0.227	0.011-4.477	
Education			0.704
No High School Diploma	1.435	0.129-16.001	
High School Graduate	1.672	0.427-6.536	
Blood Transfusion			0.578
Yes	579.037	0-3.5E+17	
No	2.387	0.699-8.147	
Tattoo			0.414
Yes	1.947	0.253-15.009	
No	3.084	0.842-11.291	
Piercing			0.82
Yes	1.482	0.242-9.087	
No	3.515	0.885-13.958	

Table 4.7 Characteristics of study subjects and HGV viremia [frequency (percentage)]

			HGV VIREMIA	
	Cases	**Controls**	**Cases**	**Controls**
Number	553	438	25(4.5)	8(1.8)
Sex				
Male	322 (58.2)	248 (56.6)	20 (6.2)	4 (1.6)
Female	231 (41.8)	190 (43.4)	5(2.2)	4 (2.1)
Age				
20-49	108 (19.5)	130 (29.7)	8 (7.4)	4 (3)
50-59	143 (25.9)	103 (23.5)	5 (3.5)	1 (1.0)
60-69	150 (27.1)	106 (24.2)	11 (7.3)	1 (1.0)
70+	152 (27.5)	99 (22.6)	1 (0.7)	2(2)
Region				
Vancouver	475 (85.9)	329 (75.1)	19 (4.0)	6 (1.8)
Victoria	78 (14.1)	109 (24.9)	6 (7.7)	2(1.8)
Ethnicity				
European	435 (78.7)	355 (81.1)	22 (5.1)	6(1.7)
Asian	59 (10.7)	36 (8.2)	0	1(2.8)
South Asian	19 (3.4)	13 (3.0)	0	1 (7.7)
Other/Mixed	24 (4.3)	20 (4.6)	3 (12.5)	0
Unknown	16 (2.9)	14 (3.2)	0	0
Education				
Less than High School	94(17.0)	62 (14.2)	3 (3.2)	4 (6.5)
High School Graduate	299 (54.1)	238 (54.3)	16 (5.4)	3 (1.3)
University Graduate	147 (26.6)	131 (30.0)	5 (3.4)	1(0.8)
Unknown	13(2.3)	7(1.6)	1 (7.7)	0

Table 4.7 Characteristics of study subjects and HGV viremia [frequency (percentage)](Continued)

			HGV VIREMIA	
	Cases	**Controls**	**Cases**	**Controls**
Ever Told Having Hepatitis				
Yes	36 (6.5)	31 (7.1)	3 (8.3)	1 (3.2)
No	486 (87.9)	403 (92)	21 (4.3)	7 (1.7)
Unknown	31 (5.6)	4 (0.9)	1 (3.2)	0
IV Drug Use				
Yes	11 (2.0)	3 (0.7)	2 (18.2)	0
No	511(92.4)	432 (98.6)	22 (4.3)	8 (1.9)
Unknown	31 (5.6)	3 (0.7)	1 (3.2)	0
Blood Transfusion				
Yes	114 (20.6)	61 (13.9)	9 (7.9)	1(1.6)
No	404 (73)	369 (84.2)	14 (3.5)	7 (1.9)
Unknown	35 (6.3)	8 (1.8)	2 (5.7)	0
Tattoo				
Yes	29 (5.2)	33 (7.5)	2 (6.9)	1 (3.0)
No	495 (89.5)	403 (92.0)	22 (0.2)	7 (1.7)
Unknown	29 (5.2)	2 (0.5)	1 (3.4)	0
Piercing				
Yes	212 (38.2)	187 (42.7)	8 (3.8)	5 (2.7)
No	314 (56.9)	250 (57.1)	16 (5.1)	3 (1.2)
Unknown	27 (4.9)	1 (0.2)	1 (3.7)	0
Family History of NHL				
Yes	25 (4.5)	11 (2.5)	0	0
No	506 (91.5)	427 (97.5)	24 (4.7)	8 (1.9)
Unknown	22(4.0)	0 (0)	1 (4.5)	0

