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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
Department of Health Care and Epidemiology

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
April 15, 2004

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ABSTRACT

Objectives: The objectives of this study were: (1) to estimate the prevalence and incidence of hepatitis C virus (HCV) infection among all gay men residing in Vancouver during the period 1982-98, (2) to identify risk factors for HCV infection and predictors of 'time to HCV seropositivity' in this population, and (3) to determine whether co-infection with HCV adversely influences the natural history of HIV infection in co-infected men.

Methods: The Vancouver Lymphadenopathy-AIDS Study (VLAS) has monitored a cohort of homosexual men since November 1982. Serum samples were obtained from 932 men during the period 1982-98, and tested for HCV antibody using EIA1, EIA2, and RIBA. HIV-antibody test results were also available. Data regarding demographic variables, sexual practices, substance use, and history of infectious diseases were obtained from self-administered questionnaires completed during 1982-85. Data regarding HIV-related disease progression were also available including clinical symptoms and signs, physical findings, CD4 cell count, diagnosis of AIDS, and survival. Risk factors for HCV infection were assessed for statistical significance using both cross-sectional comparisons of seropositive and seronegative men and prospective analyses of time to HCV seropositivity. Differences in time to HCV seropositivity, AIDS progression and survival were evaluated by stratified Kaplan-Meier analysis, and tested using the log-rank test. Both logistic regression and Cox proportional hazards regression were used to model the simultaneous effect of several variables on outcomes of interest. All p-values were two-sided.

Results: A total of 54 of 932 men (5.8%) tested positive for HCV antibody [95% CI: 4.3%, 7.3%]. HCV prevalence was significantly higher among HIV seropositive men compared to HIV seronegative men (8.8% vs. 2.6%; p<0.001). After 14 years of follow-
up, cumulative HCV incidence in the cohort was 7.9% [95% CI: 5.1%, 10.7%]. Annual infection rates ranged from 0 to 1.4 percent during the follow-up period. Men who reported using injection drugs during their lifetime were twenty times more likely to become HCV seropositive. In prospective analyses, significant elevations in risk were detected for the following sexual practices: 20 or more male sexual partners in the previous 12 months [RR = 3.1, 95% CI: 1.5, 6.3], insertive oral-anal contact [RR = 3.1, 95% CI: 1.1, 8.7], insertive fisting (RR = 2.6, 95% CI: 1.4, 4.8), and receptive anal intercourse [RR = 2.0, 95% CI: 1.0, 3.8]. In multivariate analysis, risk factors that exerted an independent effect on time to HCV seropositivity were injection drug use (p<0.001), HIV seropositivity (p=0.031), and the sexual practice of insertive fisting in combination with insertive oral-anal contact (p=0.038). In terms of HIV-related disease progression, HCV co-infection was not significantly associated with an increase in symptomatic illness, more rapid CD4 cell decline, faster progression to AIDS, or increased mortality. However, we did find strong indication of increased liver and spleen inflammation among co-infected men.

Conclusions: Prevalence and incidence of HCV infection are elevated among gay men in Vancouver, and are significantly higher among HIV-seropositive men compared to HIV-seronegative men. Not surprisingly, a history of injection drug use was the most significant risk factor for HCV seropositivity in this population. However, these data also provide evidence of sexual transmission of HCV independent of injection drug use. Sexual practices that result in rectal trauma may play a role in the spread of HCV in this population. With regard to disease progression, we did not detect an adverse affect of HCV infection on HIV disease progression among co-infected individuals but we did observe an adverse influence on progression of liver disease.
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I must be honest. I never really thought I would ever finish this project. At least not until I took some time away, dropped all the other competing priorities in my life, and focused on just this one thing. Fortunately, in the late spring and early summer of 2003, I did get a chance to do exactly this. I am extremely pleased I did. There are a lot of important people in my life who have helped me and encouraged me to complete this personal project, either directly or indirectly. I would like to thank each of these individuals for their much appreciated support and encouragement over the years. Dr. Martin Schechter has been my academic supervisor throughout this endeavor, and he has been my colleague and friend for more than twenty years. The majority of what I have learned about epidemiology during my career has been the result of working directly with Martin. I have always felt very privileged to be able to work with and learn from a world-renowned epidemiologist. I would like to express my gratitude to Dr. Michael O'Shaughnessy for asking me to take on this project back in 1997, and for providing me with the necessary funding to carry out HCV antibody testing on serum specimens obtained from approximately 900 VLAS participants. I also appreciate the patience and understanding that Dr. "O" has exhibited as I struggled to complete this project. I am indebted to Dr. Chris Sherlock and his laboratory staff for conducting the HCV antibody testing for this study. More importantly, I would like to thank Chris for providing me with much needed encouragement at times when I wanted to abandon this dissertation. I would also like to acknowledge the constant encouragement of my committee member, Dr. Robert Hogg. Mrs. Bonnie Devlin was instrumental in helping me focus my undivided attention on completion of this project. I must thank Bonnie for never letting me forget I needed to finish this thing. Most of all, I want to thank my loving wife Anne for always believing in me and supporting me during the past twenty-five years of our life together. Our children, Jenna and Jonathan, are to be commended for putting up with my antics while I was preparing this dissertation. This accomplishment is truly our success. To my brother Ian - thank you for always being there for me and providing me with a constant link to my past. To my best friend Bill Holden - I have always enjoyed our daily conversations about golf, music, and life. I appreciate the support you have shown me over the many years of our friendship. To Don Burton and Peter Vann - thanks for sharing some fine musical moments with me during the latter part of this journey. Finally, I would like to dedicate this completed work to the memory of my father Dr. Joseph Stoddart Craib, who passed away on January 30, 2003.
CHAPTER 1
INTRODUCTION

1.1 HEPATITIS C VIRUS - EPIDEMIOLOGY

Hepatitis C is recognized as a global health problem. Hepatitis C virus (HCV) is a blood-borne pathogen and was first identified in 1989.\(^1\) HCV belongs to the \textit{Flaviviridae} family of viruses, and is related to viruses that have been causally linked to yellow fever, hog cholera, and bovine diarrhea. Since its discovery, HCV has been established as an etiologic agent of acute and chronic liver disease, as well as cirrhosis of the liver. It has also been found to be associated with development of hepatocellular carcinoma in humans.\(^2,6\)

During the 1970s and 1980s, medical practitioners in the United States became increasingly aware that an unidentified agent in the blood supply was causing a small percentage of blood/blood product recipients to experience post-transfusion hepatitis. In some of these cases, cirrhosis and liver cancer was diagnosed many years later. To distinguish this disease from other known forms of hepatitis, it was described as non-A, non-B hepatitis. After the development of specific serological tests for antibody to HCV, it was demonstrated that the majority of cases of transfusion-associated non-A, non-B hepatitis were associated with HCV infection.\(^2,3\) In addition, the detection of genomic HCV sequences using PCR assays confirmed that HCV viremia was correlated with progression to chronic hepatitis in non-A, non-B patients.\(^7-14\)

In 1997, the World Health Organization published its first estimate of the global prevalence of HCV infection. Prevalence data obtained from reporting countries indicated that approximately 3 percent of the world’s population were infected with HCV.\(^15,16\) It was estimated that as many as 170 million people may be infected with HCV. Prevalence appears to be higher among countries in Africa (5.3%), the Eastern
Mediterranean region (4.6%), the Western Pacific Region (3.9%), and Southeast Asia (2.2%). In comparison to these regions, prevalence rates are lower in countries in North America (1.7%) and Europe (1.0%).

Countries with prevalence rates above 10% include Egypt (18.1%), Rwanda (17.0%), Bolivia (16.3%), Cameroon (12.5%), Burundi (11.1%), Guinea (10.7%) and Mongolia (10.7%). The following countries have reported prevalence rates between 5% and 10%: Libya (7.9%), Papua New Guinea (7.0%), Gabon (6.5%), Democratic Republic of the Congo (6.4%), Viet Nam (6.1%), Thailand (5.6%), Suriname (5.5%), Ghana (5.4%), and Palestinian Authority (5.2%).

The accuracy of current estimates of regional and global HCV prevalence remains in doubt because many countries have not yet conducted population-based studies to assess the exact magnitude of the problem. Moreover, initial estimation of HCV prevalence in many countries has been based on samples of individuals that are not representative of their respective target populations and as a result, these estimates of prevalence are subject to selection and volunteer bias. In addition, some countries have only recently gained access to serologic tests for detecting HCV antibody and viral genomic RNA in serum. HCV antibody assays first became commercially available in 1990.

HCV prevalence is lower in the United States and Canada compared to other regions of the world. HCV infection is, however, the most common chronic blood-borne infection in the United States. Prevalence in the general population has been estimated to be 1.8 percent, which corresponds to approximately 4 million infected Americans. Prevalence rates in the United States tend to vary according to gender, age and ethnicity. For example, among 30 to 49 year-olds, HCV prevalence ranges from 3 to 4 percent. In contrast, among Afro-Americans of similar age, prevalence is
estimated to be 10%.\textsuperscript{17} HCV infection is currently the primary reason for liver transplantation in the United States.

It has been estimated that the prevalence of anti-HCV positivity is approximately 0.8% (0.68\% to 0.94\%) in Canada, 0.96\% in males and 0.53\% in females.\textsuperscript{18,19} Estimates of prevalence exhibit heterogeneity between provinces. In Newfoundland and Prince Edward Island, HCV prevalence has been estimated to be 0.1\% and 0.2\%, respectively, compared to 0.9\% and 1.4\% in Ontario and British Columbia. HCV prevalence appears highest in British Columbia, Ontario, Alberta and Quebec. These four provinces account for about 90 percent of all HCV infections in Canada. HCV infection is now the second most frequently reported disease entity among 47 nationally reported diseases.\textsuperscript{20}

The majority of HCV transmission worldwide occurs via percutaneous exposure to infected blood. Transmission of HCV is associated with direct percutaneous exposures including transfusion of unscreened blood/blood products, transplantation of organs or tissues from infected donors, and sharing contaminated needles among injection drug users.\textsuperscript{21-31} Nosocomial transmission of HCV can occur during medical and dental procedures from inadequately sterilized instruments, and poor infection control practices.\textsuperscript{32-35} HCV prevalence is higher among health care workers involved in invasive procedures and among those who have frequent contact with infected blood.\textsuperscript{36,37} Some skin piercing practices such as tattooing, body piercing, and acupuncture have also contributed to the spread of HCV, particularly in developing nations.\textsuperscript{38}

Iatrogenic infections can also occur. The largest iatrogenic infection scenario known to date has taken place in Egypt where approximately 15 to 20\% of the general population is estimated to be infected with HCV. This high prevalence rate is thought to be associated with a decades-old strategy to treat schistosomiasis, a parasitic disease. In Egypt, parenteral antischistosomal therapy (PAT) was extensively practiced since the 1920s and discontinued during the late 1980s. PAT typically required 10 to 12 injections
and was usually given with reusable syringes. In a recent study conducted by Frank et al, the degree of exposure to PAT was estimated for over 8,000 Egyptians during the period 1961 to 1986, and higher levels of exposure were found to be significantly associated with seroprevalence of antibodies to HCV across four regions of the country. These data provide strong evidence that PAT played a major role in the diffusion of HCV infection throughout Egypt.

Both vertical and horizontal transmissions of HCV have been reported. The rate of transmission of HCV from an infected mother to their newborn infant is generally estimated to be 5%, but this rate is higher among mothers who are co-infected with HIV. Household contact with another member of the household who is infected with HCV has also been implicated as a route of transmission. Family studies suggest that horizontal transmission may be more important than vertical transmission.

Sexual contact has been identified as a means of transmitting HCV but the efficiency of transmission in this manner is thought to be low. Sexual transmission of HCV appears to be infrequent between heterosexual couples in long-term monogamous relationships who have no identifiable percutaneous exposures. Some epidemiological evidence of sexual transmission of HCV has been provided by observational studies of homosexual men and female sex workers. HCV seroprevalence appears to be higher in these populations compared to the population at large.

1.2 HEPATITIS C – NATURAL HISTORY

HCV is a single-stranded RNA virus and more than 100 strains have been identified. These strains have been grouped into at least 6 major genotypes or clades. Geographic clustering of genotypes has been observed globally. Genotypes 1 to 3 exhibit a worldwide distribution, whereas genotypes 4 and 5 are found primarily in
Africa. Genotype 6 clusters in Asia. In Canada and the United States, genotype 1 is more common.\textsuperscript{16}

In the majority of cases, individuals do not exhibit any acute signs or symptoms associated with HCV infection. Twenty to 30 percent of individuals with acute infection become jaundiced, and 10 to 20 percent develop symptoms.\textsuperscript{58-60} Severe hepatic failure associated with primary HCV infection is rare, but may occur among individuals with compromised immune systems.\textsuperscript{3,61} Although acute infection with HCV is usually asymptomatic, the majority of infected individuals develop chronic infection. Approximately 60 to 80 percent of HCV-infected individuals are asymptomatic and develop chronic HCV infection. The most common symptom associated with chronic HCV infection is fatigue. Other related symptoms include nausea, vomiting, mild fever, arthralgia, myalgias, loss of appetite, abdominal pain, diarrhea, and weight loss.

HCV RNA can be detected in the blood of an infected person within a few days after infection, and HCV antibodies can usually be detected within 45 to 90 days.\textsuperscript{62} The mean incubation period is estimated to be 6 to 12 weeks.\textsuperscript{3,63} Twenty to 30 percent of infected individuals will exhibit clinical symptoms. About 15 percent of individuals who test HCV RNA positive by serum during acute or post-acute HCV infection eventually become undetectable. This minority of infected individuals appears able to eradicate HCV from their bodies by their immune response.\textsuperscript{3,61,64} However, the findings of Haydon et al. cast doubts that HCV infection can be eradicated and that HCV disease can be self-limiting. In their study, 10 of 12 patients with chronic hepatitis C had HCV RNA detected in their liver but not in their serum.\textsuperscript{65}

The majority of HCV-infected individuals develop chronic hepatitis C. Data from various observational studies indicate that 75 to 85 percent of acute infections do not resolve and result in a chronic viral infection.\textsuperscript{3,64,66} No clinical features of the acute disease have been found to be predictive of chronicity. In the majority of those with
chronic hepatitis C infection, the disease typically progresses slowly without clinical symptoms or physical signs during the first two decades following infection. Roughly one-fifth of individuals with chronic hepatitis C develop non-specific symptoms including intermittent fatigue and malaise. Other symptoms may also accompany chronic infection. A recent report by Cacoub et al. found that almost three-quarters of HCV-infected individuals exhibited at least one of the following symptoms: arthralgia (joint pain), paresthesia (nerve sensation abnormalities), myalgias (muscle pain), pruritus (itching) and sicca syndrome (dry eyes, skin, and mouth). In many individuals with chronic hepatitis C symptoms first appear at the time of advanced liver disease.

The late sequelae of chronic HCV infection include cirrhosis and hepatocellular carcinoma. Cirrhosis emerges in at least 20 percent of HCV-infected individuals within twenty years of the onset of infection whereas hepatocellular carcinoma occurs in a smaller proportion of individuals (less than 5%) and takes about 10 or more years after onset of cirrhosis to emerge. Risk factors that hasten the rate of progression of chronic hepatitis C to cirrhosis include alcohol consumption, age at time of infection, and the severity of liver histology at the initial biopsy. In this context, the role of other factors such as gender, viral genotype, co-infection with other viruses like human immunodeficiency virus (HIV) and hepatitis B virus (HBV) is less well understood. Risk factors that are associated with hepatocellular carcinoma are similar to those for cirrhosis and include age over 40 years, duration of infection, and presence of cirrhosis. On the other hand, alcohol consumption and viral genotype have not been shown to be associated with progression to hepatocellular carcinoma.

Some individuals with chronic hepatitis C present with extrahepatic sequelae including essential mixed cryoglobulinemia and glomerulonephritis, aplastic anemia, and pulmonary fibrosis. Other extrahepatic sequelae thought to be associated with
chronic hepatitis C disease progression include cutaneous manifestations (lichen planus and porphyria cutanea tarda), ocular lesions (Mooren’s ulcers), sialadenitis and B-cell lymphoma. \textsuperscript{16,69-71}

Data from a small number of natural history studies have revealed that approximately 15 to 20 percent of HCV-infected individuals will eventually develop progressive end-stage liver disease. The remainder of infected individuals will die of causes unrelated to liver disease.\textsuperscript{72} Chronic liver disease is the tenth leading cause of death among adults in the United States, and accounts for approximately 25,000 deaths annually, or approximately one percent of all deaths.\textsuperscript{73} Population-based studies indicate that 40 percent of chronic liver disease is HCV-related, resulting in an estimated 8,000 to 10,000 deaths each year.\textsuperscript{74} Death rates due to HCV are expected to increase over the next 20 years. Estimates of death rates may underestimate the actual death rate because of underreporting of liver disease and underreporting of deaths due to liver disease on death certificates and in other databases.\textsuperscript{75}

1.3 RESEARCH NEEDS AND STUDY JUSTIFICATION

The accuracy of current estimates of regional and global HCV prevalence is questionable because many countries have not yet conducted population-based studies to assess the exact magnitude of the problem. In addition, many countries have only recently gained access to serologic tests for detecting HCV antibody and viral genomic RNA in serum. Initial estimates of HCV prevalence for the most part have been based on samples of individuals that are not representative of their respective target populations. As a result, these initial estimates of prevalence are subject to bias.

In Canada, the epidemiology of HCV remains largely unknown. Current estimates of HCV prevalence must be considered as hypotheses because initial estimates are based on fragmented data. Because of this, the true burden of HCV
disease in Canada is still a matter of speculation. At present, there are no ongoing studies of annual HCV incidence. There is a pressing need to better evaluate the extent and distribution of HCV infection, and to assess the burden of infection and disease in both the short and long term. Recent research in the United States suggests HCV may lead to a substantial health and economic burden over the next 10 to 20 years\(^5\). The situation in Canada will likely be no different.

Well designed, population-based observational studies are needed to arrive at more accurate estimates of local, regional and global HCV prevalence. The relative contribution of various routes of HCV transmission also needs to be elucidated. In particular, the role of sexual transmission in the spread of HCV requires clarification. Longitudinal investigations are needed to estimate the true burden of hepatitis C disease, and to estimate morbidity and mortality rates among those infected with HCV, and among those individuals co-infected with other infectious agents such as HIV. Such natural history studies could also be used to identify co-factors and predictors of disease progression in both HCV-infected and co-infected individuals.

During the 1980s, in response to the HIV/AIDS pandemic, a number of cohort studies of HIV-infected individuals were launched in a number of countries throughout the world. These longitudinal investigations were conducted in order to gain important knowledge about the epidemiology and the natural history of HIV infection. Many of these prospective studies have contributed greatly to our understanding and response to the HIV/AIDS pandemic during the past two decades. One such longitudinal study was conducted in Vancouver, British Columbia.

