CLEARANCE, REINFECTION AND RE-CLEARANCE OF HEPATITIS C

by

Nazrul Islam

MBBS, Rangpur Medical College, 2004
MPH, North South University, 2010, *Valedictorian*
MSc, CII in association with Harvard School of Public Health, 2012, *Valedictorian*

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Abstract

Purpose: Despite the availability of effective treatments for Hepatitis C virus (HCV) infection, public health experts face challenges to intervening effectively, in part due to gaps in understanding the natural history of HCV. To address these knowledge gaps, the current dissertation uses a large population-based cohort in British Columbia (BC), Canada, to examine spontaneous clearance, reinfection, and spontaneous clearance of reinfection (i.e., re-clearance) of HCV.

Methods: This study draws data from the BC Hepatitis Testers Cohort that includes all individuals tested for HCV in BC (1990-2013), with linkages to data on their medical visits, hospitalizations, and prescription drugs. HCV-positive individuals with ≥1 valid HCV-PCR test on/after HCV diagnosis (n=46,783) were included, with varying eligibility criteria for each of the three primary analyses. Logistic regression and Cox proportional hazards regression models were used, as applicable.

Results: The proportion of primary spontaneous clearance, reinfection, and re-clearance was 25.1% (11,737 of 46,783), 7.6% (452 of 5,915), and 33.9% (121 of 357), respectively. *Chapter 2* shows that the likelihood of spontaneous clearance of primary HCV infection is lower in people with primary T-cell immunodeficiency (adjusted odds ratio [aOR]: 0.55, 95% CI: 0.32-0.94) and higher in females (aOR: 1.61, 95% CI: 1.54-1.68). *Chapter 3* shows a higher reinfection risk in the spontaneous clearance group compared to sustained virological response group (adjusted Hazard Ratio [aHR]: 2.71, 95% CI: 2.0-3.68), those coinfected with HIV (aHR: 2.25, 95% CI: 1.78-2.85), and people who inject drugs [PWID] (aHR: 1.53, 95% CI: 1.21-1.92). Among PWID,

opioid substitution therapy [OST] (aHR: 0.73, 95% CI: 0.54-0.98) and mental health counseling (aHR: 0.71, 95% CI: 0.54-0.92) were associated with a lower HCV reinfection risk. *Chapter 4* shows that, among those who spontaneously cleared the primary infection, the likelihood of HCV re-clearance was 54% lower (aHR: 0.46, 95% CI: 0.24-0.86) if reinfected with a heterologous HCV genotype.

Conclusions: People with compromised immunity may be prioritized for HCV treatment allocation. The positive impacts of scaled-up HCV treatment might be enhanced if accompanied by appropriate harm reduction programs to prevent reinfections among PWID with a view to achieving World Health Organization's goal of HCV elimination.

Preface

All the works presented henceforth were conducted at the University of British Columbia (UBC), Vancouver, and at the British Columbia Centre for Disease Control (BCCDC), Vancouver, BC, Canada, and conceived, undertaken, and written by the candidate, Nazrul Islam (NI). Data for all empirical research projects have been obtained from BC Hepatitis Testers Cohort (BC-HTC) based at BCCDC. Data linkage to establish the BC-HTC was performed under the BCCDC's public health mandate. The UBC Behavioral Research Ethics Board provided ethical approval for the dissertation protocol (H14-01649).

Modified versions of Chapters 2 and 3 have been accepted for peer-reviewed publication. The co-authors made contributions only as is commensurate with the supervisory committee and co-investigator requirements. Jean Shoveller (JS) and Naveed Janjua (NJ) were the supervisory authors, and were involved throughout the projects from concept development to manuscript writing. NJ was the principal investigator of the BC-HTC who had access to all the data. NJ was the corresponding author, and takes full responsibility for the integrity of the results and the accuracy of the analyses.

A version of Chapter 2 has been published [Islam N, Krajden M, Gilbert M, Gustafson P, Yu A, Kuo M, Chong M, Alvarez M, Wong J, Tyndall MW, Janjua NZ, BC-HTC Team. Role of primary T-cell immunodeficiency and hepatitis B coinfection on spontaneous clearance of hepatitis C: The BC Hepatitis Testers Cohort. **J Viral Hepat**. 2016. doi: 10.1111/jvh.12650]. Another version was presented at the 2016 Annual Conference of the American Association for the Study of Liver Diseases (AASLD) in Boston, MA (**Hepatology**. 2016;63(S1):385A). NI was

the lead investigator responsible for concept development, study design, statistical analysis, and manuscript writing under the supervision of JS and NJ. JS, NJ, Paul Gustafson (PG), and Mark Gilbert (MG) were involved in the early stages of the concept formulation, and suggested edits to the manuscript. PG and NJ also contributed to data analysis.

A version of Chapter 3 has been published [Islam N, Krajden M, Shoveller J, Gustafson P, Gilbert M, Buxton JA, Wong J, Tyndall MW, Janjua NZ. Incidence, risk factors, and prevention of hepatitis C reinfection: a population-based cohort study. **Lancet Gastroenterol Hepatol**. 2017:2(3):200-210]. Another version was presented at the AASLD 2016 Conference (**Hepatology**. 2016;63(S1):31A). It has also been accepted for oral presentation at the 26th Annual Canadian Conference on HIV/AIDS Research (CAHR 2017) in Montreal, QC. NI was the lead investigator responsible for concept development, study design, statistical analysis, and manuscript writing under the supervision of JS and NJ. The roles of JS, NJ, PG, and MG were same as that for Chapter 2.

A version of Chapter 4 is currently under review [Islam N, Krajden M, Shoveller J, Gustafson P, Gilbert M, Buxton JA, Wong J, Tyndall MW, Janjua NZ. Hepatitis C re-clearance and lack of cross-genotype immunity in a large population cohort. *Submitted*]. Modified version has been presented at the AASLD 2016 Conference (**Hepatology**. 2016;63(S1):375A), and will be presented at CAHR 2017. NI was the lead investigator responsible for concept development, study design, statistical analysis, and manuscript writing under the supervision of JS and NJ. The roles of JS, NJ, PG, and MG were same as that for Chapter 2.

All inferences, opinions, and conclusions drawn are those of the author(s), and do not necessarily reflect the opinions or policies of the [British Columbia] Ministry of Health.

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List of abbreviations

aHR	Adjusted Hazard Ratio
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
aOR	Adjusted Odds Ratio
BCCDC	British Columbia Centre for Disease Control
BCCDC-PHL	British Columbia Centre for Disease Control Public Health Laboratory
BCCR	British Columbia Cancer Registry
BC-HTC	British Columbia Hepatitis C Testers Cohort
CI	Confidence Interval
DAA	Direct-acting Antivirals
DAD	Discharge Abstract Database
EHSSS	Enhanced Hepatitis Strain Surveillance System
ELISA	Enzyme-Linked Immunosorbent Assay
HAISYS	HIV/AIDS Information System
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
ICD	International Classification of Diseases
IDU	Injection Drug Use

iPHIS	Integrated Public Health Information System
IU	International Unit
MoH	Ministry of Health
MSM	Men Who Have Sex With Men
MSP	Medical Services Plan
NAAT	Nucleic Acid Amplification Test
OR	Odds Ratio
OST	Opioid Substitution Therapy
PCR	Polymerase Chain Reaction
PH	Proportional Hazards
PHN	Personal Health Number
PWID	People Who Inject Drugs
RNA	Ribonucleic Acid
SVR	Sustained Virological Response
TB	Tuberculosis
UBC	University of British Columbia

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To

My Baba— my Guru, and my Ma— my Hero

Chapter 1: Introduction

1.1 Background

The global burden of hepatitis C virus (HCV) infection has been estimated to be as high as 185 million, about 3% of world's population,[1-3] with about 500,000 HCV-related deaths each year.[4] Although the exact prevalence of HCV in Canada remains unknown,[5] the estimated prevalence of HCV in Canada was 0.61% to 1.34% (210,753 to 461,517 persons) in 2011.[6] Total healthcare cost of chronic hepatitis C in Canada has been projected to increase from $161.4 million in 2013 to $258.4 million in 2032.[5]

Hepatitis C virus is a member of the genus *Hepacivirus* under *Flaviviridae* family.[7] Further phylogenetic analysis led to identification of seven main 'types' of HCV, with additional subtypes such as 1a, 2b etc.[8] Genotypes 1, 2, and 3 are more widely distributed while genotypes 4, 5, and 6 are more confined to specific regions.[9, 10] HCV genotypes 1 and 3 are the predominant strains in North America, including Canada.[9, 10]

HCV transmission via unsafe blood transfusion is no longer a concern in countries where effective blood supply safety programs are in place,[11-13] while it is still a major challenge in many countries.[14-17] Sexual transmission of HCV has historically been considered a rare event.[18, 19] However, over the past decade, several studies have reported sexually acquired HCV infections in HIV-positive men who have sex with men (MSM).[20-33] The rate of HCV reinfection among HIV-positive MSM has been reported to be between 5.39 [34] and 15.2 [35] cases per 100 person-years, and a cumulative incidence of 16% over 12 years of follow-up [36] (one study found a cumulative incidence of 33% over a 2-year follow-up).[35]

In developed countries, the principal mode of HCV transmission is syringe sharing by people who inject drugs (PWID).[11, 12]

While chimpanzees are susceptible to experimental infection, humans are the only known natural host of HCV.[37] The virus replicates immediately upon entry, but people remain asymptomatic over the first 4-12 weeks after viral entry (the incubation period of the virus),[38] before showing the signs and symptoms of acute hepatitis C including elevated alanine aminotransferase (ALT) levels. It takes about 2-8 weeks to develop HCV-specific antibody.[39] The course of the disease depends on the host's ability to produce and maintain HCV-specific antibody and T-cell levels (Figure 1.1).[39] A positive HCV-antibody test indicates a previous exposure to HCV, while a positive HCV-RNA test confirms current active infection.[40] About a quarter of those infected clear the virus spontaneously, although the vast majority require HCV treatment to do so. Standard treatment of hepatitis C was combination pegylated interferon (PEG-IFN) and ribavirin until 2011 when direct-acting antivirals (DAA), with or without PEG-INF/ribavirin, were introduced with improved efficacy and tolerability and markedly reduced adverse effects.[41, 42]

Although spontaneous clearance of HCV infection occurs,[43-56] the majority of the infected people fail to clear the virus spontaneously, leading to chronic HCV infection.[39, 52, 56, 57] Chronic HCV infection results in a number of liver diseases including liver fibrosis, (decompensated) cirrhosis, and hepatocellular carcinoma (HCC).[58, 59] These people comprise one-quarter of all the cases of cirrhosis and HCC cases worldwide,[60] making HCV North America's most common indication for liver transplantation.[37, 61, 62]

Infection with HCV does not confer immunity to prevent future infection. [39, 52, 56, 57] Thus, people remain at risk of reinfection even after spontaneous clearance [43-56] or treatment-induced sustained virological response (SVR).[45, 48, 54, 55, 63-74] HCV reinfection has largely been reported in high risk populations, such as people who inject drugs (PWID).[44, 45, 47-49, 51, 53, 55, 63, 64, 67-70, 72, 75] The rate of HCV reinfection among PWID has been reported between 0 and 5 per 100 person-years following treatment-induced clearance, [45, 48, 63, 64, 67-70, 72] and between 1.8 and 46.7 per 100 person-years following spontaneous clearance.[44, 45, 47-49, 51-53, 55, 76]

Data on HCV re-clearance (i.e., spontaneous clearance of reinfection) is scarce. Some epidemiological studies,[47, 48, 52, 53, 76, 77] mostly conducted among PWID, report re-clearance rates ranging between 0 and 100%. However, previous studies in chimpanzees [78-80] and in humans [52, 53] indicate that compared to primary infection, exposure to a subsequent infection is associated with a lower peak viremia, overall shortened infection course and lower ALT levels, and a higher likelihood of re-clearance.

1.2 Rationale

While a nascent body of research on the natural history of HCV infection exists, there is a need for more population-based research to characterize spontaneous clearance, reinfection, and re-clearance of HCV. Most of the studies conducted so far have relied on relatively small sample sizes and have been conducted in specific risk groups, such as PWID [44, 45, 47-49, 51, 53, 55, 63, 64, 67-70, 72, 75] and MSM [20-33].

Spontaneous clearance of first HCV infection has been examined extensively,[36, 81-120] with most research among high-risk individuals such as post-transfusion/hemodialysis groups,[81-83, 85, 87, 90-92, 94, 96, 97, 101, 103, 104, 107, 108, 116, 118, 120] PWID,[81, 88, 93, 96, 98, 100, 117] and MSM.[36, 109, 110] These studies reported the proportion of spontaneous clearance as low as 0% to as high as 80% with a weighted mean of 26%.[50] Several host and viral factors have been identified as potential predictors of spontaneous clearance of HCV. [50, 89, 119, 121] However, the exact mechanisms that determine spontaneous clearance are not well-defined.[57, 122] Previous research suggests that cellular, particularly T-cell, host-immune response against HCV plays a role in determining HCV infection outcomes.[123-125] Spontaneous clearance of HCV has been found to be associated with sustained HCV-specific CD4+ and CD8+ T-cell responses [124-126], although epidemiological studies are yet to evaluate the impact of primary T-cell deficiency on viral clearance.[89, 119, 121, 127, 128] This dissertation aims to inform this knowledge gap.

Coinfection with hepatitis B virus (HBV) has also been suggested to be associated with spontaneous clearance of HCV in specific population subgroups, such as hemophiliacs [119, 129], HIV coinfected persons,[112, 113] MSM,[110] and PWID.[89] The current dissertation is the largest population-based study to date to examine the association of HBV coinfection and spontaneous clearance of HCV.

As is the case with spontaneous clearance, most studies on HCV reinfection have been limited by small numbers of reinfected cases and have been conducted among PWID [44, 45, 47-49, 51, 53, 55, 63, 64, 67-70, 72, 75] or MSM.[35, 36, 130, 131] Smaller numbers of reinfected cases also limited the ability of those studies to assess the factors associated with

reinfection risks. The current dissertation uses a large and robust dataset to examine reinfection rates following spontaneous clearance and SVR. This aspect of the dissertation was undertaken in an effort to inform public health policy on treatment of PWID including the role of opioid substitution therapy (OST) and mental health counseling and rehabilitation on HCV reinfection risk. This is the largest study to date to characterize reinfection risks following spontaneous clearance and SVR amongst individuals followed up for more than 19 years.

Understanding HCV re-clearance provides further insights into the dynamics of host immune system in clearing repeated exposure to HCV, and thus contributes to understanding the protective immunity to inform vaccine development strategies. Evidence from studies on chimpanzees [78-80] and humans [47, 48, 52, 53, 76, 77] is conflicting as to whether the rate at which individuals clear a second infection is any different from the rate of spontaneous clearance of first episode (the range reported was 0-100%). Published case reports or case series on re-clearance in humans report only on unadjusted estimates and with wide-ranging results (e.g., most report between 0 and 10 re-clearance cases, although one study reported 14 re-clearance cases).[76] Moreover, there is little convergence across these studies as to whether the ability to clear a previous episode with one HCV genotype offers broader protection to clear subsequent exposure to a different HCV genotype. While some of these studies reported evidence of cross-genotype immunity,[44, 51-53, 79] others reported limited protection against reinfection with a heterologous genotype.[80, 132] The current dissertation attempts to inform knowledge gaps in this area by examining the correlates of re-clearance using a large population-based dataset that includes a sufficiently large number of re-clearance cases.

1.3 Overall objectives and hypotheses

The overarching aim of this dissertation is to examine spontaneous clearance, reinfection, and spontaneous clearance of reinfection (i.e., re-clearance) and identify factors associated with these outcomes. The study objectives and hypotheses include:

(i) **To identify factors associated with spontaneous clearance of primary HCV infection with particular emphasis on primary T-cell immunodeficiency and hepatitis-B coinfection**: Chapter 2 examines spontaneous clearance of primary HCV infection and its correlates. It was hypothesized that the likelihood of spontaneous clearance would be lower in people with primary T-cell immunodeficiency, and higher among those coinfected with hepatitis B virus (HBV).

(ii) **To characterize HCV reinfection and to examine the effects of opioid substitution therapy and mental health counseling on HCV reinfection among PWID**: Chapter 3 examines HCV reinfection that includes both who cleared their primary HCV infection spontaneously and those who achieved SVR. In this chapter, the incidence rate of HCV reinfection was estimated and factors associated with HCV reinfection were identified, including the effects of OST and mental health counseling on HCV reinfection among PWID. It was hypothesized that PWID engaged with OST and/or mental health counseling would have lower likelihood of HCV reinfection.

(iii) **To characterize HCV re-clearance and to examine the effects of reinfection with a heterologous HCV genotype on re-clearance:** Chapter 4 examines factors associated with HCV re-clearance. It also examines whether reinfection with a

heterologous HCV genotype affects the likelihood of re-clearance among those who spontaneously cleared their primary infections.

1.4 Research design and methods

1.4.1 Study setting

This dissertation draws data from the British Columbia Hepatitis Testers Cohort (BC-HTC) which is housed and managed at the BC Centre for Disease Control (BCCDC). The BC-HTC is one of the largest cohorts of individuals tested for HCV in the world. The cohort includes all individuals tested for HCV or HIV at the BC Centre for Disease Control Public Health Laboratory (BCCDC-PHL) or reported cases of HCV, HBV, or HIV since 1990. These data are linked with centralized administrative data sources which provide information on demographic characteristics,[133] medical visits,[134] hospitalizations,[135] and prescription drugs[136, 137] (Table 1.1). Details of the BC-HTC including creation, linkage, characteristics, and matching have been reported previously.[138, 139] Overall, the linkage rate of the HCV cohort with the administrative databases was >85%, which approached 90% after 2006. [138, 139] BCCDC-PHL is the centralized laboratory for most serology (95%), all HCV-RNA (PCR), and genotype testing in British Columbia, Canada. This unique dataset was merged with the central database of all the prescription drugs, which offers the opportunity to monitor and assess the natural history of HCV infection along with the impact of HCV treatment and harm reduction initiatives.

1.4.2 Key definitions

To avoid repetition across the chapters in this dissertation, definitions of key terms are provided below. Where necessary, additional definitions that are specific to particular empirical analysis will be provided in the respective chapters.

HCV case: An HCV case is defined as an individual who tested positive for either HCV antibody or HCV-PCR or genotype, or who was reported as a case of HCV in the Integrated Public Health Information System (iPHIS).[138] For time-to-event analysis, HCV diagnosis date was the midpoint between the last negative and first positive HCV-PCR date (Note: This applies only to those who tested negative at first test and subsequently tested positive for HCV; the term "seroconverters" is also used to refer to this subgroup).[76]

Spontaneous clearance: Spontaneous clearance was defined as undetected HCV RNA without treatment as indicated by a negative HCV-PCR test result. To address the issue of viral suppression vs. viral clearance, additional analysis defined spontaneous clearance as two consecutive negative HCV-PCR tests, at least 28 days apart,[76] following HCV diagnosis without treatment. The date of spontaneous clearance was calculated as the midpoint between the last positive and first negative PCR following HCV diagnosis.[76]

Sustained virological response: Sustained virological response (SVR) was defined as two consecutive negative HCV-PCR tests, at least 28 days apart,[76] at ≥12 weeks post-treatment completion (the date of last dispensation of HCV treatment plus the days the drug was dispensed for).[140, 141] The date of SVR was calculated as the midpoint between the 12-weeks post-treatment date and the subsequent first negative PCR date.

HCV reinfection: HCV reinfection was defined as a positive HCV-PCR following clearance (spontaneous clearance or SVR) of first infection. The date of reinfection was calculated as the midpoint between the last negative PCR and the first positive PCR following clearance of the first infection.

HCV re-clearance: HCV re-clearance (*confirmed*) was defined as two consecutive negative PCR tests, at least 28 days apart, after reinfection. Those who had only one negative HCV-PCR, or two consecutive negative PCR within a 28-day time frame, were defined as *probable* re-clearance. The date of re-clearance was the midpoint between the last positive PCR and the first negative PCR after reinfection. Figure 1.2 illustrates natural history of HCV with estimated dates of each of the stages considered in this dissertation.

HIV cases: HIV cases were identified based on reporting to the HIV/AIDS Information System (HAISYS) as per provincial guidelines and/or a reactive HIV diagnostic lab test.[142] Additional HIV cases who tested without nominal information were identified through a previously validated algorithm based on medical visits and/or hospital admissions described elsewhere.[138]

Social and material deprivation: Social and material deprivation quintiles were based on Québec Index of Material and Social Deprivation.[143]

1.5 Ethical considerations

Data linkage to establish the BC-HTC was performed under the BCCDC's public health mandate. The Behavioral Research Ethics Board at the University of British Columbia provided ethical approval for the dissertation protocol (H14-01649).

1.6 Organization of the dissertation

Chapter 1 overviews the burden of HCV infection and summarizes the evidence gaps regarding its natural history. The chapter also outlines the study objectives and methodology. Chapter 2 examines factors associated with the spontaneous clearance with particular emphasis on primary T-cell immunodeficiency, and HBV coinfection. Chapter 3 examines HCV reinfection to identify factors potentially associated with HCV reinfection, and to examine the role of OST and mental health counseling and rehabilitation on HCV reinfection among PWID. Chapter 4 examines whether reinfection with a heterologous HCV genotype has any impact on the likelihood of re-clearance. Chapter 5 provides a summary of the key findings of the dissertation and includes a discussion of the potential implications for practice, policy and further research as well as a description of key methodological strengths and limitations.

