Illicit and prescribed drug use and liver fibrosis in HIV-hepatitis C co-infected individuals: marijuana, opioids and antiretroviral therapy

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Abstract

Liver diseases are an important cause of morbidity and are the third cause of death among persons living with HIV. Progression of liver disease is accelerated in HIV-hepatitis C co-infected persons. HCV cure remains the only effective intervention to reduce liver related morbidity and mortality in co-infected persons, but only a small proportion access treatment. It is therefore important to identify which drugs can accelerate the progression of liver disease so that physicians can counsel their patients or select safer therapies. However, comprehensive assessments of illicit drugs commonly used in this population are rare and studies of certain common prescription drugs are also limited.

The overall objective of this dissertation was to assess the risks of liver damage and liver disease associated with prescribed and illicit drug use in persons co-infected with HIV and hepatitis C. More specifically, the objectives were to:

1. Estimate the association between marijuana smoking and progression to significant liver fibrosis or end-stage liver disease;

2. Estimate the association between prescribed and illicit opioid use and changes in a marker of liver fibrosis, the aspartate-to-platelet ratio index (APRI) score or with progression to significant liver fibrosis.

3. Estimate the rate of change in APRI score associated with the class of antiretroviral drug used.

Data from the Canadian Co-infection Cohort were used. It is a prospective study of persons infected with HIV and with serological evidence of infection with hepatitis C (cleared or active) from 18 sites across Canada. Recruitment started in 2003 and participants are followed every six months.

Manuscript 1 focused on the association between marijuana smoking and liver disease progression. Survival analyses were performed with Cox Proportional Hazards models and showed no association between marijuana use and progression to either significant liver fibrosis or end-stage liver disease. The average number of joints smoked per day was not associated with a change in the ln(APRI) score as shown with a linear splines regression model.

Manuscript 2 focused on the association between opioid use and liver disease progression. A linear regression models with generalized estimating equations was applied to a sample including prevalent cases of liver fibrosis and end-stage liver disease. A survival analysis with pooled logistic regression was performed in the sample excluding prevalent cases of liver fibrosis and end-stage liver disease. No association was found between prescribed or illicit opioid use and changes in the median APRI score or with a faster progression to significant liver fibrosis.

Manuscript 3 focused on assessing the median rates of change in the APRI score among new users of protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI), while accounting for the backbone used. Individuals were matched with replacement based on their propensity score for receiving an NNRTI. Linear regression with generalized estimating

equations was used. Only abacavir/lamivudine containing regimens were associated with an increase of APRI score over time, regardless of the class of anchor agent used.

It is essential to understand which illicit and prescribed drugs are risk factors for the progression of liver diseases in co-infected persons as these exposures can be intervened on, but little research has been done in that area. The findings of this dissertation suggest that two types of illicit drugs commonly used by HIV-hepatitis C co-infected persons, marijuana and opioids, are not associated with a worsening of their liver health. Prescribed opioids, often used to treat dependence or manage pain in this population, did not appear to be associated with liver damage either. Finally, the choice of cART regimen seems to influence progression of liver fibrosis.

Résumé

Les maladies du foie représentent une des principales causes de morbidité et la troisième cause de mortalité chez les gens vivant avec le VIH. Leur progression est accélérée chez les personnes coïnfectées avec le VIH et l'hépatite C. La seule intervention efficace pour diminuer la morbidité et mortalité liées au foie est le traitement de l'hépatite C, très peu accessible. Il est donc primordial d'identifier quels médicaments et drogues peuvent accélérer la progression des maladies du foie. Cependant, les évaluations compréhensives des drogues et médicaments communément utilisés dans cette population sont limitées.

L'objectif global de cette thèse doctorale était d'évaluer le risque de dommage au foie associé à l'utilisation de drogues et médicaments chez les personnes coïnfectées par le VIH et l'hépatite C. Plus spécifiquement, les objectifs étaient de :

1. Estimer l'association entre l'utilisation de marijuana et la progression vers la fibrose du foie ou les maladies du foie en phase terminale.

2. Estimer l'association entre l'utilisation d'opiacés prescrits ou de manière illicite et des changements au score *aspartate-to-platelet ratio index* (APRI), un marqueur de la fibrose du foie, ou avec la progression vers la fibrose du foie.

3. Estimer le taux de changements du score APRI à travers le temps associé avec la classe de médicament antirétroviral utilisée.

Les données de la Cohorte canadienne de coïnfection ont été utilisées. Il s'agit d'une étude prospective constituée de personnes vivant avec le VIH, présentant une preuve sérologique

d'infection à l'hépatite C (éliminée ou active) et recrutées dans l'un des 18 sites au Canada. Le recrutement a débuté en 2003 et les visites de suivi ont lieu à chaque six mois.

Le premier manuscrit porte sur le lien entre la marijuana et la progression vers la fibrose ou les maladies du foie en phase terminales. Aucun lien n'a pu être établi par des analyses de survie employant des modèles à risques proportionnels de Cox. Un modèle de régression avec splines linéaires a démontré que l'augmentation du nombre moyen de joints fumés par jour n'était pas associée à un changement dans le score ln(APRI).

Le second manuscrit porte sur l'association entre l'utilisation d'opiacés et la progression des maladies du foie. Les données ont été analysées par régression linéaire avec équations d'estimation généralisées ainsi que par régression logistique combinée. Ni les opiacés prescrits ni ceux consommés illicitement n'étaient associés à un changement du score APRI médian ou à une progression accélérée vers la fibrose du foie.

Le troisième manuscrit porte sur les changements au score APRI à travers le temps chez les nouveaux utilisateurs d'inhibiteurs de la protéase (IP) ou d'inhibiteurs non-nucléosidiques de la transcriptase inverse (INNTI), en tenant compte de la combinaison d'analogues nucléosidiques utilisée. Les individus ont été appariés avec remplacement sur la base de leur score de propension. Un modèle de régression linéaire avec équations d'estimation généralisées a été utilisé. Seuls les combinaisons incluant abacavir/lamivudine étaient associées à un changement du score APRI médian avec le temps.

Il est essentiel de comprendre quelles drogues et quels médicaments peuvent influencer la progression des maladies du foie chez les personnes coïnfectées puisqu'il est possible

d’intervenir sur ces facteurs, mais peu de recherches ont été effectuées sur ce sujet. Les résultats obtenus dans cette thèse doctorale suggèrent que deux des drogues fréquemment utilisées par les personnes coïnfectées, soit la marijuana et les opiacés, ne sont pas associées à une détérioration de leur santé hépatique. Les opiacés sous prescription, souvent utilisés comme traitement contre la dépendance ou pour la gestion de la douleur, ne semblent pas non plus liés aux dommages au foie. Finalement, le choix de combinaison pour le traitement du VIH est associé à la progression de la fibrose du foie.

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Statement of originality

The work contained in this thesis constitutes an original contribution to the field of HIV-hepatitis C co-infection epidemiology and addresses gaps in the literature on the association between drug use and progression of liver diseases.

Manuscript 1 presents the first large longitudinal evaluation of the relationship between marijuana smoking and liver fibrosis. We performed survival analyses as well as a dose-response analysis showing the absence of an association. The medical community was dissatisfied by the quality of the three previously published cross-sectional studies. This study was therefore well received as it addressed their methodological flaws.

Manuscript 2 is a unique investigation of the association between opioid use and liver disease. It was based on a heterogeneous population including, but not limited to illicit drug users. The study of the separate associations between liver disease and opioids used either illicitly or with a prescription was novel, as was the special attention given to measurement error of the exposure through a Monte Carlo sensitivity analysis. Finally, the use of two analytical samples allowed the analysis of the progression of liver fibrosis among healthier and sicker populations.

Manuscript 3 furthers our understanding of the progression of liver fibrosis associated with different classes of anchor agents and the two recommended backbones for combination antiretroviral therapy. To date, most of the focus in the literature was on short-term hepatotoxicity events and have failed to take into account confounding by indication for selection of antiretroviral drugs used. We used a new-user design with propensity score

matching and an intention-to-treat analysis to emulate a randomized controlled trial and limit the bias introduced by confounding and carry-over effects. Liver fibrosis progression over time is a precursor to serious clinical events such as end-stage liver diseases, and the majority of person living with HIV is treated with combination antiretroviral therapy. A better understanding of how the various combinations available can affect liver fibrosis is therefore most valuable for clinical decision-making.

While I have received guidance from my committee members and co-authors on the methodological, statistical and substantive aspects of this thesis, I declare that the conception, execution and drafting of this thesis were my own.