The Vancouver Lymphadenopathy-AIDS Study (VLAS) followed and monitored a cohort of homosexual men from November 1982 to December 1998. The general objective of this longitudinal study was to contribute to our understanding of the natural history of HIV infection including risk factors for seroconversion through to
progression to AIDS and survival. The VLAS was a prospective study of gay/homosexual men who were enrolled during two recruitment periods. From November 1982 until December 1984, a total of 729 men were recruited through six primary care practices in central Vancouver. Two additional practices were added and 271 men were enrolled during the period from October 1986 to December 1987. Follow-up visits occurred approximately every six months until September 1986 after which subjects completed visits on an annual basis until December 1998.

During each visit participants completed a self-administered questionnaire which gathered information regarding demographic variables, numbers of sexual partners and sexual practices, history of sexually transmitted diseases, use of tobacco, alcohol, and illicit drugs. In addition, a physical examination and functional inquiry were performed and blood samples were drawn for immunologic and HIV antibody testing. Serum samples were frozen at minus 20 degrees Celsius. Since 1992, plasma specimens from study participants were frozen at minus 80 degrees Celsius.

In response to the epidemic of HCV infection in Canada and the need for population-based, long-term, prospective studies, the VLAS has provided a unique opportunity to respond to this need in a well-defined cohort. Finally, the VLAS has provided a cost-effective means to acquire important knowledge concerning the epidemiology and natural history of HCV infection in a well-characterized cohort of homosexual men during the period 1982-98.
1.4 OBJECTIVES, HYPOTHESES, AND THESIS ORGANIZATION

The general aim of this study was to contribute to our understanding of the epidemiology of HCV infection and its modes of transmission, and the natural history of HCV infection and its related clinical outcomes in a population-based study of homosexual men.

The first objective of this study was to estimate the prevalence and incidence of HCV infection in homosexual men in Vancouver during the period 1983 to 1998. This objective was achieved by utilizing an archive of frozen blood samples obtained from VLAS participants. Cumulative and annual HCV seroconversion rates were estimated using Kaplan-Meier, actuarial, and incidence-density methods.

It was estimated that 5 percent of HIV-negative and 15 percent of HIV-positive VLAS participants would be found to be infected with HCV. Based on the HIV serologic status of participants at the time of their last laboratory visit (540 HIV positive and 410 HIV-negative), it was projected that a total of 100 men would be found to be infected with HCV in this cohort. This projection included 20 cases among HIV-negative men and 80 cases among HIV-positive men. It was also estimated that 50 men would be HCV seroconverters, 50 would be seroprevalent, and that 80 would be co-infected with HIV. These numbers of HCV-infected men should provide sufficient statistical power for conducting meaningful cross-sectional comparisons and some prospective analyses.

The second objective was to identify risk factors for HCV transmission and assess the relative contributions of various routes of transmission including injection drug use, substance use, sexual practices, co-infection with other sexually transmitted diseases including HIV, history of blood transfusions, demographic characteristics, and other
variables. This objective was achieved by conducting nested comparisons between HCV-infected and uninfected men. Mantel-Haenszel methods and multivariate logistic regression modeling were used to assess and identify significant risk factors for HCV infection.

The third objective was to estimate the relative risk of HCV transmission for specific sexual practices among homosexual men independent of injection drug use. This objective was achieved by excluding men who reported using injection drugs, and by expanding the eligibility criteria for the comparative analysis to include VLAS participants from both recruitment periods.

The fourth objective of this investigation was to examine predictors of time to HCV-seropositivity. Because the number of incident infections of HCV in the cohort was small, both incident and prevalent infections were included as events of interest in the prospective analyses. Risk factors of significance that were identified in previous nested comparisons were assessed as potential predictors of time to HCV seropositivity. Variables of interest included: injection drug use, HIV antibody status, sexual practices, and history of other sexually transmitted diseases. Methods of survival analysis including Kaplan-Meier methods, and Cox proportional hazards regression were used to identify independent predictors of time to HCV seropositivity among homosexual men in Vancouver.

Finally, the fifth objective of this study was to assess the influence of HCV infection on the natural history of human immunodeficiency virus (HIV) infection. The effects of HCV infection on HIV-related outcomes were examined among HIV-infected participants in the VLAS cohort. This was achieved by including HIV seropositive participants from both recruitment periods in comparative analyses. Outcomes of interest in this investigation included symptoms and signs, physical exam findings,
Methods of survival analysis were used to analyze these data.

This thesis is comprised of eight chapters. The first chapter contains a brief introduction to the epidemiology and natural history of HCV. Chapter 2 presents an overview of previous studies of sexual transmission of HCV in heterosexuals and gay men. Chapter 2 also contains a detailed description of the VLAS study including the number of visits completed by the participants and rates of follow-up. Descriptions of data management procedures, statistical methods and approaches to multivariate modeling are also presented in chapter 2. Chapters 3 through 7 address each of the aforementioned objectives in sequential order. In chapter 3, the serological results of hepatitis C virus antibody testing for the VLAS cohort are presented and discussed. Chapters 4 and 5 provide an assessment of risk factors associated with HCV infection including injection drug use, sexual practices, substance use, history of blood transfusions, and HIV infection. Risk factors for HCV transmission that were identified by means of nested comparisons between seropositive and seronegative men in chapters 4 and 5, are re-examined prospectively in chapter 6. The results of an assessment of the influence of HCV infection on the natural history of HIV infection are presented and discussed in chapter 7. Each of chapters 3 through 7 has been written as an independent article and prepared for submission to a scientific journal. Alternatively, the results from one or more these chapters could be combined into a smaller number of scientific articles. The final chapter of this thesis provides a summary of the research findings and describes the strengths and limitations of this research. In addition, chapter 8 outlines the unique contributions and potential impact of the findings of this longitudinal study.

The work presented in this thesis was conceived, conducted, and disseminated entirely by the doctoral candidate unless otherwise indicated.
1.5 SUMMARY

Hepatitis C is recognized as a global health problem. The World Health Organization estimates that as many as 170 million people may be infected worldwide. In the United States, it is estimated that 4 million persons are infected with HCV. HCV infection is currently the primary reason for liver transplantation in the United States. In Canada, it is estimated that 250,000 to 300,000 individuals are infected, and HCV infection is now the second most frequently reported disease entity among 47 nationally reported diseases.

The majority of HCV transmission worldwide occurs via percutaneous exposure to infected blood. Persons at risk for HCV infection in developed countries include recipients of previously unscreened blood, blood products and organs, injection drug users, health care workers, individuals undergoing haemodialysis, children born to HCV-infected mothers, individuals who have undergone body piercing or tattooing; and intranasal cocaine users. It is currently believed that sexual and household transmission of HCV is uncommon and that risk of HCV transmission by sexual activity between monogamous partners is low.

In Canada, the epidemiology of HCV remains largely unknown and the true burden of this disease still remains mostly a matter of speculation. There are no ongoing population-based studies of HCV incidence (how do you know?). There is a pressing need to better evaluate the extent and distribution of HCV infection in Canada, and to assess the burden of infection and disease in both the short and long term. The relative contributions of various routes of transmission of HCV need to be elucidated.

Longitudinal investigations are required to estimate the true burden of hepatitis C disease, and to estimate morbidity and mortality rates among those infected with HCV, and among those individuals co-infected with other infectious diseases such as
HIV. Such natural history studies could also be used to identify co-factors and predictors of disease progression in both HCV-infected and co-infected individuals.

The Vancouver Lymphadenopathy-AIDS Study (VLAS) followed and monitored a cohort of homosexual men from November 1982. In response to the epidemic of HCV infection in Canada and the need for population-based, long-term, prospective studies, the VLAS has provided a unique opportunity to respond to this need. Using an archive of frozen blood samples obtained from VLAS participants during the period 1982-1998, HCV antibody testing was carried out on these specimens, and estimates of prevalence and annual incidence were calculated. Data regarding demographic variables, numbers of sexual partners and sexual practices, history of sexually transmitted diseases, use of tobacco, alcohol, and illicit drugs were used to identify significant risk factors associated with HCV infection. Longitudinal behavioural and clinical data from the VLAS were used to identify independent predictors of time to HCV seropositivity, as well as the effect of HCV infection on HIV-related disease progression and survival.

In summary, the VLAS has provided a cost-effective means to acquire important knowledge concerning the epidemiology and natural history of HCV infection in a well-characterized cohort of homosexual men during the period 1983-98.
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CHAPTER 2
BACKGROUND

2.1 SEXUAL TRANSMISSION OF HEPATITIS C VIRUS IN HETEROSEXUALS

The role of sexual transmission in the spread of HCV has been controversial. Controversy exists because the precise magnitude of the risk associated with sexual transmission remains unknown and the findings from previous studies have been inconsistent. This is primarily due to the fact that most studies have used different methods. Many studies were unable to adequately characterize and quantify the risk associated with sexual transmission because they did not collect adequate information from their participants about specific sexual behaviours and practices, nor did they obtain important information about past and present injection drug use, or a blood transfusion history.

CDC Sentinel county studies suggest that 15 to 20 percent of individuals with acute hepatitis C infection have a history of sexual exposure in the absence of other risk factors. In Canada, it is estimated that 27 percent of HCV infections have been acquired by some means other than injection drug use and/or receipt of blood or blood products prior to 1990. The efficiency of sexual transmission among heterosexuals is generally thought to be low, and infection is rare in long-term, monogamous partners. Early investigations of sexual transmission of HCV have collectively shown the risk of inter-spousal transmission to be very low or non existent.

In the early 1990s, reports began to appear documenting evidence of sexual transmission of HCV. In contrast to previous studies where inter-spousal transmission was low, a study in Japan identified 154 HCV-positive individuals and found that 27 percent of their spouses were HCV positive. Almost 90 percent of the individuals with
positive HCV RNA were infected with genotypes identical to those of their spouse. In this study, it was also noted that risk of HCV infection increased with duration of the marriage. Only those individuals who were married for more than 10 years were infected. It should be noted that inter-spousal studies are confounded by household (i.e. non-sexual) contact.

Another study conducted in the United States found that risk of HCV infection among heterosexuals increased with multiple lifetime sexual partners. Females in this study whose sexual partners were HCV-positive were at least three times more likely to be HCV-positive compared to females whose sexual partners were HCV-negative. For males, HCV seropositivity had no significant association with the serologic status of their female sexual partners. These results suggest that 'male to female' transmission of HCV is more efficient than 'female to male'.

A small number of studies have been carried out among female sex workers to determine whether this population is at higher risk for HCV infection. A study of over 600 sex workers in Taiwan reported an HCV prevalence of 12 percent. A history of paid sex for at least six months and previous blood transfusion were both significantly associated with HCV-seropositivity in multivariate analysis. Lissen and colleagues studied a total of 312 Spanish female sex workers and 88 of their male clients. All study participants denied prior or current use of injection drugs, and none reported receiving a blood transfusion. HCV prevalence was 6 percent among the sex workers and 7 percent among their male clients. In contrast to the findings of these two studies, a study of Peruvian sex workers reported a prevalence of less than 1 percent among 966 female sex workers who were tested.
2.2 HEPATITIS C VIRUS INFECTION IN HOMOSEXUAL MEN

The prevalence of HCV appears to be higher among men who have sex with men compared with the general population. During the period 1990 to 1998, there were numerous observational studies of homosexual men that published reports documenting HCV prevalence, and some studies provided assessments of putative risk factors for transmission in this population. In one of the earliest investigations in homosexual men, Melbye and colleagues reported an HCV prevalence of 4.1 percent.\textsuperscript{13} Two hundred and fifty-nine male members of a Danish homosexual organization were prospectively monitored in Copenhagen and Aarhus, Denmark from 1981 to 1989. In 1981, the prevalence of HCV was 1.6 percent in the cohort. Between 1981 and 1984, the cumulative incidence rate was 2.5 percent. From 1984 to 1989, no new infections were observed in this cohort. No significant associations were observed between sexual behaviour variables and HCV infection.

In 1991, Tedder et al. reported the results of a study of 1074 consecutive patients who had attended an outpatient genitourinary medicine clinic in London, England during November and December 1987.\textsuperscript{14} Among homosexual men who participated in the study, the prevalence of HCV infection was 6.9 percent. This estimate was significantly higher than the value for heterosexual participants (1.0%). Homosexual men who reported a higher number of other sexually transmitted infections in their lifetime were significantly more likely to be seropositive than men who did not.

The results of a study by Gasparini and colleagues also provided evidence that HCV prevalence is elevated in homosexual men.\textsuperscript{15} They studied 259 apparently healthy men from homosexual men’s clubs in the Venato region of Italy. Injection drug users were excluded from their study. Sera collected during the period 1987-89 were tested
for HCV and prevalence was calculated as 4.1 percent. No association was found between HCV seropositivity and anal intercourse or sexual promiscuity. In another Italian study published in 1998, Osella et al. reported a 12.9% prevalence rate of HCV among 228 homosexual men who were attending two clinical centers. While the study investigators did find hepatitis B virus infection was strongly associated with some sexual practices reported by the participants, there was no association between sexual behaviour and HCV.

Osmond and colleagues measured HCV prevalence among 735 homosexual/bisexual men who were participating in two cohort studies in San Francisco. Sera collected during the period between 1985 and 1986 were screened for HCV antibody and a prevalence of 4.6 percent was found. A history of injection drug use or blood transfusions was associated with HCV seropositivity. After adjustment for these variables, HCV infection was marginally associated with having more than 50 sexual partners in the previous year, and engaging in receptive oral intercourse and receptive anal intercourse with more than 25 partners in the previous year.

Buchbinder and her colleagues found HCV prevalence to be 9.2% among 435 homosexual men recruited from a municipal STD clinic in San Francisco. Sera for the study were obtained during 1983 and 1984. Injection drug use, anal receptive intercourse, fisting (i.e. insertion of fingers or hand into partner’s rectum), having an injection drug user as a sexual partner, history of genital herpes, HIV seropositivity, use of amphetamine and phencyclidine (PCP) were significant univariate risk factors. However, in multivariate analysis, only injection drug use was significantly associated with HCV seropositivity.

In another American study, Ndimbie and colleagues assessed HCV infection in 1058 homosexual men who were participating in a natural history study of HIV infection using sera that were obtained during 1984 and 1985. HCV prevalence was
2.9% and HCV-positive men were more likely to be HIV-positive. Univariate risk factors associated with HCV seropositivity included injection drug use, insertive anal intercourse with ejaculation, HIV infection, history of syphilis, and rectal gonorrhea. In a multivariate statistical model, injection drug use and insertive anal intercourse were association with HCV seropositivity.

An Australian study by Bodsworth and colleagues reported HCV prevalence of 7.6 percent among 1038 homosexual men participating in a prospective study established to identify risk factors for AIDS. In HIV-positive men, HCV prevalence was significantly higher (11.9%) compared to HIV-negative men (4.0%). Univariate risk factors included injection drug use, HIV infection, and history of syphilis, anogenital herpes, and gonorrhea. In multivariate analysis, only injection drug use and HIV infection were significantly associated with HCV seropositivity.

When assessing the results of previous studies of HCV infection among homosexual men, several common findings emerge. In all of these studies, HCV prevalence rates were substantially higher than the general populations from which these participants were selected. Prevalence in these studies ranged from 2.9 to 12.9 percent. There is evidence that prevalence of HCV is higher among men who are HIV-positive compared with HIV-negative men.

Prevalence of HCV among gay men also appears to be higher in those with other sexually transmitted infections such as genital herpes and rectal gonorrhea. In three American studies and one Australian study that were reviewed, injection drug use was identified as the primary risk factor for HCV acquisition. In some of these studies, specific sexual practices were identified as significant or marginal risk factors in univariate analyses, including number of sexual partners, frequency of anal intercourse, and fisting. These findings suggest that risk of sexual transmission of HCV infection may be increased with specific sexual acts or that they are confounded with injection
drug use. In contrast, the majority of European studies have not found any evidence of an association between HCV seropositivity and sexual behaviour.

2.3 NATURAL HISTORY STUDIES OF HCV/HIV CO-INFECTION

The majority of studies of individuals who are co-infected with HCV and HIV have concluded that, while the progression of HIV disease is not strongly influenced by HCV infection, hepatitis C progresses more rapidly in individuals with co-infection. Most of these studies showed that individuals with HCV/HIV co-infection experience more rapid progression to cirrhosis, and have more evidence of extensive liver damage. Staples and colleagues studied time from HIV diagnosis to AIDS, time from HIV diagnosis to death, and time from AIDS diagnosis to death among HCV/HIV co-infected individuals. They did not find any of these measures of disease progression to significantly differ between HCV/HIV co-infected and HIV-infected groups. In contrast, a study conducted by Sabin and his colleagues reported that hemophiliacs infected with HCV genotype 1 experienced more rapid progression to AIDS and AIDS-related death compared to other genotypes.

2.4 THE VANCOUVER LYMPHADENOPATHY-AIDS STUDY

2.4.1 Study Description

The Vancouver Lymphadenopathy-AIDS Study (VLAS) is a prospective cohort study involving 1,000 homosexual/bisexual men who were enrolled during two recruitment periods. From November 1982 to December 1984, a total of 729 gay and bisexual men aged 18 to 75 were recruited through six general medical practices in Vancouver. Two of the practices provided care almost exclusively to homosexual men, and the other practices had substantial numbers of homosexual male patients. During the period October 1986 to December 1987, two more practices were added to the study
and 271 additional men were recruited. Throughout these recruitment periods, any regular patient known to be predominantly or exclusively homosexual was invited by his practitioner to participate in the prospective study. The refusal rate was low, approximately five percent. Only patients who were already enrolled in these medical practices were eligible thereby minimizing self-referral of other participants who might have been aware of this study.

All participants gave informed consent. In the first cohort, follow-up visits occurred approximately every six months until September 1986, after which, participants in both cohorts completed visits on an annual basis. During each physician visit participants completed a self-administered questionnaire. Variables of interest included demographic characteristics such as age, ethnicity, income, and education; sexual behavior variables including the number of male sexual partners; frequency of receptive and insertive anal intercourse; frequency of condom use during anal intercourse; and use of tobacco, alcohol, and other drugs. Data concerning sexual practices and substance use were self-reported and described behaviors which occurred during the previous 12 months. In addition, the participants underwent a complete physical examination at each visit and blood samples were drawn for laboratory testing.

2.4.2 Follow-up of cohort

Between October 1982 and December 1984, a total of 729 participants were enrolled in the first cohort. These men were actively followed for a median of 128 months (range 26-180 months). The earliest enrolment visit in this cohort took place in October 1982 and the latest visit occurred during last cycle of physician visits in December 1998. The median number of completed visits was 9 (range 2 to 16). At the conclusion of the ninth cycle of physician visits which occurred during the period from October 1, 1988 to December 31, 1989, a total of 392 (54%) of the 729 men enrolled in the first cohort had completed the follow-up visit. Ninety-seven (13%) participants had
been diagnosed with AIDS, 95 (13%) did not attend the ninth cycle, 87 (12%) had moved away from Vancouver, 43 (6%) could not be contacted, 12 (2%) had transferred to non-study physicians and 3 (0.3%) had died from causes unrelated to AIDS.