Table 1.1 Inclusion criteria and data sources for the BC Hepatitis Testers Cohort

Criteria for inclusion in BC-HTC	
All individuals: • tested at the centralized provincial laboratory for HCV or HIV OR • reported by BC public health as a confirmed case of HCV OR • reported in BC enhanced surveillance system as a confirmed case of HIV or AIDS (all reports) OR • reported by BC public health as a confirmed case of HBV OR • included in BC Enhanced Strain Surveillance System (EHSSS) as an acute HBV or HCV case • All individuals meeting at least one the above criteria were linked internally across all their tests and case reports. Those with a valid personal health number (PHN) were then sent for deterministic linkage with province-wide Cancer and Ministry of Health (MoH) datasets	
Provincial communicable disease data sources:	**Date ranges:**
BC-PHL HIV laboratory testing datasets (tests: ELISA, Western blot, NAAT, p24, culture)	1988–2013
BCCDC-PHL HCV laboratory tests datasets (tests: antibody, HCV RNA, genotyping)	1992–2013
HIV/AIDS Information System (HAISYS) (public health HIV/AIDS case reports)	1980–2013
Integrated Public Health information System (iPHIS) (public health case reports of HCV, HBV, and TB)	1990–2013
Enhanced Strain Surveillance System (EHSSS) (risk factor data on a subset of acute HCV and acute HBV cases)	2000–2013
Cancer and MoH administrative data sources:	
BC Cancer Registry (BCCR) (primary tumour registry, excludes metastatic cancers)	1970–2012
Discharge Abstracts Dataset (DAD) (hospitalization records)[S1]	1985–2013Q1
Medical Services Plan (MSP) (physician diagnostic and billing data)[S2]	1990–2012
PharmaCare/PharmaNet (Pharma) (prescription drug dispensations)[S3, S4]	1985–2012
BC Vital Statistics (VS) (deaths registry)[S5]	1985–2013
The final BC-HTC comprises all individuals successfully linked on PHN to the MoH Client Roster[S6] (a registry of all BC residents enrolled in the publicly-funded universal healthcare system)	

HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; BC-PHL: BC Public Health Laboratory; RNA: Ribonucleic Acid; PCR: Polymerase Chain Reaction.

Supplementary References:

S1. British Columbia Ministry of Health [creator]. Discharge Abstract Database (Hospital Separations). British Columbia Ministry of Health [publisher]. Data Extract. MOH (2013). 2014. http://www.health.gov.bc.ca/data

S2. British Columbia Ministry of Health [creator]. Medical Services Plan (MSP) Payment Information File. British Columbia Ministry of Health [publisher]. Data Extract. MOH (2013). 2014. http://www.health.gov.bc.ca/data

S3. British Columbia Ministry of Health [creator]. PharmaCare. British Columbia Ministry of Health [publisher]. Data Extract. MOH (2013). 2014. http://www.health.gov.bc.ca/data

S4. British Columbia Ministry of Health [creator]. PharmaNet. British Columbia Ministry of Health [publisher]. Data Extract. MOH (2013). 2014. http://www.health.gov.bc.ca/data

S5. BC Vital Statistics Agency [creator]. Vital Statistics Deaths. BC Vital Statistics Agency [publisher]. Data Extract. BC Vital Statistics Agency (2014). 2014.

S6. British Columbia Ministry of Health [creator]. Client Roster (Client Registry System/Enterprise Master Patient Index). British Columbia Ministry of Health [publisher]. Data Extract. MOH (2013). 2014. http://www.health.gov.bc.ca/data

Figure 1.1 Changes in HCV-RNA levels upon exposure along with HCV-specific antibody and T-cell response

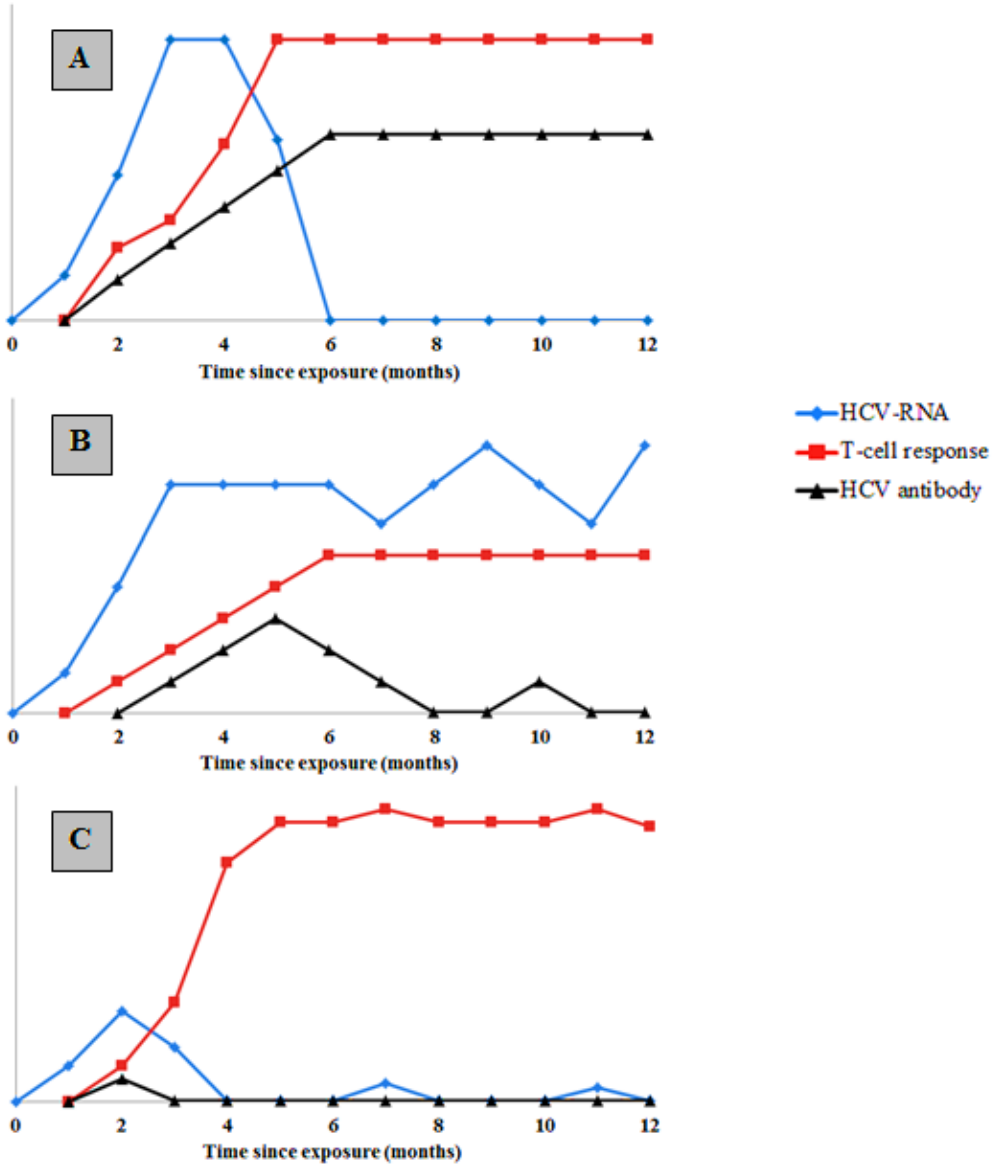
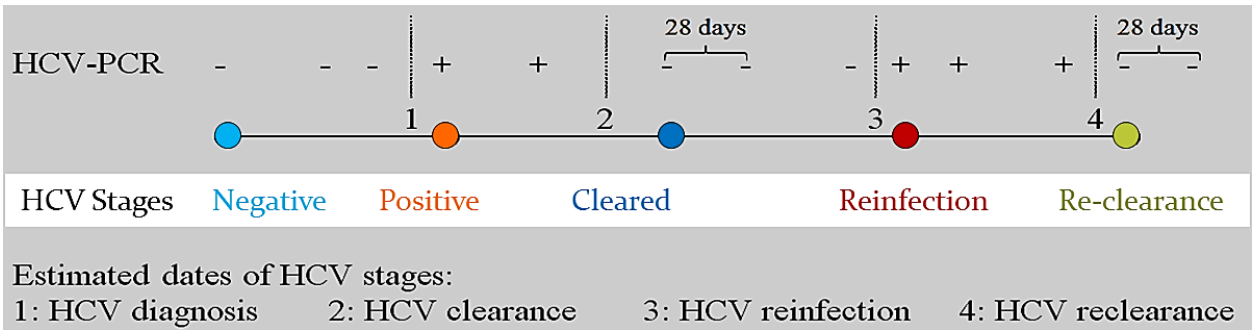


Figure legends: Changes in HCV-RNA levels upon exposure along with HCV-specific antibody and T-cell response: [A] Acute or self-limiting infection, [B] Chronic infection, and [C] Viral clearance, re-exposure and re-clearance without effective antibody production. (Adapted from Elliott *et al*., 2006 [39])

Figure 1.2 Natural history of HCV and estimated dates (midpoint) of each of the stages of HCV transitions used in this dissertation



Chapter 2: Spontaneous clearance of hepatitis C: role of primary T-cell immunodeficiency and hepatitis B coinfection

2.1 Background

As many as 185 million people have been reported to have been living with HCV globally.[2, 3] While the majority of HCV infected individuals develop persistent infection, about a quarter clear the virus spontaneously, without any treatment. [50, 88, 89, 95, 111, 115, 117] The exact mechanisms that determine these outcomes are not well-defined,[57, 122] although host, viral, and environmental factors have been suggested to influence these outcomes.[50, 89, 119, 121] A growing body of evidence suggests that cellular, particularly T-cell, host immune response against HCV plays a critical role in determining the outcomes of the infection.[123-125] Moreover, spontaneous clearance of HCV has been found to be associated with sustained HCV-specific CD4+ and CD8+ T-cell responses.[124-126] However, epidemiological research that studied the factors associated with spontaneous clearance of HCV has not examined the role of primary T-cell deficiency in viral clearance.[89, 119, 121, 127, 128] HBV coinfection has also been suggested to be associated with spontaneous clearance of HCV in specific group of population such as hemophiliacs,[119, 129] and PWID.[89] Most studies on spontaneous clearance were of relatively small size, and were conducted in specific high risk population cohort such as PWID, hemophiliacs, or MSM. Thus these studies may not be generalizable to the broader population. To address these gaps, this study was conducted on the data from BC-HTC to assess spontaneous clearance at a population level, and to examine the relationship of primary T-cell deficiency and other factors with spontaneous clearance.

2.2 Research design and methods

2.2.1 Study cohort

The data for this analysis is based on the BC-HTC as described in Chapter 1 (Table 1.1). Details of the BC-HTC database have been described in Chapter 1 and elsewhere [138, 139].

2.2.2 Case definitions

HCV case definition was described in Chapter 1.

For the logistic regression analysis, spontaneous clearance was defined as a negative HCV-PCR test following HCV diagnosis without treatment. In the sensitivity analysis, spontaneous clearance was defined as two consecutive negative PCRs, at least 28 days apart, as described in Chapter 1.

Primary T-cell Immunodeficiency was defined based on diagnostic codes for disease conditions [144] reported to the medical services plan (MSP) or discharge abstract database (DAD) (Table 2.1).

HIV cases were identified based on the methodology described in Chapter 1. Definitions of diabetes (type-2), major mental illness, injection drug use (IDU), and problematic alcohol use were based on diagnostic codes from MSP or DAD. Additional details, including the international classification of diseases (ICD) codes, are provided in Table 2.1. To address the issue of temporality, all these conditions, including the primary T-cell immunodeficiency, were defined at baseline (i.e., diagnoses of co-morbidities that occurred on/before HCV diagnosis).

Social and material deprivation quintiles were based on Québec Index of Material and Social Deprivation.[143]

2.2.3 Eligibility

This analysis included all HCV positive individuals who had at least one valid HCV-PCR (test for HCV RNA) test on or after HCV diagnosis. The HCV-PCR test results were available until December 31, 2013. Thus, to allow sufficient follow-up time to observe spontaneous clearance, the analysis was restricted to those who were diagnosed as HCV cases until December 31, 2012.

2.2.4 Data analysis

The characteristics of the eligible individuals are presented. Bivariate relationships were explored by simple logistic regression, and the unadjusted odds ratios (OR) along with 95% confidence intervals (CI) are presented. Variables based on a-priori hypotheses, [127, 145, 146] and those significant at level 0.10 in the univariate analysis were included in the multivariable models; adjusted odds ratios (aOR) are reported with 95% CI. Birth cohort, sex, and year of HCV diagnosis were included in all the models irrespective of their statistical significance in the univariate analysis; the first two were added as they are established risk factors of HCV, and the latter was used to adjust for varying testing pattern over time. To avoid non-identification problem (Age = Period − Cohort),[147] age was not included in the same adjusted models with birth cohort and HCV diagnosis year.

As most HCV infections are asymptomatic, and people generally do not test for HCV regularly, the onset date of infection is not known – raising the issue of temporality of risk

factors. However, HCV onset date is relatively more accurate among seroconverters who have a negative test result available preceding a positive test, and thus it is possible to calculate the follow-up time relatively more accurately. To address this issue, Cox proportional hazards (PH) models were fitted on seroconverters using the follow-up time from the estimated date of HCV infection (midpoint between last negative test and first positive test) to the estimated date of spontaneous clearance (the midpoint between the last positive PCR post-diagnosis and the first negative PCR),[76] and adjusted hazard ratios (aHR) and 95% CI were reported. To further validate the findings, Cox PH model was fitted among the seroconverters who had two PCRs available (n=4,610). In this sample, spontaneous clearance was defined as two consecutive negative PCR tests, at least 28 days apart. All the analyses were conducted in [SAS/STAT] Software version [9.4].[148] All the tests were two-sided at a significance level of 0.05.

2.3 Results

Figure 2.1 demonstrates the selection of the final sample. Of the 67,726 HCV positive cases identified in the BC-HTC, 46,940 individuals had at least one valid PCR test result on or after HCV diagnosis (on/before December 31, 2012). Due to the possibility of data linkage error, 157 cases were excluded from the final analysis resulting in a final sample size of 46,783 (Figure 2.1).

2.3.1 Characteristics of the participants

Table 2.2 shows the characteristics of the sample population. In brief, the majority of the individuals were 35-44 years of age (31.5%; n=14,733), born between 1945 and 1964 (61.9%; n=28,977), male (63.6%; n=29,748), and from most-deprived neighborhoods (36.3%;

n=16,969). Overall, 22.2% (n=10,395), 22.5% (n=10,509), and 16.7% (n=7,819) of the participants had a history of baseline injection drug use, problematic alcohol use, and major mental illness, respectively. The proportions of people coinfected with HIV, HBV, and primary T-cell deficiencies were 3.1% (n=1,429), 1.1% (n=516), and 0.2% (n=114), respectively. Of 3,268 participants with known HCV genotype, most (62.6%, n=2,044) were of genotype 1 followed by genotype 3 (24.5%, n=802) and genotype 2 (11%, n=258); the rest were genotype 4, 5, 6 or mixed.

2.3.2 Spontaneous clearance

A quarter (25.1%, n=11,737) of all eligible participants spontaneously cleared their infection. Females cleared more frequently (32%) than the males (21%), while people with primary T-cell immunodeficiency at baseline had much lower clearance rate (14%) compared to those without (25%). People coinfected with HBV at baseline had much higher rate of clearance (42%) compared to those without HBV (25%). Genotype 3 (17%) had more than twice the clearance rate of genotype 1 (8%) (Table 2.2).

2.3.3 Factors associated with spontaneous clearance in overall sample

Table 2.3 shows unadjusted and adjusted estimates of the effect of primary T-cell immunodeficiency on spontaneous clearance. In unadjusted analysis, all the variables except HIV, and diabetes at baseline were significantly associated with spontaneous clearance. After adjusting for birth cohort, sex, year of HCV diagnosis, HCV genotypes, HBV, major mental illness, injection drug use, problematic alcohol use, and material deprivation at baseline, people with primary T-cell immunodeficiency had 45% lower odds (aOR: 0.55, 95% CI: 0.32-0.94) of

spontaneous clearance of HCV compared to those without. People coinfected with HBV had 2.31 times the odds (aOR: 2.31, 95% CI: 1.93-2.77) of clearing the infection spontaneously, while HCV genotype 3 was found to have more than twice the odds (aOR: 2.23, 95% CI: 1.74-2.86) of spontaneous clearance compared to HCV genotype 1, and females had higher odds (aOR: 1.61, 95% CI: 1.54-1.68) of spontaneous clearance of HCV compared to males (Table 2.3).

2.3.4 Factors associated with spontaneous clearance among seroconverters

Additional analysis on seroconverters (n=6,238), who had a relatively well-defined infection onset date, showed that primary T-cell immunodeficiency was associated with a lower clearance (aHR: 0.25, 95% CI: 0.06-0.99) adjusting for all the variables mentioned above, while females were found to have higher likelihood of spontaneous clearance of HCV (aHR: 1.61, 95% CI: 1.47-1.76) (Table 2.4). These findings were similar in a sensitivity analysis (Table 2.5) among the seroconverters with two PCRs (n=4,610) where 25.6% (n=1,182) cleared the infection spontaneously (defined as two consecutive negative PCR test, at least 28 days apart). Since the interval between the last negative and first positive HCV tests (in the seroconverters) was longer (median: 2, IQR: 0.9-4.4 years), additional analysis was conducted among the seroconverters who did so within 24 months (n=3,101). The rate of spontaneous clearance in this group was 34.6% (n=1,074). However, the effect of primary T-cell immunodeficiency could not be assessed due to insufficient cases (four cases of primary T-cell immunodeficiency; none spontaneously cleared the infection).

2.4 Discussion

Using a large population-based cohort, this study shows that overall spontaneous clearance was 25%, similar to other published studies.[50] Spontaneous clearance was significantly lower among those with primary T-cell immunodeficiency and older birth cohorts. Conversely, higher clearance rates were observed among females, people with genotype 3 infections, and those coinfected with HBV. Primary T-cell immunodeficiency was associated with lower likelihood of spontaneous clearance of HCV infection both in the overall study population and among seroconverters. These findings are supported by the results from immunological studies [124-126] demonstrating that HCV-specific T-cell response is associated with successful clearance of HCV infection. The population-level prevalence of primary T-cell immunodeficiency is low, thus primary T-cell deficiency may not have substantial population level impact on HCV clearance. However, clearance is low among these patients; as a result, they require treatment to prevent progressive liver diseases. As clinical intervention models move towards treating people earlier in the course of illness, people with primary T-cell immunodeficiency remain an important priority. However, results from the analysis on seroconverters should be interpreted with caution due to very low number of spontaneous clearance cases among those with primary T-cell deficiency.

HBV coinfection was associated with a higher likelihood of spontaneous clearance of HCV which is consistent with previous findings.[121, 127, 149] The study by Grebely *et al.* on PWID [89] also found a similar trend but did not reach statistical significance, which may be due to very small number of cases with viral clearance (n=9). Previous research suggested that HBV superinfection may supress pre-existing HCV infections.[150] This is supported by in-vitro and

in-vivo evidence that interferon-γ (IFNγ), interferon-α, and tumor necrosis factor-α, released by host inflammatory cells in response to superimposed HBV infection, have been found to inhibit HCV replication.[151] Reciprocal inhibition between HBV and HCV was reported but was not extensively examined. In the analyses on seroconverters in this study, where HBV infection preceded HCV infection, the HBV infection was not found to be a statistically significant predictor of spontaneous clearance. This may largely be due to the fact that seroconverters are distinct group of people (Table 2.6). Compared to non-seroconverters, they are younger (>60% aged below 35 years vs. 22% among the non-seroconverters), more represented by females (45% vs. 35%), with a higher proportion of people with HIV coinfection (4.23% vs. 2.87%), injection drug use (47% vs. 18%), problematic alcohol use (36% vs. 20%), and major mental illnesses (33% vs. 14%). However, further research is required to disentangle the consequence of co- and super-infection of HBV on the natural history of HCV.

Hepatitis C virus genotype 3 was found to have twice the odds of spontaneously clearing the infection compared to genotype 1. This finding is consistent with previous epidemiological studies,[152, 153] while other studies reported higher clearance for genotype 1.[154-156] It was however no longer significant in the time to spontaneous clearance analysis among seroconverters. Moreover, a vast majority of the genotype was ‘unknown’, which most likely consists of genotypes 1 and 3, as these are the dominant HCV strains in Canada. It is likely that genotype 1 clears faster, and thus remains ‘untypable’ contributing highly to the ‘unknown’ category. This analysis also found females to have higher likelihood of spontaneous clearance of HCV compared to males, which was maintained across the sensitivity analyses among the seroconverters, and is consistent with the findings from other epidemiological studies.[89, 121]

This disparity in spontaneous clearance of HCV has been linked with sex hormones, particularly oestrogen.[157] For example, 17β-estradiol (the most potent physiological oestrogen) was found to inhibit mature virion production in cultured hepatic cells.[158] Moreover, oestrogen was found to have supressed the expression of hepatic scavenger receptors, critical for viral entry, while testosterone was found to have enhanced the expression of the same thus facilitating viral entry.[159] Some epidemiological studies found that the effect of IL28B polymorphism on the spontaneous clearance of HCV is affected by sex [154, 160] while other failed to find an association emphasizing on the mode of HCV acquisition.[109] However, this study was not able to assess this effect.