Contribution of authors

Manuscript 1: Marijuana smoking does not accelerate progression of liver disease in HIV-HCV co-infected individuals

Brunet L, Moodie EEM, Rollet K, Cooper C, Walmsley S, Potter M, Klein MB and the Canadian Co-infection Cohort Investigators. *Clinical Infectious Diseases* 2013.

Laurence Brunet designed the study, conducted all the analyses, interpreted the results and drafted the manuscript. Erica Moodie contributed to the design of the study and provided statistical guidance. Kathleen Rollet was responsible for managing the data and producing datasets for the analysis. Curtis Cooper, Sharon Walmsley and Martin Potter recruited and followed participants. Marina Klein contributed to the design of the study and provided input on the interpretation of results. All the authors have commented on the manuscript.

Manuscript 2: Use of opioids in a Canadian HIV/hepatitis C co-infected population and its relation to liver fibrosis

Brunet L, Moodie EEM, Cox J, Gill J, Cooper C, Walmsley S, Rachlis A, Hull M, Klein MB for the Canadian Co-infection Cohort Study Investigators.

Laurence Brunet designed the study, conducted all the analyses, interpreted the results and drafted the manuscript. Erica Moodie contributed to the design of the study and provided statistical guidance. Joseph Cox recruited participants and contributed to the study design and interpretation of the results. John Gill, Curtis Cooper, Sharon Walmsley, Anita Rachlis and Mark Hull recruited participants and contributed to the interpretation of results. Marina Klein

contributed to the design of the study and provided input on the interpretation of results. All the authors have commented on the manuscript.

Manuscript 3: Changes in APRI scores among NNRTI and PI users co-infected with HIV and hepatitis C

Brunet L, Moodie EEM, Young J, Cox J, Hull M, Cooper C, Walmsley S, Martel-Lafferrière V, Rachlis A, Klein MB for the Canadian Co-infection Cohort study

Laurence Brunet designed the study, conducted all the analyses, interpreted the results and drafted the manuscript. Erica Moodie contributed to the design of the study and provided statistical guidance. Jim Young contributed to the design of the study. Mark Hull, Curtis Cooper, Sharon Walmsley, Valérie Martel-Laferrière and Anita Rachlis recruited participants and contributed to the interpretation of results. Marina Klein contributed to the design of the study and provided input on the interpretation of results. All the authors have commented on the manuscript.

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List of abbreviations

3TC	Lamivudine
ABC	Abacavir
ALT	Alanine aminotransferase
APRI	Aspartate aminotransferase to platelet ratio index
ART	Antiretroviral therapy
AST	Aspartate aminotransferase
AUROC	Area under the receiver operating characteristic
cART	Combination antiretroviral therapy
DAA	Direct-acting antivirals
DDD	Defined daily doses
ELISA	Enzyme-linked immunosorbent assay
ESLD	End-stage Liver Disease
FTC	Emtricitabine
GEE	Generalized estimating equations
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HR	Hazard ratio
IDU	Injection drug use
MMT	Methadone maintenance treatment
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
OR	Odds ratio
PI	Protease inhibitor
SMR	Standardized mortality ratio
TDF	Tenofovir
ULN	Upper limit of normal

Chapter 1: Introduction

HIV-infected persons live longer, healthier lives now because of the discovery of antiretroviral therapy (ART) and their high efficacy when combined as "combination antiretroviral therapy" (cART). This increased longevity has been translated into a shift in the common morbidities and causes of death in this population. In high-income countries, mortality is now related to cancer, liver disease, cardiovascular disease, non-AIDS related infections, suicide and overdose much more frequently than to AIDS.[1,2] HIV-hepatitis C (HCV) co-infection is common, with 4 to 5 million co-infected persons worldwide (approximately 30% of those infected with HIV).[3] HIV infection aggravates the natural history of HCV infection, leading to a faster progression to advanced liver disease.[4,5]

Several factors contributing to the progression of liver diseases have been identified. However, the increased risk of liver disease observed cannot be fully explained by these risk factors. HIV-HCV co-infected persons are exposed to a myriad of potentially hepatotoxic illicit and prescription drugs. Among the drugs often used illicitly in this population are marijuana and opioids, both used recreationally or to self-medicate. It is generally believed that these drugs are harmful for the liver although the epidemiological evidence is scarce and of poor quality. Opioids are also often prescribed to treat addiction or pain, but it is unclear whether prescribed opioids can lead to increased liver fibrosis. The introduction of cART has led to remarkable benefits in terms of controlling the progression of HIV infection and preventing AIDS and mortality. However, there have been many reports of short-term cART-associated hepatotoxicity, and some evidence that there may be longer-term cumulative hepatotoxicity

for certain drugs. Most studies however have been of relatively short duration and/or not in the setting of HIV-HCV co-infection, making it difficult to know if certain drugs may be more hazardous for this population.

The overall objective of this doctoral dissertation is to understand how illicit and prescribed drugs can affect the progression of liver disease in HIV-HCV co-infected persons. More specifically, this doctoral work aims at:

1. Describing marijuana use in the Canadian Co-infection Cohort and estimating the association between marijuana smoking and progression to significant liver fibrosis or end-stage liver disease.

2. Describing opioids used (prescribed and illicit) in the Canadian Co-infection Cohort and assessing the association between their use and changes in a marker of liver fibrosis or progression to significant liver fibrosis.

3. Estimating the rate of change in a marker of liver fibrosis over time among new users of protease inhibitor (PI)-based and of non-nucleoside reverse transcriptase inhibitor (NNRTI)-based cART regimens.

The format of this thesis is manuscript-based. It includes three manuscripts each corresponding to an objective of the thesis. Additional chapters complement these manuscripts to form a cohesive dissertation. Each manuscript is preceded by a preamble explaining the rational for the manuscript and its relation to the corresponding thesis objective. Chapter 2 consists of a review of the literature and is followed in Chapter 3 by a description of the Canadian Co-

Infection Cohort methodology as well as a detailed description of the specific methods used in each of the manuscripts. Manuscript 1 (Chapter 4) investigates the presence of an association between marijuana smoking and liver disease. Manuscript 2 (Chapter 5) examines the relationship between illicitly used opioids, prescribed opioid use and liver fibrosis. Manuscript 3 (Chapter 6) explores the changes in a marker of liver fibrosis associated with NNRTI- and PI-based regimens. Chapter 7 consists of a discussion of the findings from this doctoral thesis as well as concluding remarks. The references to the publications cited in this work, including in the manuscripts, are provided in the References section at the end of the thesis.

Chapter 2: Literature review

2.1. Epidemiology of HIV, hepatitis C and co-infection

Despite a 33% decline in the number of new HIV infections worldwide between 2001 and 2012, HIV remains a major concern. In 2012, approximately 35.3 million persons were living with HIV world-wide.[6] In Canada, the World Health Organization estimates that between 59 and 85 thousand people were living with HIV in 2012.[6] Since the introduction of cART, life expectancy has increased considerably, but comorbidities such as cancer, cardiovascular disease and liver diseases have become common.[7,8]

HCV infection also represents a major health concern worldwide due to the important number of persons infected and the serious long-term consequences of HCV infection on the liver. In 2005, the estimated global prevalence of HCV seropositivity was 2.8% with over 185 million people with evidence of HCV infection.[9] In Canada and the United States, an estimated 1.3% of the population have anti-HCV antibodies.[9] A Canadian report estimated the prevalence of HCV infection in 2007 in Canada at 0.78%, as measured by HCV antibody testing.[10]

HIV and HCV share common routes of transmission through exposure to contaminated blood, mainly from injection drug use (IDU). Globally, approximately 4 to 5 million persons could be co-infected with HIV and HCV.[3] The prevalence of HIV-HCV co-infection is estimated to range between 20-30%.[11] However, the prevalence can be much larger in some subgroups. As many as 72-95% of persons infected with HIV who have a history of IDU show evidence of HCV infection.[3] Although sexual contact is less frequently responsible for the transmission of HCV,

the incidence of sexually acquired HCV is increasing among HIV-infected men who have sex with men.[4,12] In Canada, HIV-infected females of aboriginal ethnicity are more likely to acquire HCV than females of other ethnicities.[13]

2.2. Natural history of HIV infection

HIV is a retrovirus, an RNA virus that uses reverse transcriptase to replicate in the host cell. HIV targets the CD4 T cells, which play a key role in the immune response to pathogens. Therefore, as HIV replicates, the number of CD4 cells decreases, leading to a high vulnerability to opportunistic infection.[14]

The stages of HIV infection are defined by the Center for Disease Control and Prevention based on the CD4 cell count. Stage 1 of HIV infection in adults consists of a CD4 cell count of at least 500 cells/µL and stage 2 is defined by a CD4 cell count between 200 and 499 cells/µL.[15] A person is considered to have AIDS (stage 3) either if their CD4 cell count is below 200 cells/µL, resulting in a severely compromised immune system or if the they acquire one of 25 stage-3-defining opportunistic illnesses.[15] However, in Canada, clinical criteria are used to define AIDS, which consist in the presence of an AIDS-defining condition concurrent with positive HIV serology.[16]

Before 1996, persons living with HIV survived for a median of 4 to 13 years, depending on their age at seroconversion.[17] cART has led to an increased survival of persons living with HIV. Data from a large collaboration of European and North American HIV cohorts (Antiretroviral Therapy Cohort Collaboration) showed that at age 20 years, those who initiated cART between 1996 and

1999 had a life expectancy of 36 years. Initiating cART in 2003-2005 increased life expectancy to 49 years.[18]

The improved longevity associated with the introduction of cART has led to a shift in the types of health problems that persons living with HIV are facing. Other morbidities such as cancer, end-stage liver disease, cardiovascular disease, central nervous system complications and kidney disease have become important concerns.[19] AIDS is no longer the most important cause of premature death among HIV-infected persons. The most frequent causes of death are now cancer, liver disease, cardiovascular disease, non-AIDS related infections, suicide and overdose.[1,2] However, the importance of specific causes of death varies between the different populations studied.