A total of 271 men were enrolled in the second cohort between September 1986 and December 1987. These men were followed for a median of 75 months (range 6-128 months). In this cohort, the earliest visit occurred in September 1986 and the latest visit occurred in December 1998. The median number of physician visits completed by these men was 5 (range: 2 to 10). As of December 31 1989, a total of 197 (73%) of the 271 men had completed the follow-up visit, 25 (9%) had been diagnosed with AIDS, 18 (7%) had moved away from Vancouver, 12 (4%) could not be contacted, 9 (3%) did not attend the ninth cycle, 9 (3%) had transferred to non-study physicians and 1 (0.3%) had died from causes unrelated to AIDS.

## 2.4.3 HCV antibody testing procedure and results

All VLAS participants who completed two or more physician visits and provided blood specimens underwent HCV antibody testing. Antibody testing was carried out using stored sera obtained during the period 1982 to 1998, and the testing of these specimens was conducted between August 1999 and March 2000. HCV antibody was assayed by enzyme immunoassay (EIA), using a modified algorithm to confirm seropositivity. This testing algorithm was based on a study carried out in the University of British Columbia Diagnostic Virology and Reference Laboratory and the B.C. Centre for Disease Control Virology Laboratory. Variations of this algorithm have been widely used in diagnostic virology laboratories throughout Canada. Briefly, a micro-particle enzyme immunoassay (Abbott AxSYM HCV Version 3.0 MEIA) was used to screen all sera. Negative sera were tested no further. Positive samples, as well as samples with an indeterminate result, were further tested using a synthetic peptide-based EIA (United Biomedical Inc. HCV EIA 4.0). Sera that tested positive on both EIAs
were classified as positive and tested no further. Sera that gave discordant results were further tested by immunoblot (RIBA III; Ortho Diagnostic Systems). Sera that were negative by RIBA were classified as negative.

Sera were available for 932 of the 1000 VLAS participants. Using the testing algorithm described above, a total of 54 men were classified as HCV antibody positive. Thirty-nine infections were identified among 662 members of the first cohort, whereas 15 infections were detected among 270 participants in the second cohort. Among seropositive men in the first cohort, the numbers of prevalent and incident infections were 26 and 13, respectively. In this group, the earliest date of HCV seropositivity occurred in January 1983 and the latest date in February 1997. All 15 HCV infections identified in the second cohort were deemed prevalent. The dates of HCV seropositivity for members of the second cohort took place between October 1986 and January 1988.

2.4.4 Data management

All questionnaire, laboratory and serology data were stored on a Sun Microsystems computer. Because no identifying information on any participant was allowed to leave his physician's office, all participants were identified by a numeric code. Data for participants were stored in databases that were designed to facilitate cross-referencing. For example, the primary database contained all questionnaire and laboratory data, serologic information (HCV and HIV antibody test results), dates of seroconversion for seroincident men, dates and types of diagnoses for AIDS cases, and dates of death. The secondary database contained information regarding stored blood specimens. The third database contained information regarding completed follow-up visits. All databases were maintained and updated on an ongoing basis by study personnel. All statistical analyses presented in this dissertation were conducted using SAS statistical software (version 6.12).
2.4.5 Description of statistical methods

Methods of statistical analysis used in this dissertation were dependent on the type of data being analyzed and the study design being used. For cross-sectional comparisons of demographic and behavioural variables, both parametric and non-parametric statistical methods were used to analyze the data. Bivariate categorical data were analyzed using Pearson's chi-squared test. Fisher's exact test was used when twenty-five percent or more of the expected cell frequencies in a contingency table were less than five. Unadjusted relative risk estimates were computed using the odds ratio, and 95 percent confidence intervals were approximated using test-based limits proposed by Miettinen. Bivariate comparisons of categorical and numeric variables were conducted using Student's t-test and Wilcoxon's rank-sum test. Multivariate logistic regression analysis was used to model the independent association of several risk factors with HCV infection.

Survival analytic methods were used to investigate and describe the time to HCV seropositivity among members of the first cohort. The critical event in these analyses was defined as 'time to HCV seropositivity'. Time zero was taken to be January 1983 for prevalent men, and defined as the midpoint between the last negative and first positive HCV antibody test for seroincident men. Cumulative incidence curves were generated, stratified over various putative risk factors and compared via the log-rank test. To model the simultaneous effect of several variables progression to HCV seropositivity, Cox proportional hazards analysis was used.

A parsimonious approach was undertaken with respect to multivariate model building in this dissertation. This approach was undertaken because of the relatively small number of HCV infections identified in this study. When there is a small number of outcome events per independent variable in a multivariate model, the results from
the fitted regression model may not be accurate or precise. In logistic regression analysis, the number of outcome events is defined by the smaller number of binary outcomes (e.g. HCV seropositive vs. HCV seronegative) of the dependent variable. In proportional hazards analysis the number of outcome events is defined by the count of "failure" events (e.g. HCV infection). When the number of outcome events per variable analyzed is low, the accuracy, precision, and significance of the regression coefficients estimated by these methods will become untrustworthy. The results of proportional hazards analysis and logistic regression should be interpreted with caution when fewer than 10 events per variable are analyzed. Since the number of outcome events in cross-sectional comparisons and prospective analyses was less than 40, multivariate models presented in this dissertation contained no more than three independent variables. All demographic and behavioural variables that were found to be significantly or marginally associated with HCV seropositivity were considered for inclusion in multivariate models. Both forward selection and backward elimination procedures were used to select variables for final inclusion in multivariate models.

2.4.6 **Rationale for using both cohorts**

The decision to include or exclude the second cohort in a specific sub-analysis was made entirely by the candidate. The inclusion of both cohorts for some analyses described in this dissertation was not done to adjust or alter the overall findings in any way. The use of both cohorts was chosen for some sub-analyses for purpose of comparison, and to demonstrate consistency between estimates of HCV prevalence and incidence. The exclusion of the second cohort from some sub-analyses was primarily due to a lack of comparability between questionnaire items pertaining to sexual practices during the two different periods of participant recruitment.

In Chapter 3, results are presented separately for members of the cohort who were enrolled during the first recruitment period (Cohort #1: November 1982 to December
1984) and for those who entered the prospective study during the second period of recruitment (Cohort #2: September 1986 to December 1987). The inclusion of cohort#2 in this sub-analysis was primarily for purpose of comparison. Men who were enrolled during the second recruitment period were less likely to be Caucasian, more likely to report an annual income greater than $10,000 and more likely to have attended college or university. Despite these demographic differences and the different time periods of recruitment, the point estimates of HCV prevalence were almost identical. Furthermore, combining the two cohorts resulted in increased precision of our overall estimate of the true population prevalence.

In Chapter 4, a cross-sectional comparison between HCV-positive and HIV-negative men is presented. This sub-analysis was restricted to the first cohort. This was done in order to make use of the earliest available behavioural data, and because the format of sexual behaviour questions was markedly different from those used during the second period of recruitment. Throughout the years of follow-up of the VLAS cohort, individual questionnaire items were revised considerably to reflect new knowledge. As our knowledge of HIV/AIDS epidemiology expanded, the questions regarding sexual behaviour were revised and expanded. During the second recruitment period, questions concerning specific forms of male-to-male contact were asked separately for regular (i.e. at least once per month) and casual (i.e. less than once a month) sexual partners. No distinction was made between regular and casual partners during the first recruitment period. In addition, the measures of frequency of specific sexual practices differed between the two recruitment periods. Another reason for excluding the second cohort from the sub-analysis in Chapter 4 relates to behaviour change. After the discovery of HIV, numerous epidemiological studies of gay men were successful in identifying specific sexual practices and other behaviours that were significantly associated with increased risk of infection. Dissemination of preventive
public health messages in the gay community was followed shortly by reports of reductions in high-risk sexual behaviours, increased condom use, and lower HIV incidence rates. VLAS participants who were enrolled during the second recruitment period entered the study after the discovery of HIV and had the benefit of receiving these prevention messages designed to reduce the frequency high-risk behaviours. Consequently, their responses to questions about high-risk sexual behaviours could have been influenced by these prevention messages. Responses to questions concerning sexual behaviours, injection drug use, and substance use could be influenced by the participant’s knowledge of their HIV or HCV antibody status. However, in the first cohort, responses to these questions were obtained two to three years before the participant’s HIV antibody testing took place, and over fifteen years prior to HCV antibody testing. Thus responses to questions concerning sexual behaviours and substance use were not likely influenced by the participant’s knowledge of their HIV or HCV antibody status.

The sub-analyses described in Chapters 5 and 6 also excluded participants from the second cohort for the same reasons as in Chapter 4.

In Chapter 7, both cohorts were included in the sub-analysis because data regarding symptoms and signs, physical exam findings, CD4 cell counts, diagnosis of AIDS, and survival were available for both cohorts, and the questionnaire items for these variables were directly comparable.
2.5 REFERENCES


CHAPTER 3
PREVALENCE AND INCIDENCE OF HCV INFECTION IN A COHORT OF HOMOSEXUAL MEN IN VANCOUVER (1983-98)

3.1 FORWARD

In this chapter, the serological results of hepatitis C virus antibody testing for the entire VLAS cohort are presented and discussed. For purpose of presentation, the results are shown separately for members of the cohort who were enrolled during the first recruitment period (Cohort #1: November 1982 to December 1984) and for those who entered the prospective study during the second period of recruitment (Cohort #2: September 1986 to December 1987). Both cumulative incidence and incidence density estimates are used to quantify the rate at which new HCV infections spread through the population of homosexual males in Vancouver during the period between 1983 and 1998. Prevalence and incidence data are summarized using both tabular and graphical methods.

The candidate identified and selected serum specimens from archived samples for HCV antibody testing. The selected specimens were delivered to Dr. Chris Sherlock’s laboratory where they were tested. The candidate managed the serological data, linked the HCV test results to questionnaire data, performed statistical analyses, summarized and interpreted the findings, and wrote the summary report.
3.2 INTRODUCTION

The World Health Organization has estimated that three percent of the world’s population (approximately 170 million) is infected with Hepatitis C Virus (HCV).\(^1\) In the United States, it is estimated that four million persons are infected.\(^2\) HCV has been established as an etiologic agent of acute and chronic liver disease and cirrhosis of the liver, and has been found to be associated with development of hepatocellular carcinoma.\(^3^\)\(^-^\)\(^7\) Furthermore, the onset of cirrhosis or liver failure may be accelerated by co-infection with HIV leading to increased morbidity and mortality.\(^8^\)\(^9\)

In Canada, prevalence of HCV has been estimated to be 0.8% in the general population, and is assumed to be higher among males (0.96%) compared to females (0.53%).\(^10^\)\(^-^\)\(^11\) Higher prevalence rates have been reported among injection drug users in Vancouver\(^12\), among street youth in Montreal\(^13\) and Ottawa\(^14\), and among dialysis patients in Alberta.\(^15\) However, the true burden of hepatitis C virus (HCV) infection in Canada remains largely unknown. Population-based prevalence studies are lacking and there are no ongoing studies investigating the annual incidence of HCV.

Previous studies conducted outside Canada have reported elevated HCV prevalence rates among men who have sex with men compared to the general population.\(^16^\)\(^-^\)\(^22\) To date, no prevalence/incidence studies have been reported in the homosexual male population in Canada. The primary objective of this study was to estimate the prevalence and annual incidence of hepatitis C virus infection in the male homosexual population in Vancouver during the period from 1983 to 1998. We also investigated HCV incidence in parallel with HIV incidence during the same time period.
3.3 METHODS

3.3.1 Study Description

The Vancouver Lymphadenopathy-AIDS Study (VLAS) followed and monitored a cohort of homosexual men since November 1982. From November 1982 to December 1984, over 700 men were recruited through six general practices in central Vancouver. Two medical practices were added to the study and approximately 300 additional participants were enrolled during the period from October 1986 to December 1987. Follow-up visits occurred approximately every six months until September 1986 after which subjects completed visits on an annual basis. Active follow-up of this cohort continued until December 31, 1998.

At each visit, participants provided a blood sample for routine laboratory and serological testing. Serum samples were cryopreserved at minus 20° C. Since 1992, plasma specimens from study participants were cryopreserved at minus 80° C. All participants who completed two or more physician visits during the observation period and who provided blood specimens underwent HCV and HIV antibody testing. Testing was carried out using stored serum specimens obtained from study participants during the period 1982 to 1998. HCV antibody testing of these specimens was carried out between August 1999 and March 2000.

3.3.2 HCV Antibody Testing

HCV antibody was assayed by enzyme immunoassay (EIA), using a modified algorithm to confirm positivity. This testing algorithm was based on a study carried out in the University of British Columbia Diagnostic Virology and Reference Laboratory and the B.C. Centre for Disease Control Virology Laboratory. Variations of this algorithm have been widely used in diagnostic virology laboratories throughout
Canada. Briefly, a micro-particle enzyme immunoassay (Abbott AxSYM HCV Version 3.0 MEIA) was used to screen all sera. Negative sera were tested no further. Positive samples and those with an indeterminate result were further tested using a synthetic peptide-based EIA (United Biomedical Inc. HCV EIA 4.0). Sera that tested positive on both EIAs were classified as positive and tested no further. Sera that gave discordant results were further tested by immunoblot (RIBA III; Ortho Diagnostic Systems). For this study, the HCV antibody testing was done on the specimens collected between November 1982 and December 1998.

3.3.3 **HIV Antibody Testing**

The National Reference Laboratory in Ottawa, using the enzyme-linked immunosorbent assay (ELISA) carried out HIV antibody testing. Positive results were verified using Western blot.

3.3.4 **Statistical Methods**

Estimates of HCV prevalence were obtained using data from both separate and combined cohorts. Ninety-five percent confidence intervals were also calculated. Both cumulative incidence and incidence density were used to quantify the burden and spread of HCV in the VLAS cohort. To study cumulative incidence, we examined time to the first positive HCV-antibody test for all men who were enrolled during the first recruitment period. In this analysis, time zero was defined as January 1, 1983. Participants were right-censored at the date of their last follow-up visit.

A sub-analysis of 'time to the first positive HCV-antibody test' was also performed. In this sub-analysis, men who were found to be HCV seroprevalent at the time of their first laboratory visit were excluded. This analytic approach was undertaken to reduce prevalence bias in the estimate of the population cumulative HCV incidence rate. Kaplan-Meier product-limit methods were used to estimate cumulative and annual incidence. For the incidence density approach, incidence was expressed in
terms of person years of observation. Using this approach, person years were
calculated from January 1, 1983 for cohort #1 and from September 1, 1986 for members
of cohort #2. These incidence density estimates were not calculated as estimates of the
true values but for purposes of comparison between cohorts.

3.4 RESULTS

3.4.1 Demographic characteristics

Demographic characteristics of the VLAS cohort members were stratified by
recruitment period and presented in table 3.1. Men who were enrolled during the first
recruitment period were more likely to be Caucasian, less likely to report an annual
income above ten thousand dollars, and less likely to have attended college or
university than men belonging to cohort #2. The mean age at enrolment was similar
between the two cohorts.

3.4.2 HCV prevalence

Serum samples were available for a total of 932 men and 54 (5.8%) of these men
tested positive for HCV antibody [95% CI: 4.3%, 7.3%]. Prevalence of HCV was similar
in the two cohorts. A total of 39 (5.9%) of 662 men who were enrolled during the first
recruitment period tested HCV positive [95% CI: 4.1%, 7.7%]. Among 270 participants
who were enrolled during the second recruitment period, a total of 15 (5.6%) men tested
positive [95% CI: 2.8%, 8.3%]. Overall, approximately 1 in 17 men in the VLAS cohort
tested positive for HCV antibody.

3.4.3 HCV cumulative incidence

Figure 3.1 shows the Kaplan-Meier curve for ‘time to first positive HCV antibody
test’ for men who were enrolled during the first recruitment period. These 662 men
were followed for an average of 99 months (or 8.25 years). As previously mentioned, a
total of 39 events were ascertained in cohort #1 during the observation period. As seen
in figure 3.1, after 14 years of follow-up cumulative HCV incidence was 7.9% [95% CI: 5.1%, 10.7%]. It was noted the incidence curve rose dramatically during the first 12 to 24 months of follow-up. This increase was likely related to a significant number of prevalent infections in the cohort during this period.

Further HCV testing of all available serum specimens obtained from the 39 HCV positive men was conducted in order to distinguish between prevalent and incident infections. Further testing revealed that 2 out of 3 HCV-positive cases were prevalent infections (26 prevalent and 13 incident). Figure 3.2 shows the cumulative incidence curve when these 26 prevalent men were excluded from the analysis. This analysis included 626 men who were HCV-negative as of January 1, 1983. The revised estimate of the cumulative incidence in the population of homosexual men in Vancouver was 4.0% [95% CI: 1.5%, 6.5%]. HCV incidence is shown for five consecutive 3-year intervals in figure 3.3. During the period between 1983 and 1997, annual HCV seroconversion rates ranged from 0 to 1.4 percent.

3.4.4  **HCV incidence density estimates**

A total of 662 men in cohort #1 were followed for a total of 5,474 person years. Incidence density was estimated as 7.1 per 1,000 person years [95% CI: 4.9, 9.4]. In comparison, 270 men who joined cohort #2 were followed for 1,890 person years, and 15 were HCV positive. This yielded an incidence density estimate of 7.9 per 1,000 person years [95% CI: 3.9, 11.9]. The entire cohort of 962 men was followed for a total of 7,364 person years and the incidence density estimate was 7.3 per 1,000 person years based on 54 seropositive men in the cohort [95% CI: 5.4, 9.3].

3.4.5  **Comparison of HCV and HIV incidence**

Comparative Kaplan-Meier curves for HCV and HIV are shown in Figure 3.4. A total of 445 men were HIV-negative as of January 1, 1983. One hundred and thirty-six HIV seroconversions were documented during follow-up. HIV cumulative incidence
was approximately 8 times higher than HCV incidence during the observation period. After 14 years of follow-up, cumulative HIV incidence was 34.8% [95% CI: 29.8%, 39.7%] compared to 4.0% [95% CI: 1.5%, 6.5%] for HCV.

Rates of HCV and HIV seroconversion during 1983-97 are compared in Figure 4.5. The rate of HIV infection was highest during the period 1983-85 and declined significantly thereafter. In comparison, annual HCV infection was significantly lower throughout the duration of the study. Despite the ‘closed cohort effect’, HIV rates remained at 1 to 4 percent during the 3-year time intervals from 1989 onward. However, the pattern of incidence for HCV infection was different. Unlike HIV, there was no evidence of a decline in HCV incidence during the latter years of the study. Incidence ranged from 0.8% to 1.8% during four of the five 3-year intervals shown in Figure 4.5. The highest HCV seroconversion rate occurred during the period 1995-97.