Of all the HCV positive individuals, 29% did not further test for HCV-PCR (and ultimately were not eligible for this analysis. These individuals were compared to those who were included in the analyses. No significant differences were observed between these two groups (Table 2.7). Estimated date of HCV seroconversion was calculated by taking the midpoint of last negative and first positive PCR, and estimated date of HCV clearance by taking the midpoint of the last positive and first negative PCR following HCV diagnosis, as has been the standard practice in the HCV literature so far.[44, 76] In practice, this is actually a case of interval censoring, and thus regression models addressing the interval-censored data would appear to be a better fit. However, to maintain comparability of the results with the existing literature, interval censoring was not applied. Moreover, the non-informative censoring assumptions behind the standard methods for censored data are questionable in this context. Generally, the assumption is independence of censoring times and event times given the covariates included in the model. But when the censoring times are testing times, there may be

violation of this assumption. Moreover, it has been shown that using midpoint is as robust as using the earliest day of HCV transitions.[161]

The sensitivity of different HCV-PCR assays changed over time, and may impact classification of spontaneously cleared cases. Most of the quantitative HCV-PCR tests were validated by a more sensitive qualitative test up until 2007, after which quantitative PCR assays were as sensitive (RNA detection level: 10-15 IU/mL) as the qualitative test. Between 2000 and 2006, a small proportion (2.95% of those tested for RNA) of quantitative test results with lower limit of RNA detection of 615 IU/mL (all negative) were not verified by a qualitative test. In this analysis, 227 negative HCV-PCR test results (HCV RNA <615 IU/mL) were not validated by a qualitative test. Since this was the test used in practice back then, potentially all of them could well be HCV negative. However, this study attempted to estimate the maximum number of HCV cases (assuming all of them had RNA levels between 15 and 615 IU/mL) that might have been HCV positive but test results were negative due to inability of the less sensitive assays to detect them. Therefore, the lowest estimate of spontaneous clearance rate would be 24.6% (n=11,510).

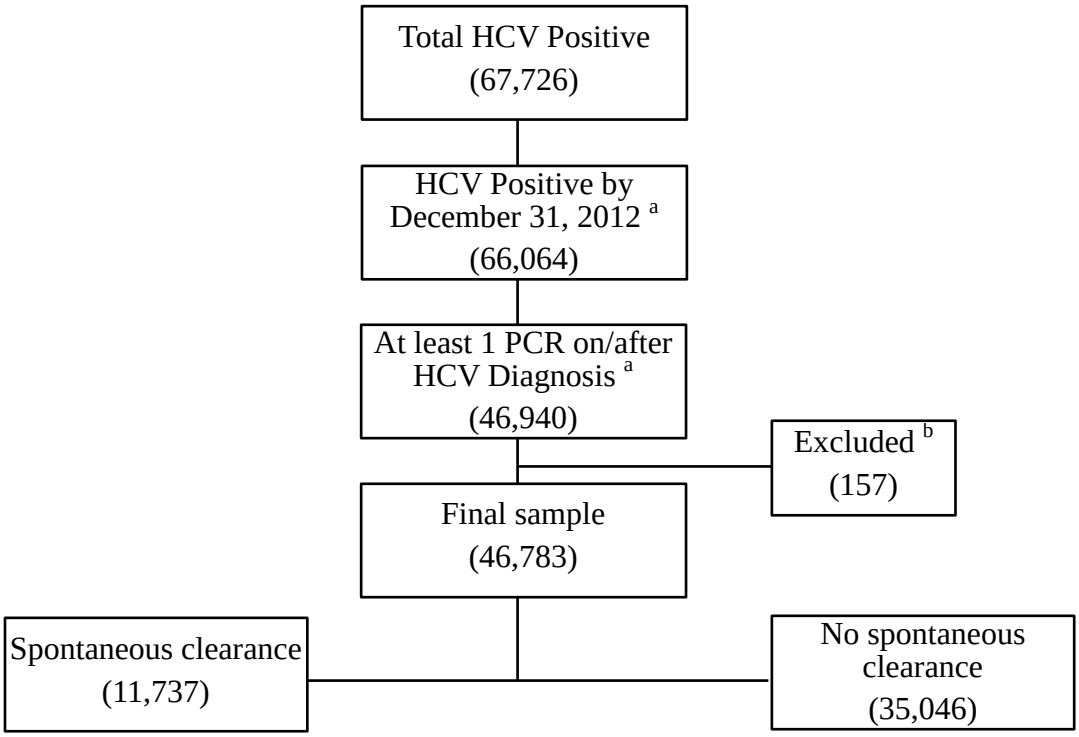
This is the largest population-based study to date with sufficient follow-up time to examine the effect of primary T-cell immunodeficiency, HBV coinfection, and other factors on the spontaneous clearance of HCV. Covariates used in the analysis were defined at baseline to disentangle the temporal issues of comorbidities and other risk factors. Moreover, several sensitivity analyses were conducted to validate the primary findings. Injection drug use variable was previously validated against interview-based data [162] while problematic alcohol use, diabetes, and major mental illness variables were derived from administrative codes validated by others. However, since these variables were defined using health services utilization codes, there

is a possibility of underestimation and misclassification depending on health care utilization patterns, miscoding, and physician billing practices. Another limitation of this study is a lack of access to ethnicity data. While ethnicity was reported to be associated with spontaneous clearance of HCV,[89] it was not found to be a significant predictor in other studies[127] including a systematic review and meta-analysis conducted earlier.[50]

2.5 Conclusion

In conclusion, 25% of HCV infected individuals cleared infection spontaneously. Primary T-cell immunodeficiency was associated with a lower likelihood of spontaneous clearance, while coinfection with Hepatitis B, females, and infections with HCV genotype 3 were associated with a higher likelihood of spontaneous clearance of HCV infections. These findings coincide with existing theories of the role of immune system in HCV clearance. They will also be useful in informing follow-up and treatment decisions in the era of highly effective direct-acting antivirals, pointing to population subgroups who could be prioritized to receive HCV treatment.

Figure 2.1 Schematic presentation of analytic sample for the study of spontaneous clearance of hepatitis C in British Columbia, Canada



[a] Date of HCV diagnosis was restricted to December 31, 2012 to allow sufficient time to observe spontaneous clearance; [b] Excluded are those who received treatment when they were HCV negative (possibly data linkage error); HCV: Hepatitis C Virus; PCR: Polymerase Chain Reaction

Table 2.1 Definitions of comorbid conditions for the BC Hepatitis Testers Cohort and current analysis (spontaneous clearance)

Primary T-cell immunodeficiency Primary T-cell immunodeficiency was diagnosed at the first occurrence of 1 MSP or 1 hospitalization codes for Combined immunodeficiencies, Wiskott–Aldrich syndrome, Di George's syndrome, Common variable immunodeficiency with predominant immunoregulatory T-cell disorders, Common variable immunodeficiency with autoantibodies to B- or T-cells, Lymphocyte function antigen-1 defect, Friedreich's ataxia, or other spinocerebellar diseases including Ataxia-telangiectasia. Physician Billing Data: MSP ICD-9 diagnostic codes: starting with 2791, or exact codes 2792, 3340, 3348 Hospitalization Data: DAD1/ICD-9-CM: exact codes 27906, 27910-2, 2792, 3340, 3348; DAD2/ICD-10-CA: starting with D81, or exact codes D82.0, D82.1, D83.1, D83.2, D84.0, G11.1, G11.3, G11.8
Diabetes Diabetes was defined at the first occurrence of 2 MSP or 1 hospitalization codes for diabetes-specific diagnostic codes. Physician Billing Data: MSP ICD-9 diagnostic codes: starting with 250. Hospitalization Data: DAD1/ICD-9-CM: starting with 250; DAD2/ICD-10-CA: starting with E10-4
Major Mental Illness Major mental illness was flagged at the first occurrence of either 1 hospitalization diagnostic code or 2 MSP diagnostic codes from a psychiatrist visit for schizophrenic, bipolar, delusional, nonorganic psychotic, adjustment, anxiety, dissociative, personality and major depressive disorders. Physician Billing Data: MSP ICD-9 diagnostic codes: starting with 295-298, 300-301, 308-309, 311 AND claim specialty = 3 Hospitalization Data: DAD1/ICD-9-CM: starting with 295-298, 300-301, 308-309, 311; DAD2/ICD-10-CA: starting with F20-F25, F28-F34, F38-F45, F48, F60-F61
Injection Drug Use Injection drug use was defined at the first occurrence of 1 MSP or 1 hospitalization diagnostic codes for major drug-related diagnoses involving addiction, dependence, and drug-induced mental disorders; illicit drug use most likely to be injectables (e.g., excluding cannabis), or illicit use of prescribed drugs including: hallucinogens, barbituates/tranquillizers, sedatives, hypnotics, anxiolytics, opioids, cocaine, amphetamine, volatile solvents; or discharge to drug rehabilitation, counselling, surveillance or methadone/buprenorphine substitution treatment. **Physician Billing Data**: MSP ICD-9 diagnostic codes: starting with 292, 3040, 3042, 3044, 3046-9, 3054-7, 3059, 6483, 9650, 9697, 970, E8500, or exact codes V6542 or fee item = 39 **Hospitalization Data**: **DAD1/ICD-9-CM**: starting with 292, 3040, 3042, 3044, 3046-9, 3054-7, 3059, 6483, 9650, 9697, 970, E8500; **DAD2/ICD-10-CA**: starting with F11, F13-5, F18, F19, T42, or exact codes T401, T402, T404-6, T436, T438, T439, T507.
Problematic Alcohol Use Problematic alcohol use was defined at the first occurrence of 2 MSP or 1 hospitalization codes for major alcohol-related diagnoses including alcoholic mental disorders and dependence/abuse syndromes; alcoholic polyneuropathy, myopathy, cardiomyopathy; pseudo Cushing's syndrome; or discharge to alcohol rehabilitation, counselling, or surveillance. Physician Billing Data: MSP ICD-9 diagnostic codes: starting with 291, 303, 3050, 3575, 4255 Hospitalization Data: DAD1/ICD-9-CM: starting with 291, 303, 3050,3575, 4255; DAD2/ICD-10-CA: starting with F10, E244, G312, G621, G721, I426, Z502, Z714

Table 2.2 Characteristics of HCV positive cases with at least one PCR test on/after HCV diagnosis in British Columbia, Canada

Characteristics	Total (n=46,783)	Spontaneous clearance (n=11,737)	% Spontaneous Clearance
Age at HCV diagnosis (years)			
<25	3278 (7.0)	1309 (11.2)	39.9
25-34	9271 (19.8)	2817 (24.0)	30.4
35-44	14733 (31.5)	3513 (29.9)	23.8
45-54	12877 (27.5)	2678 (22.8)	20.8
≥55	6624 (14.2)	1420 (12.1)	21.4
Median age [IQR]	42 [34-50]	39 [31-48]	
Birth Cohort			
<1945	3391 (7.2)	710 (6.1)	20.9
1945-1964	28977 (61.9)	6370 (54.3)	22.0
1965-1974	9106 (19.5)	2678 (22.8)	29.4
≥1975	5309 (11.3)	1979 (16.9)	37.3
Female	17035 (36.4)	5366 (45.7)	31.5
Year of HCV diagnosis			
1990-1994	3200 (6.8)	873 (7.4)	27.3
1995-1999	15662 (33.5)	3869 (33.0)	24.7
2000-2004	12916 (27.6)	2912 (24.8)	22.6
2005-2009	10507 (22.5)	2860 (24.4)	27.2
2010-2013	4498 (9.6)	1223 (10.4)	27.2
HCV Genotypes			
Genotype 1	2044 (4.4)	159 (1.4)	7.8
Genotype 3	802 (1.7)	135 (1.2)	16.8
Genotype- other*	422 (0.9)	36 (0.3)	8.5
Genotype- unknown	43,515 (93.0)	11407 (97.8)	26.2
HIV**	1429 (3.1)	333 (2.8)	16.4
Primary T-cell immunodeficiency**	114 (0.2)	16 (0.1)	14.0
Hepatitis B**	516 (1.1)	216 (1.8)	41.9
Diabetes (type-2)**	783 (1.7)	207 (1.8)	26.4
Major mental illness**	7819 (16.7)	2098 (17.9)	26.8
Injection drug use**	10395 (22.2)	2904 (24.7)	27.9
Problematic alcohol use**	10509 (22.5)	2782 (23.7)	26.5
Material deprivation quintile**			
Q1 (most privileged)	6209 (13.3)	1532 (13.1)	24.7
Q2	7236 (15.5)	1760 (15.0)	24.3
Q3	8312 (17.8)	2030 (17.3)	24.4
Q4	10523 (22.5)	2569 (21.9)	24.4
Q5 (most deprived)	13395 (28.6)	3522 (30.0)	26.3
Unknown	1108 (2.4)	324 (2.8)	29.2
Social deprivation quintile**			
Q1 (most privileged)	5042 (10.8)	1213 (10.3)	24.1
Q2	6018 (12.9)	1492 (12.7)	24.8
Q3	7859 (16.8)	1927 (16.4)	24.5
Q4	9787 (20.9)	2423 (20.6)	24.8
Q5 (most deprived)	16969 (36.3)	4358 (37.1)	25.7
Unknown	1108 (2.4)	324 (2.8)	29.2

*: Other includes genotype 2, 4, 5, 6, and Mixed; **: on/before HCV diagnosis; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; IQR: Interquartile range

Table 2.3 Unadjusted and adjusted odds ratios for factors associated with spontaneous clearance in British Columbia, Canada

Characteristics	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Age at HCV diagnosis (years)		<0.0001		
<25	2.44 (2.22-2.67)			
25-34	1.60 (1.49-1.72)			
35-44	1.15 (1.07-1.23)			
45-54	0.96 (0.90-1.04)			
≥55	*Ref*			
Birth Cohort		<0.0001		<0.0001
<1945	0.45 (0.40-0.49)		0.44 (0.40-0.49)	
1945-1964	0.47 (0.45-0.51)		0.50 (0.47-0.54)	
1965-1974	0.70 (0.65-0.75)		0.72 (0.67-0.78)	
≥1975	*Ref*		*Ref*	
Female	1.69 (1.62-1.76)	<0.0001	1.61 (1.54-1.68)	<0.0001
Year of HCV diagnosis		<0.0001		<0.0001
1990-1994	1.01 (0.91-1.11)		1.23 (1.10-1.36)	
1995-1999	0.88 (0.82-0.95)		1.03 (0.96-1.12)	
2000-2004	0.78 (0.72-0.84)		0.87 (0.81-0.94)	
2005-2009	1.00 (0.93-1.08)		1.04 (0.96-1.13)	
2010-2013	*Ref*		*Ref*	
HCV Genotypes		<0.0001		<0.0001
Genotype 1	*Ref*		*Ref*	
Genotype 3	2.40 (1.88-3.07)		2.23 (1.74-2.86)	
Genotype- other*	1.11 (0.76-1.61)		1.13 (0.78-1.66)	
Genotype/unknown	4.21 (3.58-4.96)		4.19 (3.55-4.94)	
HIV**	0.91 (0.80-1.02)	0.1140		
Primary T-cell immunodeficiency**	0.49 (0.29-0.83)	0.0076	0.55 (0.32-0.94)	0.0292
Hepatitis B**	2.17 (1.82-2.59)	<0.0001	2.31 (1.93-2.77)	<0.0001
Diabetes (type-2)**	1.08 (0.92-1.26)	0.3719		
Major mental illness**	1.12 (1.06-1.18)	<0.0001	0.98 (0.92-1.04)	0.4517
Injection drug use**	1.21 (1.15-1.27)	<0.0001	1.03 (0.97-1.09)	0.3417
Problematic alcohol use**	1.10 (1.05-1.15)	0.0002	1.09 (1.03-1.15)	0.0015
Material deprivation quintile**		<0.0001		0.0003
Q1 (most privileged)	*Ref*		*Ref*	
Q2	0.98 (0.91-1.06)		0.97 (0.89-1.05)	
Q3	0.99 (0.91-1.07)		0.97 (0.90-1.05)	
Q4	0.99 (0.92-1.06)		0.96 (0.89-1.03)	
Q5 (most deprived)	1.09 (1.02-1.17)		1.05 (0.98-1.13)	
Unknown	1.26 (1.10-1.45)		1.25 (1.08-1.44)	
Social deprivation quintile**		0.0025		
Q1 (most privileged)	*Ref*			
Q2	1.04 (0.95-1.14)			
Q3	1.03 (0.94-1.11)			
Q4	1.04 (0.96-1.12)			
Q5 (most deprived)	1.09 (1.01-1.17)			
Unknown	1.31 (1.13-1.51)			

*: Other includes genotype 2, 4, 5, 6, and Mixed; **: on/before HCV diagnosis; HCV: Hepatitis C Virus; HBV: Hepatitis B Virus; HIV: Human Immunodeficiency Virus; OR: Odds Ratio: CI: Confidence Interval

Table 2.4 Cox proportional hazards model examining the factors associated with spontaneous clearance among HCV seroconverters in British Columbia, Canada

(N=6,238)

Characteristics	Spontaneous clearance (N=2,054)	% spontaneous clearance	Adjusted HR (95% CI)	p-value
Birth Cohort				<0.0001
<1945	31 (1.51)	33.0	1.15 (0.80-1.65)	
1945-1964	474 (23.08)	28.7	0.78 (0.69-0.88)	
1965-1974	635 (30.92)	30.3	0.78 (0.70-0.86)	
≥1975	914 (44.5)	38.1	*Ref*	
Female	1115 (54.28)	40.1	1.61 (1.47-1.76)	<0.0001
Year of HCV diagnosis				<0.0001
1990-1994	57 (2.78)	46.72	0.38 (0.28-0.52)	
1995-1999	397 (19.33)	30.56	0.26 (0.21-0.31)	
2000-2004	774 (37.68)	33.20	0.35 (0.30-0.42)	
2005-2009	641 (31.21)	33.08	0.47 (0.40-0.55)	
2010-2013	185 (9.01)	33.76	*Ref*	
HCV Genotypes				<0.0001
Genotype 1	48 (2.34)	16.96	*Ref*	
Genotype 3	46 (2.24)	25.84	1.43 (0.96-2.15)	
Genotype- other*	8 (0.40)	13.56	0.70 (0.33-1.48)	
Genotype-/unknown	1952 (95.03)	34.14	2.40 (1.80-3.20)	
Primary T-cell immunodeficiency**	2 (0.1)	7.69	0.25 (0.06-0.99)	0.049
Hepatitis B**	47 (2.29)	30.32	1.02 (0.76-1.37)	0.892
Major mental illness**	637 (31.01)	30.80	0.91 (0.83-1.01)	0.085
Injection drug use**	930 (45.28)	31.72	0.91 (0.83-1.00)	0.048
Problematic alcohol use**	730 (35.54)	32.12	1.03 (0.94-1.14)	0.482
Material deprivation quintile**				0.258
Q1 (most privileged)	272 (13.24)	35.23	*Ref*	
Q2	276 (13.44)	30.80	0.84 (0.71-1.00)	
Q3	336 (16.4)	34.11	0.94 (0.80-1.10)	
Q4	465 (22.6)	31.31	0.86 (0.74-1.00)	
Q5 (most deprived)	670 (32.6)	33.94	0.94 (0.82-1.08)	
Unknown	35 (1.7)	27.78	0.88 (0.62-1.25)	

*: Other includes genotype 2, 4, 5, 6, and Mixed; **: on/before HCV diagnosis; HCV: Hepatitis C Virus; PCR: Polymerase Chain Reaction; HR: Hazard Ratio: CI: Confidence Interval; Seroconverters: who had at least one negative HCV test recorded before testing positive for HCV.

Table 2.5 Cox proportional hazards model examining the factors associated with spontaneous clearance (defined as two consecutive negative PCR, ≥28 days apart) among HCV seroconverters in BC Hepatitis Testers Cohort (n=4,610)

Characteristics	Adjusted HR (95% CI)	p-value
Birth Cohort		<0.0001
<1945	1.10 (0.68-1.76)	
1945-1964	0.71 (0.62-0.82)	
1965-1974	0.74 (0.66-0.83)	
≥1975	*Ref*	
Female	1.62 (1.46-1.79)	<0.0001
Year of HCV diagnosis		<0.0001
1990-1994	0.43 (0.30-0.62)	
1995-1999	0.29 (0.23-0.37)	
2000-2004	0.38 (0.31-0.47)	
2005-2009	0.51 (0.41-0.62)	
2010-2013	*Ref*	
HCV Genotypes		<0.0001
Genotype 1	*Ref*	
Genotype 3	1.42 (0.95-2.13)	
Genotype- other*	0.71 (0.34-1.51)	
Genotype-/unknown	2.34 (1.75-3.12)	
Primary T-cell immunodeficiency**	0.77 (0.57-1.03)	0.074
Hepatitis B**	0.99 (0.71-1.39)	0.95
Major mental illness**	0.92 (0.82-1.03)	0.145
Injection drug use**	0.88 (0.79-0.98)	0.02
Problematic alcohol use**	1.04 (0.93-1.16)	0.512
Material deprivation quintile**		0.18
Q1 (most privileged)	*Ref*	
Q2	0.82 (0.67-0.99)	
Q3	0.88 (0.73-1.07)	
Q4	0.85 (0.71-1.01)	
Q5 (most deprived)	0.96 (0.81-1.13)	
Unknown	0.87 (0.56-1.34)	

*: Other includes genotype 2, 4, 5, 6, and Mixed; **: on/before HCV diagnosis; HCV: Hepatitis C Virus; PCR: Polymerase Chain Reaction; HR: Hazard Ratio: CI: Confidence Interval; Seroconverters: who had at least one negative HCV test recorded before testing positive for HCV.