Table 4.8 HGV viremia association with NHL

Histologic Subtype	Cases HGV+/HGV-	Controls HGV+/HGV-	OR	95%CI
All NHL	25/528	8/430	3.16	1.38-7.27
All B-cell	24/464		3.42	1.48-7.90
Diffuse large cell	12/127		5.73	2.25-14.60
Follicular	3/135		1.27	0.31-5.15
Marginal zone	2/71		1.80	0.35-9.33
Other B-Cell	7/131		3.48	1.16-10.43
MCL	2/29		4.06	0.78-21.11
SLL/CLL	1/24		2.92	0.31-27.04
LPL	0/29		-	-
MISC BCL	4/49		4.86	1.34-17.64
All T-cell	1/64		0.93	0.11-7.78
MF	0/37		-	-
PTCL/MISC TCL	1/27		2.09	0.25-17.75

Table 4.9 HGV viremia association with NHL adjusted for age, region and blood transfusion

Histologic Subtype	Cases HGV+/HGV-	Controls HGV+/HGV-	OR	95%CI
All NHL	23/495	8/422	2.84	1.22-6.59
All B-cell	22/433		3.04	1.30-7.09
Diffuse large cell	12/121		5.35	2.092-13.69
Follicular	3/126		1.33	0.33-5.36
Marginal zone	1/62		1.05	0.12-8.89
Other B-Cell	6/124		2.98	0.95-9.32
MCL	1/28		2.33	0.27-20.16
SLL/CLL	1/24		2.85	0.30-26.68
LPL	0/28		-	-
MISC BCL	4/44		5.05	1.4-18.22
All T-cell	1/62		1.02	0.12-8.54
MF	0/35		-	-
PTCL/MISC TCL	1/27		2.14	0.25-18.19

Table 4.10 HGV interaction adjusted for age, region and blood transfusion

	OR	95%CI	Interaction P- value
Age			0.622
<60	3.65	1.213-10.986	
60+	2.228	0.596-8.329	
Sex			0.251
Male	3.838	1.267-11.628	
Female	2.052	0.482-8.744	
Ethnicity			0.473
European	3.467	1.322-9.090	
Non European	1.496	0.228-9.826	
Education			0.051
No High School Diploma	0.468	0.097-2.169	
High School Graduate	6.282	1.693-23.309	
Injection Drug Use			0.916
Yes	-	-	-
No	2.662	1.130-6.269	
Tattoo			0.676
Yes	1.446	0.107-19.463	
No	2.988	1.222-7.310	
Piercing			0.306
Yes	2.188	0.650-7.367	
No	4.243	1.201-14.988	

Table 4.11 Characteristics of HCV and HGV infection in NHL cases and controls

NO.	CASE/ CONTROL	AGE	SEX	HISTOLOGY	REGION	EDUCATION	IDU	TRANS FUSION	PIERCING	TATTOO
1	Case	53	M	DLBCL	Victoria	High school graduate	Y	N	N	N
2	Case	48	M	DLBCL	Vancouver	<High school	Y	Y	Y	Y
3	Control	42	M	N/A	Vancouver	<High school	N	N	Y	N

5 Discussion

5.1 Overview

To the best of our knowledge, the present study is the largest case-control study in Canada to study the association between HCV and NHL, and the largest case-control study worldwide on the possible association between HGV and NHL. The findings that patients were 3.6 times more likely to be infected with HCV or 3.4 times more likely to be infected with HGV than the controls suggest that an association does exist.

5.2 HCV

These results support the hypothesis that exposure to HCV increases the risk of NHL, and are consistent with four meta-analyses and systematic reviews performed in 2003-2006 which include Dal Maso et al [98], Matsuo et al [99], Gisbert et al [101], and Negri et al [100]. The present study also confirms the higher risk for B-cell lymphoma than for T-cell lymphoma. The odds ratios were 3.4 for B-cell and 1.8 for T-cell lymphoma, whereas Matsuo et al calculated the odds ratios of 5.0 and 2.5, respectively. Restricting the analysis to non-blood donor controls resulted in an odds ratio of 4.7 for B-cell lymphoma, closer to the odds ratio of 3.6 observed in this study. Dal Maso et al reported a sex/age adjusted estimate of the association between HCV seropositivity and NHL of 2.5.

The authors also reported a large difference in the relative risk of NHL between studies in high and low endemic areas (≥5% and <5% HCV prevalence) of 3.0 and 1.9, respectively. The prevalence of 1.9 in low endemic areas was much lower than our study result. Gisbert et al reported an OR of 10.8 from 48 studies of which the mean prevalence of HCV was 13%; this estimated risk was three times higher than our finding. In sum, the present study agreed with these four meta-analyses and systematic reviews as well as another systematic review published in 1997 [107] that HCV infection might increase risk in B-NHL with both indolent and aggressive subtypes.