3.5 DISCUSSION

Using stored serum specimens, we documented a high prevalence of HCV among homosexual men residing in Vancouver during 1983-98. The prevalence observed in our study was approximately 6 percent, and this value was consistent with rates of HCV prevalence reported in other studies of homosexual men conducted in other geographic locations.17,19,22 Prevalence observed in the Vancouver cohort was 7 times higher than the estimated HCV prevalence in the general Canadian population, and 6 times greater than prevalence estimated for Canadian males.10,11 Our study confirms findings from previous observational studies that HCV prevalence is substantially higher among men who have sex with men compared to the general population.

To our knowledge, this is the first population-based, prospective study to report rates of HCV incidence in homosexual men in Canada. Annual infection rates among
men enrolled in the study remained less than one percent for each year of observation with the exception of 1997. The cumulative incidence rate observed in the Vancouver cohort (about 4 percent) was in agreement with the results reported by a Danish study of homosexual males.¹⁶ The pattern of HCV incidence was stable and remained low throughout the duration of the study. However, there was no evidence of a decline in HCV infections despite the closed cohort effect. This observation was even more prominent when comparing trends between HCV and HIV incidence during 1983-98. Rates of HIV infection were significantly higher among homosexual men in Vancouver throughout these years and tended to decrease over time whereas HCV rates remained fairly stable.

We have documented elevated prevalence and incidence of HCV infection in a cohort of homosexual men in Vancouver during 1983-98. During this period, we found approximately 1 in 17 men to be HCV positive. The reasons for this observed elevation in prevalence among gay men need to be further explored including the relative contribution of various routes of HCV transmission. In addition to injection drug use, the role of sexual transmission in the spread of HCV in this population requires clarification. Documentation of specific risk factors is necessary in order to elucidate various ways HCV can enter the human body, and to identify specific sexual practices that carry the greatest risk of HCV transmission.
Table 3.1: Comparison of VLAS cohorts with respect to demographic characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cohort #1</th>
<th>Cohort #2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>632 (97.5)</td>
<td>251 (94.4)</td>
<td>0.016*</td>
</tr>
<tr>
<td>Non-Caucasian</td>
<td>16 (2.5)</td>
<td>15 (5.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Annual Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$10,000</td>
<td>161 (24.3)</td>
<td>50 (18.4)</td>
<td>0.052</td>
</tr>
<tr>
<td>$10,000</td>
<td>501 (75.7)</td>
<td>221 (81.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attended College or University</td>
<td>291 (44.0)</td>
<td>162 (59.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grade 12 or less</td>
<td>371 (56.0)</td>
<td>109 (40.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Age at enrollment (in years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>32.1</td>
<td>32.2</td>
<td>0.713†</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>7.0</td>
<td>6.3</td>
<td></td>
</tr>
</tbody>
</table>

* Based on Pearson’s chi-squared test
† Based on t-test for independent samples
Figure 3.1: Cumulative Incidence of Hepatitis C Infection in the VLAS cohort (1983-1998)*

* Time to first positive test (seroprevalent men included)
Cohort #1: n = 662 participants (39 events)
Figure 3.2: Revised Cumulative Incidence of HCV Infection in the VLAS cohort (1983-1998)*

* Time to first positive test (seroprevalent men excluded)
Cohort #1: n = 636 participants (13 events)
Figure 3.3: HCV seroconversion in a cohort of 626 initially seronegative men in the VLAS cohort (1983-97)*

* Based on actuarial estimates of seroconversion

![Bar chart showing seroconversion rates over time periods: 0.8% in 1983-85, 0.2% in 1986-88, 0.9% in 1989-91, 0.8% in 1992-94, and 1.8% in 1995-97.]
Figure 3.4: Comparison of HCV and HIV cumulative incidence in the VLAS cohort (1983-98)
Figure 3.5: Comparison of HCV and HIV incidence in the VLAS cohort (1983-97)

- HCV
- HIV

* Based on actuarial estimates of seroconversion
3.6 REFERENCES


9 Ragni M. Impact of HIV on progression to end-stage liver disease in HCV co-infected hemophiliacs. Program and abstracts of the 7th Conference on Retroviruses and Opportunistic Infections; January 30-February 2, 2000; San Francisco, California; Abstract 281.


CHAPTER 4
RISK FACTORS FOR HEPATITIS C VIRUS INFECTION IN THE VLAS COHORT

4.1 FORWARD

This chapter has been prepared as a manuscript for submission to a scientific journal. The title of the manuscript is "Risk factors for hepatitis C virus infection in a cohort of homosexual men." Co-authors of this study include Drs. Mark Tyndall, Christopher Sherlock, Robert Hogg, Michael O'Shaughnessy and Martin Schechter. Drs. Schechter, Hogg, and Sherlock are members of the supervisory committee. Dr. O'Shaughnessy provided funding for the hepatitis C antibody testing of stored blood specimens from the VLAS cohort, and Dr. Sherlock supervised the virology laboratory staff that conducted the testing of the specimens.

The candidate is the first author on this manuscript, and formulated the study design, and performed all data collection and management, statistical analysis, as well as writing the initial draft and final manuscript.

4.2 INTRODUCTION

The World Health Organization has estimated that three percent of the world’s population (approximately 170 million) is infected with Hepatitis C Virus (HCV).\textsuperscript{1} In the United States, it is estimated that four million persons are infected.\textsuperscript{2} In Canada, HCV infection ranks second among nationally reported diseases with an estimated 225,000 to 300,000 individuals currently infected.\textsuperscript{3}

HCV has been established as an etiologic agent of acute and chronic liver disease and cirrhosis of the liver, and has been found to be associated with development of
hepatocellular carcinoma. Furthermore, the onset of cirrhosis or liver failure may be accelerated by co-infection with HIV leading to increased morbidity and mortality. 

In both the United States and Canada, the majority of HCV infections has been associated with injection drug use or with transfusion with blood or blood products prior to 1990. In contrast to the efficient transmission by parenteral exposure, there is conflicting evidence regarding the sexual transmission of HCV despite the presence of viral particles and HCV RNA in saliva and genital fluids.

Some evidence of sexual transmission of HCV has been provided by studies of heterosexuals and female sex workers. In addition, CDC Sentinel county studies have suggested that 15 to 20 percent of patients with acute hepatitis C infection have a history of sexual exposure in the absence of other risk factors. However, other studies of heterosexuals have shown that transmission rates are low among monogamous, stable sexual partners of individuals with chronic HCV infection.

Evidence of sexual transmission of HCV has also been provided by studies of homosexual men. A study in Italy conducted among homosexual males who did not use injection drugs, reported that prevalence of HCV was eleven times higher compared to male blood donors from the same area (18.9% vs. 0.6%). An American study concluded that HCV infection in the male homosexual population is associated with sexual practices such as insertive anal intercourse. In contrast, other studies have concluded that sexual practices appear to play a minor role in its transmission, and sexual transmission is rare in this population.

The objectives of this study were to determine the prevalence of HCV infection in a cohort of sexually active homosexual men and to identify risk factors associated with
HCV infection including injection drug use, sexual practices, substance use, history of blood transfusions, and HIV infection.

4.3 METHODS

4.3.1 Study Description

The Vancouver Lymphadenopathy-AIDS Study (VLAS) recruited 729 homosexual men aged 18 to 75 into a longitudinal study over the period November 1982 to December 1984. Participants were recruited through six primary care practices in central Vancouver. Follow-up visits occurred approximately every six months until September 1986, after which, participants completed visits on an annual basis. During each physician visit, participants completed a self-administered questionnaire that elicited information regarding demographic variables, sexual practices, substance use and other variables. In addition, blood samples were drawn for immunologic and HIV antibody testing. HIV antibody testing was routinely carried out for all participants throughout the follow-up period. Serum samples were stored at minus 20° C.

4.3.2 Sources of data

The latest available serum specimen was selected for each participant for HCV antibody testing. A total of 662 specimens were available for antibody testing, and these specimens were tested between August 1999 and March 2000. A total of 67 men did not have specimens available for HCV antibody testing. These participants either failed to provide blood samples during the period of follow-up, or their specimens had been depleted as a result of previous laboratory testing. HCV antibody was assayed by enzyme immunoassay (EIA) using a modified algorithm to confirm positives. This algorithm was based on a study carried out in the University of British Columbia
To assess differences in sexual behaviors and other risk factors for HCV infection, we compared HCV-positive and HCV-negative men using data obtained from their enrolment questionnaire. This questionnaire was completed between November 1982 and December 1984. We chose this study visit for comparison because this was the earliest visit from which information on demographics, sexual behaviour, and history of recreational and injection drug use was available. Behavioural data from the enrolment questionnaire was used for these analyses for both prevalent and incident infections. For prevalent infections, putative risk behaviours could have occurred at any time prior to the enrolment visit. Data from the enrolment questionnaire was used for incident infections because comparable questionnaire items were not available from subsequent physician visits.

Data regarding history of blood transfusions were not available from the enrolment questionnaire and were obtained from two follow-up visits, which occurred within twelve months of the enrolment visit.

4.3.3 Statistical Methods

Categorical variables were compared between HCV-positive and HCV-negative men using Pearson’s chi-squared test. Contingency tables that contained one or more expected counts of less than five were analyzed by Fisher’s exact test. Comparisons of quantitative variables were carried out using Wilcoxon’s rank-sum test. Odds ratios (unadjusted and adjusted) and 95 percent confidence intervals were calculated for all variables of interest. Variables that were significantly or marginally associated with HCV infection in bivariate analyses were considered for inclusion in multivariate
statistical models. Multivariate logistic regression analysis was used to model the independent association of several risk factors with HCV infection.

4.4 RESULTS

4.4.1 HCV Prevalence

A total of 39 of 662 men with available sera were identified as HCV-positive, yielding a prevalence of 5.9 percent [95% CI: 4.1, 7.7]. Prevalence of HCV infection was significantly higher among HIV-positive men compared to HIV-negative men (p<0.001). Thirty-one of 352 (8.8%) HIV-positive participants were HCV-positive [95% CI: 6.6, 11.0], whereas only 8 of 310 (2.6%) HIV-negative men were identified as HCV-positive [95% CI: 1.4, 3.8].

4.4.2 Demographic characteristics

HCV-positive men were similar to HCV-negative men with respect to demographic characteristics including age, ethnicity, and annual income (Table 4.1). Although HCV-positive men were less likely to report having attended university or college, this difference was not statistically significant. However, HCV-positives were significantly more likely to be infected with HIV compared to HCV-negative men.

4.4.3 Injection drug use

Fewer than ten percent of study participants reported using injection drugs. A total of 41 (6.2%) reported injection drug use during their lifetime. Injection drug use was a highly significant risk factor for HCV infection (Table 4.2). Men who reported using injection drugs during their lifetime were thirty times more likely to be HCV positive.

4.4.4 Use of psychoactive drugs

HCV-positive men were more likely than HCV-negative men to report using the following psychoactive drugs during the previous twelve months: methylene-dioxy amphetamine (MDA), lysergic acid diethyl amide (LSD), amphetamine, and cocaine.
(Table 4.2). Men who reported using one or more of these drugs during the past 12 months were 2.5 times more likely to be HCV positive [95% CI: 1.3, 5.1]. The prevalence of heroin use among study participants was low (<2%) and was similar in both groups.

4.4.5 Use of tobacco and alcohol

Men who were HCV-positive were twice as likely to report using tobacco (Table 4.3). Over ninety percent of study participants reported consuming alcohol and over seventy percent reported using marijuana during the previous 12 months. No significant differences between HCV-positive and HCV-negative men were observed with regard to alcohol or marijuana use. HCV-positive men were twice as likely to report using nitrite inhalants or poppers, but this association was of marginal significance.

4.4.6 Sexual practices

HCV-positive and HCV-negative men were compared with respect to numbers of male sexual partners and specific sexual practices (Table 4.3). Elevated numbers of male sex partners over the lifetime and during the previous year were both positively associated with HCV infection in univariate analyses. Positive associations were also noted for active oral-anal contact (rimming), and insertive fisting (i.e. insertion of fingers or hand into partner's rectum). Men who reported engaging in these sexual practices were three times more likely to be HCV positive. Weaker positive associations were observed with respect to frequency of receptive oral sex, receptive fisting, and passive oral-anal contact. No significant associations were detected with respect to frequency of insertive oral sex or frequency of insertive anal intercourse.

4.4.7 History of blood transfusion

A total of 610 of the 662 participants (92.1%) completed at least one follow-up questionnaire in the year following their enrolment into the study. Participants who completed one or more of these follow-up visits were asked if they ever received a
blood transfusion. No significant difference was observed between HCV-positive and HCV-negative men with respect to their history of blood transfusions (p=0.796). Three of 35 HCV-positive men (8.6%) reported receiving a blood transfusion in their lifetime, compared to 57 of 557 (9.9%) in the HCV-negative group.

4.4.8 Multivariate Analysis

The results of the multivariate logistic regression model are shown in Table 4.4. Independent risk factors for HCV infection included injection drug use (adjusted OR=27.3; 95% CI: 12.4, 60.4), HIV-infection (adjusted OR=2.4; 95% CI: 1.0, 5.8), and insertive fisting (adjusted OR=2.2; 95% CI: 1.0, 4.8). When HIV-infection was excluded from this multivariate model, both injection drug use and insertive fisting remained statistically significant but the estimates of risk shown above did not change appreciably (data not shown).

4.5 DISCUSSION

These data indicate an overall prevalence rate of HCV seropositivity of 5.9 percent among the 662 men tested. Our estimate is similar to prevalence rates reported in studies of gay and homosexual men in different geographic locations and conducted during comparable time periods.\textsuperscript{25-29} Prevalence rates in these studies ranged from 2.9 to 9.2 percent. Our finding that HCV prevalence was significantly higher among HIV-positive men is also consistent with the findings of other studies.\textsuperscript{26,28,29}

Our study confirms the role of injection drug use as the primary risk factor for HCV infection among gay and homosexual men. This finding is concordant with the results of previous studies, which found both univariate and multivariate associations between injection drug use and hepatitis C infection in this population.\textsuperscript{26-29} However, there was a low prevalence of self-reported injection drug use among our study participants. Almost one-half of the HCV-positive men in our study did not report a history of injection drug use.
After controlling for both injection drug use and HIV-antibody status, we found an association between HCV infection and insertive fisting. A study of homosexual men recruited from a municipal STD clinic in San Francisco, reported an association with HCV positivity and fisting, but no association was observed with respect to oral-anal contact.29

Sexual practices such as insertive fisting may cause damage to the mucosal barrier of the rectum, and result in tearing and bleeding. Lesions produced by such trauma could serve as a source or passage for pathological micro-organisms like HCV. Moreover, individuals who engage in this sexual practice in conjunction with other sexual practices such as oral-anal contact, may increase their likelihood of contact with HCV-infected blood or fluids.

We found an elevated risk for HCV infection among men who reported using psychoactive drugs. Previous studies have reported positive associations between HCV infection and use of amphetamines and cocaine (26).26,29 Our study of homosexual men found increased risk among users of LSD and tobacco in univariate analyses, but these risks did not remain statistically significant after adjustment for other risk factors.

In contrast to two studies which failed to detect an association between HCV positivity and HIV infection,25,27 our data may suggest that concurrent HIV infection may facilitate sexual transmission of HCV, independent of injection drug use. Other HCV prevalence studies of homosexual men conducted in San Francisco, Pittsburgh, and Sydney, Australia have also reported HIV infection as a significant risk factor for HCV-positivity.26,28,29 Only one of these studies found HIV infection to be associated with HCV positivity in multivariate analysis.28 The failure of HIV infection to retain significance in multivariate analysis in these studies could be due to residual confounding.

We detected no association between HCV-infection and a history of blood
transfusion among our participants. This finding is in contrast to a study of homosexual men conducted by Osmond et al, which reported history of injection drug use and blood transfusion to be independent risk factors for HCV positivity. However, our results are compatible with the those reported by Ndimbie et al. who found similar percentages of HCV-positive and HCV-negative men receiving a blood transfusion during the previous five years (5.3% vs. 2.9%).

An important limitation in assessing whether a specific sexual practice is associated with HCV transmission is the inadequate control of confounders or differential misclassification within the variables under consideration for inclusion in the multivariate statistical model. Although insertive fisting remained significantly associated with HCV seropositivity after adjustment for injection drug use and HIV seropositivity in our multivariate model, the possibility must be acknowledged that insertive fisting was not directly associated with HCV infection but rather with other residual lifestyle factors not fully captured in the multivariate model. For example, if insertive fisting was associated with other parenteral exposures such as tattooing or body piercing this could explain its retention in the multivariate model. We were unable to assess the risk associated with tattooing, ear or body piercing, and needle borrowing/sharing, because these variables were not included as items in our enrolment and early, follow-up questionnaires. In addition, we did not evaluate occupational and nosocomial exposures. The role of horizontal exposure in the spread of HCV was not addressed in this study.

For prevalent infections in this study, risk behaviours of interest could have occurred at any time prior to their enrolment visit. It is also possible that for seroincident men, high-risk behaviours could have occurred after their enrolment visit. As a result, our estimates of risk associated with specific sexual practices might be distorted.
The results of this study are based on prevalent HCV infections and self-reported behaviours. Responses to questions concerning sexual behaviours, injection drug use, and substance use may be influenced by the participant’s knowledge of their HIV or HCV antibody status. However, in our study, responses to these questions were obtained two to three years before the participant’s HIV antibody testing took place, and over fifteen years prior to HCV antibody testing. Neither HIV nor HCV antibody testing was available prior to 1984. Thus responses to questions concerning sexual behaviours and substance use were not likely influenced by the participant’s knowledge of their antibody status in this study.

In order to assess the effect of HIV infection on HCV transmission it is important to establish the temporal sequence of these infections in longitudinal studies. Distinguishing between prevalent and incident cases of HCV infection will permit estimation of the incidence rate of HCV seroconversion in risk groups of interest. Prospective analyses of risk factors identified in previous cross-sectional studies will help to identify and confirm predictors of HCV seroconversion. We are presently addressing these objectives in the Vancouver Lymphadenopathy-AIDS Study cohort.