Between 24 and 80% of HIV-infected persons experience pain of nociceptive or neuropathic nature.[20-24] Pain can be caused either by HIV infection itself, its complications (opportunistic infections, malignancies), HIV treatment, debilitation diseases (pressure sores), wasting, or other causes unrelated to HIV infection (nutritional deficiencies, alcohol related neuropathies).[20,24] In a survey of 153 persons living with HIV, close to 20% of those with an asymptomatic infection experienced pain, whereas 79% of those with advanced to severe HIV-associated symptoms reported pain, although it was impossible to determine the aetiology of the pain at the time of the visit in 70% of the cases.[23]

2.3. Hepatitis C infection

2.3.1. Natural history of hepatitis C infection

HCV is also an RNA virus of the *Flaviviridae* family. It infects the liver primarily and there are no vaccines available to prevent infection. The first phase of HCV infection is acute infection, which is usually asymptomatic.[25] Some clear the infection spontaneously between 3 months and 2 years after acquisition,[26] but 70-80% of HCV mono-infected persons develop chronic infection,[27] defined as detectable HCV RNA six months after seroconversion.[28] Factors such as younger age, female sex, HCV genotype 1,[29] and certain genetic haplotypes like IL-28B single-nucleotide polymorphism[30] are predictors of spontaneous clearance. Hepatitis C virus and the immune process leading to the necroinflammation associated with HCV infection can cause fibrosis. Among those who develop a chronic infection, 67 to 85% develop liver fibrosis after 10 years of infection. Severe fibrosis can in turn progress to liver cirrhosis: about 20% of persons with chronic hepatitis C infection develop cirrhosis after 20 years.[28] Cirrhosis can then lead to liver failure and hepatocellular carcinoma, although some persons presenting with little to no liver fibrosis have hepatocellular carcinoma.[28,31] Figure 2.1 illustrates the progression of HCV from acute infection to development of hepatocellular carcinoma (HCC). Evidence shows that successful treatment of the underlying infection can result in a significant regression of fibrosis.[32]

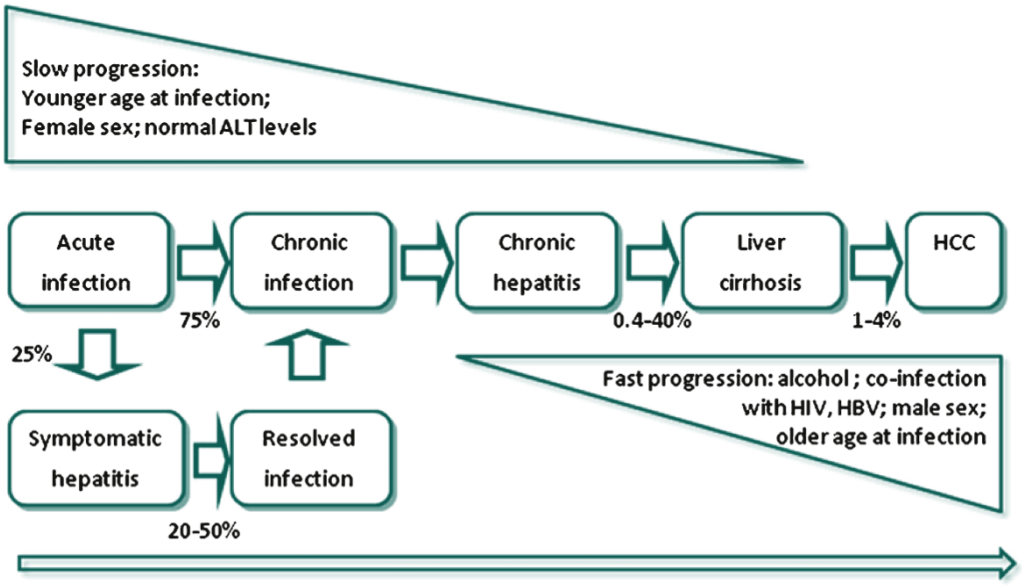

Figure 2. 1 Natural history of hepatitis C infection

Source: Wursthorn et al., *Best Pract Res Clin Gastroenterol,* 2008.[28]

Persons infected with HCV also often experience pain. Among 8 224 veterans diagnosed with HCV between 2000 and 2004, 67% received a pain related diagnosis during that period.[33] In a survey of HCV-infected persons, as many as 85% of participants reported having pain symptoms. From a list of HCV-related symptoms, 52% reported experiencing muscle or joint pain and 31% reported abdominal pain.[34] Depression severity could explain at least part of the pain experienced by HCV-infected persons.[35]

2.3.2. Treatment of hepatitis C

The World Health Organization recommends that all chronically infected persons be assessed for HCV antiviral treatment. Until recently, the recommended treatment for genotype 2 and 3 was a 24 weeks course of pegylated interferon in combination with ribavirin. For genotype 1, the recommended treatment was 48 weeks of either telaprevir or boceprevir in addition to

pegylated interferon and ribavirin.[36] However, new direct-acting antivirals (DAA) are becoming available. They are effective for genotypes 1, 2, 3 and 4, with only 12 to 24 weeks of treatment and have high sustained virological response rates, while being associated with fewer side effects. These new agents are now the first choice to treat HCV[36] but are not yet widely available. Due to their enormous cost ($67 000-$125 000 per treatment course) the vast majority of HCV infected persons worldwide will not be treated and thus remain at risk for ongoing liver injury from HCV and other hepatotoxic exposures.[37,38]

2.4. Impact of co-infection on the natural history of HIV and hepatitis C

It is unclear how HCV infection influences the natural history of HIV.[39] Some studies reported no association between HCV infection and the risk of AIDS and overall mortality in HIV-infected persons.[27,40,41] However, there have been reports of a faster progression to AIDS or death[42-44] and CD4 response to cART appears reduced[42] in co-infected compared to HIV mono-infected persons.

HIV infection is known to aggravate the natural history of HCV infection. Progression to chronic HCV infection following the acute phase occurs in about 90% of co-infected persons, but only in 70-80% of HCV mono-infected persons.[27] In the pre cART era, co-infected persons progressed to cirrhosis on average 7 years after HCV infection, compared to 23 years for HCV mono-infected persons.[39] Co-infected persons are also more likely than HCV mono-infected persons to have higher HCV viral load and progress more rapidly to advanced liver disease.[4,5] For example, progression to hepatocellular carcinoma takes 28 years in HCV mono-infected persons and only 18 years after HCV infection in co-infected persons.[39] There is little data on the natural history

of HCV in persons living with HIV in the post cART era. Control of HIV infection with cART could reduce the long-term risk of liver disease, although this could be counteracted by the toxicity of treatment.