Table 2.6 Comparison between the seroconverters and non-seroconverters in BC Hepatitis Testers Cohort

	Seroconverters (n=6,238)		Non-seroconverters (n=40,545)	
Characteristics	**% Total**	**% Spontaneous Clearance**	**% Total**	**% Spontaneous Clearance**
Age at HCV diagnosis (years)				
<25	22.12	39.93	4.68	39.94
25-34	38.51	32.47	16.94	29.65
35-44	26.71	29.35	32.23	23.14
45-54	9.63	30.62	30.28	20.32
≥55	3.03	26.46	15.87	21.29
Birth Cohort				
<1945	1.51	32.98	8.13	20.59
1945-1964	26.43	28.74	67.40	21.57
1965-1974	33.62	30.28	17.29	29.15
≥1975	38.44	38.12	7.18	36.59
Female	44.63	40.05	35.15	29.83
Year of HCV diagnosis				
1990-1994	1.96	46.72	7.59	26.51
1995-1999	20.82	30.56	35.42	24.17
2000-2004	37.37	33.20	26.11	20.2
2005-2009	31.07	33.08	21.13	25.9
2010-2013	8.78	33.76	9.74	26.28
HCV Genotypes				
Genotype 1	4.54	16.96	4.34	6.3
Genotype 3	2.85	25.84	1.54	14.26
Genotype- other/unknown*	92.61	33.93	94.12	24.85
HIV**	4.23	20.83	2.87	23.86
Primary T-cell immunodeficiency **	0.42	7.69	0.22	15.91
Hepatitis B**	2.48	30.32	0.89	46.81
Diabetes (type-2)**	1.51	31.91	1.70	25.69
Major mental illness**	33.15	30.80	14.18	25.4
Injection drug use**	47.00	31.72	18.41	26.45
Problematic alcohol use**	36.44	32.12	20.31	24.92
Material deprivation quintile**				
Q1 (most privileged)	12.38	35.23	13.41	23.17
Q2	14.36	30.80	15.64	23.41
Q3	15.79	34.11	18.07	23.12
Q4	23.81	31.31	22.29	23.28
Q5 (most deprived)	31.64	33.94	28.17	24.97
Unknown	2.02	27.78	2.42	29.43
Social deprivation quintile**				
Q1 (most privileged)	8.13	33.53	11.19	23
Q2	10.77	31.99	13.19	23.89
Q3	14.83	31.46	17.10	23.59
Q4	20.15	33.97	21.04	23.4
Q5 (most deprived)	44.10	33.30	35.07	24.21
Unknown	2.02	27.78	2.42	29.43

*: Other includes genotype 2, 4, 5, 6, and Mixed; **: on/before HCV diagnosis; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus. Seroconverters: who had at least one negative HCV test recorded before testing positive for HCV.

Table 2.7 Comparison of characteristics of participants at the BC Hepatitis Testers Cohort who had a valid HCV-PCR on/after HCV diagnosis to those who didn't

Characteristics	PCR on/after HCV diagnosis	
	Yes	**No**
	46,783 (71.0)	**19,124 (29.0)**
Age at HCV diagnosis (years)		
<25	3278 (7.0)	1092 (5.7)
25-34	9271 (19.8)	3986 (20.8)
35-44	14733 (31.5)	6385 (33.4)
45-54	12877 (27.5)	4406 (23.0)
≥55	6624 (14.2)	3255 (17.0)
Birth Cohort		
<1945	3391 (7.3)	2887 (15.1)
1945-1964	28977 (61.9)	11324 (59.2)
1965-1974	9106 (19.5)	3561 (18.6)
≥1975	5309 (11.4)	1352 (7.1)
Female	17035 (36.4)	5927 (31.0)
Year of HCV diagnosis		
1990-1994	3200 (6.8)	2325 (12.2)
1995-1999	15662 (33.5)	9479 (49.6)
2000-2004	12916 (27.6)	4246 (22.2)
2005-2009	10507 (22.5)	2102 (11.0)
2010-2012	4498 (9.6)	972 (5.1)
HIV*	1429 (3.1)	602 (3.2)
Primary T-cell immunodeficiency *	114 (0.2)	29 (0.2)
Hepatitis B*	516 (1.1)	338 (1.8)
Diabetes (type-2)*	783 (1.7)	481 (2.5)
Major mental illness*	7819 (16.7)	2761 (14.4)
Injection drug use*	10395 (22.2)	4080 (21.3)
Problematic alcohol use*	10509 (22.5)	4665 (24.4)
Material deprivation quintile*		
Q1 (most privileged)	6209 (13.3)	2216 (11.6)
Q2	7236 (15.5)	2856 (14.9)
Q3	8312 (17.8)	3150 (16.5)
Q4	10523 (22.5)	4016 (21.0)
Q5 (most deprived)	13395 (28.6)	5571 (29.1)
Unknown	1108 (2.4)	1315 (6.9)
Social deprivation quintile*		
Q1 (most privileged)	5042 (10.8)	1728 (9.0)
Q2	6018 (12.9)	2196 (11.5)
Q3	7859 (16.8)	2928 (15.3)
Q4	9787 (20.9)	3778 (19.8)
Q5 (most deprived)	16969 (36.3)	7179 (37.5)
Unknown	1108 (2.4)	1315 (6.9)

*: on/before HCV diagnosis; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus

Chapter 3: Incidence, risk factors, and prevention of hepatitis C reinfection

3.1 Background

As described previously, infection with hepatitis C virus is a major global public health problem.[2, 3] In developed countries, the principal mode of HCV transmission is injection drug use.[11, 12] New DAAs are well-tolerated with higher cure rates (95%). The introduction of these new treatments for HCV is expected to reduce morbidity and mortality. However, because neither spontaneous nor treatment-induced clearance of the virus confers immunity, reinfection remains a concern.[52, 56, 57, 72, 74] The high cost of DAAs poses concomitant concerns regarding potential reinfection risks, and this has fuelled debates regarding approaches to scaling-up treatment access [163, 164] especially amongst high-risk groups (e.g., PWID).[165-168]

Most studies assessing HCV reinfection rates were conducted in cohorts of high risk populations, e.g., PWID.[44, 45, 47-49, 51, 53, 55, 63, 64, 67-70, 72, 75] Studies on HCV reinfection following spontaneous clearance [44, 45, 47-49, 51-53, 55, 76] or SVR [45, 48, 63, 64, 67-70, 72, 77] reported a wide range of reinfection estimates. These reports were also limited by small numbers of reinfected cases limiting their ability to assess the factors associated reinfection risks. Thus, the validity of the inferences drawn from these studies may be prone to greater uncertainties. Furthermore, there is a need to assess factors increasing or decreasing the risk of reinfection to inform strategies to scale up newer HCV treatments for people at potentially higher reinfection risk. Co-occurring risk factors such as IDU and mental illnesses are associated with increased risk of HCV infection, and thus addressing these conditions are

paramount to reducing HCV reinfection risk.[169-171] This study estimated the reinfection rate and evaluated potential intervention options including the role of OST and mental health counseling on HCV reinfection risk among PWID. It was hypothesized that engagement with these services would reduce HCV reinfection risk. This is the largest population-level study to date to characterize reinfection risks following spontaneous clearance and SVR amongst individuals followed up for more than 19 years.

3.2 Research design and methods

3.2.1 Study cohort

The data for this analysis is based on BC-HTC as described in Chapter 1 (Table 1.1). Details of the BC-HTC database has been described in Chapter 1 and elsewhere [138, 139].

3.2.2 Case definition

The definition of an HCV case was described in Chapter 1. Spontaneous clearance was defined as two consecutive negative HCV-PCR tests, ≥28 days apart,[76] following HCV diagnosis without treatment. In the primary analysis, the date of spontaneous clearance was calculated as the midpoint between the last positive and first negative PCR following HCV diagnosis.[76] The first negative PCR date was used in the sensitivity analysis.

SVR was defined as two consecutive negative HCV-PCR tests, ≥28 days apart, 12 weeks after completion of treatment.[172] For this analysis, data was available only for interferon-based treatments. In the primary analysis, the date of SVR was calculated as the midpoint between 12-week post-treatment date and the date of the first negative PCR after this

date. The first negative PCR date was used in the sensitivity analysis.

Reinfection was defined as a positive HCV-PCR following clearance (spontaneous or SVR). In the primary analysis, the date of reinfection was calculated as the midpoint between the last negative and first positive PCR following clearance.[76] The first positive PCR date after clearance was used in the sensitivity analysis.

HIV diagnosis was defined as described in Chapter 1 and elsewhere.[138] The date of HIV diagnosis was the earliest date a person was diagnosed as a case of HIV. Mental health counseling, IDU, OST, and problematic alcohol use were defined based on ICD diagnostic and/or procedure codes and/or fee item codes from MSP or DAD or prescription databases, as applicable (Table 3.1). OST assessment is based on record of dispensed prescriptions in the centralized prescription database, PharmaNet, which records all prescriptions dispensed in the province. For the main analysis, mental health counseling was defined as any mental health counseling visit during the follow-up time. In the sensitivity analysis, it was defined as number of visits per year during the follow-up time to explore the association between the level of engagement with healthcare services and HCV reinfection risk. Material and social deprivation was based on Québec Index of Material and Social Deprivation.[143] Those with missing information on the material and social deprivation were classified as ‘unknown’.

3.2.3 Eligibility

The analysis included all HCV positive individuals who cleared their primary HCV infection spontaneously, or achieved SVR following HCV treatment, and those who had at least one valid HCV-PCR following spontaneous clearance or SVR. The laboratory results on HCV-

PCR were available until December 31, 2013. Therefore, to allow sufficient follow-up time to observe reinfections, the date of HCV spontaneous clearance was restricted up to December 31, 2012, and the treatment completion date up to July 16, 2012. The last date of follow-up was the date of reinfection for those who developed reinfection, and the last negative PCR on/before December 31, 2013 for those who did not develop reinfection. After applying these criteria, the enrollment period was from November 07, 1992 to December 31, 2013.

3.2.4 Data analysis

The incidence rates of HCV reinfection (per 100 person-years of follow-up), and corresponding 95% confidence intervals (CI), were calculated assuming a Poisson distribution. Bivariate relationships were explored by Cox proportional hazards (PH) models, and the unadjusted hazard ratios (HR) along with 95% CIs are presented. Variables based on a-priori hypotheses, and those significant at level 0.10 in the univariate analysis were included in the multivariable models, and adjusted HR (aHR) with 95% CIs are reported. Birth cohort, sex, and year of HCV diagnosis were included in all the models irrespective of their statistical significance in the univariate analysis; the first two were added as they are established risk factors of HCV, and the latter was used to adjust for varying testing pattern over time. To avoid non-identification problem (Age = Period − Cohort),[147] age was not included in the same adjusted models with birth cohort and HCV diagnosis year. Variables in the final multivariable model were also evaluated in the additional Cox PH models fitted separately in the spontaneous clearance, and the SVR group. Finally, the effects of mental health counseling, and OST were assessed among people with a history of injection drug use during the follow-up time by fitting another Cox PH model. Over the course of the study period, cohort members can be on OST and

off OST (defined as not taking OST for more than seven consecutive days); thus, this variable was used as a time-varying covariate. HIV status also was used as a time-varying covariate in all the analyses. To assess the robustness of using mid-points as the date of HCV transitions, as used in the primary analysis, the earliest date of transition was used in the sensitivity analysis. In observational studies, people who receive interventions are usually different from those who do not, which introduces treatment indication bias or confounding by indication. To correct for treatment indication bias, inverse probability of treatment weighting (IPTW) was applied. The propensity scores (PS) of receiving mental health counseling or OST (at each time-point) were computed using multivariable logistic regression. Propensity scores were used to construct the IPTW which were applied to the intervention (IPTW=1/PS) and control (IPTW=1/(1-PS)) groups.[173, 174] IPTW-weighted Cox PH models were fitted to estimate the effects of OST and mental health counseling on reinfection risk among PWID. All the analyses were conducted in [SAS/STAT] Software version [9.4].[148] All the tests were two-sided at a significance level of 0.05.

3.3 Results

Figure 3.1 shows the selection of participants in this study. After exclusion (n=14; excluded because they had two consecutive negative PCR tests, but the gap between the two tests was <28 days), a total of 5,915 cases with at least one valid HCV-PCR following primary clearance (3,690 cases of spontaneous clearance, and 2,225 cases of SVR) were included in this analysis. They were followed up for a median of 5.4 (IQR: 2.9-8.7) years, and the median time to reinfection was 3.0 (IQR: 1.5-5.4) years. Individuals included in this study had a median of 9 (IQR: 6-12) HCV-PCR tests with a median testing interval of 7.4 (IQR: 2.8-18.5) months. The

median (IQR) number of tests was 8 (6-10), and 10 (8-13) in those who spontaneously cleared, and those who achieved SVR, respectively. Median (IQR) testing interval was 8.7 (3.0-22.4), and 6.3 (2.8-14.2) months, in those who spontaneously cleared, and those who achieved SVR, respectively.

3.3.1 Characteristics of the study populations

A total of 452 (7.6%) cases developed reinfection, of which 402 (10.9%) were among those who cleared the primary infection spontaneously, and the rest (2.2%; n=50) were among those who achieved SVR following HCV treatment. Table 3.2 summarizes the characteristics of the overall sample, and also by the clearance type of their primary HCV episodes. The overall sample was predominantly younger (53% ages below 45 years) at HCV clearance, born before 1965 (63%; n=3,711), and male (59%; n=3,471). Proportions of PWID, and people coinfected with HIV (any time during the follow-up time), and those with ≥1 mental health counseling visits were 42% (n=2,493), 9% (n=533), and 27% (n=1,582), respectively

3.3.2 Incidence rate of HCV reinfection

The overall reinfection rate in this study was 1.27 (95% CI: 1.15-1.39) per 100 person-years of follow-up over a total of 35,672 person-years of follow-up, with higher rates (per 100 person-years) in spontaneous clearance group (1.59, 95% CI: 1.44-1.76) than in the SVR group (0.48, 95% CI: 0.36-0.63). Higher rates of reinfection were observed in people younger than 35 years, males, people coinfected with HIV, PWID, people with a history of problematic alcohol use, and those from most-deprived neighborhood, while lower rates were observed in those who were engaged with mental health counseling services. The reinfection rates among

PWID were 1.88 (95% CI: 1.66-2.12), and 1.14 (95% CI: 0.77-1.63) per 100 person-years, among those who cleared their previous HCV episode spontaneously, and those who achieved SVR, respectively (Table 3.3). In this analysis, stringent criteria were used to define spontaneous clearance and SVR (two consecutive negative PCR, ≥28 days apart). In additional analysis using one negative PCR to define spontaneous clearance and SVR, the rates of reinfection were higher than these estimates (Table 3.4). The cumulative incidence rates are shown in Figures 3.2-3.4 by clearance type of first infection, IDU status, and HIV coinfection status, respectively.

3.3.3 Factors associated with HCV reinfection

In the multivariable Cox PH model, birth cohort, female sex, spontaneous clearance, HIV coinfection, and injection drug use were significantly associated with HCV reinfection. After adjusting for other potential confounders, females had significantly lower likelihood of HCV reinfection (aHR: 0.57, 95% CI: 0.47-0.70). Compared to the SVR group, the risk of HCV reinfection was 2.71 times (aHR: 2.71, 95% CI: 2.0-3.68) for the spontaneous clearance group. The likelihood of HCV reinfection was significantly higher among people coinfected with HIV (aHR: 2.25, 95% CI: 1.78-2.85), and PWID (aHR: 1.55, 95% CI: 1.23-1.95) (Table 3.5). In both the spontaneous clearance and the SVR group, females had lower likelihood of reinfection while PWID and people coinfected with HIV had higher likelihood of reinfection (Table 3.6).

3.3.4 Effects of OST and mental health counseling and rehabilitation in PWID

In the adjusted Cox PH model, PWID who were on OST had a 27% lower likelihood (aHR: 0.73, 95% CI: 0.54-0.98) of HCV reinfection, and those who ever received mental health counseling services during the follow-up time were 29% less likely (aHR: 0.71, 95% CI: 0.54-

0.92) to develop HCV reinfection (Table 3.7). The joint effect (interaction) of OST and mental health counseling was found non-significant (p=0.326).

3.3.5 Sensitivity analysis

Further sensitivity analysis was conducted using the number of mental health counseling visits per year during the follow-up time. About 20% (n=1,185) of the participants had at least one mental health counseling visit per year during the follow-up time; 8.5% (n=501) had one visit, and the rest (11.6%, n=684) had ≥2 visits per year. In the adjusted Cox PH model, compared to those who received no visits, those with one visit per year had a 68% reduced risk (aHR: 0.32, 95% CI: 0.2-0.51) of reinfection while those with ≥2 mental health counseling visits had a 33% reduced risk (aHR: 0.67, 95% CI: 0.48-0.93) of HCV reinfection (Table 3.8). The joint effect (interaction) of OST and mental health counseling was non-significant as in the original analysis (p=0.885). Additional Cox PH models using single negative PCR for the assessment of clearance showed similar results as found in the main analysis (Table 3.9). Analysis using the earliest date of HCV transitions also yielded similar findings as reported in the main analysis (Table 3.10). In the IPTW-weighted analysis of mental health counseling, the aHR (95% CI) was 0.70 (0.58-0.84), and 0.71 (0.58-0.87), for mental health counseling and OST, respectively, while these estimates were 0.71 (0.59-0.86), and 0.73 (0.59-0.90), respectively, with IPTW of OST receipt.

3.4 Discussion

The incidence rate of HCV reinfection was higher among those who cleared their primary infections spontaneously compared to those who achieved SVR following HCV

treatment. The risk of HCV reinfection was much lower in females, while it was higher in the spontaneous clearance group, those coinfected with HIV, and among PWID. Being on OST and being engaged with mental health counseling services were independently associated with a significantly lower likelihood of HCV reinfection among PWID. These findings have important implications for post-clearance follow-up, and interventions for prevention of reinfections in the DAA era when HCV treatment is being scaled-up to PWID in many countries across the world.

The post-SVR reinfection estimate among PWID in this study was 1.14 (95% CI: 0.77-1.63) per 100 person-years, while the estimates reported in earlier smaller studies were wider ranging between 0 and 5 per 100 person-years.[45, 48, 63, 64, 67-70, 72, 77] Wider range of reinfection rates reported in these studies may be due to varying sample size and study population in addition to any differences in the availability of harm reduction programs and population risk activities.

The HCV reinfection rate among the PWID in the spontaneous clearance group was 1.88 (95% CI: 1.66-2.12) per 100 person-years in this study with a higher estimate using single negative PCR for clearance. Earlier studies reported a wide range of estimates between 0 and 46.8 per 100 person-years.[44, 45, 47-49, 51-53, 55, 76] This study found a lower reinfection rate in the SVR group compared the spontaneous clearance group, which is consistent with the findings from previous smaller reports including a meta-analysis though different from a recent study on HIV positive MSM in which reinfection rate was lower among those with spontaneous clearance.[57, 165, 175, 176] The difference in the reinfection rates between the spontaneous clearance and the SVR groups is likely due to differences in their characteristics including their risk factors. Compared to the SVR group, participants in the spontaneous clearance group were

younger (< 45 years: 66% vs. 31%), with higher proportions of female (44% vs. 37%), PWID (52% vs. 25%), people coinfected with HIV (11% vs. 5.7%), people with a history of problematic alcohol use (44% vs. 26%), and people from lower socioeconomic status (39% vs. 30%) (Table 3.2). People with HIV coinfection and substance use were less likely to be treated with interferon-based treatments due to potential toxicity, tolerability and adherence concerns.[172] Thus, there was an under-representation of PWID in the SVR group in this study. Restriction to treatment access has been documented in other studies.[42, 177, 178] Highly effective, well-tolerated DAA treatments open up opportunities to reduce disease burden, and potentially reduce transmission providing overall population benefits in addition to individual health benefits, especially in PWID. However, as seen in the spontaneous clearance group, reinfection rate among PWID following SVR in DAA era could increase unless accompanied by appropriate interventions to prevent reinfection.[179] Future research in the era of DAA will delineate this issue in terms of long-term benefits of treatment coupled with harm reduction services.

Within the context of expansion of HCV treatment with DAA to high-risk populations such as PWID, this study provides important evidence on the impacts of OST and mental health counseling on HCV reinfection. The results from this study showed that engagement with these harm reduction initiatives is associated with significant reductions in HCV reinfection risk. This is the first study to examine the association of mental health counseling with HCV reinfection risk among PWID, which is important in light of higher risk of HCV infection among PWID with psychiatric comorbidities.[180] Building on evidence previously established through mathematical modelling studies,[168, 181] the findings of the

current study show that the reinfection risk could be reduced if treatment is accompanied by OST or treatment is provided with the OST programs. In addition, other harm reduction activities (e.g., syringe distribution, expansion of safer injection facilities) may need to be scaled-up to reduce risk of HCV reinfection among injection non-opioid users and to provide broader public health benefits as well as new access points to low-threshold healthcare services for people at high risk of HCV (re)infection. Further research in the era of DAA would be helpful in understanding the changing risk behavior and potential benefits of these harm reduction initiatives.

In further analysis (Table 3.8) to examine the impact of level of engagement with mental health counseling, it was found that an increase in the number of visits was not associated with a linear increase in the reduction of reinfection risk. This is because more visits to mental health counseling may be associated with higher risk factors instead, rather than representing those who are more health aware and engaged with the healthcare services and utilization. People who are accessing multiple counseling sessions may represent those at higher behavioral risk of infection. Thus, the relationship between mental health counseling and reinfection risk does not appear to be linear, as observed in this study. This requires further in-depth investigation.