We conclude that hepatitis C virus can be spread by sexual transmission among gay and homosexual men, independent of injection drug use. We speculate that sexual practices that cause rectal trauma in combination with oral-anal contact, may increase the risk of exposure to HCV-infected blood and fluids.
Table 4.1: Demographic characteristics and HIV antibody status of 39 HCV-positive and 623 HCV-negative men

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCV-positive n (%)</th>
<th>HCV-negative n (%)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>39 (100)</td>
<td>593 (97)</td>
<td>0.616†</td>
</tr>
<tr>
<td><strong>Annual Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$10,000</td>
<td>10 (26)</td>
<td>151 (24)</td>
<td>0.843</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>College/University</td>
<td>17 (44)</td>
<td>354 (57)</td>
<td>0.106</td>
</tr>
<tr>
<td><strong>HIV antibody status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>31 (79)</td>
<td>321 (52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Age (at time of HCV testing)</strong></td>
<td>Years</td>
<td>Years</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
<td>40</td>
<td>.713‡</td>
</tr>
<tr>
<td>Range</td>
<td>26-51</td>
<td>21-69</td>
<td></td>
</tr>
</tbody>
</table>

* Based on Pearson’s chi-squared test
† Based on Fisher’s exact test
‡ Based on Wilcoxon’s rank sum test
Table 4.2: Comparison of HCV-positive and HCV-negative participants with respect to self-reported use of injection drugs, psychoactive drugs, tobacco, alcohol, and other substances.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCV-positive n (%)</th>
<th>HCV-negative n (%)</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection drug use during lifetime</td>
<td>20 (51)</td>
<td>21 (3)</td>
<td>&lt;0.001*</td>
<td>30.2</td>
<td>17.3, 52.6</td>
</tr>
<tr>
<td>Methylenedioxyamphetamine (MDA)</td>
<td>25 (64)</td>
<td>228 (37)</td>
<td>&lt;0.001</td>
<td>3.0</td>
<td>1.6, 5.9</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>11 (28)</td>
<td>84 (14)</td>
<td>0.013</td>
<td>2.5</td>
<td>1.2, 5.0</td>
</tr>
<tr>
<td>Tobacco</td>
<td>26 (67)</td>
<td>296 (48)</td>
<td>0.027</td>
<td>2.1</td>
<td>1.1, 4.2</td>
</tr>
<tr>
<td>Lysergic acid diethylamide (LSD)</td>
<td>19 (49)</td>
<td>158 (26)</td>
<td>0.002</td>
<td>2.0</td>
<td>1.4, 5.1</td>
</tr>
<tr>
<td>Cocaine</td>
<td>18 (46)</td>
<td>180 (30)</td>
<td>0.029</td>
<td>2.0</td>
<td>1.1, 3.9</td>
</tr>
<tr>
<td>Heroin</td>
<td>1 (3)</td>
<td>8 (1)</td>
<td>0.429*</td>
<td>2.0</td>
<td>0.3, 15.7</td>
</tr>
<tr>
<td>Nitrite inhalants</td>
<td>28 (72)</td>
<td>341 (56)</td>
<td>0.052</td>
<td>2.0</td>
<td>1.0, 4.1</td>
</tr>
<tr>
<td>Marijuana</td>
<td>32 (82)</td>
<td>470 (77)</td>
<td>0.480</td>
<td>1.4</td>
<td>0.6, 3.1</td>
</tr>
<tr>
<td>Alcohol</td>
<td>34 (87)</td>
<td>554 (91)</td>
<td>0.407*</td>
<td>0.7</td>
<td>0.3, 1.9</td>
</tr>
</tbody>
</table>

* Based on Fisher’s exact test
Table 4.3: Comparison of HCV-positive and HCV-negative participants with respect to number of sexual partners and sexual practices

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCV-positive n (%)</th>
<th>HCV-negative n (%)</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of male sexual partners in previous year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20 partners</td>
<td>29 (74)</td>
<td>294 (48)</td>
<td>0.002</td>
<td>3.1</td>
<td>1.5, 6.3</td>
</tr>
<tr>
<td>Number of male sexual partners in lifetime</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 100</td>
<td>32 (82)</td>
<td>391 (64)</td>
<td>0.024</td>
<td>2.5</td>
<td>1.1, 5.7</td>
</tr>
<tr>
<td>Oral-anal contact (active)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (90)</td>
<td>443 (73)</td>
<td>0.019</td>
<td>3.3</td>
<td>1.2, 8.9</td>
</tr>
<tr>
<td>Oral-anal contact (passive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (95)</td>
<td>522 (85)</td>
<td>0.100</td>
<td>3.2</td>
<td>0.8, 12.4</td>
</tr>
<tr>
<td>Insertive fisting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (49)</td>
<td>162 (27)</td>
<td>0.003</td>
<td>2.6</td>
<td>1.4, 4.9</td>
</tr>
<tr>
<td>Receptive fisting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (23)</td>
<td>81 (13)</td>
<td>0.087</td>
<td>2.0</td>
<td>0.9, 4.2</td>
</tr>
<tr>
<td>Oral sex (receptive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of encounters</td>
<td>31 (79)</td>
<td>401 (66)</td>
<td>0.076</td>
<td>2.0</td>
<td>0.9, 4.4</td>
</tr>
<tr>
<td>Oral sex (insertive)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of encounters</td>
<td>29 (74)</td>
<td>444 (73)</td>
<td>0.818</td>
<td>1.1</td>
<td>0.5, 2.3</td>
</tr>
<tr>
<td>Receptive anal intercourse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of encounters</td>
<td>24 (62)</td>
<td>274 (45)</td>
<td>0.043</td>
<td>2.0</td>
<td>1.0, 3.8</td>
</tr>
<tr>
<td>Insertive anal intercourse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25% of encounters</td>
<td>23 (59)</td>
<td>339 (55)</td>
<td>0.670</td>
<td>1.2</td>
<td>0.6, 2.2</td>
</tr>
</tbody>
</table>
Table 4.4: Multivariate logistic regression model of risk factors for HCV infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted OR</th>
<th>95% CI</th>
<th>Adjusted OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection drug use (during lifetime – yes vs. no)</td>
<td>30.2</td>
<td>17.3, 52.6</td>
<td>27.3</td>
<td>12.4, 60.4</td>
</tr>
<tr>
<td>HIV infection (yes vs. no)</td>
<td>3.6</td>
<td>1.7, 7.7</td>
<td>2.4</td>
<td>1.0, 5.8</td>
</tr>
<tr>
<td>Insertive fisting (yes vs. no)</td>
<td>2.6</td>
<td>1.4, 4.9</td>
<td>2.2</td>
<td>1.0, 4.8</td>
</tr>
</tbody>
</table>
4.6 REFERENCES


10. Ragni M. Impact of HIV on progression to end-stage liver disease in HCV coinfected hemophiliacs. Program and abstracts of the 7th Conference on Retroviruses and Opportunistic Infections; January 30-February 2, 2000; San Francisco, California; Abstract 281.


CHAPTER 5

SEXUAL PRACTICES ASSOCIATED WITH HCV POSITIVITY AMONG NON-INJECTION DRUG USERS IN THE VLAS COHORT

5.1 FORWARD

In this chapter, results of a comparison between HCV-positive and HCV-negative men who did not use injection drugs are presented and discussed. The quantification and estimation of risk associated with specific sexual practices that cause rectal trauma and increase the likelihood of exposure to HCV-infected blood and fluids is explored more thoroughly. This was achieved by excluding men who reported using injection drugs. The material that is presented in this chapter is intended to serve as an addendum to the results of the previous chapter.

The candidate formulated the study design and hypothesis, performed all data management, conducted statistical analyses, summarized and interpreted the findings, and wrote the manuscript.

5.2 INTRODUCTION

The role of sexual contact in the spread of hepatitis C virus has been controversial. Controversy has existed because of inconsistencies among previous studies. The efficiency of sexual transmission among heterosexuals is generally thought to be low, and infection is rare in long-term, steady partners. Some evidence of sexual transmission of HCV has been provided by observational studies of men who have sex with men. However, more data are needed to identify high-risk practices that are associated with HCV transmission between sexual partners.
In the previous chapter, it was concluded that HCV could be spread by sexual contact among gay and homosexual men, independent of injection drug use. Sexual practices such as insertive fisting may cause damage to the mucosal barrier of the rectum, and result in tearing and bleeding. Lesions produced by such trauma could serve as a source or passage for pathological micro-organisms like HCV. Moreover, individuals who engage in this sexual practice in conjunction with other sexual practices such as oral-anal contact (or rimming) may increase their likelihood of contact with HCV-infected blood or fluids. The objective of this investigation was to estimate the relative risk of HCV transmission for specific sexual practices that cause rectal trauma and involve oral-anal contact among homosexual men who were not injection drug users.

5.3 METHODS

5.3.1 Study Description

The Vancouver Lymphadenopathy-AIDS Study (VLAS) was an observational study of 729 gay men who were recruited through their general practitioners in central Vancouver between November 1982 and December 1984. Follow-up visits occurred approximately every six months until September 1986, after which participants completed visits on an annual basis. During each visit, participants completed a self-administered questionnaire, which gathered information regarding demographic variables, injection drug use, and sexual practices. In addition, blood samples were drawn for HCV and HIV antibody testing. For this study the HCV antibody testing was done on the specimen collected from the latest visit.
5.3.2 Study Variables

Demographic variables of interest in this investigation included age, ethnic group, education and annual income. Participants in the study were asked whether they had ever used injection drugs during their lifetime. Sexual behaviour variables of interest included numbers of male sexual partners during the participant's lifetime and the past year. Specific sexual practices that were studied included fisting (i.e. insertion of fingers or hand into the partner's rectum), oral-anal contact, and anal intercourse. We also studied the sexual practice of fisting in combination with oral-anal contact between sexual partners. This composite variable was of particular interest because it involved rectal trauma of one sexual partner, and potential oral exposure of the other partner to their sexual partner's HCV-infected blood or fluids. Study participants were asked to indicate whether they engaged in these sexual practices with their male partner(s) during the preceding 12 months. All demographic and behavioural data were obtained from the questionnaire administered at the time of the first physician visit.

5.3.2 Statistical Methods

Bivariate comparisons between HCV-positive and HCV-negative men were carried out using Pearson’s chi-square test for categorical variables. Fisher’s exact test was used when more than 25 per cent of the expected cell frequencies were less than five. Unadjusted relative risk estimates were computed using odds ratios, and 95% confidence intervals were approximated using test-based limits. Multivariate comparisons were conducted using logistic regression analysis. For all comparisons, participants with missing or unknown values for the variable(s) of interest were excluded from the analysis. All reported p-values are two-sided.
5.4 RESULTS

5.4.1 Injection drug use

In total, 662 VLAS participants had serum specimens available and were tested for HCV antibody. Of these, 39 (5.9%) men tested positive. Previous injection drug use was reported by 41 (6.1%) of 662 men, and by 20 of those who were HCV-positive. Men who reported using injection drugs during their lifetime were 30.2 times more likely to be HCV positive (95% CI: 17.3, 52.6). A total of 19 (48.7%) of the 39 men who tested positive did not report using any lifetime injection drugs use. To assess the risk of specific sexual practices for HCV transmission, we excluded men who reported using injection drugs in the study. Thus, comparisons between HCV-positive and HCV-negative men with respect to sexual practices were based on 621 participants. Among men who did not report using injection drugs in their lifetime, 19 (3.1%) were HCV-positive.

5.4.2 Demographic characteristics

HCV-positive men were similar to negative men with respect to demographic characteristics including age at enrolment (33 vs. 32 years; p=0.629), Caucasian ethnicity (100% vs. 97%; p=0.999), annual income less than $10,000 (16% vs. 24%; p=0.586), and post-secondary education (37% vs. 42%; p=0.632). A higher percentage of HCV-infected men were HIV-positive compared to uninfected men and this difference was marginally significant (74% vs. 51%; p=0.053).
5.4.3 Sexual practices

A higher percentage of HCV-positive men reported having 20 or more sexual partners during the previous 12 months (68% versus 48%; $p = 0.078$) yielding an odds ratio of 2.4 [95% CI: 0.9, 6.1]. We compared HCV positive and negative men with respect to specific sexual practices (Table 5.1). A higher percentage of HCV-positive men reported having practiced receptive anal intercourse in more than 25% of sexual encounters during the previous 12 months (63% versus 44%; $p = 0.099$) giving rise to an odds ratio of 2.2 (95% CI: 0.8, 5.6). Both active oral-anal contact and insertive fisting were found to be significantly associated with increased risk of HCV infection. Men who reported these sexual practices were 6.9 and 3.1 times more likely to be HCV-positive, respectively. In addition, those who reported engaging in both practices with their sexual partners during the previous 12 months were 10.6 times more likely to be HCV positive compared to men who reported neither practice [95% CI: 2.0, 56.8]. No significant differences between the two serologic groups were observed with respect to frequency of insertive anal intercourse ($p=0.832$), the practice of receptive fisting ($p=0.728$), or receptive oral-anal contact ($p=0.999$).

5.4.4 Multivariate Analysis

All behavioural variables found to be significantly or marginally associated with HIV seropositivity were considered for inclusion in a multivariate logistic regression model. Using a stepwise approach to model building, the only variable that retained statistical significance in multivariate analysis was insertive fisting ($p=0.012$). Because of the marginal association of HCV-positivity with the number of sexual partners during the previous 12 months, and because this variable was likely to be confounded with the various sexual practices studied, we carried out further analysis with
adjustment for number of sexual partners. After such adjustment, insertive fisting (AOR=2.6, 95% CI: 1.0, 6.8) remained marginally associated with HCV positivity.

5.5 DISCUSSION

We examined the prevalence of HCV-antibody in an observational study of homosexual men in Vancouver, with the specific objective to estimate the relative risk of HCV infection for specific sexual practices independent of injection drug use. Our study confirms that HCV prevalence is elevated in homosexual males. Prevalence of HCV in homosexual men has generally been found to be significantly higher than that observed in the general population. Our study also confirms that injection drug use is the primary risk factor for HCV acquisition in men who have sex with men.

It is important to recognize that not all HCV seropositive cases observed in this cohort were related to injection drug use. Of the 39 HCV seropositive men identified in this study, 20 (51%) were related to injection drug use but 19 (49%) were not. The effect of injection drug use on the spread of HCV is related to both the relative risk and the proportion of the population who use injection drugs. In this observational study, the prevalence of injection drug use was 6 percent and the relative risk associated with injection drug use was equal to 30. Using these estimates, we calculated the attributable risk for injection drug use to be 0.635. We estimate that approximately 64 percent of HCV infections occurring among homosexual men in Vancouver could be eliminated by reducing the risk associated with injection drug use. However, about 36 percent of HCV seropositive cases identified in this study were attributable to other risk factors other than injection drug use.

The role of sexual contact in the global spread of HCV should not be underestimated. For example, if 35 to 45 percent of all HCV infections were attributable to sexual contact, then this would account for 59.5 to 76.5 million infections worldwide.
(WHO global estimate of number of HCV-infected is 170 million). Sexual behaviour may be an important mode of spread of HCV if the pool of asymptomatic and infectious carriers is large. Because HCV infection becomes chronic in the majority of individuals and sub-clinical hepatitis may be common, there is reason to believe that in some countries and sub-populations, this carrier pool could be large. Even a low level of sexual transmission may result in a substantial ‘attributable’ risk.\(^5\)

Of considerable interest in our analysis was the association of specific sexual acts with increased risk of HCV positivity among men who did not use injection drugs. Significant elevations in risk were detected in univariate analyses for the practice of insertive fisting, insertive oral-anal contact, and receptive anal intercourse. The highest elevation in risk was observed for men who practiced both insertive fisting and insertive oral-anal contact with their sexual partners. After adjustment for number of partners, elevations in risk persisted for these sexual practices. To our knowledge, this is the first observational study of homosexual men to exclude injection drug users and document specific sexual practices as significant risk factors for HCV acquisition.

The sexual practices identified in our study involve rectal trauma and potential exposure to a sexual partner’s infected blood or fluids. Both anal intercourse and fisting may cause damage to the mucosal lining of the rectum, and result in tearing and bleeding. Lesions produced by receptive anal intercourse could serve as a passage for the semen of an HCV-infected partner. Injury to the rectum caused by insertive fisting could serve as a source of HCV-infected blood for a partner who also engages in oral-anal contact. Chmiel and colleagues, who examined numerous types of sexual behaviour between homosexual men, have reported similar findings with respect to HIV.\(^9\) In their observational study, the factor most strongly associated with prevalent HIV infection was rectal trauma.
There are a number of caveats regarding these data which should be discussed. Although individual participants provided estimates of their number of sexual partners during the previous 12 months, the number of HCV-infected sexual partners to which an individual was exposed during this period was not taken into account. The extent of exposure to each partner (duration and type of exposure) and the degree of infectivity of each partner are unknown. In addition, there may be recall problems associated with the reporting of past sexual behavior. Although the effect of these recall problems is difficult to estimate, several studies have shown that interview-administered and self-administered questionnaires can provide reasonably reliable data concerning sexual behavior in homosexual men when the recall period is relatively short.\textsuperscript{10,11} In our study, information regarding sexual practices was obtained from the questionnaire at the enrollment visit. All participants in this study were unaware of their HCV status at the time of the questionnaire completion. This might have reduced the amount of recall bias in our study.

Our findings provide evidence that HCV can be spread by sexual contact among gay and homosexual men in the absence of injection drug use. Sexual practices that cause rectal trauma and involve oral-anal contact can increase the risk of exposure to HCV-infected blood and fluids. These findings not only have important implications for sexually active homosexual men, but heterosexuals as well. Sexual practices that result in vaginal or rectal trauma may play a role in the spread of HCV and other sexually transmitted diseases in the heterosexual population. Large population-based observational studies are needed to confirm these findings and determine which sexual practices place individuals at increased risk of contracting HCV.
Table 5.1: Comparison of 19 HCV-positive and 602 HCV-negative men who did not report injection drug use with respect to specific sexual practices

<table>
<thead>
<tr>
<th>Sexual Practice</th>
<th>HCV-positive n (%)</th>
<th>HCV-negative n (%)</th>
<th>p-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anal intercourse</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertive</td>
<td>10 (53)</td>
<td>325 (55)</td>
<td>0.832</td>
<td>0.9</td>
<td>0.4, 2.3</td>
</tr>
<tr>
<td>Receptive</td>
<td>12 (63)</td>
<td>260 (44)</td>
<td>0.099</td>
<td>2.2</td>
<td>0.8, 5.6</td>
</tr>
<tr>
<td><strong>Oral-anal contact</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertive</td>
<td>18 (95)</td>
<td>466 (72)</td>
<td>0.029</td>
<td>6.9</td>
<td>1.2, 39.8</td>
</tr>
<tr>
<td>Receptive</td>
<td>17 (89)</td>
<td>502 (85)</td>
<td>0.999†</td>
<td>1.5</td>
<td>0.3, 6.5</td>
</tr>
<tr>
<td><strong>Fisting</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertive</td>
<td>10 (53)</td>
<td>173 (26)</td>
<td>0.012†</td>
<td>3.1</td>
<td>1.3, 7.4</td>
</tr>
<tr>
<td>Receptive</td>
<td>3 (16)</td>
<td>77 (13)</td>
<td>0.728†</td>
<td>1.2</td>
<td>0.4, 4.4</td>
</tr>
</tbody>
</table>

* Percentage of sexual encounters that included this practice exceeded 25 percent.
† Based on Fisher's exact test
5.6 REFERENCES


CHAPTER 6
PREDICTORS OF TIME TO FIRST POSITIVE HCV ANTIBODY TEST IN THE VLAS COHORT

6.1 FORWARD

In this chapter, results of prospective analyses of 'time to event' data are presented and discussed. Risk factors for HCV transmission that were identified by means of nested comparisons between seropositive and seronegative men in previous chapters, are re-examined prospectively. HIV serologic status and a number of self-reported sexually transmitted diseases are also assessed as potential predictors of time to HCV seropositivity in the VLAS cohort. These objectives were achieved by utilising longitudinal serologic data from members of the cohort who were enrolled during the first recruitment period, and by focusing on the dependent variable 'time to HCV seropositivity from January 1, 1983'. Methods of survival analysis were used to analyze the data.