Authors of the systematic review/meta-analysis suggested that the genetic and/or environmental factors possibly involved in the pathogenesis of B-cell lymphomas, may account for the variation in risk estimates [101]. In Europe, there seemed to be a gradient in risk from northern to southern Europe; strongest in Italy [85, 102, 104, 212, 213], but weaker in France [92, 109, 214] and the UK [90]. This variation also appeared within the same countries. In the US study in southern California [215], where majority of the residences were Hispanic, the association was significant, but not in the study conducted in the Midwestern United States where most of the residents were European descent [93]. The two Canadian studies did not find an association [28, 29], but the present study demonstrated a significant association between HCV infection and NHL with the risk of three times higher than the HCV seronegative individuals. These conflicting results suggest that other factors such as genetic, environmental, diet

etc. may co-exist in combination with HCV in order to promote the pathogenesis of NHL.

The risk according to NHL subgroups is difficult to determine from previously conducted studies due to changing classification systems and the small number of cases in the different studies. From the recent studies (with sufficient numbers of cases, seropositive subjects), subgroup results have not been consistent. Our finding that more of an 8 fold increased risk for diffuse large cell (DLC) lymphoma and 4.5 fold increased risk for marginal zone B-cell lymphomas (MZ) agreed with the findings of three studies in Italy [102, 112]. However, the two US studies [95, 96] and four Italian studies [79, 86, 102, 112] showed the highest risk for follicular lymphomas (FL). The two US studies also showed increased risk for marginal zone (MZ) and mucosa-associated lymphoid tissue (MALT) [95, 96] that agreed with our findings. Finally, our study observed a significantly high risk (OR=6.9) for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) that was reported as insignificant in most of these studies except one in Italy [87]. After adjusting for injection drug use, the odds ratios for all the results were attenuated but remained statistically significant for all B-cell, diffuse large cell and marginal zone lymphoma.

Our data showed the highest risk with diffuse large cell, the most common type of high-grade aggressive lymphoma, while other studies reported a higher risk for follicular lymphoma (the most common type of low-grade indolent

lymphoma [87, 95, 96, 109-111, 216]. Other studies showed increased risk in both low grade and high grade lymphoma [82, 102, 105, 112]. Chronic infection may explain the pathogenesis mechanism how HCV infection attributes to the risk of B-cell NHL, but does not seem to explain why the presence of the virus to the indolent and aggressive NHL subtypes because these two types of NHL represent different stages of cell differentiation. Future studies should prospectively evaluate the association between HCV and indolent or aggressive lymphomas.

The HCV prospective cohort study in Sweden [103] created a model to estimate the date of infection based on the data of the Swedish intravenous drug database suggesting that the exposure of HCV in most cases occurred long before the notification. The authors assumed that individuals born in 1930, were likely to have been infected through IDU at the age of 35 years (e.g. 1965 for a case diagnosed in 2000); and people born in 1955 or later considered to have been infected at the age of 20 years (e.g. 1975). In our study, the mean age of the positive HCV seropositive was 53.7 and 52.2 years for cases and controls, respectively. Applying the model of Duberg et al to our data, 7 of the 19 HCV seropositivity NHL individuals who were at the age of 45 to 55 years and had been exposed to IDU might have been infected during the 1970s.

IDU is the single most important route of HCV transmission in Canada, accounting for at least 60% of all HCV transmission [193]. Fifty percent of the

HCV related NHL males in the present study reported they had used injection drugs. Although the prevalence of HCV infection in Canada is very low (<1%), the proportion of NHL attributable to HCV in the population may be relatively small. Nevertheless, the notion that HCV infection may be a risk factor to NHL should not be undermined.