In this study, HIV coinfection was found to increase the risk of HCV reinfection, which is supported by a study on prisoners.[72] HIV could impact reinfection risk by affecting immune response, or could be a proxy for high-risk IDU or high-risk sexual behavior among MSM.[36, 131, 182] Due to common route of transmission, and greater HCV reinfection risk, HIV-HCV coinfected individuals may benefit more from harm reduction efforts.

The rate of reinfection was higher among people coinfected with HIV than the rate among PWID, which is consistent with the results from earlier studies.[35, 131, 183] However, comparing the rates between these two groups of people, as reported in the published literature, may require considerations of the characteristics of the participants, HCV infection status, and type of risk factors they are involved in.[179] Most of the studies conducted in PWID involved cases of chronic HCV without ongoing risk behavior, while those conducted in HIV-coinfected MSM mostly included cases of acute HCV infection with ongoing risk behavior.[179] Another important difference is that the MSM often have multiple risk factors, injection drug use being one of them. Moreover, HIV coinfection is associated with compromised immunity, which is a biological risk factor for HCV. Thus, compared to PWID, HIV-HCV coinfected MSM are probably at greater risk of HCV disease progression.[179]

Overall incidence of reinfection among PWID reported in these analyses appears to be lower than that published in the smaller reports, including those among MSM. Besides the issues of sample size, underlying population risk behavior, study design, HCV disease stages (acute vs. chronic), this difference may be attributed to varying definitions used to identify PWIDs. First, injection drug use is a stigmatized risk behavior which is often underreported.[184] Second, the definition used in this analysis cannot perfectly identify all the PWIDs. The sensitivity and specificity of the PWID definition used in this study was 78.2%, and 72.3%, respectively. Together with underreporting, many of the PWIDs might have been misclassified as non-PWIDs and vice versa. This also explains why the incidence rate of reinfection among non-PWIDs in this study was higher than expected. This was also evident when the reinfection incidence was higher when spontaneous clearance was defined based on

one negative PCR. Previous study from the same cohort reported that PCR testing was poorer in specific group of people including men.[172] Therefore, using more stringent criteria of two consecutive negative PCR ≥28 days apart might have missed many PWIDs.

Females were found have a lower likelihood of HCV reinfection. Both biological and behavioral factors might have contributed to this phenomenon. As noted in Chapter 2, females were more likely to spontaneously clear their first infections. This has been linked with sex hormones, particularly oestrogen, which was found to inhibit mature virion production, and to reduce the likelihood of viral entry by suppressing the expression of hepatic scavenger receptors. [157, 158] On the contrary, testosterone was found to have facilitated viral entry by enhancing the expression of the same receptor.[159] Besides these biological factors, gender disparities in the HCV risk behaviors and comorbidities might have played a significant role as well. As reported earlier, compared to women, men are more likely to use almost all types of illicit drugs and alcohol.[185, 186] Studies also suggest that women tend to use smaller amounts of heroin and for shorter period of time.[187] They are also less likely to inject heroin.[187] With regard to comorbidities, the reported rates of mental health illnesses including depression among women are much higher than that in men.[188-190] This becomes yet more complicated due to gender disparities in sexual behavior (e.g., condom use),[189, 191] coping styles,[192] and condom-negotiation capacity.[193] These factors also are associated in multidimensional ways.[194-197] For example, coping has been reported to interact with stress,[192] condom negotiation skill has been reported to interact with depression,[193] and gender interacts in the association between mental health and sexual risk behavior.[189] More research regarding the

biological and social mechanisms underlying disparities between males and females in terms of spontaneous clearance and HCV reinfection is warranted.

In both univariate and multivariate analysis, earlier years of HCV diagnosis were associated with a lower reinfection risk. Intuitively, it would be expected that the longer a person in the study, the higher the number of HCV tests, and thus the higher the likelihood of being detected as a case of reinfection. While this variable was added to the multivariable models to structurally adjust for this, the findings are likely related to changing risk behavior patterns over time. In earlier days (particularly before 1998), most of the HCV cases were a mix of those acquired via blood transfusion and injection drug use. Over the period of time, as people age, their drug use pattern may have changed. Earlier studies found older and experienced PWID to be less likely to share needles compared to younger PWID.[198, 199] Previous research based on HC-HTC data [200] showed that HCV incidence was much lower in older birth cohorts compared to younger birth cohorts, which is also supported by several other studies that found a lower rate of reinfection in older populations.[48, 63, 69, 175]

This is the largest population-based study with the longest follow-up time to examine HCV reinfections, both among those who spontaneously cleared the primary HCV infection and those who achieved SVR. HCV testing is centralized at BCCDC-PHL, which enhances the completeness of the testing data. However, there could be missing tests because of non-linkage if identifiers were not available. Furthermore, HCV tests were not done at regular intervals which may have missed some episodes of clearance and reinfections in the intervals between testing as suggested by mathematical modeling.[201] As a result, the estimates of reinfection from this study may be an underestimation. While HCV testing at regular intervals

would improve the accuracy of estimating the time at reinfection, this study provides information on the real-world scenario of clinical practice with the largest sample size to date. The primary analysis in this study required two negative RNA tests for HCV clearance. In clinical practice two RNA tests are not always performed for confirmation of clearance and may have led to underestimation of reinfection incidence as reported in Table 3.4.

The sensitivity of different HCV-PCR assays changed over time, and might have impacted the classification of cases, especially the cases of spontaneous clearance and SVR. Most of the quantitative HCV-PCR tests were validated by a more sensitive qualitative test up until 2007, after which quantitative PCR assays were as sensitive (RNA detection level: 10-15 IU/mL) as the qualitative test. Between 2000 and 2006, a small proportion (2.95% of the overall cohort) of quantitative test results with lower limit of RNA detection of 615 IU/mL (all negative) were not verified by a qualitative test. However, in this analysis, eight cases of clearance (negative HCV-PCR test results; HCV RNA <615 IU/mL) were not validated by a qualitative test. This was the test used in practice back then, and potentially all of them could well be HCV negative. Moreover, the potential error rate is negligible (0.14%) in this study even if it is assumed that all of them had RNA levels between 15 and 615 IU/mL but test results were negative due to inability of the less sensitive assays to detect them. Thus, this may not have had any significant impact on the analysis and/or inference drawn from this study.

The concern of distinguishing between relapse and reinfection is paramount in HCV reinfection studies. However, this may not be substantial in this study. First, this study used two consecutive negative PCR tests after 12-week post-treatment (SVR12), at least 28 days apart. Thus, relapse soon after SVR12 may be ruled out by a second negative test $\geq$ 28 days

apart, as used in previous studies.[76] Moreover, those with a second negative test which was found within a 28-day time frame were excluded. A similar approach was applied to the spontaneous clearance group as well. Second, as reported in the results section, the median (IQR) time to reinfection (from the date of clearance to the date of reinfection) was quite longer in this study— median: 3 years (IQR: 1.5-5.4 years). Moreover, earlier studies showed that late relapse post-SVR is very rare (<1%).[175, 202] Thus, issue of relapse may not be a serious concern in this study.

All prescriptions dispensed in BC, both covered by public and private insurance including HCV treatments and OST, are recorded in a centralized database thus capturing all dispensed OST and HCV treatments. Mental health counselling is covered through the Medical Services Plan which includes all services that are billed by healthcare providers. In this case, a service provided by a non-fee-for-service provider would not be captured and would lead to under-ascertainment of mental health counselling. In addition, the current study is subject to the usual caveats of using administrative data in defining some covariates such as IDU, and problematic alcohol use. Optimal definition was used in this study, which is based on validation performed by BC-HTC team or others,[162] however, issue of some level of misclassification and underestimation still remains. It is expected that misclassification is non-differential leading to underestimation of associations. Drawing causal inference on intervention effects from observational data is prone to biases. Attempts were taken to delineate this further by applying propensity score methods to correct for non-comparability of those who received and did not receive OST or mental health counselling. Although, this analysis yielded similar results to those from the main analysis, some unmeasured confounding may remain. Thus, further studies

complemented with appropriate analytic strategies within causal inference framework are recommended to validate these findings. Caution should also be exercised when interpreting the stratified models in the SVR group due to small number of outcome events (n=50), however, sensitivity analysis using single negative PCR yielded higher sample size and more stable results (Table 3.9). The use of midpoints as the date of HCV transitions, which has been the standard practice in HCV literature, has been found to be robust in the sensitivity analysis using the earliest date of HCV transition (Table 3.10).

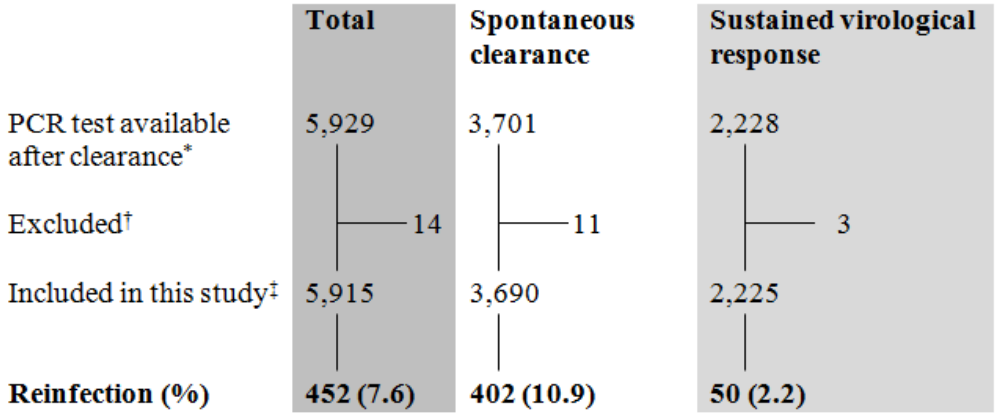
As is the case, the difference in healthcare settings with varying access to healthcare services, especially to those at risk, may have different results. It is expected that the results from this study will be comparable to other developed countries with comparable healthcare settings. However, differential access to healthcare, especially to PWID, and those coinfected with HIV, has been reported in developed countries as well (e.g., USA, Canada).[42, 177, 178] Thus, more research from diverse healthcare settings will add invaluable evidence to HCV literature.

3.5 Conclusion

The rate and risk of HCV reinfection were significantly higher in the spontaneous clearance group compared to the SVR group, those coinfected with HIV, and among PWID. Higher reinfection risk in the spontaneous clearance group calls for post-clearance follow-up of PWID, and provision of harm reduction services to minimize HCV reinfection and transmission. Consistent with previous mathematical models,[201] the current study showed that engagement with opioid substitution therapy, as well as mental health counseling, is associated with a significant reduction in HCV reinfection risk among PWID. In light of this, the positive impacts

of scaled-up HCV treatment might be enhanced, if accompanied by appropriate harm reduction programs to prevent reinfections among PWID with a view to achieving World Health Organization's goal of HCV elimination.[203]

Figure 3.1 Selection of participants for HCV reinfection analysis in British Columbia, Canada



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* Clearance was defined as two consecutive negative PCRs after HCV diagnosis without treatment (spontaneous clearance group), or ≥12 weeks post-treatment (for the SVR group), as applicable.
† Excluded because the difference between the two negative PCRs was <28 days.
‡ Subjects with two consecutive negative PCRs ≥28 days apart who had ≥1 valid PCR post-clearance.

HCV = Hepatitis C virus; PCR = Polymerase Chain Reaction; SVR: Sustained Virological Response

Table 3.1 Definitions for comorbid conditions for the BC Hepatitis Testers Cohort and current analysis (reinfection)

Mental health counseling Mental health counseling was defined at the first occurrence of 1 medical services plan (MSP) fee item code for consultations and psychotherapy sessions related to mental health problems within the study period. **MSP fee item code:** 600-699, 60607-45
Injection drug use Injection drug use was defined at the first occurrence of 1 MSP or 1 hospitalization diagnostic codes for major drug-related diagnoses involving addiction, dependence, and drug-induced mental disorders; illicit drug use most likely to be injectables (e.g., excluding cannabis), or illicit use of prescribed drugs including: hallucinogens, barbiturates/tranquillizers, sedatives, hypnotics, anxiolytics, opioids, cocaine, amphetamine, volatile solvents; or discharge to drug rehabilitation, counselling, surveillance or methadone/buprenorphine substitution treatment. **Physician Billing Data**: MSP ICD-9 diagnostic codes: starting with 292, 3040, 3042, 3044, 3046-9, 3054-7, 3059, 6483, 9650, 9697, 970, E8500, or exact codes V6542 or fee item = 39 **Hospitalization Data**: **DAD1/ICD-9-CM**: starting with 292, 3040, 3042, 3044, 3046-9, 3054-7, 3059, 6483, 9650, 9697, 970, E8500; **DAD2/ICD-10-CA**: starting with F11, F13-5, F18, F19, T42, or exact codes T401, T402, T404-6, T436, T438, T439, T507.
Problematic alcohol use Problematic alcohol use was defined at the first occurrence of 2 MSP or 1 hospitalization codes for major alcohol-related diagnoses including alcoholic mental disorders and dependence/abuse syndromes; alcoholic polyneuropathy, myopathy, cardiomyopathy; pseudo Cushing's syndrome; or discharge to alcohol rehabilitation, counselling, or surveillance **Physician Billing Data:** MSP ICD-9 diagnostic codes: starting with 291, 303, 3050, 3575, 4255 **Hospitalization Data:** DAD1/ICD-9-CM: starting with 291, 303, 3050,3575, 4255; DAD2/ICD-10-CA: starting with F10, E244, G312, G621, G721, I426, Z502, Z714
Opioid substitution therapy Opioid substitution therapy was defined at the first occurrence of 2 MSP or 1 PharmaNet DIN/PIN code for methadone/buprenorphine substitution treatment. **Physician Billing Data:** MSP ICD-9: fee item = 39 **PharmaNet DIN/PIN**: 2242963, 2242964, 99792, 66999990-3, 66999997, 66999999, 67000000, 2295695, 2295709.

*DIN = Drug Identification Number, PIN = Product Identification Numbers

Table 3.2 Characteristics of the participants in hepatitis C reinfection analysis in British Columbia, Canada

	Spontaneous clearance*		SVR*		Total	
	Overall (n=3690)	Reinfection (n=402)	Overall (n=2225)	Reinfection (n=50)	Overall (n=5915)	Reinfection (n=452)
Age at clearance (years)						
< 35	1216(33)	180 (44.8)	248(11.1)	9 (18)	1464(24.8)	189 (41.8)
35-44	1224(33.2)	151 (37.6)	443(19.9)	16 (32)	1667(28.2)	167 (37)
≥ 45	1250(33.9)	71 (17.7)	1534(68.9)	25 (50)	2784(47.1)	96 (21.2)
Median (IQR)	40 (32-47)	36 (28-42)	50 (42-55)	45 (36-53)	43 (35-51)	37 (30-43)
Birth cohort						
< 1965	1992(54)	157 (39.1)	1719(77.3)	32 (64)	3711(62.7)	189 (41.8)
1965-1974	998(27)	140 (34.8)	333(15)	15 (30)	1331(22.5)	155 (34.3)
≥ 1975	700(19)	105 (26.1)	173(7.8)	3 (6)	873(14.8)	108 (23.9)
Sex						
Female	1622(44)	154 (38.3)	822(36.9)	10 (20)	2444(41.3)	164 (36.3)
Male	2068(56)	248 (61.7)	1403(63.1)	40 (80)	3471(58.7)	288 (63.7)
Year of HCV diagnosis						
1990-97	1213(32.9)	137 (34.1)	654(29.4)	15 (30)	1867(31.6)	152 (33.6)
1998-04	1552(42.1)	180 (44.8)	1131(50.8)	30 (60)	2683(45.4)	210 (46.5)
2005-13	925(25.1)	85 (21.1)	440(19.8)	5 (10)	1365(23.1)	90 (19.9)
HIV coinfection**						
Yes	407(11)	79 (19.7)	126(5.7)	12 (24)	533(9)	91 (20.1)
No	3283(89)	323 (80.4)	2099(94.3)	38 (76)	5382(91)	361 (79.9)
≥1mental health counseling visit***						
Yes	1168(31.7)	119 (29.6)	414(18.6)	16 (32)	1582(26.7)	135 (29.9)
No	2522(68.3)	283 (70.4)	1811(81.4)	34 (68)	4333(73.3)	317 (70.1)
Injection drug use‡						
Yes	1928(52.2)	268 (66.7)	565(25.4)	30 (60)	2493(42.1)	298 (65.9)
No	1762(47.8)	134 (33.3)	1660(74.6)	20 (40)	3422(57.9)	154 (34.1)
Problematic alcohol use‡						
Yes	1615(43.8)	210 (52.2)	586(26.3)	19 (38)	2201(37.2)	229 (50.7)
No	2075(56.2)	192 (47.8)	1639(73.7)	31 (62)	3714(62.8)	223 (49.3)
Material deprivation quintile†						
Q1 (most privileged)	492(13.3)	42 (10.5)	321(14.4)	11 (22)	813(13.7)	53 (11.7)
Q2	500(13.6)	62 (15.4)	373(16.8)	7 (14)	873(14.8)	69 (15.3)
Q3	580(15.7)	67 (16.7)	453(20.4)	7 (14)	1033(17.5)	74 (16.4)
Q4	804(21.8)	82 (20.4)	484(21.8)	12 (24)	1288(21.8)	94 (20.8)
Q5 (most deprived)	1183(32.1)	132 (32.8)	577(25.9)	13 (26)	1760(29.8)	145 (32.1)
Unknown	131(3.6)	17 (4.2)	-	-	148(2.5)	17 (3.8)
Social deprivation quintile†						
Q1 (most privileged)	359(9.7)	27 (6.7)	384(17.3)	6 (12)	743(12.6)	33 (7.3)
Q2	418(11.3)	39 (9.7)	348(15.6)	5 (10)	766(13)	44 (9.7)
Q3	586(15.9)	72 (17.9)	377(16.9)	10 (20)	963(16.3)	82 (18.1)
Q4	748(20.3)	89 (22.1)	431(19.4)	11 (22)	1179(19.9)	100 (22.1)
Q5 (most deprived)	1448(39.2)	158 (39.3)	668(30)	18 (36)	2116(35.8)	176 (38.9)
Unknown	131(3.6)	17 (4.2)	-	-	148(2.5)	17 (3.8)

* Clearance type of first HCV diagnosis (reference group: SVR); ** HIV diagnosis before the end of the study; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance; SVR: Sustained virological response; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus.

Table 3.3 Incidence rates of HCV reinfection among those who cleared primary infections (defined as two consecutive negative PCR, ≥28 days apart) in British Columbia, Canada

Characteristics	Number of reinfection	Incidence rate (95% CI); per 100 person-years
Overall	**452**	**1.27 (1.15-1.39)**
Spontaneous clearance		
Total	402	1.59 (1.44-1.76)
PWID	268	1.88 (1.66-2.12)
SVR		
Total	50	0.48 (0.36-0.63)
PWID	30	1.14 (0.77-1.63)
Age at clearance (years)		
< 35	189	1.99 (1.71-2.29)
35-44	167	1.49 (1.27-1.73)
≥ 45	96	0.64 (0.52-0.79)
Birth cohort		
< 1965	189	0.82 (0.71-0.95)
1965-1974	155	1.88 (1.60-2.20)
≥1975	108	2.43 (2.00-2.94)
Sex		
Female	164	1.03 (0.88-1.20)
Male	288	1.46 (1.30-1.64)
Year of HCV diagnosis		
1990-97	152	1.03 (0.87-1.20)
1998-04	210	1.28 (1.11-1.47)
2005-13	90	2.01 (1.62-2.47)
HIV coinfection**		
Yes	91	2.56 (2.06-3.14)
No	361	1.12 (1.01-1.25)
≥1 mental health counseling visit***		
Yes	135	1.16 (0.97-1.37)
No	317	1.32 (1.18-1.47)
Injection drug use‡		
Yes	298	1.77 (1.57-1.98)
No	154	0.82 (0.69-0.96)
Problematic alcohol use‡		
Yes	229	1.53 (1.34-1.74)
No	223	1.08 (0.94-1.23)
Material deprivation quintile†		
Q1 (most privileged)	53	1.14 (0.85-1.49)
Q2	69	1.30 (1.01-1.64)
Q3	74	1.23 (0.97-1.54)
Q4	94	1.19 (0.96-1.45)
Q5 (most deprived)	145	1.31 (1.11-1.54)
Unknown	17	2.37 (1.38-3.79)
Social deprivation quintile†		
Q1 (most privileged)	33	0.79 (0.55-1.11)
Q2	44	0.98 (0.72-1.32)
Q3	82	1.37 (1.09-1.70)
Q4	100	1.43 (1.16-1.74)
Q5 (most deprived)	176	1.32 (1.13-1.53)
Unknown	17	2.37 (1.38-3.79)

* Clearance type of first HCV diagnosis (reference group: SVR); ** HIV diagnosis before the end of the study; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance.