The candidate's contributions to this work consisted of formulation of the study design, data management, performing statistical analysis, summarizing and interpreting the data, and writing the manuscript.

6.2 INTRODUCTION

The prevalence of HCV infection appears to be elevated among men who have sex with men. Previous observational studies of gay men in different geographic locations have reported prevalence rates ranging from approximately 3 to 13 percent. Cross-sectional comparisons of HCV-infected and uninfected men have revealed injection drug use as the predominant mode of transmission in this population.
Evidence for sexual transmission of HCV among gay has been provided by several cohort studies conducted in the United States. Osmond et al. found that HCV infection was marginally associated with more than 50 sexual partners a year. Buchbinder and colleagues reported receptive anal intercourse, fisting, having a sexual partner with a history of injection drug use, a self-reported history of genital herpes, and being HIV positive as significant univariate risk factors for HCV infection. However, injection drug use was the only significant risk factor in their multivariate analysis. In another study, Ndimbie et al. identified insertive anal intercourse with ejaculation, douche or enema before receptive anal intercourse, self-reported history of rectal gonorrhea, as well as syphilis as statistically significant univariate risk factors. In contrast, other cross-sectional studies of gay men did not find a significant association between sexual practices and HCV seropositivity. 

Previous studies of HCV transmission in homosexual men have all been cross-sectional. In these studies, HCV antibody has been determined at a single point in time and comparisons of risk factors between infected and uninfected men are subsequently conducted. Such analyses are useful for generating hypotheses but are potentially misleading because they do not focus on the event of seroconversion. For this reason, risk factors for HCV acquisition identified in previous cross-sectional studies need to be re-examined prospectively. The objective of this investigation was to examine predictors of time to HCV-seropositivity in a cohort of homosexual men in Vancouver. Because the number of incident infections of HCV in the cohort was small, both incident and prevalent infections were included as events of interest in prospective analyses. Variables of interest in this study included: injection drug use during lifetime, HIV antibody status, sexual practices, and self-reported history of other sexually transmitted diseases.
6.3 METHODS

6.3.1 Study Description
The Vancouver Lymphadenopathy-AIDS Study (VLAS) has previously been described in detail. In summary, during the period November 1982 to December 1984, over 700 homosexual men were recruited and enrolled in a prospective study through six primary care practices located in central Vancouver. At each physician visit, participants completed self-administered questionnaire, which contained items pertaining to demographic characteristics, sexual practices, and history of sexually transmitted diseases including gonorrhea, syphilis, and genital herpes. Participants also provided blood samples for serologic testing.

Participants who entered the VLAS between November 1982 and December 1984, and for whom HCV and HIV serologic results were available for at least one follow-up visit were eligible for this analysis. Thus, the serologic status of each participant was based on at least two and as many as seventeen successive tests for HCV and HIV antibody.

6.3.2 HCV and HIV antibody testing
HCV antibody was assayed by enzyme immunoassay (EIA), using a modified algorithm to confirm positivity. This testing algorithm was based on a study carried out in the University of British Columbia Diagnostic Virology and Reference Laboratory and the B.C. Centre for Disease Control Virology Laboratory. Briefly, a micro-particle enzyme immunoassay (Abbott AxSYM HCV Version 3.0 MEIA) was used to screen all sera. Negative sera were tested no further. Positive samples, as well as samples with an indeterminate result, were further tested using a synthetic peptide-based EIA (United Biomedical Inc. HCV EIA 4.0). Sera that tested positive on both EIAs were classified as positive and tested no further. Sera that gave discordant results were further tested by immunoblot (RIBA III; Ortho Diagnostic Systems). For this study, the
HCV antibody testing was done on specimens collected between January 1983 and December 1998. HIV antibody testing was conducted using the enzyme-linked immunosorbent assay (ELISA) and equivocal results were further tested using the Western blot technique.

6.3.3 Statistical methods

Methods of survival analysis were used to analyze the data. The event of interest was time to the first positive HCV antibody test. The starting point (or time zero) for this prospective analysis was defined as January 1, 1983. Participants who consistently remained HCV-antibody negative were considered as right-censored at the time of their most recent test result. The time to the first seropositive test was calculated as the number of months elapsed from January 1983 until HCV-antibody was detected.

Cumulative rates of time to HCV seropositivity were plotted using Kaplan-Meier methods. In these plots, prevalent and incident HCV infections were distinguished from one another. Data regarding risk factors were obtained from the enrolment questionnaire and categorized using previously published levels.\textsuperscript{10} Tests of association between putative predictors and subsequent HCV seropositivity were performed by comparing the cumulative rates of seropositivity associated with the strata of the predictor variable using the log-rank test. Risk ratios and 95 percent confidence intervals were obtained for variables of interest. Cox proportional hazards regression was used to obtain both unadjusted estimates of relative risk, and to assess the independent effect of predictors on time to HCV seropositivity in the cohort. Variables that were identified as either significantly or marginally associated with time to HCV seropositivity in unadjusted analyses were considered for inclusion in multivariate Cox regression models. Forward stepwise regression was used to model the effects of selected predictors on time to seropositivity. All p-values were two-sided.
6.4 RESULTS

6.4.1 Demographic characteristics

A total of 662 VLAS participants met the entry criteria for statistical analysis. The median duration of follow-up of these men was 91 months (inter-quartile range: 47 to 168). With regard to demographic characteristics, the majority of men were Caucasian (98%) with a median age of 31 years at entry into the study (range: 17 to 60). Forty-four percent of men attended college or university, and 24 percent reported having annual incomes less than $10,000.

6.4.2 HCV cumulative incidence

Thirty-nine events of HCV seropositivity were documented during the observation period, which yielded a crude cumulative rate of 5.9 percent. The product limit estimate of the probability of becoming HCV seropositive during the 15-year period of observation was 7.9% [95% CI: 5.1%, 10.7%]. The 39 events of HCV seropositivity were comprised of 26 prevalent and 13 incident infections. Prevalent infections were identified on the first laboratory visit occurring between February 1983 and November 1985. Among incident infections, the earliest event of HCV seropositivity occurred in May 1983, and the latest event occurred in February 1997.

6.4.3 HCV cumulative incidence stratified by injection drug use

Figure 6.1 shows the Kaplan-Meier curves for time to first positive HCV-antibody test stratified by self-reported injection drug use. The cumulative incidence rate of seropositivity was considerably higher among those who reported injection drug use during their lifetime compared to those who did not (p<0.001). Among 41 men who reported injection drug use, the product limit estimate of the cumulative rate of HCV infection was 52.0 percent (standard error = 8.4%) at 15 years, compared to 4.8 percent (standard error = 1.3%) among 621 men who did not inject drugs. Of the 19 events observed in the group that did not use injection drugs, 8 events were documented as
'incident' HCV infections compared to 5 of 20 events that occurred in the group that did not inject drugs. As seen in figure 6.1, six of the eight incident infections occurring in men who did not inject drugs, were documented in 1984 or later. Incident infections continued to occur throughout the observation period among men in this group.

6.4.4  **HCV cumulative incidence stratified by HIV antibody status**

Figure 6.2 shows the Kaplan-Meier curves for time to first positive HCV-antibody test stratified by HIV antibody status. The majority of HCV infections occurred in men who were HIV seropositive, 31 (79%) versus 8 (21%) infections in HIV seronegative men. The cumulative rate of HCV infection was significantly higher among men who were HIV seropositive compared to men who were HIV-negative (p<0.001). The product limit estimate of cumulative HCV infection in the HIV-infected group was 14.3% (standard error = 3.4%) at 15 years, compared to 2.6% (standard error = 0.9%) in the HIV-negative group.

6.4.5  **HCV cumulative incidence stratified by fisting and oral-anal contact**

Significant disparities between cumulative rates of HCV infection were also observed between strata for a number of sexual practices that were assessed. Figure 6.3 displays the cumulative event curves stratified according to whether the participant reported engaging in both insertive fisting and insertive oral-anal contact with their sexual partner(s) during the previous 12 months. Among men who did engage in this combination of sexual practices, the estimate of the cumulative rate of HCV infection at 15 years was 16.4% compared to 5.2% in the group that did not (p<0.001).

6.4.6  **Relationship between HCV infection and other sexual practices**

Other sexual practices that were significantly associated with increased risk of HCV seropositivity included: 20 or more male sexual partners in the previous year (p<0.001), anal receptive intercourse with more than 25 percent of sexual encounters (p=0.030), insertive fisting (p=0.003), and insertive oral-anal contact (p=0.024). A
marginal elevation in risk was detected for receptive fisting (p=0.074). No association with time to HCV seropositivity was observed with regard to anal insertive intercourse (p=0.705), receptive oral-anal contact (p=0.120), insertive oral-genital contact (p=0.100) or receptive oral-genital contact (p=0.784). For the purpose of brevity, Kaplan-Meier curves have not been illustrated for all sexual practices that were assessed.

Table 6.1 shows unadjusted estimates of relative risk and 95 percent intervals for injection drug use, HIV-antibody status, and several sexual practices that were significantly associated with time to HCV seropositivity. Men who reported using injection drugs during their lifetime were about 20 times more likely to have tested seropositive during the observation period [95% CI: 10.9, 39.4]. HIV-seropositive men were almost 4 times more likely to have tested HCV antibody positive during follow-up. For each of the sexual practices shown in table 6.1 there was an approximate two to three-fold increase in risk of HCV infection.

6.4.7 Relationship between HCV infection and history of infectious diseases

The relationship between lifetime history of other infectious diseases and time to first positive HCV antibody test was also investigated. Table 6.2 displays the relative risk estimates for 10 self-reported infectious diseases. Men who reported a history of non-specific urethritis were 2.5 times more likely to test seropositive during the observation period compared to men without such a history (p=0.013). A marginal increase in risk of HCV infection was noted for a history of gonorrhea (p=0.055). No significant elevation in risk was observed for any of the other infectious diseases that were reported.

6.4.8 Multivariate Analysis

Because injection drug use, sexual practices, and history of infectious diseases were likely confounded with each other, multivariate analysis was conducted using Cox regression to model the simultaneous effect of these variables on time to HCV
seropositivity. All variables found to be significantly or marginally associated with HCV infection in univariate Cox regression models were considered for inclusion in the final model (p<0.10). Table 6.3 shows the final multivariate model of this analysis. The risk factors that exerted an independent effect on time to HCV seropositivity were injection drug use (p<0.001), HIV seropositivity (p=0.031), and the sexual practice of insertive fisting in combination with insertive oral-anal contact (p=0.038).

6.5 DISCUSSION

Previous cross-sectional studies of gay men have consistently implicated injection drug use as the primary risk factor for HCV seropositivity. Other risk factors that have been less consistently associated with HCV infection include HIV seropositivity, sexual practices, and history of other sexually transmitted infections. In contrast, some observational studies have not found evidence of an association between sexual behaviour and HCV seropositivity among gay men.

In our prospective study of homosexual men in Vancouver, we have confirmed that injection drug use is the primary risk factor for HCV infection in this population. Other risk factors found to be independently associated with HCV seropositivity in multivariate analysis included HIV seropositivity, and the sexual practice of insertive fisting in combination with insertive oral-anal contact. To our knowledge, this is the first prospective study of gay men to report a specific sexual practice as a significant predictor of time to HCV seropositivity independent of injection drug use.

Our finding that HIV antibody positive men were more likely to be infected with HCV, independent of injection drug use, is consistent with the results reported by Bodsworth et al. While a number of other studies have also reported elevated HCV infection rates among HIV seropositive gay men in univariate analysis, HIV antibody status did not retain significance in multivariate analysis.
Univariate analysis revealed several sexual practices to be associated with risk of HCV seropositivity in our cohort including twenty or more sexual partners in the prior year, frequency of receptive anal intercourse, insertive fisting, and insertive oral-anal contact. Both insertive fisting and receptive anal intercourse can result in rectal trauma and oral-anal contact can result in exposure to a sexual partner's HCV-infected blood or fluids. It is noteworthy that a history of non-specific urethritis and gonorrhea were either statistically or marginally associated with HCV seropositivity in univariate analysis. These sexually transmitted infections could also promote infection with HCV by disruption of rectal or mucosal barriers.

It should be acknowledged there is a considerable amount of overlap between the results presented in this chapter and results presented in chapters 4 and 5. In previous chapters, behavioural data were obtained from the enrolment visit and cross-sectional comparisons were carried out between HCV seropositive and seronegative men to identify significant risk factors associated with HCV acquisition. In this chapter, we adopted a different analytic approach and focused on comparisons of the distributions of time to first positive HCV-antibody test between strata of the predictor variable of interest. Behavioural data for these prospective analyses were also obtained from the enrolment questionnaire. Thus the sources of behavioural data were the same for both cross-sectional and prospective analyses.

There are limitations that should be acknowledged with regard to our analysis. The number of incident infections of HCV observed in the cohort (n=13) did not provide enough statistical power to adequately assess comparisons of interest and draw generalizable conclusions. In order to increase the statistical power for the prospective analysis, HCV seroprevalent men were also included. Men who were HCV seropositive at their enrolment visit were likely at highest risk for HCV infection. The inclusion of the 26-seroprevalent men in this report likely resulted in a group that was probably less
homogenous with regard to their risk factors. Secondly, the results of our study are based on both prevalent and incident HCV infections, and self-reported behaviours. It is possible that responses to questions concerning sexual behaviours, and injection drug use may be influenced by the participant’s knowledge of their HCV or HIV antibody status. However, in our study, responses to these questions were obtained two to three years before the participant’s HIV antibody testing took place, and over fifteen years prior to HCV antibody testing. HCV antibody testing for this study was carried out during 1999 and 2000. Responses to questions concerning sexual behaviours and injection drug use in our study could not have been influenced by the participant’s knowledge of their HCV antibody status.

We conclude that injection drug use was the primary risk factor for HCV infection in homosexual men in Vancouver during 1983-98. Our findings also provide confirmatory evidence that HCV can be spread by sexual contact among gay and homosexual men independent of injection drug use. Sexual practices that cause rectal trauma in combination with oral-anal contact increase the risk of exposure to HCV-infected blood and fluids.
Figure 6.1: Time to first positive HCV-antibody test among 662 men stratified by injection drug use during lifetime (1983-98)

Injection drug users (n=41, 20 events)

- No IDU - Seroprevalent
- No IDU - Seroincident
- IDU - Seroprevalent
- IDU - Seroincident

Log-rank p-value < 0.001

Did not use injection drugs (n=621, 19 events)

Months since January 1983
Figure 6.2: Time to first positive HCV-antibody test among 662 men stratified by HIV antibody status (1983-98)

- □ HIV negative (HCV prevalent)
- ● HIV negative (HCV incident)
- △ HIV positive (HCV prevalent)
- ▲ HIV positive (HCV incident)

Log-rank p-value < 0.001

HIV antibody positive (n=352, 31 events)

HIV antibody negative (n=310, 8 events)

Months since January 1983
Figure 6.3: Time to first positive HCV-antibody test among 662 men stratified by composite variable: insertive fisting and insertive oral-anal contact (1983-98)

- No (HCV prevalent)
- No (HCV incident)
- Yes (HCV prevalent)
- Yes (HCV incident)

Log-rank p-value < 0.001

No (n=484, 20 events)
Yes (n=165, 19 events)

Months since January 1983
Table 6.1: Predictors of time to first positive HCV antibody test in the VLAS cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection drug use during lifetime</td>
<td>20.5</td>
<td>10.9, 39.4</td>
</tr>
<tr>
<td>HIV antibody positive</td>
<td>3.8</td>
<td>1.7, 8.3</td>
</tr>
<tr>
<td>20 or more male sexual partners during the previous 12 months</td>
<td>3.1</td>
<td>1.5, 6.3</td>
</tr>
<tr>
<td>Insertive oral-anal contact (yes vs. no)</td>
<td>3.1</td>
<td>1.1, 8.7</td>
</tr>
<tr>
<td>Insertive fisting and insertive oral-anal contact (yes vs. no)</td>
<td>2.9</td>
<td>1.5, 5.4</td>
</tr>
<tr>
<td>Insertive fisting (yes vs. no)</td>
<td>2.6</td>
<td>1.4, 4.8</td>
</tr>
<tr>
<td>Receptive anal intercourse during more than 25% of sexual encounters</td>
<td>2.0</td>
<td>1.0, 3.8</td>
</tr>
</tbody>
</table>
Table 6.2: Estimates of relative risk of HCV infection for self-reported history of infectious diseases among 662 men in the VLAS cohort

<table>
<thead>
<tr>
<th>Infectious disease</th>
<th>Risk Ratio</th>
<th>[95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific urethritis</td>
<td>2.3</td>
<td>1.2, 4.5</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>2.2</td>
<td>1.0, 5.0</td>
</tr>
<tr>
<td>Scabies</td>
<td>1.7</td>
<td>0.9, 3.2</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>1.6</td>
<td>0.6, 4.0</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>1.5</td>
<td>0.7, 2.9</td>
</tr>
<tr>
<td>Syphilis</td>
<td>1.4</td>
<td>0.8, 2.8</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>1.2</td>
<td>0.6, 2.4</td>
</tr>
<tr>
<td>Genital warts</td>
<td>0.7</td>
<td>0.4, 1.4</td>
</tr>
<tr>
<td>Pubic lice</td>
<td>0.7</td>
<td>0.3, 1.5</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>0.5</td>
<td>0.2, 1.7</td>
</tr>
</tbody>
</table>
Table 6.3: Multivariate predictors of time to first positive HCV antibody test in the VLAS cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>Chi-square p-value</th>
<th>Adjusted Risk Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection drug use during lifetime (yes vs. no)</td>
<td>2.845</td>
<td>0.324</td>
<td>&lt;0.001</td>
<td>17.2</td>
<td>9.1, 32.5</td>
</tr>
<tr>
<td>HIV antibody positive</td>
<td>0.883</td>
<td>0.410</td>
<td>0.031</td>
<td>2.4</td>
<td>1.1, 5.4</td>
</tr>
<tr>
<td>Insertive fisting and insertive oral-anal contact (yes vs. no)</td>
<td>0.680</td>
<td>0.328</td>
<td>0.038</td>
<td>2.0</td>
<td>1.0, 3.8</td>
</tr>
</tbody>
</table>
6.6 REFERENCES


CHAPTER 7

HIV DISEASE PROGRESSION AND MORTALITY AMONG MEN IN THE VLAS COHORT WHO ARE CO-INFECTED WITH HCV

7.1 FORWARD

In this chapter, the results of an assessment of the influence of HCV infection on the natural history of human immunodeficiency virus (HIV) infection are presented and discussed. The effects of HCV infection on HIV-related outcomes are examined among HIV-infected participants in the VLAS cohort. Outcomes of interest in this investigation included symptoms and signs, physical exam findings, CD4 cell count, progression to AIDS, and survival.