In Europe, a cross sectional study showed 29% of HIV-HCV co-infected persons had significant fibrosis (stages F2-F4 on the Metavir staging scale) as compared to 15% of HCV mono-infected persons.[45] In a study of 78 co-infected persons with no significant fibrosis, 42 (54%) progressed to significant fibrosis after a median of three years between biopsies.[46] Hepatic steatosis also occurs more frequently among co-infected persons and can accelerate the progression of liver disease and decrease HCV treatment effectiveness.[47] Moreover, a meta-analysis has shown that the risk of progression to cirrhosis is twice as high in HIV-HCV co-infected than in HCV mono-infected persons, and the risk of liver failure is six times higher.[5] Co-infected persons are also less likely to achieve sustained virologic response with first generation HCV treatment than HCV mono-infected ones.[47]

Finally, compared to HIV mono-infection, HIV-HCV co-infection appears to be associated with higher odds of pain that interferes with daily living, muscle or joint pain and headaches. However, co-infection is not associated with increased odds of peripheral neuropathy.[48]

2.5. Assessing the presence of liver damage and liver disease

2.5.1. Liver biopsy

The gold standard for the diagnosis of liver fibrosis is biopsy. Several scales have been developed to describe the different stages of liver fibrosis. Stages 0 through 4 on the Batts-Ludwig scale and F0 through F4 on the Metavir scale correspond to no fibrosis, mild fibrosis

(portal fibrosis without septa), moderate fibrosis (portal fibrosis and few septa), severe fibrosis (numerous septa without cirrhosis), and cirrhosis, respectively.[49]

Liver biopsy is an invasive and costly procedure that is prone to sampling error[50] and can lead to serious complications.[51] Therefore, studies of liver fibrosis using biopsy to measure the outcome are prone to selection bias because it can only be performed in persons with a medical indication for liver biopsy. Moreover, performing repeated biopsies for research purposes is unethical on a large scale and repeated biopsies are seldom used in clinical follow-up. All these reasons explain the wide use of imperfect measures in the literature. Biochemical markers, composite scores and non-invasive procedures can be used to indicate liver damage. These markers are associated with various types of liver damage. A non-exhaustive list of the markers available follows and includes the markers used in the studies reported in this review of the literature.

2.5.2. Transient elastography

Transient elastography is a non-invasive and rapid technique measuring liver stiffness. It is a marker of liver fibrosis using ultrasounds and low frequency vibration to measure liver stiffness.[52,53] This method performs well with an area under the receiver operating characteristic curve (AUROC) of 0.84 (95% CI: 0.83, 0.86) for significant liver fibrosis, and 0.94 (95% CI: 0.93, 0.95) for liver cirrhosis.[54] Optimal cut-off values corresponding to the Metavir scale have been determined and are similar for HCV mono-infected and HIV-HCV co-infected persons.[52] However, older age and obesity of the patient, experience of the person performing the test and position of the probe can reduce its success rate.[52,53] The measure itself can be

affected by the presence of acute hepatitis, biliary obstruction, hepatic venous congestion, hepatic amyoloidosis and food consumption.[53] *FibroScan* was licensed for use in Canada in 2009,[55] but the equipment necessary to perform transient elastrography measurement is expensive and not available everywhere.

2.5.3. Biochemical markers

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are markers of hepatocyte integrity. These enzymes catabolize amino acids.[56]

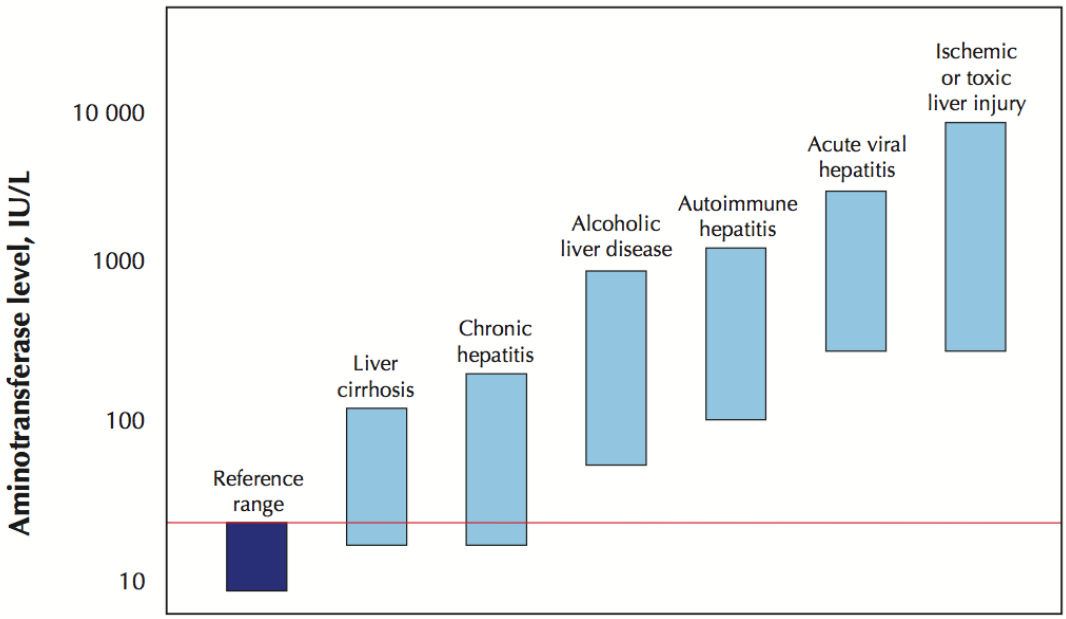

Figure 2. 2 Serum aminotransferase levels in various liver diseases

Source: Giannini et al., *CMAJ,* 2005.[56]

Figure 2.2 shows the variations in serum aminotransferase level in different liver diseases. It is of note that the range found among persons with liver fibrosis and chronic hepatitis overlap with the reference range.

Changes in AST and ALT levels are often used to define acute hepatotoxicity but are not sensitive for diagnosing chronic liver damage (fibrosis or cirrhosis). Most studies of drug-induced hepatotoxicity in HIV-infected populations use a set of criteria developed by the AIDS Clinical Trials Group and modified by Sulkowski et al[57] and is presented in Table 2.1. The grade of change is determined based on a comparison with the upper limit of normal or the baseline aminotransferase value, depending if the person has a pre-treatment level within or above the normal range. Severe hepatotoxicity is defined as a grade 3 or 4 elevation.[57]

Table 2. 1 Changes in alanine aminotransferase and aspartate aminotransferase in persons with HIV

Grade	Persons with pre-treatment serum ALT and AST levels within normal range	Persons with elevated pre-treatment serum ALT and AST (>ULN*)
0	<1.25 × ULN*	<1.25 × baseline value
1	1.25-2.5 × ULN	1.25-2.5 × baseline value
2	2.6-5 × ULN	2.6-3.5 × baseline value
3	5.1-10 × ULN	3.6-5 × baseline value
4	>10 × ULN	>5 × baseline value

* ULN: Upper limit of normal, where the normal range is <31 U/L for alanine aminotransferase and <35 U/L for aspartate aminotransferase.

Cytoplasmic lactate dehydrogenase, an enzyme found in all organs, but mainly present in the liver is another marker of hepatocellular injury. Elevations of lactate dehydrogenase levels can occur with minor tissue injury and it has poor sensitivity and specificity to detect liver damage.[58]

Gamma-glutamyltransferase is a marker of cholestatic injury, but it is not liver-specific. It is a very sensitive marker, but it lacks in specificity as elevations can be caused by minor liver injuries and are not always associated with liver disease.[58]

Glutathione, glutathione peroxidase, glutathione s-transferase, superoxyde dismutase and catalase are involved in the defense against oxidative stress. Levels can be affected when large amounts of oxidative stress is present due to drug metabolism.[58]

2.5.4. Aspartate-to-platelet ratio index

A marker was developed in 2003 in chronic hepatitis C patients using readily available laboratory results to predict liver fibrosis and cirrhosis: the asparatate aminotransferase to platelet ratio (APRI). It is calculated using the following formula: APRI = 100[AST/upper limit of normal]/platelet count (10^9/L). An APRI score ≥1.5 has been validated compared with liver biopsy for significant liver fibrosis (F2 or more on the Metavir scale) and a score ≥2 indicates cirrhosis (F4 on the Metavir scale).[59]

The APRI score has been validated against liver biopsy in both HCV-mono-infected and HIV-HCV co-infected populations. A recent meta-analysis of 23 studies (4 502 patients) concluded that the AUROC for significant fibrosis was 0.77. The cut-off of 1.5 demonstrated poor sensitivity (0.37, 95% confidence interval (CI): 0.35, 0.39), but very good specificity (0.93, 95% CI: 0.91, 0.94). The AUROC for cirrhosis is 0.83 and the sensitivity and specificity for an APRI score of 2 are 46% and 91% respectively.[60] This meta-analysis showed a non-significant decrease in accuracy of APRI in HIV-HCV co-infected persons with an AUROC of 0.75.[60]

High specificity is clinically important to avoid unnecessary biopsies. Despite the low sensitivity, this cut-off parallels important outcomes in co-infected persons. For example, an APRI score ≥1.5 at the last visit before death is associated with a standardized mortality ratio (SMR) of 16.2 (95% CI: 11.1, 21.3). This SMR is similar to that observed in those with end-stage liver disease

(ESLD) (SMR: 17.0, 95% CI: 11.7, 22.3).[61] The APRI score has been shown to perform similarly to other markers of liver fibrosis.[62]