The HCV genotype is a predictive indicator in term of response to antiviral therapy in HCV infected patients with chronic hepatitis [217]. It may also be possible that different HCV genotypes may have affect different subtypes of NHL. In the Italian studies, the prevalence of HCV genotype 2a/c was significantly increased among B-cell NHL patients (48.4%) compared with the control group (9%) [218]. Subtypes 1a (35%) and 3 (31%) were the most common HCV genotypes in Sweden [103] and they concluded that the risks of B-cell NHL was significantly increased. Although genotyping was not included in the cohort study, it is reasonable to assume that these two common HCV genotypes were highly correlated with B-cell NHL. All HCV related NHL was genotype 1b in the Spanish study [105]. In the North America and Western Europe, genotypes 1a and 1b are the most common, followed by genotypes 2 and 3 [38]. A Canadian study also reported that type I was predominant in the general population with subtypes 1b and 1c most common [66]. In our study we did not examine the HCV genotypes, however the HCV genetic characteristics of the study samples might reflect the genomic characteristic of the study regions (GVRD and CRD), and therefore we can hypothesize that HCV genotypes 1a and 1b may be the underlying causal

factors in our study. Although the recent meta-analysis [98] did not find a difference in risk for NHL between different HCV genotypes, genotype analysis may still worth to take into consideration in future study.

Overall, the prevalence of HCV infection in the United States and Canada are low, 1.8% and 0.8%, respectively. This study and the recent American case-control studies [95, 96] confirmed that HCV may be associated with increased risk in B-cell NHL. Our findings are consistent with those in the high endemic regions increase the probability that HCV infection is a causal factor in NHL. The association between HCV infection and indolent NHL has well been established. Our study described a very high risk in DLBC, an aggressive subtype. Further research effort should aim at understanding the molecular and cellular routes of HCV infection in fast grow aggressive B-NHL particularly diffuse large B-cell lymphoma.

5.3 HGV

We found a statistically significant relationship between HGV infection and NHL with a higher risk for HGV viremia positive subjects compared to viremia negative subjects. In particular, there was a strong association between HGV infection and B-cell lymphoma with an odds ratio of 3.4. The study results support the findings of the meta-analysis by Wiwanitkit [172], although the overall HGV positivity in controls was lower in the meta-analysis compared to our study (0.8%, 3/355 vs. 1.8%, 8/438).

Data obtained in the present study suggest that HGV viremia may play a major role in the pathogenesis of NHL especially in B-cell lymphomas and other/ unknown B-cell neoplasms with both indolent and aggressive subtypes. The largest increased risks associated with HGV were observed for diffuse large cell with more than 5 fold increase, and a nearly 5 fold increase in risk for other/unknown B-cell lymphomas. Due to the very small samples, no subtype analysis was provided by the meta-analysis. Ellenrieder et al [88] reported an increased risk of NHL in patients with low grade NHL whereas no patients with high grade NHL were found positive for HGV-RNA. Minton et al, 1998 [169] reported an increased risk in high grade NHL, but their sample size was too small to provide reliable estimates of risk. Other studies which reported an association between HGV and lymphomas did not conduct NHL subtypes analysis. Our study is the first study to provide a systematic pathologic subtype examination of HGV risk.

Hepatitis viruses have been proposed to have possible role in the pathogenesis of many disorders. HBV and HCV are the main risk factors contributing to the increased incidence of hepatocellular carcinoma. The role of HCV in promoting risk for malignant B-cell NHL has been supported by a number of epidemiologic, experimental and clinical studies. Since HGV is closely related to HCV, it has been hypothesized that it may also be involved in lymphomagenesis. HGV shares genetic and biological features with HCV, in

particular the E2 sequences of these related viruses are functionally equivalent, and therefore preserve some structural similarity. Persistent HCV infection is associated with a range of immune-related conditions, including essential mixed cryoglobulinemia, a low-grade lymphoproliferative disorder that can progress to NHL [191, 219]. Similarly, the prevalence of HGV was observed significantly higher in patients with B-cell lymphoproliferative disorders (B-LPD) HGV [151]. In addition, HCV, HGV and their protein products have been detected in lymphocytes. One possible mechanism may be that HCV and HGV, as chronic antigenic stimuli, cause lymphoid hyperplasia which, in the later phase of the process, leads to the clonal expansion of lymphocytes and to the development of malignant lymphomas [191, 220].

Some studies reported a very high rate of HGV co-infection with other hepatitis infection. Both Januszkiewicz-Lewandowska et al [211] and Li et al [210] observed the an HGV co-infection rate of 22% for patients with chronic HCV infection. The authors investigated the prevalence of HGV co-infection on HCV-RNA from the peripheral blood of 144 hemodialysis patients. After 2.5 years follow up, the 18 of the 80 patients who remained HCV-RNA-positive were found to be HGV positive. Li et al studied 72 HCV-RNA positive individuals [210]. They found that HGV-RNA was positive in plasma of 11 patients, in PBMCs of 15 patients, and simultaneously in both of plasma and PBMCs of 10 patients. However, the studies that reported this exceptionally high co-infection rate of HGV and HCV were neither population-based nor examined the associated with

NHL. Table 2.1 reports all published population-based and some case-only HGV related NHL studies. Of these studies, only Giannulis et al reported co-existing HBV in 3, and HCV in 1 NHL patients [174]. In two case-control studies, Kaya et al did not find co-infection with other hepatitis disease [173]. Only 1 of 70 control person was exposed to HGV with no record of co-infection. Pavlova et al reported one case co-infected with HCV, none in the controls [150]. The case study of Ellenrieder did not find co-infection with HGV in NHL patients [88].