The candidate was responsible for formulating the study design, data management, statistical programming and analyses, summarizing and interpreting the data, and writing the manuscript.

7.2 INTRODUCTION

Both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are global health problems. Because both viruses share similar routes of transmission, co-infection with HCV and HIV is common. In the United States, it is estimated that 240,000 people are infected with both HCV and HIV. It is estimated that 11,000 individuals are co-infected in Canada. Compared to the general population, the prevalence of co-infection is higher among injection drug users, and homosexual men.
The majority of studies of individuals who are co-infected with HCV and HIV have concluded that HIV disease progression is not strongly influenced by HCV infection, but hepatitis C progresses more rapidly in individuals with co-infection.\cite{9,14} Individuals with HCV/HIV co-infection experience more rapid progression to cirrhosis, and have more evidence of extensive liver damage.\cite{16} Staples et al. studied time from HIV diagnosis to AIDS, time from HIV diagnosis to death, and time from AIDS diagnosis to death among HCV/HIV co-infected individuals.\cite{14} They did not find any of these measures of disease progression to significantly differ between HCV/HIV co-infected and HIV-infected groups.

The objective of this study was to determine whether co-infection of HCV and HIV influences the natural history of HIV infection in a cohort of HIV-infected homosexual men in Vancouver. HIV-related outcomes were compared between men who were co-infected with HCV and HIV and those who were infected only with HIV. Outcomes of interest for these comparisons included clinical symptoms and signs, physical findings, CD4 cell count, progression to AIDS, and survival.

### 7.3 METHODS

#### 7.3.1 Study Description

The methods and aims of the Vancouver Lymphadenopathy-AIDS Study (VLAS) have been described previously.\cite{16,17} Briefly, the VLAS has monitored a cohort of 1,000 homosexual men since November 1982. From November 1982 to December 1984, a total of 729 men were recruited and enrolled through six general practices in central Vancouver. Two medical practices were added to the study and 271 additional participants were enrolled during the period October 1986 to December 1987. Follow-up visits occurred approximately every six months until September 1986, after which subjects completed visits on an annual basis.
7.3.2 Sources of data

During each study visit, a physical examination was performed and a functional inquiry was administered. The physical exam included an assessment of the size of the liver and spleen (normal, enlarged, small), and measurement of lymph nodes at each of the following sites: occipital, scalene, upper neck, supraclavicular, axilla, tonsillar, groin, and antecubital. Generalized lymphadenopathy was defined as the presence of lymph nodes greater than 1 centimeter in diameter at two or more extrainguinal sites. The physician administered the functional inquiry. Study participants were asked whether they experienced any of the following symptoms and/or signs during the past year: shortness of breath (unrelated to smoking), cough (unrelated to smoking), night sweats (at least two times per week for one month), fever (for at least 1 week), unintentional weight loss, excessive fatigue, arthralgias (multiple joint pains), diarrhea (for at least 1 week), oral thrush, oesophageal candida, herpes simplex (cold sores), herpes zoster (shingles), and presence of skin infections. Data regarding symptoms, signs, and physical findings were obtained from follow-up visits that occurred subsequent to the date of the first positive HCV and HIV antibody tests. For each participant, we determined the number of visits in which a specific symptom, sign, or physical finding was reported.

7.3.3 HCV and HIV antibody testing

Both HIV and HCV antibody testing were conducted using serum specimens. HIV antibody tests were performed at the Laboratory Centre for Disease Control in Ottawa. The enzyme-linked immunosorbent assay (ELISA) test was used with equivocal results tested by Western blot. HCV antibody tests were performed at the University of British Columbia Diagnostic Virology and Reference Laboratory at St Paul’s Hospital in Vancouver. HCV antibody was assayed by enzyme immunoassay
(EIA), using a modified algorithm to confirm seropositivity. Variations of this algorithm have been widely used in diagnostic virology laboratories throughout Canada. Briefly, a micro-particle enzyme immunoassay (Abbott AxSYM HCV Version 3.0 MEIA) was used to screen all sera. Negative sera were tested no further. Positive samples as well as samples with an indeterminate result, was further tested using a synthetic peptide-based EIA (United Biomedical Inc. HCV EIA 4.0). Sera that tested positive on both EIAs were classified as positive and tested no further. Sera that gave discordant results were further tested by immunoblot (RIBA III, Ortho Diagnostic Systems). Sera that were negative by RIBA were classified as negative. For this study, the HCV antibody testing was done on specimens collected between January 1983 and December 1998.

7.3.4 Laboratory methods

Laboratory methods of the VLAS have been described previously. Serial measurements of CD4 cell count were obtained for the majority of HIV-infected participants. For HIV-infected men with two or more CD4 cell counts following enrolment, the annual rate of CD4 cell decline was estimated using the method of least squares.

7.3.5 Eligibility criteria

All HIV seropositive men who were enrolled in the VLAS during the period November 1982 to December 1986, and who completed at least two visits during the period January 1983 to December 1998, were eligible for statistical analysis. This group included men who were HIV seropositive at enrolment, denoted ‘HIV seroprevalent’, and those who seroconverted during the observation period, denoted ‘HIV seroincident’. These men were further classified and denoted as ‘HCV seroprevalent’ and ‘HCV seroincident’.
7.3.6  Outcomes of interest

The diagnosis of AIDS in the cohort was defined according to criteria of the Centers for Disease Control (CDC), in Atlanta. Cases of AIDS were ascertained through physician reports and verified by record linkages with a national registry. Deaths of cohort members were also determined by physician reports and through record linkages with British Columbia Vital Statistics.

7.3.7  Statistical methods

Bivariate categorical data (e.g. co-infection and symptoms) were analyzed using Pearson’s chi-squared test. Fisher’s exact test was used when 25 percent or more the expected values in a contingency table were less than 5. Rates of CD4 cell decline were compared between groups using Student’s t-test for independent samples.

To analyze data regarding progression to AIDS and mortality, we used statistical methods for survival analysis, which take into account varying lengths of observation for study participants. Differences in AIDS progression and survival rates between HCV/HIV co-infected and HIV-infected men were evaluated by stratified Kaplan-Meier analysis, and tested using the log-rank test. The starting point for these prospective analyses was defined as the date of the first positive HIV and HCV antibody test for seroprevalent men and the mid-point between the last negative and first positive antibody test result for seroincident men. The events of interest in these analyses were ‘time to AIDS diagnosis’ and ‘time to death’. Participants who were event-free were right-censored as of December 31, 1998.

For all statistical comparisons, participants with missing or unknown values for the variable(s) of interest were excluded from the analysis. All reported p-values are two-sided.
7.4 RESULTS

7.4.1 Demographic characteristics

A total of 563 men were eligible for statistical analysis. This group included 408 (72%) HIV seroprevalent men and 155 (28%) HIV seroincident men. A total of 46 (8%) of the 563 men who met the entry criteria were HCV antibody positive. This group was comprised of 35 (76%) HCV seroprevalent and 11 (24%) HCV seroincident men. Table 7.1 shows the demographic characteristics and follow-up features of the cohort stratified by HCV antibody status. HCV seropositive and HCV seronegative men were similar with respect to average age at enrolment (p=0.988), ethnicity (p=0.785), education (p=0.788), income (0.616), and average duration of follow-up (p=0.405).

7.4.2 Symptoms and signs

Table 7.2 displays a comparison of the co-infected and control groups with respect to clinical symptoms and signs. No statistically significant differences were observed for any of the individual clinical symptoms included as part of a functional inquiry. However, a marginal association was observed between HCV infection and diarrhea. HCV seropositive men were more likely than HCV seronegative men to report the occurrence of this symptom during the observation period were (53% vs. 41%; p=0.097).

7.4.3 Physical findings

Table 7.3 shows the comparative results of physical findings between men who were co-infected with HCV and those who were not. As seen in this tabular summary, significantly more HCV co-infected men were diagnosed with an enlarged liver (31% vs. 13%; p<0.001) and an enlarged spleen (24% vs. 11%; p=0.011). There was no significant difference between the groups with respect to the occurrence of generalized lymphadenopathy (p=0.527).
7.4.4 CD4 cell decline

We studied HIV disease progression as measured by the number of CD4 cells measured at baseline, and the rate of CD4 cell decline. The HCV co-infected and comparison groups did not significantly differ with respect to baseline CD4 count (p=0.495) or the rate of CD4 cell decline (mean annual decline: 72 versus 82 cells per year; p=0.870).

7.4.5 Progression to AIDS

A total of 315 cases of AIDS were documented in the cohort during the observation period January 1983 to December 1998. Of these, 27 cases occurred among the HCV seropositive men and 288 cases in HCV negative men. The crude AIDS attack rate was similar in both groups (58% vs. 56%; p=0.696). Figure 7.1 shows the Kaplan-Meier curves of progression to AIDS among co-infected men and those infected with HIV only. After 14 years of follow-up, the estimated cumulative AIDS progression rate was 64 percent [95% CI: 49%, 78.8%] in the co-infected group compared to 58.5 percent [95% CI: 53.9%, 63.1%] in men who were not co-infected. Cumulative AIDS progression was slightly higher among co-infected men but the difference was not statistically significant (p=0.451).

We also carried out a sub-analysis in which the co-infected group was restricted to only HCV seroprevalent men (n=33). This restriction was implemented to ensure that the diagnosis of AIDS did not occur prior to the date of HCV infection among HCV seroincident men. In this sub-analysis, the cumulative AIDS progression rate after 12 years of follow-up was 67.7 percent [95% CI: 51.2%, 84.2%] in the co-infected group compared to 55.5 [95% CI: 50.9%, 60.0%] percent in the comparison group. Although the AIDS progression rate was higher among co-infected men, this difference did not achieve statistical significance (p=0.243).
7.4.6 Mortality

During the observation period, a total of 265 deaths in the cohort were reported. Twenty-four deaths occurred in the HCV seropositive group and 241 deaths were reported among HCV seronegative men. Crude death rates did not significantly differ between the respective groups (52% vs. 47%; p=0.469). Kaplan-Meier mortality curves are displayed in Figure 7.2. The estimated cumulative mortality rate at 14 years was 55.0 percent [95% CI: 39.7%, 70.2%] in men who were co-infected with HCV. In comparison, among men who were not co-infected, the 14-year cumulative mortality rate was 49.0 percent [95% CI: 44.3%, 53.7%]. Although the 14-year cumulative mortality rate was higher among men who were HCV seropositive, overall the difference was not statistically significant (p=0.314).

7.5 DISCUSSION

In this longitudinal study of HIV seropositive men in Vancouver, we found that co-infection with HCV did not significantly influence the natural history of HIV infection. In particular, HCV-HIV co-infection was not significantly associated with an increase in symptomatic illness, more rapid CD4 cell decline, faster progression of HIV disease to AIDS, or increased mortality. To our knowledge, this is the first longitudinal study in Canada to compare HIV disease progression rates at 14 years among individuals who are co-infected with HCV with men who are infected with HIV only. Another important finding of our prospective study is that mortality did not significantly differ between HCV seropositive and HCV seronegative men. To date, comparable data have not been reported in this population.

We found no significant difference between HCV co-infected men and those who were infected with HIV only with regard to the occurrence of numerous non-specific, clinical symptoms and signs. These data provide evidence that HCV infection does not result in any significant increase of symptomatic illness among HIV-infected
individuals. However, we did find strong indication of increased liver and spleen inflammation as evidenced from our physical findings among HCV co-infected men. This result is consistent with previous studies that have reported evidence of increased liver damage among HCV/HIV co-infected individuals.\textsuperscript{15}

To assess HIV disease progression we first compared the rate of CD4 cell decline in the two serologic groups of interest. We did not find any significant difference between baseline CD4 cell counts and rates of CD4 cell decline of HCV seropositive and seronegative men our cohort. These findings are in agreement with the results of a previous cross-sectional study and an Italian cohort study of HCV co-infection in HIV seroconverters.\textsuperscript{20,21} In contrast, another cross-sectional study reported that CD4 lymphocyte counts were higher among HCV-positive compared to HCV-negative patients.\textsuperscript{22}

The rate of progression to AIDS was not significantly different among HCV-seropositive and seronegative men in our study. This result corroborates the findings of other prospective studies of HCV co-infection.\textsuperscript{20,21} In addition, we found no significant increase in the rate of mortality among men co-infected with HCV in our cohort. This result is in agreement with the findings of an earlier observational study, which also reported HCV infection did not shorten survival among individuals with HIV infection.\textsuperscript{9}

There are several limitations that should be addressed concerning the findings of this investigation. First, the absolute number of HCV-seropositive men in the cohort was relatively small. Consequently, our estimates of cumulative AIDS progression and mortality in this group were subject to considerable variability. This was evidenced by much wider interval estimates of these parameters in the HCV-seropositive group compared to the HCV-seronegative group. Second, we are unable to adequately comment on progression of HCV disease in this cohort because HCV-related outcomes
were not well studied. For example, no prospective data regarding liver function were available (e.g. liver enzymes). Third, we are unable to comment on the causes of death among HCV co-infected men at this time. It is quite possible that significantly more HCV-related deaths occurred in the co-infected group. The results of a recently conducted death record linkage with British Columbia Vital Statistics are currently under review and will be the subject of a future report.

In conclusion, this prospective study of gay men in Vancouver has demonstrated the natural history of HIV disease is not strongly influenced by HCV infection. These findings provide confirmation of results reported in previous observational studies. The prevalence of HCV-HIV co-infection in homosexual men appears to be elevated but HCV does not appear to affect HIV progression or survival.
Table 7.1: Demographic and follow-up characteristics of 563 HIV seropositive men stratified by HCV antibody status (46 HCV seropositive and 517 HCV seronegative men)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HCV-seropositive (%)</th>
<th>HCV-seronegative (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>95</td>
<td>96</td>
<td>0.785*</td>
</tr>
<tr>
<td>Annual Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; $10,000</td>
<td>27</td>
<td>23</td>
<td>0.616</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attended College/University</td>
<td>56</td>
<td>53</td>
<td>0.788</td>
</tr>
<tr>
<td>Age (at enrolment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>32</td>
<td>32</td>
<td>.988†</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>92</td>
<td>103</td>
<td>.405†</td>
</tr>
<tr>
<td>Range</td>
<td>15-192</td>
<td>20-193</td>
<td></td>
</tr>
</tbody>
</table>

* Based on Fisher’s exact test
† Based on Student’s t-test
‡ Duration between date of first positive HIV-antibody test and participant’s death date or December 31, 1998 if participant was alive
Table 7.2: Comparison of 46 HCV+/HIV+ and 517 HCV-/HIV+ men with respect to clinical symptoms and signs

<table>
<thead>
<tr>
<th>Clinical symptom and sign*</th>
<th>HCV-seropositive (%)</th>
<th>HCV-seronegative (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>43</td>
<td>52</td>
<td>0.247</td>
</tr>
<tr>
<td>Multiple joint pains</td>
<td>34</td>
<td>32</td>
<td>0.771</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>53</td>
<td>41</td>
<td>0.097</td>
</tr>
<tr>
<td>Unintentional weight loss</td>
<td>36</td>
<td>34</td>
<td>0.764</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>22</td>
<td>24</td>
<td>0.835</td>
</tr>
<tr>
<td>Night sweats</td>
<td>29</td>
<td>29</td>
<td>0.970</td>
</tr>
<tr>
<td>Fever</td>
<td>27</td>
<td>21</td>
<td>0.364</td>
</tr>
<tr>
<td>Cough (unrelated to smoking)</td>
<td>36</td>
<td>36</td>
<td>0.996</td>
</tr>
<tr>
<td>Herpes simplex</td>
<td>67</td>
<td>62</td>
<td>0.494</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>47</td>
<td>36</td>
<td>0.170</td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>24</td>
<td>27</td>
<td>0.702</td>
</tr>
<tr>
<td>Oesophageal candida</td>
<td>16</td>
<td>23</td>
<td>0.287</td>
</tr>
<tr>
<td>Skin infections</td>
<td>69</td>
<td>73</td>
<td>0.575</td>
</tr>
</tbody>
</table>

*Reported at one or more follow-up visits subsequent to the date of the first positive HCV/HIV antibody tests
Table 7.3: Comparison of 46 HCV+/HIV+ and 517 HCV-/HIV+ men with respect to physical findings

<table>
<thead>
<tr>
<th>Physical finding*</th>
<th>HCV-seropositive (%)</th>
<th>HCV-seronegative (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlarged liver</td>
<td>31</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Enlarged spleen</td>
<td>24</td>
<td>11</td>
<td>0.011</td>
</tr>
<tr>
<td>Generalized lymphadenopathy</td>
<td>62</td>
<td>67</td>
<td>0.527</td>
</tr>
</tbody>
</table>

*Reported at one or more follow-up visits subsequent to the date of the first positive HCV/HIV antibody tests
Figure 7.1: Kaplan-Meier AIDS progression curves for 563 HIV seropositive men stratified by HCV antibody status

- HCV seropositive (n=46, 27 events)
- HCV seronegative (n=517, 288 events)

\[ \text{Log-rank p-value} = 0.451 \]
Figure 7.2: Kaplan-Meier curves of cumulative mortality for 563 HIV seropositive men stratified by HCV antibody status

- HCV seropositive (n=46, 24 deaths)
- HCV seronegative (n=517, n=241 deaths)

Log-rank p-value = 0.391
7.6 REFERENCES


19. Centers for Disease Control Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR 1987,36 (suppl):1-5.


8.1 SUMMARY OF STUDY FINDINGS

In this study, we have documented elevated prevalence and incidence of HCV infection in a cohort of homosexual men residing in Vancouver. During the period 1983-98, approximately 1 in 17 men in the cohort or about 6 percent were HCV antibody positive. Prevalence observed in this cohort was seven times higher than the estimated HCV prevalence in the general Canadian population, and six times greater than prevalence estimated for Canadian males.\textsuperscript{12} This result is consistent with prevalence rates reported by other observational studies of gay men.\textsuperscript{3-6}

Annual infection rates among men enrolled in this prospective study remained on average, at slightly less than one percent for each year of observation with the exception of 1997, in which it was slightly higher. The pattern of HCV incidence observed in this cohort was stable and remained so throughout the duration of the observation. There was no evidence of a decline in HCV infections despite the closed cohort effect. This pattern was even more prominent when comparing HCV and HIV incidence trends between 1983 and 1998. Rates of HIV infection were significantly higher among homosexual men in Vancouver throughout this time period and tended to decrease. In comparison, HCV rates remained fairly stable.