2.6. Factors associated with progression of liver disease

Several factors have been found to be associated with fibrosis progression such as current age, age at infection, male gender, duration of infection, heavy alcohol use and tobacco smoking.[25,63] Certain co-morbidities, including diabetes and obesity, could also be associated with liver fibrosis.[63] In persons with chronic HCV infection, co-infection with HIV or hepatitis B and immunosuppression have also been linked to liver fibrosis.[63]

While these factors may partly explain more rapid fibrosis, co-infected persons experience a number of other potentially hepatotoxic exposures that could contribute to liver disease. A high proportion of co-infected persons use illicit drugs through injection or other routes of administration. As many as 50% of adults living with HIV have reported using drugs in the past month; 12% reported using marijuana only and 38% reported using other drugs.[64] Despite this, only a few studies have looked at the association between the use of specific illicit drugs and liver disease. Marijuana and opioids are often used recreationally or to self-medicate in this population, but data on their effect on the liver is limited or of poor quality. Co-infected persons also use many prescription medications that could potentially accelerate the progression of liver disease. Opioids are often prescribed for addiction treatment and pain management. For example, it has been estimated that 46% of HCV-infected American veterans had received a prescription for opioids in the past three years.[33] In addition, the vast majority of persons living with HIV are taking cART to control the infection. Although some studies have

been conducted, it remains unclear whether or not these medications could be associated with progression of liver disease.

2.7. Marijuana use and liver diseases

2.7.1. Marijuana and its use in North America

Marijuana, also called cannabis, is a drug derived from the plant *Cannabis sativa* L.[65] A total of 489 natural compounds have been identified in the composition of *C. sativa* L: 70 cannabinoids and 419 other constituents.[65] Δ^9-THC is the best known cannabinoid as it is the most psychoactive constituent of cannabis.[65] In vitro, this compound has anti-inflammatory effects: it suppresses macrophage function and antigen presentation and it inhibits macrophage nitric oxide production, T lymphocytes proliferative responses, and cytotoxic T cell activity.[66,67]

Cannabidiol is a type of non-psychoactive cannabinoid comprising seven compounds found in the marijuana plant.[65] Cannabidiol also has potential anti-inflammatory properties such as chemokine production suppression by human B cells and regulation of tumour necrosis factor, interleukin I and interferon gamma production by human peripheral blood mononuclear cells.[66]

Marijuana is widely used in Canada: in a 2005 survey, an estimated 44% of Canadians reported cannabis use in their lifetime and 14% reported use in the past year.[68] In a study of marijuana use among 104 HIV patients in Ontario, 43% of patients reported marijuana use in the past year and 29% reported medicinal use of marijuana.[69]

Although use of marijuana as a therapeutic product is not approved in Canada, on December 31, 2012, 28 115 persons held an “Authorization to Possess Dried Marihuana [*sic*]” under the

Marihuana Medical Access Regulations.[70] Until March 31, 2014 the *Marihuana Medical Access Regulations* allowed seriously ill individuals to possess marijuana for their own medical use, with the support of a medical practitioner.[71] A new regulation came in effect on April 1st, 2014 authorizing certain producers to distribute marijuana to people with valid prescriptions. However, individuals are no longer allowed to produce marijuana for their personal use.[72] As a result of this new regulation, it is now illegal to possess marijuana even for those who have an authorization. There is ongoing litigation regarding the restriction of safe access to marijuana and the authorizations issued before March 31, 2014 have been extended until a verdict is pronounced.[73]

The conditions for which marijuana is believed to be helpful and is recognized by Health Canada include severe pain/persistent muscle spasms caused by multiple sclerosis or spinal cord injury, severe pain/cachexia/anorexia/weight loss/severe nausea caused by HIV/AIDS or cancer, severe pain caused by arthritis, and seizures caused by epilepsy.[74] The reasons frequently reported by persons living with HIV for using medicinal marijuana are appetite stimulation or weight gain (70%), sleep or relaxation (37%), nausea or vomiting (36%), pain management (20%), anxiety or depression (20%), and/or stimulation or energy (10%).[69] A randomized controlled trial has shown that marijuana can be beneficial to treat neuropathic pain and improve daily functioning in persons living with HIV.[75] In observational studies, marijuana use improved adherence to ART in persons living with HIV[76] and sustained virologic response following HCV treatment with interferon and ribavirin was more likely among marijuana users.[77]

2.7.2. Marijuana and the liver

Experimental data suggests that cannabidiol could serve as a therapeutic agent for fibrosis and liver injuries. In cell cultures, it induces death of hepatic stellate cells, activation of which contributes to development of fibrosis.[78] In mice, cannabidiol decreases liver inflammation, oxidative/nitrative stress and cell death,[79] and normalizes liver enzymes.[80] Cannabinoids seem to have a transient effect on the immune system cell functions, suggesting the need for long-term administration, if cannabinoids are to be used for therapy.[67]

Cannabidoids receptors are CB1 and CB2 receptors. Whereas CB2 receptors seem to have an antifibrogenic effect, CB1 receptors seem profibrogenic.[81-83] When a CB1 receptor antagonist is administered to cirrhotic animals, fibrosis is significantly reduced.[84] Administration of a CB2 receptor agonist also reduces fibrosis in cirrhotic rats.[85,86] Moreover, expression of both receptors is increased in persons with chronic hepatitis or cirrhosis, in cells with chronic liver injury and in cirrhotic rats.[81,84,87] Chronic hepatitis C is associated with an up-regulation of CB1 receptors.[88]

Only three small cross-sectional studies have estimated the effect of daily cannabis use on liver diseases among chronic HCV patients. A French study of 270 participants found that daily marijuana users were more likely than non-users to have severe fibrosis (odds ratio (OR): 2.5, 95% CI: 1.1-5.6) and have a rapid progression rate (OR: 3.6, 95% CI: 1.5-7.5).[89] The same team found that daily cannabis use compared to no use was associated with steatosis (OR=2.1, 95% CI: 1.0-4.5) among 315 chronic HCV patients.[90] In the US, a cross-sectional study of 204 patients found increased odds of severe fibrosis in daily cannabis smokers compared with non-daily

users (OR=6.8 [1.9-24.3]).[91] In contrast, a cohort study of 58 HIV/AIDS patients with 12 months of follow-up reported no statistically significant change in liver enzymes in marinol and/or marijuana users over the span of one year.[92]

The contradictory results between the experimental studies and the three cross-sectional studies could be explained by many factors. It is possible that the quality or purity of lab cannabinoids versus street marijuana could have an influence on liver toxicity. The route of drug delivery (injected vs. smoked) could also impact the results. Finally, the harmful effect observed in cross-sectional studies could also be a result of protopathic bias if sicker patients use marijuana to relieve symptoms of advanced liver disease. This theory is consistent with the absence of difference in liver enzymes found in the only cohort study published.

2.8. Opioid use and liver diseases

2.8.1. Opioids and their use in North America

The term opiate is used for opium and its derivatives and comprises natural and semi-synthetic compounds. Opioid is the general term used for both natural and synthetic drugs with properties similar to that of morphine (but not necessarily similar with respect to the chemical structures).[93] These compounds bind to receptors located mainly in the brain and spinal cord. When activated by an agonist, the response consists of analgesia, physical dependence, euphoria, respiratory depression and decreased gastrointestinal motility.[94] Those responses are blocked when an antagonist binds to the receptor. Opioids can be used to treat mild to severe pain, for anaesthesia, to suppress cough or to control addiction.[93] Both therapeutic use and

misuse of opioids have escalated over last few decades and opioids are among the most misused prescription drugs.[95]

Canada accounts for approximately 6% of the global morphine consumption.[93] Canada and the US have the highest overall opioid consumption in the world. In 2009, over 20 000 defined daily doses (DDD) per million inhabitants were consumed per day in Canada and 40 000 DDD per million inhabitants per day were consumed in the United States.[93] The number of regular illegal opioid users in Canada was estimated at more than 80 000 in 2003, which represents an average of about 500 users per 100 000 people between 15 and 49 years of age. Approximately 26% were enrolled in a methadone maintenance treatment (MMT) programme, although this is likely an under-estimate.[96]

2.8.2. Opioid receptor agonists and the liver

Heroin, codeine, morphine, hydromorphone, oxycodone, diphenoxylate, fentanyl, meperedine, methadone and tramadol are all opioid receptor agonists. Most studies on their effect on liver diseases are experimental.