In the present study, only two NHL HGV-positive cases and one HGV-positive control were co-infected with HCV. The two cases were diagnosed with DLBC lymphoma, and had self-reported injection drug use. One of them also reported a prior blood transfusion and piercing. The control subject reported that he had never used injection drugs, or had blood transfusion; however, did report prior piercing. Our study as well as other studies on Table 2.1 do not support the hypothesis that the co-infection rate of HGV with other hepatitis infection is high in NHL individuals or population controls. This may suggest that HGV has a different pathogenesis pathway from HCV. Further large epidemiologic studies to evaluate co-infection of HGV and HCV in population controls and B-cell NHL may help us to better understand the joint effect of these two viruses. In addition, since HGV does not contain a hypervariable region, and has less mutation over the entire genome in compare to the HCV [136], molecular studies of HGV may optimize an experimental platform to better understand the molecular and genetic mechanism of HCV.

HGV-RNA testing provides evidence of present HGV infection, but cannot provide evidence of past infection. Approximately 50-75% of the HGV exposed individuals clear the infection [121] therefore testing by HGV-RNA alone may underestimate the true prevalence of exposure. Only HGV-RNA testing was used to observe the prevalence of HGV infection in our study. In Canada, HGV viremia in blood donors of 1.1% and anti-E2 of 7.3% [123]. HGV viremia in our controls was 1.8% near to the national infection rate. It is unclear whether previous HGV infection followed by clearance of virus is associated with the development of lymphoproliferative disorders or NHL. Future studies may consider assessing anti-E2 to estimate the prevalence of cleared infection, and the possible clinical impact of previous HGV infection in the development of B-cell NHL or other clinical disorders.

HGV has been confirmed to not be a hepatotropic virus, and the major replication sites of HGV are not well defined. Until recently, its potential role in NHL has been suspected, but the results are inconsistent. Our study provides evidence of a role of HGV in NHL.

5.4 Strengths and Limitations

A primary strength of this study is that the cases and controls were population based, which greatly reduces the possibility of bias in the observed association. The two previous Canadian HCV and HGV studies utilized clinic

based lymphoma cases and convenience controls (health care workers or blood donors) [28, 29]. Our study utilized a population-based study design which eliminates this possible source of bias.

Second, we used a frequency matched control group. Controls were similar to cases by age, region, sex, which allowed us to directly compare the prevalence of HCV or HGV with NHL to comparable subjects from the same population. Prevalence of the HCV or HGV in the controls mirrored national estimates [130, 221] further supporting our approach.

A third strength of our study was the large size, with over seven times the number of cases as either of the two previous HCV Canadian studies which allow us to estimate the effect of HCV and HGV with more precision than smaller studies. The previous HGV Canadian study evaluated seventy cases and seventy controls, 20% the number of cases as in our study. Its sample size may be too small to allow the appropriate statistical analyses and to provide adequate power to answer the question with regard to the risk of HGV infection in HNL.

Fourth, cases with HIV or prior transplant were excluded, thus eliminating potential confounding in the risk estimates from the associations of these factors with NHL and HCV or HGV. Fifth, only histologically confirmed cases were included in the parent NHL study to minimize information bias results from disease misclassification. Finally, 93% of NHL cases and 80% of controls of our

study provided a blood sample to the parent NHL study, and that enabled us to perform testing for both HCV and HGV, on almost our entire study sample.