Table 3.4 Incidence rates of HCV reinfection among those who cleared primary infections (defined as a single negative PCR) in British Columbia, Canada
(N=10,408)

Characteristics	Number of reinfection	Incidence Rate (95% CI) per 100 person-years
Overall	**1231**	**2.42 (2.29-2.56)**
Injection drug use		
Yes	858	3.34 (3.12-3.56)
No	373	1.48 (1.33-1.63)
Clearance type of first infection		
Spontaneous clearance	1106	2.88 (2.71-3.05)
Injection drug use		
Yes	784	3.53 (3.29-3.79)
No	322	1.99 (1.78-2.22)
SVR	125	1.00 (0.83-1.18)
Injection drug use		
Yes	74	2.11 (1.66-2.65)
No	51	0.57 (0.42-0.75)
HIV		
Yes	122	4.17 (3.43-4.91)
No	1109	2.31 (2.18-2.45)

HIV: Human Immunodeficiency Virus; SVR: Sustained virological response

Figure 3.2 Cumulative incidence of HCV reinfection by clearance type of previous episode

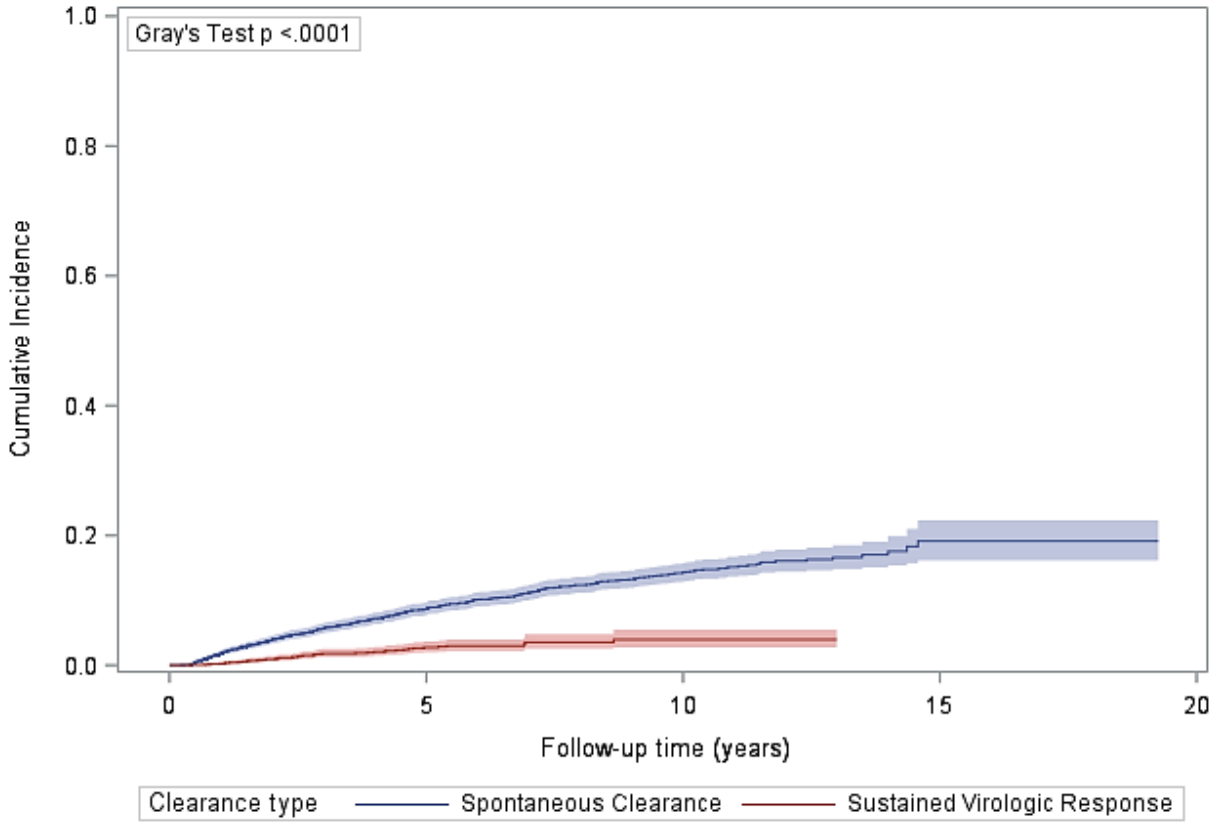

Figure 3.3 Cumulative incidence of HCV reinfection by injection drug use status

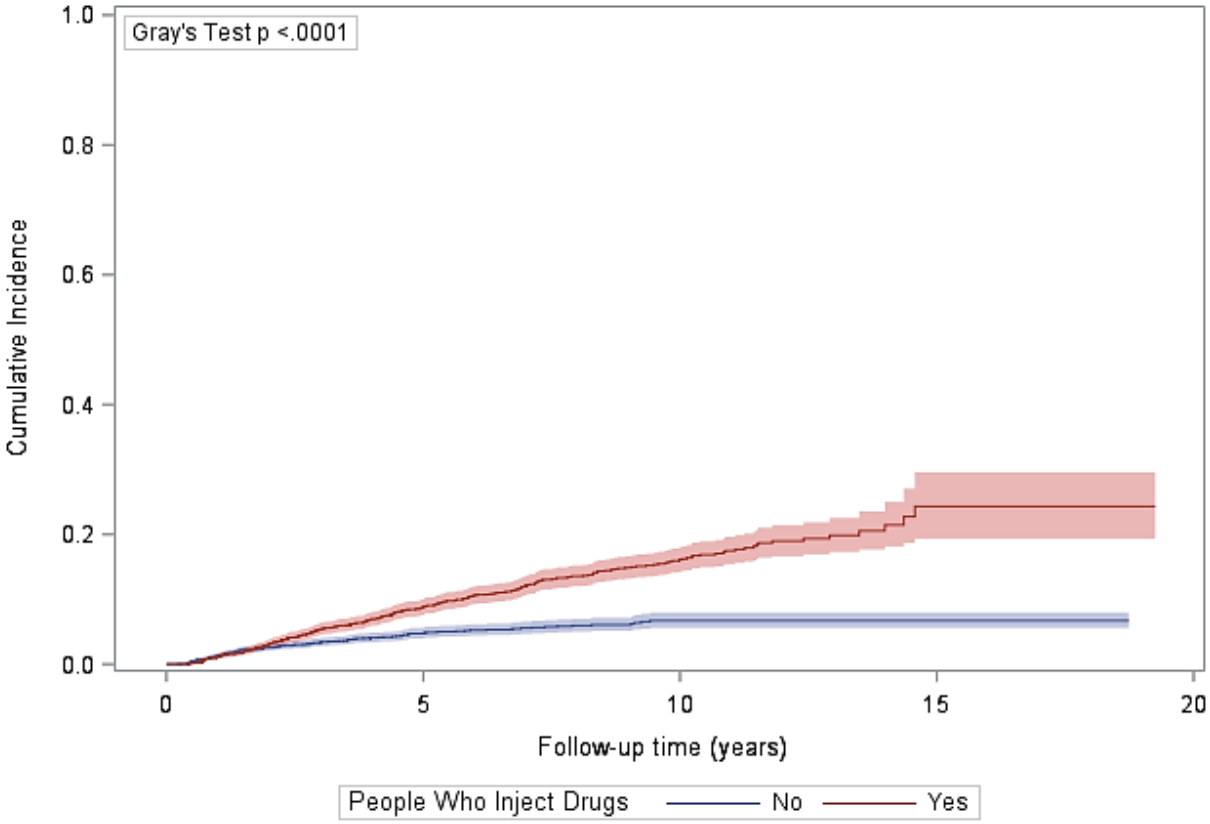

Figure 3.4 Cumulative incidence of HCV reinfection by HIV coinfection status

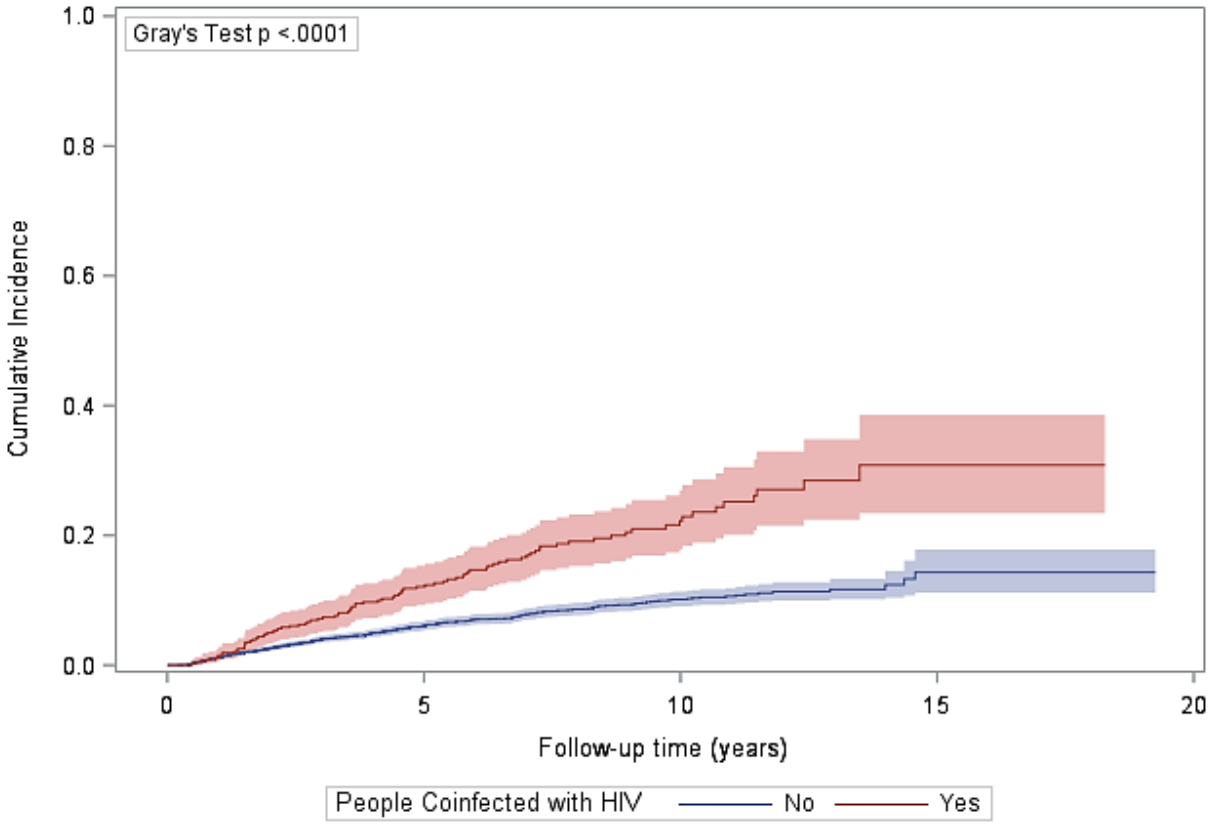

Table 3.5 Unadjusted and adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection in British Columbia, Canada

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at clearance (years)		<0.0001		
< 35	3.18 (2.49-4.07)			
35-44	2.39 (1.86-3.08)			
≥ 45	*Ref*			
Birth cohort		<0.0001		<0.0001
< 1965	0.35 (0.27-0.44)		0.48 (0.37-0.63)	
1965-1974	0.79 (0.62-1.01)		0.87 (0.68-1.13)	
≥1975	*Ref*		*Ref*	
Female	0.71 (0.59-0.86)	0.0006	0.57 (0.47-0.70)	<0.0001
Year of HCV diagnosis		<0.0001		0.002
1990-97	0.54 (0.41-0.71)		0.60 (0.44-0.80)	
1998-04	0.66 (0.51-0.85)		0.74 (0.57-0.96)	
2005-13	*Ref*		*Ref*	
Spontaneous clearance*	3.63 (2.70-4.89)	<0.0001	2.71 (2.00-3.68)	<0.0001
HIV coinfection**	2.77 (2.20-3.49)	<0.0001	2.25 (1.78-2.85)	<0.0001
≥1 mental health counseling visit***	0.90 (0.74-1.10)	0.315		
Injection drug use‡	2.21 (1.82-2.69)	<0.0001	1.53 (1.21-1.92)	<0.001
Problematic alcohol use‡	1.45 (1.21-1.75)	<0.0001	1.04 (0.84-1.28)	0.726
Material deprivation quintile†		0.164		
Q1 (most privileged)	*Ref*			
Q2	1.15 (0.80-1.64)			
Q3	1.08 (0.76-1.53)			
Q4	1.05 (0.75-1.47)			
Q5 (most deprived)	1.16 (0.85-1.59)			
Unknown	2.08 (1.20-3.58)			
Social deprivation quintile†		0.002		0.121
Q1 (most privileged)	*Ref*			
Q2	1.25 (0.80-1.96)		1.16 (0.74-1.82)	
Q3	1.75 (1.17-2.63)		1.45 (0.97-2.18)	
Q4	1.82 (1.23-2.70)		1.39 (0.93-2.06)	
Q5 (most deprived)	1.69 (1.16-2.45)		1.20 (0.82-1.75)	
Unknown	3.00 (1.67-5.39)		2.04 (1.13-3.68)	

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; SVR: Sustained virological response.

Table 3.6 Adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection, stratified by clearance type, in British Columbia, Canada

	Spontaneous clearance*		SVR*	
Characteristics	**Adjusted HR (95% CI)**	**p-value**	**Adjusted HR (95% CI)**	**p-value**
Birth cohort		<0.0001		0.546
< 1965	0.45 (0.35-0.60)		1.32 (0.39-4.50)	
1965-1974	0.84 (0.64-1.09)		1.78 (0.50-6.29)	
≥1975	*Ref*		*Ref*	
Female	0.60 (0.48-0.73)	<0.0001	0.47 (0.23-0.96)	0.037
Year of HCV diagnosis		0.003		0.598
1990-97	0.58 (0.43-0.79)		0.78 (0.27-2.25)	
1998-04	0.71 (0.54-0.94)		1.08 (0.41-2.88)	
2005-13	*Ref*		*Ref*	
HIV coinfection**	2.14 (1.66-2.75)	<0.0001	3.37 (1.68-6.76)	<0.001
Injection drug use‡	1.34 (1.05-1.70)	0.019	3.94 (2.00-7.76)	<0.0001
Problematic alcohol use‡	1.07 (0.86-1.34)	0.536	0.86 (0.46-1.61)	0.631
Social deprivation quintile†		0.152		0.956
Q1 (most privileged)	*Ref*		*Ref*	
Q2	1.22 (0.74-1.99)		0.68 (0.20-2.25)	
Q3	1.45 (0.93-2.26)		1.17 (0.42-3.28)	
Q4	1.40 (0.91-2.15)		0.91 (0.32-2.56)	
Q5 (most deprived)	1.20 (0.80-1.81)		0.90 (0.34-2.36)	
Unknown	2.09 (1.14-3.85)		-	

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; SVR: Sustained virological response

Table 3.7 Unadjusted and adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection among current injection drug users in British Columbia, Canada

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at clearance (years)		<0.001		
< 35	2.47 (1.58-3.86)			
35-44	1.80 (1.13-2.87)			
≥ 45	*Ref*			
Birth cohort		<0.0001		<0.0001
< 1965	0.39 (0.28-0.55)		0.47 (0.33-0.69)	
1965-1974	0.71 (0.52-0.98)		0.89 (0.63-1.25)	
≥ 1975	*Ref*		*Ref*	
Female	0.82 (0.63-1.07)	0.143	0.71 (0.54-0.93)	0.013
Year of HCV diagnosis		<0.0001		<0.0001
1990-97	0.24 (0.16-0.36)		0.27 (0.17-0.42)	
1998-04	0.46 (0.32-0.67)		0.47 (0.32-0.69)	
2005-13	*Ref*		*Ref*	
Spontaneous clearance*	1.52 (0.90-2.58)	0.119		
HIV coinfection**	2.11 (1.59-2.81)	<0.0001	2.39 (1.79-3.19)	<0.0001
≥1 mental health counseling visit***	0.72 (0.55-0.94)	0.014	0.71 (0.54-0.92)	0.011
Problematic alcohol use‡	0.92 (0.69-1.22)	0.548		
Opioid substitution therapy**	0.74 (0.55-1.00)	0.05	0.73 (0.54-0.98)	0.038
Material deprivation quintile†		0.677		
Q1 (most privileged)	*Ref*			
Q2	1.45 (0.82-2.56)			
Q3	1.62 (0.93-2.82)			
Q4	1.37 (0.81-2.32)			
Q5 (most deprived)	1.32 (0.80-2.18)			
Unknown	1.40 (0.41-4.72)			
Social deprivation quintile†		0.186		
Q1 (most privileged)	*Ref*			
Q2	1.46 (0.73-2.93)			
Q3	1.76 (0.95-3.26)			
Q4	1.49 (0.81-2.77)			
Q5 (most deprived)	1.14 (0.64-2.04)			
Unknown	1.37 (0.39-4.80)			

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; SVR: Sustained virological response.

Table 3.8 Unadjusted and adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection among current injection drug users in British Columbia, Canada (sensitivity analysis using mental health counseling as number of visits per year)

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at clearance (years)		<0.001		
< 35	2.47 (1.58-3.86)			
35-44	1.80 (1.13-2.87)			
≥ 45	*Ref*			
Birth cohort		<0.0001		<0.0001
< 1965	0.39 (0.28-0.55)		0.46 (0.32-0.67)	
1965-1974	0.71 (0.52-0.98)		0.86 (0.61-1.21)	
≥1975	*Ref*		*Ref*	
Female	0.82 (0.63-1.07)	0.143	0.72 (0.55-0.95)	0.012
Year of HCV diagnosis		<0.0001		<0.0001
1990-97	0.24 (0.16-0.36)		0.28 (0.18-0.43)	
1998-04	0.46 (0.32-0.67)		0.48 (0.33-0.71)	
2005-13	*Ref*		*Ref*	
Spontaneous clearance*	1.52 (0.90-2.58)	0.119		
HIV coinfection**	2.11 (1.59-2.81)	<0.0001	2.47 (1.85-3.30)	<0.0001
Problematic alcohol use‡	0.92 (0.69-1.22)	0.548		
Mental health counseling visit per year***		<0.0001		<0.0001
0	*Ref*			
1	0.32 (0.20-0.51)		0.32 (0.20-0.51)	
≥ 2	0.71 (0.51-0.98)		0.67 (0.48-0.93)	
Opioid substitution therapy**	0.74 (0.55-1.00)	0.05	0.72 (0.53-0.97)	0.028
Material deprivation quintile†		0.677		
Q1 (most privileged)	*Ref*			
Q2	1.45 (0.82-2.56)			
Q3	1.62 (0.93-2.82)			
Q4	1.37 (0.81-2.32)			
Q5 (most deprived)	1.32 (0.80-2.18)			
Unknown	1.40 (0.41-4.72)			
Social deprivation quintile†		0.186		
Q1 (most privileged)	*Ref*			
Q2	1.46 (0.73-2.93)			
Q3	1.76 (0.95-3.26)			
Q4	1.49 (0.81-2.77)			
Q5 (most deprived)	1.14 (0.64-2.04)			
Unknown	1.37 (0.39-4.80)			

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; SVR: Sustained virological response.

Table 3.9 Adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection in British Columbia, Canada (sensitivity analysis using single negative PCR for clearance)

Characteristics	Full cohort (n=10,408)	Spontaneous clearance* (n=6874)	SVR* (n=3534)	PWID (n=4626)
Birth cohort				
< 1965	0.54 (0.46-0.63)	0.52 (0.44-0.61)	0.99 (0.49-1.98)	0.50 (0.42-0.61)
1965-1974	0.77 (0.66-0.90)	0.73 (0.62-0.86)	1.58 (0.78-3.20)	0.89 (0.75-1.05)
≥1975	*Ref*	*Ref*	*Ref*	*Ref*
Female	0.77 (0.68-0.86)	0.78 (0.69-0.88)	0.71 (0.48-1.06)	0.83 (0.73-0.96)
Year of HCV diagnosis				
1990-97	0.70 (0.59-0.83)	0.68 (0.57-0.82)	0.89 (0.48-1.65)	0.39 (0.32-0.48)
1998-04	0.77 (0.66-0.90)	0.75 (0.64-0.87)	1.08 (0.62-1.89)	0.56 (0.47-0.67)
2005-13	*Ref*	*Ref*	*Ref*	*Ref*
Spontaneous clearance*	2.62 (2.16-3.17)	-	-	-
HIV coinfection**	2.03 (1.74-2.38)	1.96 (1.66-2.32)	2.63 (1.62-4.25)	1.89 (1.60-2.23)
Injection drug use‡	1.72 (1.49-1.98)	1.59 (1.37-1.85)	2.94 (1.92-4.50)	-
Problematic alcohol use‡	1.01 (0.89-1.14)	1.01 (0.88-1.14)	1.03 (0.69-1.53)	-
≥1 mental health counseling visit***	-	-	-	0.61 (0.53-0.71)
Opioid substitution therapy**	-	-	-	0.80 (0.69-0.94)
Social deprivation quintile†				
Q1 (most privileged)	*Ref*		*Ref*	-
Q2	1.03 (0.80-1.33)	1.08 (0.83-1.42)	0.68 (0.33-1.40)	-
Q3	1.05 (0.83-1.32)	1.09 (0.85-1.39)	0.70 (0.35-1.39)	-
Q4	1.08 (0.87-1.35)	1.08 (0.85-1.37)	1.00 (0.55-1.81)	-
Q5 (most deprived)	0.98 (0.79-1.20)	0.97 (0.78-1.21)	0.96 (0.55-1.67)	-
Unknown	1.44 (0.97-2.12)	1.44 (0.97-2.16)	1.43 (0.19-10.77)	-

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; PWID: People who inject drugs; SVR = Sustained virological response.