A large number of experimental studies of opioid agonists studied the effect of morphine on the liver in mice and rats or on human hepatocytes. In animal models, all these studies suggest increased liver enzyme levels and liver damage. In mice and rats, morphine was shown to increase the levels of AST,[97] ALT[97-100] and lactate dehydrogenase,[97] as well as the proportion of DNA damaged cells.[99] Morphine was also shown to decrease the hepatic cellular antioxidant defence in mice, although the liver glutathione S-transferase activity was increased.[101]

Fatty accumulation in the liver,[100,102] histopathologic abnormalities,[97] hepatocyte apoptosis,[101,102] hepatocyte necrosis, hepatic inflammation and fibrosis have also been observed following administration of morphine to mice and rats.[102]

In cultures of HIV-HCV co-infected human hepatic cells, morphine aggravates the disruption of host immune defences.[103] It also enhances expression of HCV RNA in cell cultures.[104] The administration of either ascorbic acid or exogenous glutathione with morphine seems to decrease its hepatotoxicity by lowering the ALT activity.[99] Morphine administration to mice and rats results in reduced levels of glutathione.[98,99,105]

Cytotoxic effects have been observed only following the administration of approximately 100 times the therapeutic dose of morphine, meperedine or methadone to human hepatocytes, suggesting that the therapeutic dose might not be sufficient to result in irreversible liver damage.[106]

Opioid agonists other than morphine such as propoxyphene, levo-α-acetylmethadol and l-alpha-[O,O'-3H2]-acetylnormethadol also lower hepatic glutathione in mice.[98] Evidence of fibrosis has been found in liver biopsies of heroin administered rats.[107] Administration of heroin or methadone to human liver cell cultures results in a dose-dependent toxicity manifested by a reduction of cytoplasmic lactate dehydrogenase. This effect is enhanced by ethanol in a concentration equivalent to plasma levels following intoxication.[108] Heroin also decreases urea synthesis.[108] Glutathione content is reduced by high doses of heroin only, although ethanol potentiates it.[108] Administration of tramadol to rats results in the elevation of ALT levels.[97]

One experimental study of codeine in rats failed to detect a toxic effect on the liver.[109] Moreover, when animals are tolerant to narcotics, administration of morphine, hydromorphone or methadone does not result in hepatotoxicity in mice.[100]

A small number of observational studies have looked at the effect of opioid receptor agonist use on the liver, all among current or ex-illicit drug users; none was truly prospective in design. Histological examination of liver biopsies of heroin users show signs of hepatotoxicity such as inflammation and fibrosis.[110] Users of heroin and other drugs also demonstrate elevated levels of serum ALT more frequently than non-users.[111] Autopsies of cases of death by opioid overdose have shown a high prevalence of liver diseases. Indeed, clinically significant levels of liver fibrosis, cirrhosis, hepatomegaly or necrosis were observed in 5 to 11% of the cases. Steatosis and lymphocytic infiltrates of the hepatic portal tract were found in 37% and 43% of the cases, respectively. Of the 841 cases studied, 71% were HCV-positive and 3% were HIV-positive.[112] No association was found between methadone use and transaminase levels or advanced fibrosis in a cross-sectional study of 571 HCV infected male veterans in the United States.[113]

2.8.3. Opioid receptor antagonists and the liver

Naloxone and naltrexone are two opioid antagonists prescribed to treat addiction or opioid overdose. Buprenorphine is a mixed agonist-antagonist because it possesses both antagonist and agonist properties. It is also used to treat addiction or opioid overdose.

Experimental studies of the effect of opioid receptor antagonists show that their administration at the same time as morphine could prevent the decrease in hepatic glutathione, hepatic

glutathione S-transferase activity[98,101] and HCV RNA expression[104] observed when morphine is administered alone. Moreover, administration of the opioid-like compound JKB-119 in vitro can lower levels of serum ALT and its administration to rats can reduce inflammation and liver damage by decreasing tumour necrosis factor alpha levels and hepatic polymorphonuclear leukocytes infiltration.[114] In rats, naloxone appears to reduce liver fibrosis[115] and naltrexone reduces the activation of activated hepatic stellate cells, increases glutathione levels and also prevents development of liver fibrosis.[116]

A few trials and longitudinal observational studies have been conducted with opioid-receptor antagonists. Among 114 persons with HIV, the mean levels of ALT and AST decreased over the course of treatment with naltrexone.[117]

The effect of treating opioid addiction with buprenorphine on liver enzymes was assessed in a study of 120 patients. The 72 patients with hepatitis B or C had elevated AST and ALT levels after at least 40 days of treatment, whereas liver enzyme levels remained unaffected in the 48 patients with no evidence of hepatitis. Only the effect on AST levels seemed to be dose-dependent. Among hepatitis patients, a trend toward reduction of liver enzyme levels was observed during detoxification.[118]

In another study, 152 persons treated for opioid dependence with buprenorphine did not show evidence of transaminase abnormalities.[119] Among 123 HCV infected persons, ALT levels did not change, but AST levels decreased slightly but significantly after treatment with buprenorphine or naloxone compared to before starting the treatment.[120] Reversible elevation of liver

enzymes has been noted following high doses of naltrexone administered for a prolonged period.[121]

However, in an randomized placebo-controlled trial of naltrexone for alcohol dependence treatment, mean values of gamma glutamyltransferase and ALT decreased over the 12 weeks of the trial.[122] Another randomized trial comparing naltrexone vs. placebo to treat opioid dependence found no significant difference in levels of AST, ALT and gamma glutamyltransferase between the two groups.[123] Two randomized controlled trials assigned participants to either methadone or buprenorphine to treat opioid dependence and found no significant differences in terms abnormal liver function tests.[124,125]

Most of the literature on opioid use and liver disease points towards a harmful effect of the opioid receptor agonists. However, the majority of epidemiological studies focused on exposure to illicit opioids and were of a cross-sectional nature. When comparing opioid receptor agonists and antagonists or mixed agonist-antagonists in randomized controlled trials, there did not seem to be a difference in liver enzymes elevation between these types of drugs, suggesting that prescription opioids might not be a major concern in humans, although illicit opioid use might be.

2.9. Antiretroviral therapy and liver diseases

2.9.1. History of HIV treatments and their mechanisms of action

The first five cases of AIDS were described on June 5, 1981 by the Centers for Disease Control and Prevention[126] and HIV was identified as its causative agent in 1983.[127] Only three years later, the first anti-HIV drug, zidovudine, was tested in a trial in 1986 and approved by the Food

and Drug Administration in 1987. The second drug available for HIV treatment, didanosine, was approved in 1991.[128] At the moment, 25 drugs acting on HIV through different mechanisms are available to treat HIV.

Antiretroviral drugs are classified based on the phase of the virus' life cycle it disrupts. The first phase is the entry of HIV in the cell. The entry process can be separated in three distinct steps: (1) HIV attaches to the CD4 T cell receptor through the viral protein gp120; (2) gp120 binds to a co-receptor (CCR5 or CXCR4); (3) the viral and cellular membranes fuse.[129] Co-receptor inhibitors impair the ability of the virus to bind with the receptors of the cell, which prevents its entry. Maraviroc, a CCR5 receptor agonist, is the only drug approved affecting the binding of the virus to the cell. Fusion inhibitors affect the virus' fusion to the cellular membrane, thus preventing its entry in the cell. Enfuvirtide is the only fusion inhibitor currently approved.

The second phase in retroviruses' life cycle is the reverse transcription during which the single strand of RNA contained in the virus is converted in double-stranded DNA.[130] The first drugs developed were all part of the nucleoside reverse transcriptase inhibitors (NRTI) class, also called nucleoside analogues. These drugs are analogs of the nucleosides naturally used in the formation of DNA. When incorporated to the forming DNA strands, DNA polymerization is terminated and the DNA synthesis is blocked.[130] Abacavir, zidovudine, stavudine, didanosine, emtricitabine, tenofovir and lamivudine are the drugs in the NRTI class approved for use in Canada.

Non-nucleoside reverse transcriptase inhibitors (NNRTI) also affect the RNA transcription into DNA by binding to the reverse transcriptase enzyme, impairing its activity.[130] The drugs in this

class that have been approved for use in Canada are delavirdine, efavirenz, etravirine, nevirapine and rilpivirine.

During the integration phase, the HIV DNA is inserted in the cell's DNA by the integrase enzyme. The viral DNA is primed by the integrase enzyme, which trims the ends of the strand and remains attached to it to form the pre-integration complex. The viral DNA is then inserted onto the host chromosome.[131] Elvitegravir, dolutegravir and raltegravir are drugs in the integrase inhibitors class that block the integrase enzyme, preventing this phase of the HIV life cycle.