The main limitation of this study was that the high rate of non-response could have introduced bias if cases and controls differentially participated based their knowledge of prior HCV or HGV infection. We cannot rule out the possibility that hepatitis infection may discourage the controls to respond to the questionnaires. It is possible that controls who engaged in high risk activities such as injection drug use may not have chosen to participate. However, the estimates of risk for HCV and HGV infection did not change after adjusting for education, history of injection drug use, blood transfusion, and tattooing/ piercing. The results of our studies are also consistent with other studies of HCV. These considerations argue against a major response bias. The HCV study is by far the largest Canadian study, the HGV study is the largest study internationally. However, because of the low prevalence of exposure to HCV and HGV, the study still has limited power to detect associations for specific NHL subtypes, or to detect interactions.

One second potential major limitation of our study is the assessment of HCV and HGV status after diagnosis of NHL. Determining whether viral exposure precedes the onset of NHL can be problematic for a retrospective study since the date of primary infection is unknown. We found 6 of the 19 HCV seropositive cases had notification of HCV infection before the diagnosis of NHL. For the

remaining cases, 7 were identified through the Registry and 6 through serology. Six of the 7 Registry cases were identified within 2 months and 5 of the 6 serology cases were identified within 3 months of diagnosis. Since the sensitive of anti-HCV testing is poor for exposure less than 3-6 months previous, it is likely that all of these individuals were infected prior to diagnosis. For HGV, it is more difficult to exclude the possibility of late infection. The median time from diagnosis to sample collection was less than 3 months, with 75% of the samples collected within 4 months. Therefore, the possibility that the cases were infected after diagnosis cannot be excluded.

To observe the risk of chronic infection in HCV, the present HCV study evaluated the presence of HCV serology, but we did not examine the genotype of HCV, and thus could not analyze the genetic patterns of risk. Also, the risk of HGV was determined by testing HGV-RNA in the present HGV study and that provided the presence of HGV infection. Since most of the past epidemiologic studies only tested HGV-RNA, we can compare our results to other studies. However, we failed to examine HGV serology to analyze the impact of previously cleared infection to NHL.

5.5 Conclusion

Despite controversy in the literature, the molecular and epidemiologic evidence strongly suggests a pathogenetic link between HCV and NHL. Our study has shown a strong association between HCV infection and NHL. Not only

is this the first study to show an association between HCV and NHL in Canada, but it also further confirmed that HCV-NHL occurs in a low-endemic area.

The present HGV study is by far the largest epidemiologic study internationally showing a strong association between HGV infection and NHL, and to conduct a systematic subtype analysis which illustrated that HGV infection increased risk for B-cell NHL with both indolent and aggressive subtypes. Little is known about the viral pathogenicity and the clinical impacts of HGV, however, our study nonetheless provides strong evidence on the health significance of HGV infection.

6 Conclusion and Recommendation for Future Work

6.1 Summary

Infectious agents, chiefly viruses, are accepted causes of diverse malignancies worldwide. Viruses account for up to 20% of all cancers [222]. Incident rates of NHL have increased dramatically over the past few decades throughout the world especially in the North America and Europe. Despite great efforts to uncover possible environmental and genetic risk factors for NHL, the current evidence has not explained the majority of this increase of NHL. Identification of infectious agents as risk factors for NHL may have a great impact in terms of planning public health policies for prevention and treatment of NHL.

6.2 Implications

Supported by an abundance of evidence, the association of HCV and NHL is well established. Although HCV related NHL only makes up of a small proportion of NHL cases in Canada, HCV infection is an alterable risk factor so a large effort to promote the preventing of HCV infection should be put in to reduce the harmful effect of infection.

Despite the higher prevalence of HGV in the population, and knowing that blood transfusion and blood products are the major route to the infection, HGV screening has not be recommended. HCV screening has been available since

the mid 1980s but the screening program was not initiated until 1990. Because of this a substantial number of individuals had been infected due to blood transfusion between 1986 and 1990. Recently, the federal government announced a plan to compensate some of the HCV infected individuals due to blood transfusion. To avoid repeating the same mistake leading to a high social and economic cost to both individual and public levels, HGV screening to all blood products may need to be re-evaluated. Now that there is epidemiologic and experimental evidence that HGV may be related to NHL, more research initiative to investigate the impact of HGV on other clinical disorders should be encouraged and funded.

6.3 Future Research Directions

Although the sample size of our study is relatively large, it still lacks sufficient power to determine the risk to the rare subtypes of NHL. Larger population based studies or pooled analyses need to be conducted in order to examine risk by NHL subtype. Furthermore, the future studies should focus on the risk associated with different HCV genotypes. Cohort studies with longitudinal testing will be essential to further understand the latency period between the exposure of the infection and onset of NHL.

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