Table 3.10 Unadjusted and adjusted hazard ratios from Cox proportional hazards model for time to HCV reinfection in British Columbia, Canada (sensitivity analysis using the earliest date of HCV transitions, instead of the mid-points as used in the main analysis)

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at clearance (years)		<0.0001		
< 35	3.22 (2.52-4.12)			
35-44	2.43 (1.89-3.13)			
≥ 45	*Ref*			
Birth cohort		<0.0001		<0.0001
< 1965	0.35 (0.28-0.45)		0.49 (0.38-0.63)	
1965-1974	0.81 (0.63-1.03)		0.87 (0.68-1.13)	
≥1975	*Ref*		*Ref*	
Female	0.72 (0.59-0.87)	0.0008	0.58 (0.47-0.70)	<0.0001
Year of HCV diagnosis		0.0004		0.01
1990-97	0.58 (0.44-0.77)		0.64 (0.48-0.85)	
1998-04	0.67 (0.52-0.87)		0.75 (0.58-0.98)	
2005-13	*Ref*		*Ref*	
Spontaneous clearance*	3.73 (2.77-5.01)	<0.0001	2.75 (2.03-3.74)	<0.0001
HIV coinfection**	2.74 (2.19-3.44)	<0.0001	2.22 (1.76-2.80)	<0.0001
Injection drug use‡	2.24 (1.85-2.73)	<0.0001	1.52 (1.21-1.92)	0.0003
Problematic alcohol use‡	1.48 (1.23-1.78)	<0.0001	1.05 (0.85-1.29)	0.665
≥1 mental health counseling visit***	0.91 (0.75-1.12)	0.372		
Material deprivation quintile†	1.45 (1.21-1.75)	<0.0001		
Q1 (most privileged)	*Ref*	0.17		
Q2	1.15 (0.81-1.65)			
Q3	1.08 (0.76-1.54)			
Q4	1.05 (0.75-1.48)			
Q5 (most deprived)	1.17 (0.86-1.61)			
Unknown	2.06 (1.19-3.56)			
Social deprivation quintile†				0.119
Q1 (most privileged)	*Ref*	0.002		
Q2	1.25 (0.80-1.96)		1.16 (0.74-1.83)	
Q3	1.76 (1.17-2.63)		1.45 (0.97-2.18)	
Q4	1.82 (1.23-2.70)		1.38 (0.93-2.06)	
Q5 (most deprived)	1.69 (1.17-2.46)		1.19 (0.82-1.73)	
Unknown	2.97 (1.65-5.33)		2.02 (1.12-3.64)	

* Clearance type of first HCV diagnosis (reference group: SVR); ** used as a time-varying covariate; *** reported between the date on HCV clearance and the last day of follow-up; ‡ ever reported in the cohort; † at the time of HCV clearance; HR: Hazard ratio; CI: Confidence interval; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; SVR: Sustained virological response.

Chapter 4: Hepatitis C re-clearance and cross-genotype immunity

4.1 Background

About a quarter of people with HCV infections clear their primary episodes spontaneously, without treatment [89, 204]. However, ability to clear an HCV episode does not confer protection against a future infection.[39, 52, 56, 57] Thus, people remain at risk of reinfection even after successfully clearing the first infection.[48, 68, 72, 74, 161]

Newly released direct acting antiviral drugs are highly effective in treating HCV infections, but their cost creates a strong economic barrier to increasing treatment uptake.[163, 164] On the other hand, since successful treatment does not prevent future infections either,[48, 68, 72] an effective vaccine is highly desirable in preventing reinfection and achieving the World Health Organization's goal of HCV elimination. However, development of an effective vaccine has been complicated by the diversity of HCV genotypes, lack of suitable animal model other than chimpanzees, and complexities in HCV immunological responses.[205]

While an effective vaccine is yet to be in use, studies on humans and chimpanzees (the only non-human animal known to be susceptible to HCV infection) provide important insights into developing one such vaccine.[39] Previous studies in chimpanzees, [78-80] and in humans,[52, 53] indicated that compared to primary infection, exposure to a subsequent infection was associated a lower peak viremia, overall shortened infection course and lower ALT, and a higher likelihood of viral clearance. While these findings are important in understanding the natural history of HCV to lend support to vaccine development, these, and other epidemiological studies,[47, 48, 52, 53, 77] mostly conducted among people who inject drugs (PWID), were

smaller studies that may have compromised their generalizability and their ability to examine factors determining the outcome following HCV reinfection. The studies on humans published so far reported the cases of re-clearance (clearance of reinfection) as case reports or case series (ranging between 0 and 10 cases) except one study [76] that only reported unadjusted estimates, and was unable to examine factors associated with HCV re-clearance adjusting for other potential confounders due to very small sample size (n=14). Recently, one study on HIV positive MSM reported adjusted estimates of re-clearance (N=21).[176] Moreover, findings from these studies have been inconsistent with regard to estimates of re-clearance [range reported: 0-100%], and testing intervals. This study examined the factors associated with spontaneous clearance of HCV reinfection. This study also examined if reinfection with a heterologous HCV genotype had any impact on re-clearance among those who cleared their previous episode spontaneously.

4.2 Research design and methods

4.2.1 Study cohort

The data for this analysis is based on the BC Hepatitis Testers Cohort as described earlier (Table 1.1). Details of the BC-HTC database have been described in Chapter 1 and elsewhere [138, 139].

4.2.2 Case definitions

Case definitions of HCV case, spontaneous clearance, SVR, reinfection, and re-clearance have been described in Chapter 1.

Definitions of HIV, major mental illness, IDU, and problematic alcohol use were

defined based on diagnostic codes described in Chapter 2 (Table 2.1). The heterologous genotype was defined as reinfections with an HCV genotype other than the one in the first infection. Social and material deprivation was assessed based on Québec Index of Material and Social Deprivation as described in Chapter 1.[143]

4.2.3 Eligibility

The general eligibility described in Chapter 1 applies here too. Current analysis included all HCV reinfected individuals who had at least one valid HCV-PCR after reinfection.

4.2.4 Data analysis

The characteristics of the eligible individuals are presented overall, and by their re-clearance outcome (confirmed/probable/no). The rates of HCV re-clearance per 100 person-years of follow-up (presented separately for confirmed, and probable plus confirmed re-clearance), and corresponding 95% confidence intervals (CI), were calculated assuming a Poisson distribution. Variables based on a-priori hypotheses, and those significant at level 0.10 in the unadjusted analysis were included in the multivariable Cox proportional hazards (PH) models. As described earlier, birth cohort, sex, and year of HCV diagnosis were included in all the models irrespective of their statistical significance in the univariate analysis. Age was not included in the same adjusted models with birth cohort and HCV diagnosis year with a view to avoiding non-identification problem (Age = Period − Cohort),[147].

For the main analyses, time to confirmed re-clearance was the main outcome variable (probable and no re-clearance cases were censored).

To assess the role of reinfection with a heterologous genotype on re-clearance, the analysis was restricted to those who cleared their first infection spontaneously. In the sensitivity analysis, the confirmed and probable re-clearance groups were merged as re-clearance. HIV was used as a time-varying covariate in all the analyses. Earlier reports suggested a higher clearance rate in subsequent HCV infection episodes. Thus, to compare, the rate of clearance of first infection was calculated among the seroconverters (who had a relatively well-defined first HCV diagnosis date required for time-to-clearance analysis) [204] assuming a Poisson distribution. All the analyses were conducted in [SAS/STAT] Software version [9.4].[148] All the tests were two-sided at a significance level of 0.05.

4.3 Results

Of 452 cases of reinfection, 357 participants had at least one valid HCV-PCR test after reinfection. They were followed up for a median of 26.7 (IQR: 12.2-53.8) months. Almost half (49%; n=175) of them had at least one negative HCV-PCR after reinfection; 121 of them had two consecutive negative PCR at least 28 days apart (*confirmed* re-clearance), and the rest (n=54) had either only one negative PCR, or two consecutive negative PCR <28 days apart (*probable* re-clearance). Individuals included in this study had a median of 12 (IQR: 9-16) HCV-PCR tests overall with a median of 5 (IQR: 3-8) HCV-PCR tests after reinfection. The median testing interval post-reinfection was 5.5 (IQR: 2.2-12.1) months.

4.3.1 Characteristics of the participants

Table 4.1 shows the characteristics of the sample population. The majority of the participants were male (64%; n=228), and 35-44 years old at reinfection (39%; n=139). The

proportion of PWID, and people coinfected with HIV in this sample was 39% (n=140), and 21% (n=76), respectively. More than a fifth (22%; n=78) had an ongoing history of problematic alcohol use, and almost a quarter (24%; n=86) had a current history of mental health illness. Most of them (90%; n=323) cleared their first infection spontaneously, and 18% (n=63) had a reinfection episode with a heterologous HCV genotype.

4.3.2 Rate of re-clearance

Overall rate of confirmed re-clearance was 12.36 (95% CI: 10.26-14.77) per 100 person-years. The rate (per 100 person-years) of re-clearance was higher among those who cleared their first infection spontaneously (12.79, 95% CI: 10.55-15.36) compared to those who achieved SVR (8.02, 95% CI: 3.23-16.53), while the rate was much lower in reinfection with a heterologous HCV genotype (6.5 [95% CI: 3.36-11.36] vs. 13.72 [95% CI: 11.27-16.55]). The rate was lower in people coinfected with HIV, major mental illness, and with a history of ongoing injection drug use, and problematic alcohol use (Table 4.2). In the same cohort (BC-HTC), the overall rate of spontaneous clearance of first infection among the seroconverters was much lower (3.69, 95% CI: 3.48-3.90) per 100 person-years.

4.3.3 Factors associated with re-clearance

In the adjusted analysis, reinfection with a heterologous HCV genotype (adjusted Hazard Ratio [aHR]: 0.42, 95% CI: 0.23-0.78), and ongoing history of problematic alcohol use (aHR: 0.47, 95% CI: 0.28-0.77) were significantly associated with a lower likelihood of HCV re-clearance (Table 4.3). The results were similar in the sensitivity analysis combining probable and confirmed re-clearance (Table 4.4).

4.3.4 Effect of reinfection with heterologous genotype on re-clearance among those who spontaneously cleared the first infection

Among those who cleared their previous episode spontaneously, reinfection with a heterologous genotype was associated with a 54% lower likelihood (aHR: 0.46, 95% CI: 0.24-0.86) of HCV re-clearance adjusting for other potential confounders (Table 4.5).

4.3.5 Sensitivity analysis

Further analysis was conducted to see if re-clearance was associated with the sequence of HCV Genotypes (GT). Compared to those who were infected with GT1 in both of their episodes, those infected with GT2/3 in both their infection episodes showed a slightly reduced likelihood of re-clearance but it did not reach statistical significance (aHR: 0.56, 95% CI: 0.21-1.51). Further, compared to those without reinfection with a heterologous HCV genotype, those who were infected with GT1 followed by a second infection with GT2/3 showed a higher likelihood of re-clearance (aHR: 1.31, 95% CI: 0.47- 3.65), but those who were infected with GT2/3 followed by a second infection with GT1 showed a lower likelihood of re-clearance (aHR: 0.62, 95% CI: 0.23-1.70). Again, none of these reached statistical significance.

4.4 Discussion

This is the largest study thus far to examine HCV re-clearance in humans that includes both PWID and non-PWID population. This study showed that the rate of spontaneous clearance of reinfection was much higher than that of first infection. The current study also showed that reinfection with a heterologous HCV genotype was associated with a significant reduction in the likelihood of re-clearance among those who cleared their previous HCV

episodes spontaneously indicating a lack of cross-genotype protective immune response against subsequent infections.

In this study, the proportion of confirmed re-clearance was 34%. Earlier studies reported a wide range of estimates (0-100%).[52, 53, 76, 77, 176] Even though the rate of re-clearance (per person-years) has not been previously reported, the proportion of re-clearance was reported to be higher than the clearance of the first infection in smaller studies in chimpanzees and humans. These findings align with the results from this study and those from Chapter 2.[76, 77, 176, 204]

This study found a significantly reduced likelihood of HCV re-clearance when reinfected with a heterologous genotype, and among those who had an ongoing history of problematic alcohol use. Though not evaluated in the context of HCV re-clearance, problematic alcohol use was reported to be associated with reduced odds of spontaneous clearance of HCV. [127] Problematic alcohol use was found to worsen the clinical outcomes of HCV in humans by altering the immune response to HCV, particularly by inhibiting the T-cell activating capacity.[206, 207] Previous study reported females with the IFNL4 rs12979860 CC genotype a significant predictor of HCV re-clearance, even though it was not adjusted for other potential confounders.[76] While female sex was not a significant predictor in the adjusted analysis, data on IFNL4 were not available, and so this study was not able to examine this. Further studies with sufficient sample size to adjust for potential confounders are required to validate these findings.

Among those who cleared their previous HCV episodes spontaneously, this study found that reinfection with a heterologous genotype results in a significantly lower likelihood of re-clearance. While earlier case studies reported similar findings,[52, 53] including cross-

neutralizing antibodies,[52] this has not been examined in any population-based studies before. However, animal studies reported seemingly opposing results.[79, 80] Some animal data reported cross-genotype immunity,[79] while other findings suggested limited protection against heterologous HCV viral strains.[80, 132] Further studies on humans with larger sample size are required to disentangle this crucial issue.

While an effective vaccine to offer sterilizing immunity is yet to be in practice, it is probably more pragmatic to develop a prophylactic vaccine that augments spontaneous clearance of HCV inducing partial protective immunity.[205] Lessons from other vaccines (e.g., those against hepatitis B, human papilloma, influenza, and varicella zoster) may be helpful in the context of HCV.[208] These vaccines, instead of providing sterilizing immunity, protect against persistent infections and result in a weakened course of infections. Within the context of limited protection against heterologous genotypes shown in this study, immunization with prophylactic vaccine, and boosting with different HCV genotypes have been suggested for a broader and more effective protection.[132]

As discussed in Chapters 1 and 2, the midpoint of HCV transitions was used as is the standard practice in the HCV literature so far.[44, 76] Moreover, it has been shown that using midpoint is as robust as using the earliest day of HCV transitions.[161]

This is the first population-based comprehensive study with the largest sample size to examine spontaneous clearance of HCV reinfection. Data were collected on other potential confounders to examine the factors associated with HCV re-clearance making it the first study to do so. However, some of the limitations of this study include the inability to examine the impact of ethnicity, and genetic markers including IFNL4. Other limitations include varying sensitivity

of HCV-PCR assays which changed over time, and irregular (and potentially longer) HCV-PCR testing intervals; the former may impact classification of re-clearance cases. As described earlier, most of the quantitative HCV-PCR tests were validated by a more sensitive qualitative test up until 2007. Quantitative PCR assays used thereafter were as sensitive (RNA detection level: 10-15 IU/mL) as the qualitative assays. Between 2000 and 2006, a small proportion (2.95% of the overall cohort) of quantitative test results with lower limit of RNA detection of 615 IU/mL (all negative) were not verified by a qualitative test. In this analysis, 33 negative HCV-PCR test results (HCV RNA <615 IU/mL) from 22 unique individuals were not validated by a qualitative test. However, none of them had all their tests unverified (i.e., they had other test results that were verified by a qualitative test). Nevertheless, HCV testing was not as uniform, frequent, and controlled as it would have been in a controlled experimental study. A mathematical modeling study suggested that testing intervals longer than one month may result in inaccurate estimates due to the possibility of missing cases of re-clearance.[201] In this study, median testing interval post-reinfection was 5.5 (IQR: 2.2-12.1) months. Thus, while it is possible that the re-clearance estimate from this study is an underestimation of true re-clearance, the estimates generated in this analysis reflect data gathered through a population-based cohort as opposed to data derived in a controlled laboratory/trial setting.

4.5 Conclusion

In conclusion, this study examined HCV re-clearance with the largest population-based sample to date. The study found that the rate of re-clearance was higher than that of the first clearance. This study also found that reinfection with heterologous genotype and an ongoing history of problematic alcohol use are associated with significantly lower likelihood of re-

clearance. These have important policy implications with regard to public health monitoring, surveillance, and resource allocation. The lack of cross-genotype immunity lends support to the existing literature on a prophylactic vaccine boosted with different HCV genotypes.

Table 4.1 Characteristics of participants assessed for HCV re-clearance in British Columbia, Canada

Characteristics	Total	Confirmed* re-clearance	Probable† re-clearance	No re-clearance
Age at HCV reinfection (years)				
< 35	100 (28)	37 (30.6)	14 (25.9)	49 (26.9)
35-44	139 (38.9)	47 (38.8)	25 (46.3)	67 (36.8)
≥ 45	118 (33.1)	37 (30.6)	15 (27.8)	66 (36.3)
Birth Cohort				
< 1964	162 (45.4)	54 (44.6)	27 (50)	81 (44.5)
1965-1974	113 (31.7)	38 (31.4)	14 (25.9)	61 (33.5)
≥ 1975	82 (23)	29 (24)	13 (24.1)	40 (22)
Sex				
Female	129 (36.1)	39 (32.2)	28 (51.9)	62 (34.1)
Male	228 (63.9)	82 (67.8)	26 (48.2)	120 (65.9)
Year of HCV diagnosis				
1990-1997	123 (34.5)	50 (41.3)	15 (27.8)	58 (31.9)
1998-2004	169 (47.3)	48 (39.7)	25 (46.3)	96 (52.8)
2005-2013	65 (18.2)	23 (19)	14 (25.9)	28 (15.4)
HCV heterologous genotype				
Yes	63 (17.7)	12 (9.9)	8 (14.8)	43 (23.6)
No	294 (82.4)	109 (90.1)	46 (85.2)	139 (76.4)
Clearance type‡				
Spontaneous clearance	323 (90.5)	114 (94.2)	50 (92.6)	159 (87.4)
Sustained virological response	34 (9.5)	7 (5.8)	4 (7.4)	23 (12.6)
HIV**				
Yes	76 (21.3)	24 (19.8)	8 (14.8)	44 (24.2)
No	281 (78.7)	97 (80.2)	46 (85.2)	138 (75.8)
Major mental illness***				
Yes	86 (24.1)	26 (21.5)	14 (25.9)	46 (25.3)
No	271 (75.9)	95 (78.5)	40 (74.1)	136 (74.7)
Injection drug use***				
Yes	140 (39.2)	47 (38.8)	24 (44.4)	69 (37.9)
No	217 (60.8)	74 (61.2)	30 (55.6)	113 (62.1)
Problematic alcohol use***				
Yes	78 (21.9)	19 (15.7)	16 (29.6)	43 (23.6)
No	279 (78.2)	102 (84.3)	38 (70.4)	139 (76.4)
Material deprivation quintile at reinfection				
Q1 (most privileged)	46 (12.9)	16 (13.2)	3 (5.6)	27 (14.8)
Q2	58 (16.3)	19 (15.7)	11 (20.4)	28 (15.4)
Q3	48 (13.5)	18 (14.9)	6 (11.1)	24 (13.2)
Q4	81 (22.7)	32 (26.5)	14 (25.9)	35 (19.2)
Q5 (most deprived)	108 (30.3)	30 (24.8)	16 (29.6)	62 (34.1)
Unknown	16 (4.5)	6 (5)	4 (7.4)	6 (3.3)
Social deprivation quintile at reinfection				
Q1 (most privileged)	27 (7.6)	12 (9.9)	4 (7.4)	11 (6)
Q2	36 (10.1)	11 (9.1)	5 (9.3)	20 (11)
Q3	41 (11.5)	14 (11.6)	6 (11.1)	21 (11.5)
Q4	69 (19.3)	30 (24.8)	11 (20.4)	28 (15.4)
Q5 (most deprived)	168 (47.1)	48 (39.7)	24 (44.4)	96 (52.8)
Unknown	16 (4.5)	6 (5)	4 (7.4)	6 (3.3)

* Two consecutive negative PCR, ≥28 days apart; † Either one negative PCR, or two consecutive negative PCR but the difference between them was less than 28 days; ‡ Clearance type of the first HCV infection; ** Any time before the last day of follow-up; *** Any time during the study follow-up time; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus.

Table 4.2 Rates (95% CI) of HCV re-clearance per 100 person-years in British Columbia, Canada

Characteristics	Confirmed*	Probable† + Confirmed*
Total	**12.36 (10.26-14.77)**	**16.02 (13.74-18.58)**
HCV heterologous genotype		
Yes	6.5 (3.36-11.36)	10.33 (6.31-15.95)
No	13.72 (11.27-16.55)	17.25 (14.64-20.19)
Age at HCV reinfection (years)		
< 35	14.99 (10.56-20.67)	18.59 (13.84-24.44)
35-44	12.45 (9.15-16.55)	16.78 (13.13-21.13)
≥ 45	10.44 (7.35-14.39)	13.38 (9.99-17.54)
Birth Cohort		
< 1964	10.55 (7.92-13.76)	14.01 (11.13-17.42)
1965-1974	13.41 (9.49-18.4)	16.83 (12.57-22.07)
≥ 1975	15.82 (10.59-22.72)	20.46 (14.75-27.65)
Sex		
Female	12.41 (8.83-16.97)	17.71 (13.72-22.49)
Male	12.34 (9.81-15.31)	15.13 (12.41-18.27)
Year of HCV diagnosis		
1990-1997	11.8 (8.76-15.56)	13.99 (10.79-17.83)
1998-2004	10.19 (7.51-13.51)	13.8 (10.81-17.35)
2005-2013	27.44 (17.39-41.17)	37.64 (26.5-51.88)
Clearance type‡		
Spontaneous clearance	12.79 (10.55-15.36)	16.4 (13.99-19.11)
Sustained virological response	8.02 (3.23-16.53)	11.9 (5.94-21.3)
HIV**		
Yes	10.48 (6.71-15.59)	12.74 (8.71-17.98)
No	12.94 (10.49-15.78)	17 (14.33-20.03)
Major mental illness***		
Yes	10.05 (6.56-14.72)	13.57 (9.69-18.48)
No	13.2 (10.68-16.13)	16.93 (14.19-20.04)
Injection drug use***		
Yes	11.63 (8.55-15.47)	14.95 (11.67-18.85)
No	12.88 (10.11-16.17)	16.85 (13.77-20.42)
Problematic alcohol use***		
Yes	7.93 (4.78-12.39)	11.63 (8.1-16.18)
No	13.8 (11.25-16.75)	17.69 (14.88-20.88)
Material deprivation quintile at reinfection		
Q1 (most privileged)	11.47 (6.56-18.63)	13.27 (7.99-20.72)
Q2	13.67 (8.23-21.35)	18.33 (12.37-26.17)
Q3	11.92 (7.07-18.84)	14.88 (9.53-22.14)
Q4	14.53 (9.94-20.52)	18.18 (13.31-24.25)
Q5 (most deprived)	10.25 (6.92-14.63)	13.97 (10.23-18.64)
Unknown	16.44 (6.03-35.79)	23.85 (11.44-43.87)
Social deprivation quintile at reinfection		
Q1 (most privileged)	14.49 (7.49-25.31)	17.84 (10.19-28.96)
Q2	11.16 (5.57-19.98)	15.07 (8.61-24.47)
Q3	13.33 (7.29-22.37)	17 (10.38-26.25)
Q4	16.36 (11.04-23.35)	19.98 (14.34-27.1)
Q5 (most deprived)	10.16 (7.49-13.47)	13.55 (10.6-17.06)
Unknown	16.44 (6.03-35.79)	23.85 (11.44-43.87)

* Two consecutive negative PCR, ≥28 days apart; † Either one negative PCR, or two consecutive negative PCR but the difference between them was <28 days; ‡ Clearance type of the first HCV infection; ** Any time before the last day of follow-up; *** Any time during the study follow-up time; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; CI: Confidence interval.