This study confirms the role of injection drug use as the primary risk factor for HCV infection among gay and homosexual men. However, less than ten percent of the study cohort reported using injection drugs. Almost one-half of the HCV-positive men identified in our study did not report a history of injection drug use. After controlling for both injection drug use and HIV-antibody status, we found an association between HCV infection and insertive fisting.
Among non-injection drug users, we investigated specific sexual practices as putative risk factors for HCV infection including fisting, oral-anal contact, and anal intercourse. We also estimated the relative risk associated with the sexual practice of insertive fisting in combination with oral-anal contact. This composite variable was of special interest because it involved rectal trauma of one sexual partner, and potential oral exposure of the other partner to their sexual partner's HCV-infected blood or fluids. Significant elevations in risk were detected for each of the individual sexual practices: insertive fisting, insertive oral-anal contact, and receptive anal intercourse. The highest elevation in risk was observed for men who practiced both insertive fisting and insertive oral-anal contact with their sexual partners. HCV positive men in our study were also more likely to report higher numbers of sexual partners during the previous 12 months. After adjustment for number of partners, significant elevations in risk persisted for the each of the sexual practices mentioned above.

We did not observe a significant association between HCV-infection and a history of blood transfusion among our participants. We were unable to assess the risk associated with tattooing, body piercing, needle borrowing/sharing, and condom use, because these variables were not included as items in most of our survey questionnaires.

We did not find that co-infection with HCV significantly influenced the natural history of HIV infection. HCV co-infection was not significantly associated with an increase in symptomatic illness, more rapid CD4 cell decline, faster progression of HIV disease to AIDS, or increased mortality among HIV-infected men. In addition, we found no significant difference between HCV co-infected men and the comparison group with regard to the occurrence of numerous non-specific, clinical symptoms and signs. However, we did find strong indication of increased liver and spleen
inflammation as evidenced from the physical findings among HCV co-infected men in the cohort.

8.2 UNIQUE CONTRIBUTIONS, IMPACT, AND IMPLICATIONS

The series of articles comprising this dissertation has contributed to our understanding of the epidemiology of HCV infection, and the natural history of HCV-HIV co-infection and its related outcomes. Using frozen serum specimens from a cohort study of homosexual men in Vancouver, we were able to carry out HCV antibody testing for approximately 900 participants and estimate the prevalence and incidence of HCV infection among all gay men residing in Vancouver during the period 1983 to 1998. Using self-administered questionnaire data obtained from these men, we were able to identify specific sexual practices that are associated with increased risk of HCV acquisition in this population. Significant risk factors that were identified in cross-sectional comparisons were further examined and confirmed by conducting prospective analyses of 'time to event' data. Long-term follow-up of this cohort enabled us to assess the influence of HCV infection on outcomes associated with human immunodeficiency virus (HIV) infection including progression to AIDS and survival.

The results of this epidemiological study have provided important data for assessing the burden of HCV in the population of gay men in Vancouver. We have documented elevated prevalence and incidence of HCV infection in this population during 1983-98. This finding highlights the need for improved health intervention programs that are designed to control HCV infection in homosexual men. To our knowledge, this is the first population-based, prospective study to report cumulative incidence rates of HCV infection in Canada. In addition, this is the first study to compare HCV and HIV incidence rates in gay men.
The results of this study have also addressed an urgent need to identify behavioural risk factors associated with HCV infection. Not surprisingly, our findings confirm that injection drug use is the primary risk factor for HCV infection in this population. Of greater interest in this research, was to determine the strength of the association between specific sexual acts and risk of HCV infection among individuals who did not use injection drugs.

This study provides epidemiological evidence that HCV can be spread by sexual contact in the absence of injection drug use. After adjustment for number of sexual partners, significant elevations in risk persisted for insertive fisting, insertive oral-anal contact, and receptive anal intercourse. The highest elevation in risk was observed for men who practiced both insertive fisting and insertive oral-anal contact with their sexual partners. Sexual practices such as insertive fisting can cause damage to the mucosal barrier of the rectum, and result in tearing and bleeding. Lesions produced by such trauma can serve as a source or passage for pathological microorganisms like HCV. Moreover, individuals who engage in this sexual practice in conjunction with other sexual practices including oral-anal contact may increase their likelihood of contact with HCV-infected blood or fluids. To our knowledge, this is the first observational study of homosexual men to document specific sexual practices as significant risk factors for HCV acquisition among men who did not use injection drugs.

The findings of this research have contributed to our understanding of the influence of HCV infection among men who are also infected with HIV. We found that co-infection with HCV did not significantly influence the natural history of HIV disease. However, we did find evidence of increased liver and spleen inflammation among co-infected individuals. These physical findings are indicative of chronic viral hepatitis. To our knowledge, this is the first longitudinal study of HIV seropositive men in Canada to estimate HIV-related disease progression rates at 14 years among individuals
who are co-infected with HCV, and to identify evidence of liver damage in this population.

There are a number of implications that arise from these findings. First, the elevated prevalence of HCV infection, and the low but stable incidence observed throughout the period 1983-98, suggests HCV infections continue to occur in this population. In addition to injection drug use, sexual behaviour may be an important mode of spread of HCV among gay men.

Prevalence of HCV infection was significantly higher among injection drug users in the VLAS cohort compared to those who did not inject drugs (20/41=48.8% versus 19/621=2.9%; p<0.001). Therefore, HCV prevalence observed among non-injection drug users in the Vancouver cohort was approximately 3 times greater than the estimate for Canadian males.²

It is important to recognize that not all HCV seropositive cases observed in this cohort were related to injection drug use. Of the 54 HCV seropositive men identified in this study, 32 (59%) were related to injection drug use but 22 (41%) were not. The effect of injection drug use on the spread of HCV is related to both the relative risk and the proportion of the population who use injection drugs. In this study, the prevalence of injection drug use was 6 percent and the risk ratio associated with injection drug use was equal to 21. Using these estimates, we calculated the attributable risk percent for injection drug use. We found 56 percent of HCV infections occurring in the population of homosexual men in Vancouver could be eliminated by reducing the risks associated with injection drug use. However, up to 44 percent of HCV seropositive cases identified in this cohort were attributable to factors other than injection drug use.

Our finding that specific sexual practices were significant risk factors for HCV seropositivity among homosexual men has implications concerning sexual transmission of HCV among heterosexuals. Sexual practices that result in vaginal or rectal trauma
may also play a role in the spread of HCV and other sexually transmitted diseases in the heterosexual population. For example, the practice of ‘dry sex’ is common in many African countries, and this practice may be associated with an increased prevalence of sexually transmitted diseases among heterosexuals in this geographic location. The practice of dry sex refers to the drying and tightening of the vagina for sexual intercourse. This practice has been adopted in some cultures to increase sexual pleasure. The methods for this practice include insertion of a substance into the vagina, drying with cloths, vaginal douching, and drinking preparations believed to cause drying effects on the vagina. Some of these methods used for dry sex can cause an inflammatory response and epithelial damage. Genital lesions have been shown to increase the risk of HIV transmission. In an observational study by Beksinska and colleagues, dry sex practices were reported by 60 percent of men and 40 percent of women. Dry sex practice was associated with an increased prevalence of self-reported sexually transmitted diseases among men in the study. The relationship between dry sex and risk of HCV transmission has not been investigated.

The role of sexual contact in the global spread of HCV should not be underestimated. If 35 to 45 percent of all HCV infections (WHO global prevalence estimate = 170 million people) were attributable to sexual contact, then this would account for 59.5 to 76.5 million infections worldwide.

In order to develop appropriate primary prevention messages regarding the potential risk of acquiring HCV by sexual transmission it is important to have knowledge of the sexual practices in the targeted population. Larger observational studies are needed in both developed and developing countries to elucidate the role of sexual transmission of HCV and to identify specific sexual practices that place individuals at increased risk of exposure.
Our assessment of men who were co-infected with HCV and HIV in this cohort indicates that progression of HIV disease is not strongly influenced by HCV infection. However, co-infected men showed significantly more evidence of liver inflammation. Similar results have been reported by other observational studies. HCV co-infection among HIV-infected individuals is increasingly becoming a common problem. It is estimated that one-third to one-half of HIV-infected individuals in industrialized countries are also infected with HCV. In the next ten to twenty years, a marked increase in morbidity and mortality related to HCV disease is likely to occur among HIV-infected individuals.

8.3 STRENGTHS AND LIMITATIONS

8.3.1 Strengths

This is a timely study. The burden of HCV on the health care system in Canada is expected to increase substantially in the next decade. Therefore, it is important to acquire an improved understanding of HCV infection through population-based research. Our study has directly addressed some urgent issues facing HCV epidemiological research in Canada. This longitudinal investigation targeted the population of gay men, estimated the burden of HCV in this population, and identified high-risk sexual practices that are associated with increased risk of acquiring HCV. This study also assessed the effect of HCV co-infection on both HIV-related and HCV-related outcomes. To our knowledge, no other study in Canada has addressed all of these research objectives at one time.

This study has several methodological strengths. The major strength of this research lies in the fact the findings are based on the results of a prospective study. The prospective component of this study permitted the calculation of 14-year cumulative incidence rates of HCV infection, and also allowed comparisons of incidence rates
between groups of men who reported specific behavioural factors of interest and those who did not. Because this was a longitudinal study with active follow-up of its participants, we were able to assess HIV-related disease progression and mortality among HCV co-infected individuals. Outcomes of interest including diagnosis of AIDS and death were verified through record linkages with both national and provincial registries. Another methodological strength of this study was that both cross-sectional and prospective statistical analyses were used to estimate the risk of HCV infection for specific sexual practices. The results that were obtained using these two analytic approaches were consistent with one another.

The results of this study are based on prevalent HCV infections and self-reported behaviours. Responses to questions concerning sexual behaviours, injection drug use, substance use, and history of infectious diseases may be influenced by the participant’s knowledge of their HCV or HIV antibody status. However, in our study, responses to these questions were obtained two to three years before the participant’s HIV antibody testing took place, and over fifteen years prior to HCV antibody testing. Neither HIV nor HCV antibody testing was available prior to 1984. Thus responses to questions concerning sexual behaviours and substance use could not have been influenced by the participant’s knowledge of their antibody status in this study.

This research study has provided a cost-effective means of acquiring important knowledge about the epidemiology and natural history of HCV. This was accomplished by having access to epidemiological data from a well-characterized cohort of homosexual men. The VLAS is the largest and longest-running population-based natural history study of HIV infection in Canada. It continues to serve as a valuable resource for answering important research questions about HIV and HCV infection as well as other infectious diseases.
8.3.2 Limitations

There are a number of limitations that should be acknowledged concerning the interpretation of the results of this study. First, the serologic results were based on frozen specimens obtained during the period 1983-98, and it is possible that some degradation of early specimens might have occurred. This degradation may have interfered with ELISA testing for HCV antibody. The presence of HIV infection can also diminish the accuracy of HCV antibody assays. There is an increased risk of receiving false-negative results from HCV screening antibody tests in people with HIV infection.\textsuperscript{16,22,23} There exists a possibility the presence of both of these factors could have led to under-estimation of the true rate of HCV prevalence and incidence in this study.

The observed number of HCV seropositive men in our cohort was fifty percent lower than what was hypothesized in the preliminary planning phase of this study. The small number of incident HCV infections in the cohort failed to provide adequate statistical power to assess comparisons of interest in this group alone. In order to increase statistical power for comparisons of interest, HCV seroprevalent men were also included in our prospective analyses. It is likely that men who were HCV seropositive at enrolment were also at highest risk for HCV infection. The inclusion of these seroprevalent men likely resulted in a sample that was less homogenous with respect to risk factors.

Sera were not available for HCV antibody testing for a number of VLAS participants. Approximately ten percent of participants either failed to provide blood samples during the period of follow-up, or their specimens had been depleted as a result of previous laboratory tests. To determine whether persons who did not have serum were different from those who did, a comparative analysis of demographic and behavioural variables was undertaken. Participants who did not have sera for HCV
antibody testing tended to be younger, were less likely to have attended college or university, and more likely to report an annual income of less than ten thousand dollars. Participants without sera were also more likely to be an injection drug user. The groups were similar with respect to the self-reported sexual behaviours that were studied. The results of this comparative analysis suggest that men in this cohort who did not undergo HCV antibody testing were at higher risk for infection. Consequently, the results of this longitudinal study likely led to under-estimation of the true prevalence and incidence of HCV infection in the population of gay men in Vancouver.

Due to the small number of men in the cohort who were co-infected with HCV and HIV, our estimates of cumulative AIDS progression and mortality in this group were subject to considerable variability. This was evidenced by much wider interval estimates of these parameters in the HCV-seropositive group. Because of these small numbers, we were unable to draw firm conclusions as to whether people with HIV more easily acquire HCV, and conversely, whether people with HCV more easily acquire HIV.

The results of this study are based on self-reported behaviours and the potential for misclassification and recall bias exists. Responses to questions concerning sexual behaviours, and injection drug use may be influenced by the participant’s knowledge of their HCV or HIV antibody status. However, in our study, responses to these questions were obtained two to three years before the participant’s HIV antibody testing took place, and over fifteen years prior to HCV antibody testing. HCV antibody testing for this study was carried out during 1999 and 2000. Responses to questions concerning sexual behaviours and injection drug use in our study could not have been influenced by the participant’s knowledge of their HCV antibody status.

Although individual participants provided estimates of their number of sexual partners during the previous 12 months, the number of HCV-infected sexual partners to
which they were exposed during this period was not taken into account. The extent of exposure to each partner (duration and type of exposure) and the degree of infectivity of each partner are unknown. In addition, there may be recall problems associated with the reporting of past sexual behavior. The effect of these recall problems is difficult to estimate. However, several studies have shown that interview-administered and self-administered questionnaires can provide reasonably reliable data concerning sexual behavior in homosexual men when the recall period is relatively short. In our study, information regarding sexual practices was obtained from the questionnaire at the enrollment visit. As mentioned previously, all participants in this study were unaware of their HCV status at the time of the questionnaire completion. This might have reduced the amount of recall bias in our study.

Another important limitation of this study involves the interpretation of associations between HCV transmission and specific sexual practices. The possibility must be acknowledged that sexual practices identified as significant risk factors in this study were not directly associated with HCV infection but rather confounded with other unmeasured risk factors. For example, we were unable to assess the risk associated with several putative parenteral and non-parenteral risk factors in this study. Tattooing, ear or body piercing, and needle borrowing/sharing, were not included as variables in our enrolment and early, follow-up questionnaires. Snorting of cocaine and sharing of paraphernalia used for this practice (e.g. straws) were not studied. In addition, we did not evaluate occupational and nosocomial exposures. The role of horizontal exposure in the spread of HCV was not addressed in this study.

We are unable to comment in any great detail on progression of HCV disease in this cohort because HCV-related outcomes were not well studied. No prospective data regarding liver function were available. We are also unable to comment on the causes of death among HCV co-infected men at this time. It is quite possible that significantly
more HCV-related deaths have occurred in the co-infected group. The results of a recently conducted record linkage study with British Columbia Vital Statistics are presently being reviewed and will be the subject of a future report.

The men who participated in this prospective study may not have been representative of the target population of all gay men in Vancouver. Homosexual men who choose to visit a primary care physician, whose practice is comprised of predominantly homosexual men, may be different from the "general" male population. Many male homosexuals do not openly acknowledge their sexual orientation and hence were not eligible to participate in this cohort study.

Finally, it is important to recognize the findings of this study are based on a cohort of gay men that were recruited almost twenty years ago. Data regarding sexual behaviour and substance use obtained during this time period may no longer be representative of current behavioural practices of younger gay men in Vancouver. This limitation underscores the need for continued research in this population to determine the risk of HCV transmission associated with current behavioural practices.

8.4 CONCLUSIONS

Four principal conclusions have been drawn from the results of this study. First, we conclude the prevalence and incidence of HCV infection is elevated among gay men in Vancouver. Prevalence and incidence of HCV are both significantly higher among HIV-infected men compared to HIV-negative men in this population. These findings highlight the need for improved primary health intervention programs that are designed to reduce and control HCV infection in this community. Second, we conclude that injection drug use is the primary risk factor for HCV infection among homosexual men in Vancouver. The role of injection drug use in the spread of HCV has been well established. While the relative risk associated with injection drug use was extremely high in this study, the prevalence of self-reported injection drug use was low among the
participants. Approximately one-half of men who were HCV antibody positive in this study did not use injection drugs. Third, we conclude that HCV can be spread by sexual transmission independent of injection drug use. Sexual practices that cause rectal trauma in combination with oral-anal contact increase the risk of exposure to HCV-infected blood and fluids. Fourth, we conclude HCV infection does not adversely affect HIV disease progression among co-infected individuals but does adversely influence progression of liver disease.

8.5 RECOMMENDATIONS

The results and findings of this study give rise to several recommendations regarding HCV research initiatives in Canada. First, surveillance of HCV needs to be dramatically improved. Current estimates of HCV prevalence must be considered as hypotheses because these estimates are based on fragmented data. There is a pressing need to better evaluate the extent and distribution of HCV infection, and to assess the burden of infection and disease in both the short and long term. In order to determine accurately the true burden of HCV in Canada, it is recommended that population-based prevalence and incidence studies be conducted in regions throughout our entire country. These observational studies need to be targeted at both high and low risk populations. It is also crucial these investigations include prospective monitoring of participants as part of their research protocols.

In Canada, it is estimated that between 10 and 40 percent of HCV-infected individuals contracted the disease by a mode of transmission that is unknown or cannot be positively identified. Further research is urgently required to elucidate the role of sexual contact in the spread of HCV in both homosexual and heterosexual populations. To help achieve this objective, it is recommended that future observational studies incorporate behavioural research components as part of their research agendas.
The results of this study suggest that specific sexual practices may place individuals at increased risk of contracting HCV. It is recommended that primary prevention programs and intervention programs that are designed to reduce and control HCV infection should address the potential risk of exposure to HCV related to high-risk sexual behaviours including number of sexual partners and sexual practices that cause rectal or vaginal trauma.

Previous observational studies of gay men have been limited in terms of their statistical power to identify specific sexual practices as significant risk factors for HCV infection. Small numbers of HCV seropositive men in these studies have made it difficult to identify specific sexual practices as independent risk factors in multivariate analysis. For example, in an observational study reported by Buchbinder et al., numerous sex acts were identified as significant risk factors in univariate analyses but only injection drug use was significant in multivariate analysis. Similar difficulties have been encountered in other observational studies of gay men.

In order to advance our understanding of the epidemiology of HCV it is recommended that an effort be made to establish an international collaborative project with researchers in Canada, the United States, Australia, and the Netherlands. During the 1980s, in response to the HIV/AIDS pandemic, cohort studies of HIV-infected individuals were launched in each of these countries. These longitudinal investigations were conducted in order to gain important knowledge about the epidemiology and the natural history of HIV infection. Like the VLAS, many of these studies have carried out HCV testing later using frozen blood specimens obtained from their study participants many years ago. A pooled data set comprised of demographic and behavioral variables for such individuals would be extremely useful in helping to identify specific sexual practices that are associated with HCV transmission. In addition, a pooled data set containing virologic, immunologic, treatment and endpoint variables from these
cohorts could be used to identify patterns of HCV and HIV disease progression, to estimate the duration from HCV seroconversion to critical events and diagnoses, and to investigate behavioral cofactors related to disease progression.
8.6 REFERENCES