The transcription phase occurs when the viral DNA is converted into mRNA, which is then translated into pre-cursors viral proteins at the cell surface. These viral particles leave the cell and acquire an outer layer and an envelope during the budding phase of HIV life cycle.[132] During this phase, long chains of HIV proteins are cut into individual proteins by the retroviral protease enzyme.[130] Protease inhibitors (PI) disrupt the cleavage action of the enzyme protease. Immature virions accumulate as the production of infectious viral proteins is inhibited by protease inhibitors.[130] The PIs available in Canada are atazanavir, darunavir, fosamprenavir, indinavir, lopinavir, nelfinavir, saquinavir and tipranavir. Because these molecules are large, they often require the co-administration of another PI, ritonavir. Ritonavir at low doses acts as a "boosting" agent that increases the bioavailable concentration of the PI it is administered with by preventing its metabolism.[133]

The first antiretroviral drugs discovered were all in the NRTI class. Since 1995, several drugs in the NNRTI and PI classes have been developed.[128] These three classes of drugs have been available the longest and are therefore the most frequently used. The discovery of these new

classes of drugs led to the introduction of cART in 1995.[128] HIV treatment is categorized as cART when a minimum of two drugs from different classes is used, although cART generally comprises three or more drugs. These regimens most commonly comprise a backbone of two NRTIs complemented with an anchor agent, typically a PI or an NNRTI. Since 2003, new classes of drugs affecting different steps of the HIV life cycle have increased the diversity of the cART regimens available.

Typically, the CD4 cell count of uninfected adults is higher than 500 cells/µl. With untreated HIV infection, this count can drop much lower than 200 cells/µl, the AIDS-defining threshold. When the immune system is compromised, the person becomes much more susceptible to opportunistic infections and cancers.[134] High levels of HIV viral replication result in depletion of CD4 cells, and increased risks of drug resistance.[134] Therefore, the primary objectives of cART are to suppress viral replication and bring the CD4 cell count up to at least 500 cells/µl. The use of cART dramatically reduces the risk of developing AIDS or dying.[135-137] The life expectancy at age 20 increased from 36 years in 1996-1999 to 45 years in 2003-2005.[138]

The current US Department of Health and Human Services guidelines recommend several potential regimens for treatment naïve persons initiating cART. Two combinations of NRTI are proposed for the backbone: tenofovir (TDF)/emtricitabine (FTC) or abacavir (ABC)/lamivudine (3TC). Several anchor agents are proposed and the combinations recommended vary depending on the patient characteristics.[139] The recommended combinations are presented in Table 2.2.

Table 2. 2 Department of Health and Human Services recommended cART regimens for treatment naïve persons

Source: Department of Health and Human Services, 2014.[139]

Anchor		Backbone	
		Tenofovir/emtricitabine	**Abacavir/lamivudine**
NNRTI	**Efavirenz**	ALL*	VL<100 000†
	Rilpivirine	VL<100 000	
PI	**Atazanavir/ritonavir**	ALL	VL<100 000
	Darunavir/ritonavir	ALL	Alternative‡
	Lopinavir/ritonavir	Alternative	Alternative
Integrase inhibitor	**Dolutegravir**	ALL	ALL
	Elvitegravir/cobicistat	ALL	
	Raltegravir	ALL	Alternative

* Recommended initial cART regimens for all patients, regardless of pre-cART viral load or CD4 cell count.

† Recommended initial cART regimens only for patients with pre-cART plasma HIV RNA <100 000 copies/mL

‡ Alternative initial cART regimens that are effective and tolerable, but with potential disadvantages or less data from randomized clinical trials. These can be the preferred regimen for some patients.

2.9.2. Potential harmful effects of antiretroviral therapy on the liver

There is a potential for both acute direct hepatotoxicity and long-term cumulative toxicity of cART because of the metabolic effects of antiretroviral drugs. Direct hepatotoxicity occurring in the weeks following treatment initiation has been studied extensively for drugs in all classes. However, data on long-term liver outcomes associated with cART remains inconclusive. The following sections of this literature review will focus on available data regarding the hepatotoxicity of NRTIs, NNRTIs and PIs as these are the drug classes relevant in the context of this thesis.

2.9.2.1. Nucleoside reverse transcriptase inhibitors and the liver

There have been several reports of disruption of mitochondrial function by NRTIs.[140,141] The mitochondrial toxicity of NRTIs could result from several mechanisms including DNA polymerase γ inhibition, disruption of mitochondria DNA replication, mutation of mitochondrial DNA and oxidative stress.[142] Mitochondrial toxicity can notably lead to hepatic steatosis (the accumulation of triglycerides in the liver)[140] and liver failure.[141]

Zalcitabine, didanosine and stavudine are NRTI drugs with the highest level of mitochondrial toxicity.[141,143] Didanosine and stavudine cause a decrease in mitochondrial DNA and hepatocyte growth as well as an increase in intracellular lipids and lactate levels.[144] ABC and zidovudine also increase intracellular lipids[144] and lactate levels and reduce hepatocyte proliferation.[143,144] However, FTC only reduces hepatocyte proliferation moderately while TDF and 3TC cause no mitochondrial toxicity.[143,144] Together, TDF and FTC reduce hepatocyte proliferation without producing mitochondrial toxicity.[144]

ABC is contraindicated for patients with moderate or severe hepatic impairment.[145] In 2008, the Food and Drug Administration issued a “boxed warning” recommending that patients be screened for the presence of the HLA-B*5701 allele before starting treatment with ABC due to an increased risk of serious and potentially fatal hypersensitivity reaction caused by ABC in persons with this allele.[146] ABC hypersensitivity reactions occur in less than 4% of users and in 93% of the cases, within six weeks of initiating ABC.[147] The most frequent symptoms are fever, rash, gastrointestinal symptoms, fatigue and malaise.[145,147] Hypersensitivity reaction is

associated with abnormal liver function tests and liver failure can occur.[145,148] ABC use has also been associated with cases of lactic acidosis and severe hepatomegaly with steatosis.[145]

NRTI-associated liver enzyme elevation has been described in several randomized controlled trials and in a few observational studies. In a randomized controlled trial of 208 PI- and stavudine-naïve persons, those randomized to a regimen of saquinavir/ritonavir with stavudine were five times more likely to experience hepatotoxicity than those randomized to saquinavir/ritonavir only.[149] However, those initiating a stavudine-containing regimen were 70% less likely to experience hepatotoxicity than those initiating a zidovudine-containing regimen in a study of 1255 naïve HIV-infected persons.[150] A randomized controlled trial of TDF with 3TC and efavirenz compared to stavudine with 3TC and efavirenz showed the same proportion of in AST and ALT abnormalities in both groups.[151] No difference in AST and ALT abnormalities was found in another trial comparing a backbone of TDF/FTC and zidovudine/3TC, with all participants using efavirenz.[152] A trial comparing the two recommended backbones (TDF/FTC and ABC/3TC), an elevation of liver enzymes was observed after 96 weeks of treatment in the TDF/FTC group only.[153] A combined analysis of two randomized controlled trials comparing these two backbones showed no difference in AST and ALT levels between the two groups.[154]

A cohort of nevirapine users showed no statistically significant association between either stavudine or zidovudine use and a three-fold increase in AST or ALT.[155] In another cohort of HIV mono-infected persons, the use of regimens containing didanosine, stavudine, TDF or FTC were associated with chronic liver enzyme elevation, measured as an elevation of ALT in at least two

visits separated by six months to two years.[156] No association was found with regimens containing zidovudine, 3TC or ABC.[156]

Among HIV-HCV co-infected persons, use of dideoxynucleotides (zalcitabine, didanosine and stavudine) were associated with an elevated risk of incident liver steatosis in persons infected with any HCV genotype[157] or genotypes other than 3 only.[158] Ever being exposed to stavudine was also associated with a five-fold increase in the risk of hepatic steatosis compared to never having been exposed to it in a study of 112 experienced HIV-HCV co-infected persons.[159]

2.9.2.2. Non-nucleoside reverse transcriptase inhibitors and the liver

The results of a study evaluating the hepatotoxicity associated with the use of any NNRTI suggested that this class of drugs is associated with severe hepatotoxicity, defined as an increase in ALT levels of five times the upper limit of normal (ULN) and of at least 100 U/l from the baseline level.[160]

Most studies have focused on liver outcomes following nevirapine use. It has been suggested that the risk of hepatotoxicity increased with each additional year of antiretroviral therapy exposure among nevirapine users.[155] However, most studies showed a short-term risk only. Current nevirapine use or starting nevirapine in the past 12 weeks were associated with an increased risk of grade 4 hepatotoxicity (AST and/or ALT elevations to at least 10 times the ULN),[161,162] of grade 3-4 hepatotoxicity (at least five times the ULN),[161,163-165] or shorter time to discontinuation of cART due to hepatotoxicity.[162]