Table 4.3 Unadjusted and adjusted hazard ratios for factors associated with HCV re-clearance (confirmed) in British Columbia, Canada

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at HCV reinfection (year)		0.579		
< 35	1.27 (0.81-2.01)			
35-44	1.11 (0.72-1.70)			
≥ 45	*Ref*			
Birth Cohort		0.661		0.147
< 1965	0.81 (0.52-1.28)		0.75 (0.46-1.23)	
1965-1974	0.89 (0.55-1.44)		1.16 (0.70-1.93)	
≥ 1975	*Ref*		*Ref*	
Female	0.86 (0.59-1.26)	0.444	0.76 (0.51-1.14)	0.187
Year of HCV diagnosis		0.01		0.017
1990-1997	0.67 (0.41-1.12)		0.71 (0.41-1.21)	
1998-2004	0.47 (0.28-0.77)		0.49 (0.29-0.82)	
2005-2013	*Ref*		*Ref*	
HCV heterologous genotype	0.47 (0.26-0.86)	0.014	0.42 (0.23-0.78)	0.007
Spontaneous clearance‡	1.70 (0.79-3.64)	0.176		
HIV**	0.86 (0.55-1.34)	0.498		
Major mental illness***	0.71 (0.46-1.09)	0.117		
Injection drug use***	0.79 (0.55-1.15)	0.219		
Problematic alcohol use***	0.50 (0.30-0.81)	0.005	0.47 (0.28-0.77)	0.003
Material deprivation quintile at reinfection		0.803		
Q1 (most privileged)	*Ref*			
Q2	0.95 (0.48-1.86)			
Q3	1.03 (0.52-2.04)			
Q4	1.03 (0.56-1.88)			
Q5 (most deprived)	0.74 (0.40-1.36)			
Unknown	1.05 (0.41-2.69)			
Social deprivation quintile at reinfection		0.344		
Q1 (most privileged)	Ref			
Q2	0.68 (0.30-1.55)			
Q3	0.83 (0.38-1.80)			
Q4	0.98 (0.50-1.92)			
Q5 (most deprived)	0.61 (0.32-1.14)			
Unknown	0.85 (0.32-2.26)			

‡ Clearance type of the first HCV infection (ref.: sustained virological response); ** used as a time-varying covariate; *** Any time during the study follow-up time; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; HR: Hazard Ratio: CI: Confidence Interval.

Table 4.4 Unadjusted and adjusted hazard ratios for factors associated with HCV re-clearance (probable + confirmed) in British Columbia, Canada

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at HCV reinfection (year)		0.429		
< 35	1.27 (0.86-1.87)			
35-44	1.21 (0.85-1.73)			
≥ 45	*Ref*			
Birth Cohort		0.537		0.555
< 1965	0.81 (0.56-1.19)		0.90 (0.60-1.34)	
1965-1974	0.83 (0.55-1.25)		1.10 (0.72-1.69)	
≥ 1975	*Ref*		*Ref*	
Female	1.13 (0.83-1.53)	0.44	1.03 (0.74-1.42)	0.877
Year of HCV diagnosis		0.0001		0.001
1990-1997	0.51 (0.34-0.77)		0.52 (0.34-0.81)	
1998-2004	0.43 (0.28-0.64)		0.45 90.30-0.68)	
2005-2013	*Ref*		*Ref*	
HCV heterologous genotype	0.56 (0.35-0.89)	0.014	0.57 (0.35-0.93)	0.024
Spontaneous clearance‡	1.54 (0.84-2.84)	0.176		
HIV**	0.77 (0.53-1.13)	0.185		
Major mental illness***	0.77 (0.54-1.09)	0.138		
Injection drug use***	0.85 (0.63-1.15)	0.295		
Problematic alcohol use***	0.66 (0.45-0.95)	0.027	0.61 (0.42-0.89)	0.011
Material deprivation quintile at reinfection		0.689		
Q1 (most privileged)	*Ref*			
Q2	1.25 (0.7-2.23)			
Q3	1.15 (0.63-2.12)			
Q4	1.24 (0.72-2.13)			
Q5 (most deprived)	0.95 (0.56-1.63)			
Unknown	1.48 (0.69-3.2)			
Social deprivation quintile at reinfection		0.332		
Q1 (most privileged)	*Ref*			
Q2	0.77 (0.39-1.55)			
Q3	0.91 (0.47-1.76)			
Q4	1.03 (0.58-1.84)			
Q5 (most deprived)	0.69 (0.4-1.19)			
Unknown	1.09 (0.5-2.41)			

‡ Clearance type of the first HCV infection (ref.: sustained virological response); ** used as a time-varying covariate; *** Any time during the study follow-up time; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; HR: Hazard Ratio: CI: Confidence Interval.

Table 4.5 Unadjusted and adjusted hazard ratios for factors associated with HCV re-clearance (confirmed) in British Columbia, Canada (restricted to those who spontaneously cleared their first HCV infection)

Characteristics	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Age at HCV reinfection (year)		0.541		
< 35	1.3 (0.81-2.1)			
35-44	1.19 (0.75-1.87)			
≥ 45	*Ref*			
Birth Cohort		0.47		0.078
< 1965	0.78 (0.49-1.24)		0.73 (0.44-1.21)	
1965-1974	0.96 (0.59-1.57)		1.23 (0.74-2.06)	
≥ 1975	*Ref*		*Ref*	
Female	0.83 (0.56-1.22)	0.337	0.73 (0.48-1.10)	0.129
Year of HCV diagnosis		0.019		0.02
1990-1997	0.63 (0.38-1.06)		0.65 (0.38-1.12)	
1998-2004	0.48 (0.29-0.8)		0.48 (0.28-0.81)	
2005-2013	*Ref*		*Ref*	
HCV heterologous genotype	0.57 (0.32-1.04)	0.069	0.46 (0.24-0.86)	0.015
HIV**	0.91 (0.58-1.42)	0.672		
Major mental illness***	0.79 (0.5-1.23)	0.287		
Injection drug use***	0.87 (0.6-1.26)	0.459		
Problematic alcohol use***	0.53 (0.33-0.87)	0.012	0.49 (0.30-0.82)	0.006
Material deprivation quintile at reinfection		0.868		
Q1 (most privileged)	*Ref*			
Q2	0.92 (0.46-1.86)			
Q3	1 (0.49-2.01)			
Q4	1.01 (0.54-1.88)			
Q5 (most deprived)	0.74 (0.39-1.39)			
Unknown	0.98 (0.38-2.54)			
Social deprivation quintile at reinfection		0.25		
Q1 (most privileged)	*Ref*			
Q2	0.78 (0.34-1.8)			
Q3	0.71 (0.3-1.66)			
Q4	1.22 (0.61-2.44)			
Q5 (most deprived)	0.69 (0.36-1.33)			
Unknown	0.9 (0.33-2.43)			

** used as a time-varying covariate; *** Any time within the study follow-up time; HCV: Hepatitis C Virus; HIV: Human Immunodeficiency Virus; HR: Hazard Ratio: CI: Confidence Interval

Chapter 5: Conclusion

5.1 Summary of key findings

The overarching objective of this dissertation was to examine the natural history of HCV infections from the perspective of spontaneous clearance, reinfection, and re-clearance, with a view to identifying potential factors affecting each of these three stages. A summary of the major findings of the dissertation is included below:

Chapter 2 examines the factors associated with spontaneous clearance of primary HCV infection, with particular emphasis on primary T-cell immunodeficiency and HBV coinfection in a large population-based cohort of HCV positive individuals. The study found that a quarter of HCV positive individuals clear the primary infection spontaneously (i.e., without any treatment). After adjusting for potential confounders, primary T-cell immunodeficiency was associated with a lower likelihood of spontaneously clearing the infection. Females, HBV coinfection, and HCV genotype 3 (compared to genotype 1) were associated with a higher likelihood of spontaneous clearance. Except for HBV coinfection, these findings were similar among the seroconverters as well.

Chapter 3 examines HCV reinfection in a large cohort of population that includes those who cleared their first infection spontaneously, and those who achieved sustained virological response after a successful course of HCV treatment. This study found that the rate of reinfection was much higher in the spontaneous clearance group compared to the SVR group. After adjusting for potential confounders, the risk of HCV reinfection was significantly lower in females, and higher in the spontaneous clearance group (compared to the SVR group), those

coinfected with HIV, and in people who inject drugs. Among PWID, engagement with OST and mental health counseling services, as hypothesized, was associated with a significant reduction of HCV reinfection risk.

Chapter 4 examines HCV re-clearance among those who were reinfected following successful clearance of their primary HCV infection (either spontaneously, or after achieving SVR). In the largest study on HCV re-clearance to date, this study found that the rate of spontaneous clearance of reinfection (i.e., re-clearance) is much higher than that of the primary infection. After adjusting for the potential confounders, reinfection with a heterologous HCV genotype and ongoing problematic alcohol use were negatively associated with re-clearance. Among those who cleared their first infection spontaneously, the likelihood of re-clearance was significantly lower among those who were reinfected with a heterologous HCV genotype.

To summarize, this study identified host factors (e.g., older birth cohorts, sex, immunodeficiency), comorbid conditions (e.g., HIV, HBV), risk behaviors (e.g., injection drug use, problematic alcohol use), and potentially effective harm reductions initiative (e.g., OST, mental health counseling) that appear to play important roles along the natural history of HCV infections. These factors inform several aspects of ongoing monitoring, surveillance, and healthcare priority settings, intervention, and resource allocation, including prioritizing HCV treatment allocation, expanding treatment access to PWID along with provisions for harm reduction interventions e.g., OST and mental health counseling.

5.2 Study contributions and strengths

Research evidence generated from this very large, population-based cohort includes data gathered in high-risk populations and uses rigorous methodological (e.g., adopting stringent case definitions) and analytic strategies (e.g., time-varying covariates, propensity score methods), complemented with multiple sensitivity analyses to verify the primary research findings. This dissertation adds novel contributions to the existing HCV literature by using methodologically rigorous empirical studies and a very large population-based cohort of HCV positive individuals. Consistent with the earlier studies, this study showed that about a quarter spontaneously clear their primary HCV infection.[50, 88, 89, 95, 111, 115, 117, 204] It is the first study to examine the role of primary T-cell immunodeficiency on the spontaneous clearance of HCV in a population-based study. Compatible with the existing research evidence from immunological studies,[124-126] this study found that primary T-cell immunodeficiency is associated with a significantly lower likelihood of spontaneous clearance. As described in Chapter 2, the prevalence of primary T-cell immunodeficiency is low in the population. Therefore, the population-level impact may not be substantive. However, as the likelihood of spontaneous clearance is low, this group of people may be prioritized to receive HCV treatment to prevent progressive liver diseases, which has implications for the healthcare system.

The study on HCV reinfection described in Chapter 3 includes the largest available number of reinfection cases to date.[161] The estimates reported from the current study will add valuable evidence to the existing HCV literature where a wide range of reinfection estimates have been reported in earlier smaller studies.[36, 44, 45, 47-49, 51, 53, 55, 63, 64, 67-70, 72, 75, 77, 176, 179, 209] This study also found that post-SVR reinfection rate was lower compared to

reinfection following spontaneous clearance of primary infection. While this is consistent with previous studies (including a meta-analysis), some other studies have reported a higher post-SVR reinfection rate among MSM coinfected with HIV.[57, 165, 175, 176] These seemingly opposing results may actually provide significant evidence to expand our understanding of HCV epidemiology and public health responses in ways that reflect the importance of the mode of HCV acquisition and HCV-related risk behaviors.[179] As described in Chapter 3, this finding has important implications from public health policy perspectives, particularly from expansion of HCV treatment to high-risk population groups such as PWID, MSM, and incarcerated people. Consistent with previous studies,[45, 63, 68, 69, 75, 165, 209, 210] the post-SVR reinfection rate among PWID was lower compared to the spontaneous clearance group. Restriction to treatment access for PWID and the HIV coinfected population was reported in earlier studies.[42, 165, 172, 177, 178, 211, 212] This study is the first of its kind to rigorously and comprehensively examine the role of these interventions in the context of HCV reinfection in the interferon era. Furthermore, , the finding that engagement with OST and mental health counseling was associated with a lower reinfection risk establishes a rationale for incorporating interventions aimed at behavioral change to prevent HCV reinfection among PWID.[179, 212-215] Thus, the findings from this study further support that PWID should not be excluded from the treatment due to perceived risk of higher reinfection rates following SVR. However, many important questions remain, including those that examine whether reinfection rates may be higher due to higher cure rate and lower adverse events in the era of DAA.[179] More analyses informed by data gathered during the DAA era will provide significant and much needed additions to the knowledge base in this area.

The study on HCV re-clearance contributes significantly to the existing literature amid conflicting evidence with regard to cross-genotype immunity.[79, 80, 132] Results from this study show that there is limited evidence of cross-genotype immunity, and people who are reinfected with a heterologous genotype are significantly less likely to spontaneously clear the reinfection episode, even among those who were able to spontaneously clear their previous HCV episodes. This study also reinforces the existing literature on the negative role of problematic alcohol use on HCV outcomes.[127, 206, 207]

5.3 Study limitations

While this dissertation offers significant contributions to the existing HCV literature, it has some limitations as is the case with all research. Limitations relevant to specific studies have been elaborated in the respective chapters (2-4). To summarize, first, administrative databases were used to define some of the comorbidities and risk factors. This is less of a concern for the comorbidities, especially because healthcare in British Columbia is single-payer publicly-funded insurance-based and thus the comorbidities used in this dissertation e.g., HIV, HBV were reported to the central healthcare databases. However, risk factors such as injection drug use and problematic alcohol use may have been underreported. This means, even after using validated algorithms to define these risk factors, this study may have misclassified some of the people with these risk factors. However, it is expected that the misclassification is non-differential resulting in an underestimation of the effects. Another major limitation of this study is the lack of information on ethnicity and IL28B polymorphism. While research shows mixed evidence on the effects of both of these variables,[50, 89, 109, 127, 154, 160] these could not be evaluated in this study. Another limitation of this study is the irregular testing with longer

intervals which, while providing important data on the real-life scenario, may have missed some episodes of reinfection and re-clearance. Variation in the sensitivity of HCV testing assays over time could have misclassified some of the active cases of HCV as non-cases (especially between 2000 and 2006 when the lower limit of detection of viral particles was higher). However, a rigorous attempt was taken to examine those test results. As reported in the respective chapters (2-4), only a few cases might have been misclassified, and these low numbers may not have had substantial impact on the inferences drawn in this dissertation. Reinfection was not defined based on genotyping as data on this was not available for most of the cases. While previous studies showed that late relapse post-SVR is very rare (<1%),[175, 202] and that most of the post-SVR recurrence can be attributed to reinfection,[179, 216] validating the reinfection cases with genotype or sequencing data will be helpful which is expected to be widely available in the era of DAA.

Being an observational study, drawing causal inference based on findings from this study is prone to bias. Even though, an advanced bias-adjusting technique (propensity score) has been applied, there might still be some unmeasured confounding. Thus, caution should be exercised in drawing causal inference from this study. Caution should also be exercised in interpreting the results from some of the stratified analysis (e.g., in the seroconverters [Chapter 2] and in the SVR group [Chapter 3]) due to smaller number of outcome events.

5.4 Research implications

Practical implementations of the research findings have been described in the empirical Chapters 2-4. The section summarizes the implications of these results. The findings

from Chapter 2 lend support to existing theories of the role of the immune system in HCV clearance which will be useful in informing public health surveillance, monitoring, and treatment decisions in the era of highly effective direct-acting antivirals as to who could be prioritized to receive HCV treatment. Results from Chapter 3 show that compared to those who achieve SVR, people who spontaneously clear their first infection are at greater risk of HCV reinfection essentially due largely to the fact that people with ongoing risk behaviors were less likely to receive older interferon-based treatments. Thus, those who clear the primary HCV infection spontaneously should be monitored for future infections along with people who inject drugs and those coinfected with HIV. Chapter 3 also shows that provision of harm reduction interventions such as OST and mental health counseling may be helpful in reducing reinfection risk among PWID. The findings from Chapter 4 show that reinfection with heterologous genotype, and an ongoing history of problematic alcohol use are associated with significantly lower likelihood of re-clearance which have important policy implications with regard to public health monitoring, surveillance, and resource (particularly, treatment) allocation. The lack of cross-genotype immunity lends support to the existing literature on a prophylactic vaccine boosted with different HCV genotypes.

5.5 Recommendations and future research directions

The results from the study on the spontaneous clearance of primary HCV suggests that people with primary T-cell immunodeficiency would benefit if they are prioritized for HCV treatment. However, due to low prevalence of this condition in the population, the number of cases examined in this study was smaller. Therefore, the study was unable to examine the association of primary T-cell deficiency and spontaneous clearance among those who

seroconverted within 24 months (Chapter 2). Larger studies, probably taking cases from multiple jurisdictions/study centers, would help disentangle this link further. As suggested by other studies,[110, 112, 113, 119, 128] HBV coinfection was associated with a higher likelihood of spontaneous clearance of HCV. However, previous results reported a weaker association among those who do not inject drugs,[110] while this was not found to be a significant predictor of spontaneous clearance among the seroconverters in this study. Thus, concomitant risk behaviors and/or mode of HCV acquisition may play an important role in clearing HCV infection spontaneously [110]. More research is required to unravel this further.

Results from Chapter 3 indicate that those who spontaneously cleared the primary HCV episode had higher reinfection risk compared to their SVR counterparts. This is presumably due to a higher proportion of people who inject drugs in the spontaneous clearance group. Newly launched highly effective DAAs are now being scaled up to include more PWID. Higher cure rates coupled with scaling-up of the DAAs, it is anticipated that more at-risk populations (e.g., PWID) will be treated and cured who would potentially be at risk of HCV reinfection.[179] Data from chapter 3 also showed a protective effect of harm reduction initiatives (OST, and mental health counseling) on HCV reinfection. Based on these findings, it could be postulated that enragement with harm reduction interventions would prevent reinfection in the DAA era. However, more research is required to see if these findings hold in the era of DAA. Research examining the level of engagement with OST with a view to understand the optimal level of engagement with harm reductions (e.g., OST, mental health counseling, and needle/syringe distribution) will add significant contributions to the HCV literature. Future research should also examine other harm reduction interventions such as needle and/or syringe

distribution, and how these interventions perform in PWID with varying drug use behaviors (e.g., stimulants, opioids, poly drugs etc.). Multilevel studies to examine the role of structural effects (e.g., access to healthcare resources, drug supply) will also be beneficial in understanding the HCV epidemiology. Further larger studies are also recommended in other high-risk groups e.g., MSM and incarcerated populations. Besides adopting rigorous methodologies, application of rigorous analytic strategies such as marginal structural models will also be helpful in drawing causal inference from observational data.

In the context of conflicting evidence from earlier studies, [79, 80, 132] results from Chapter 4 indicate the possible benefit of a prophylactic vaccine boosted with different HCV genotypes as suggested by earlier finding [132]. However, more research is required to answer the requirement for the number of booster doses, the gap between successive booster doses, the comparative efficacy of simultaneous vs. sequential use of multiple HCV strains, the order of HCV genotypes in the vaccine shots that might enhance cross-genotype immunity (in case of proven superiority of sequential use of different HCV strains) etc.

5.6 Final conclusions

This dissertation aimed at identifying potential factors associated with different stages of HCV natural history. Some of the major findings of this study include a lower likelihood of spontaneous clearance of primary HCV infection in people with primary T-cell immunodeficiency, a higher likelihood of HCV reinfection in people who cleared their previous HCV episode spontaneously, a significantly lower likelihood of HCV reinfection among people who inject drugs if they are engaged with harm reduction initiatives such as opioid substitution

therapy and mental health counseling, and a significantly lower likelihood of re-clearance if reinfected with a heterologous HCV genotype even among those who were able to spontaneously clear their primary HCV infection. Besides contributing to the existing literature, these findings will help inform public health policy including surveillance, monitoring, and designing preventive strategies. These will also help advance HCV vaccine development strategies.

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