In a randomized controlled trial of 468 persons, grade 3-4 hepatotoxicity occurred in 17% of those randomized to nevirapine and in none of those randomized to efavirenz.[166] Another randomized controlled trial of 1 166 persons comparing the use of nevirapine or efavirenz as the anchor agent, also showed a higher frequency of grade 3-4 hepatotoxicity events among nevirapine users.[167] In a cohort of 287 persons on efavirenz and 258 persons on nevirapine, those treated with nevirapine progressed to hepatotoxicity faster over 18 months of follow-up.[168] A systematic review of all analyses from five cohort studies found no significant difference in the rates of serious hepatic events between nevirapine and efavirenz users.[169]

In a cross-sectional study of 152 HIV-HCV co-infected patients, nevirapine use was found to be predictive of advanced fibrosis (F3-F4 fibrosis stage on biopsy) and a history of nevirapine use was associated with a faster fibrosis progression rate (ratio between the fibrosis stage and the estimated duration of HCV infection in years, excluding F4 –cirrhosis).[170] However, a review of analyses of 8 711 persons from five cohort studies concluded that nevirapine use was not associated with an elevated risk of liver failure or hepatic-related mortality.[169] Although nevirapine was associated with symptomatic hepatic events in a meta-analysis of nine randomized controlled trials including data on 3 642 persons, the risk was significantly reduced after the first six weeks of treatment.[171]

Based on this body of evidence, hepatotoxicity does not appear to be a class effect, but to be associated with nevirapine use only. The risk of nevirapine-associated hepatotoxicity appears elevated in the first 12 weeks of therapy only and to be more frequent among women with higher CD4 cell counts.[161,166,172] There is limited evidence that nevirapine could be responsible

for long-term harmful effects on the liver.[173] The limited chronic toxicity observed could be explained by the absence of undesirable metabolic effects of nevirapine, which is in fact associated with a favourable lipid profile, with increases in HDL-cholesterol and apo A1 plasma levels.[173]

2.9.2.3. Protease inhibitors and the liver

The use of PIs has been associated with a number of metabolic adverse effects (increases in lipids, lipodystrophy, insulin resistance, endothelial dysfunction) leading to an increased risk of morbidities such as cardiovascular disease.[174,175] It has been suggested that, among other proposed mechanisms, PI-associated endothelial disruption could be due to an increase in oxidative stress and mitochondrial injury.[176] It is therefore possible that PIs could affect the progression of liver disease through similar mechanisms. Moreover, because the liver is an insulin responsive organ, insulin resistance, stimulation of lipogenesis and free fatty acid accumulation can lead to steatosis and liver toxicity.[177]

In terms of liver diseases, the literature on PI use is conflicting. Many studies have found evidence of PI-associated hepatotoxicity. Several studies have focused on hepatotoxicity associated with the use of full dose ritonavir. In a study of 381 persons prescribed a new HIV treatment, the risk of grade 3-4 hepatotoxicity was close to nine times higher among ritonavir users compared regimens containing other PIs or two NRTIs.[57] In another study of 560 persons initiating their first HIV treatment, starting ritonavir within 12 weeks was associated with a five-fold increase in the risk of grade 4 liver enzyme elevations.[161] Among 394 persons initiating cART with a PI-based regimen, use of a full dose of ritonavir was associated with a non-

significant increased risk of grade 3-4 liver enzyme elevations compared to indinavir-based regimens.[178] The review of all analyses performed on four observational cohort studies revealed that full dose ritonavir was the only PI associated with an increased risk of hepatotoxicity.[172] The increased risk of hepatotoxicity associated with ritonavir has only been observed when a full dose is used, not when a boosting dose is used. However, small doses of ritonavir as a booster over a long period could potentially contribute to the metabolic effects of PIs.

Among the studies that attempted to isolate the effect of saquinavir, its use was linked to hepatotoxicity in some, but not all, studies. In a cohort of 1 161 PI-naïve persons initiating treatment with a PI, saquinavir/ritonavir-based regimens were associated with an increased risk of grade 3-4 hepatotoxicity compared to nelfinavir-based regimens.[179] In 262 HIV-HCV co-infected persons, only the use of saquinavir/ritonavir was associated with a five-fold increase in grade 3-4 liver toxicity.[180] However, the risk of grade 3-4 liver enzyme elevation was not higher among saquinavir or saquinavir/ritonavir users compared to indinavir users in a study of 394 persons initiating ART with a PI-based regimen.[178] Initiation of saquinavir in the prior 12 weeks was not associated with grade 4 liver enzyme elevation among treatment naïve persons either.[161]

PI naïve persons initiating cART with an indinavir-based regimen (with or without ritonavir) seem more likely to experience grade 3-4 hepatotoxicity compared to those initiating nelfinavir-based regimens.[179] Among 755 persons treated with lopinavir/ritonavir, hepatotoxicity was infrequent (0.59 events/100 person-year), suggesting limited hepatotoxicity.[181]

Among 210 HIV-HCV co-infected persons, no association between use of a drug in the PI class and the hepatic activity grade compared to no antiretroviral treatment could be detected.[182] Another study of co-infected persons could not demonstrate an associated between any PI/ritonavir use and grade 3-4 hepatotoxicity compared to efavirenz use.[180] Among new users of nevirapine or efavirenz (both NNRTIs), concurrent use of a PI was associated with a two-fold increase in the risk of severe hepatotoxicity. However, these likely represent a sicker population requiring a more complex regimen combining NNRTIs and PIs.[163]

Several studies have focused on the risks of acute hepatotoxicity associated with PI use. Taken all together, these reports suggest that PI use could potentially increase the risk of hepatotoxicity. However, most of the studies were conducted in HIV mono-infected populations and reported short periods of follow-up, presenting the risk of severe hepatotoxicity in the first year of cART use only.

Longer-term clinical liver outcomes have been the object of four studies of HIV-HCV co-infected persons. Among 112 co-infected persons treated for HIV for at least two years, hepatic steatosis was marginally associated with a cumulative exposure to PIs.[159]

A protective effect of PI use has been reported in three studies in which the outcome was assessed through biopsies of persons who likely had an indication to undergo this procedure. In a cross-sectional study of 152 co-infected persons, ever having been on a PI-based regimen appeared protective against advanced liver fibrosis (stages 3-4) and was associated with a slower fibrosis progression rate compared to never having used a PI.[170] However, the comparison group of those who had never used a PI consisted mostly of treatment naïve

persons or treated persons not on cART, whose HIV might not have been as well controlled. These analyses were adjusted for CD4 counts ≤250 cells/ml.

In another study of 683 co-infected persons, advanced fibrosis was 50% less likely and fibrosis progression rates were slower in those on a PI-based regimen compared to those not on cART.[183] Again, despite adjusting for CD4 counts <300 cells/ml, inadequate control of the HIV infection in the comparison group could be an important driver of the protective effect reported.

Finally, PI-based therapy was associated with a decreased risk of cirrhosis and a reduced rate of fibrosis progression compared to absence of PIs in 182 co-infected persons.[184] The cirrhosis analysis was controlled for a CD4 cell count <200 cells/ml and the fibrosis progression rate analysis was adjusted for a CD4 cell count <200 cells/ml and an HIV viral load >200 copies/ml. However, the non-PI group consisted mainly of persons on dual NRTI only regimens and untreated persons. This could have resulted in the protective effect of PIs observed as those using a regimen consisting of two NRTIs plus a PI would have a better-controlled HIV infection and thus less liver damage than the comparison group.

2.9.3. Comparing non-nucleoside reverse transcriptase inhibitors and protease inhibitors

Very few studies have compared NNRTI and PI use directly with regards to liver outcomes. One study of HIV-HCV co-infected persons compared the risk of liver toxicity between PI users and efavirenz users (an NNRTI). No difference in acute grade 3-4 hepatotoxicity was detected between groups starting a new regimen containing efavirenz or a PI/ritonavir.[180]

A study investigated the rates of changes in the natural logarithm of the aspartate-to-platelet ratio index score (ln(APRI)) associated with PI or NNRTI use. Among HIV mono-infected persons, no difference was shown between PI use (change in ln(APRI) of 0.09 units over three years (95% CI: 0.05 to 0.13)), and NNRTI use (0.10 units over three years (95% CI: 0.05 to 0.16)).[185] However, among HIV-HCV co-infected persons, the changes in ln(APRI) was 0.22 units per three years (95% CI: 0.13 to 0.31) among PI users compared to 0.12 units per three years (95% CI: -0.08 to 0.32) among NNRTI users,[185] suggesting that the rate of changes in ln(APRI) could differ between PI and NNRTI users although the confidence intervals overlap.

The evidence concerning the potential effect on the liver of the two classes of anchor agents most frequently used to treat HIV infection is mixed. Although there is evidence of short-term hepatotoxicity associated with drugs in both classes, the data on long-term outcomes is conflicting and it is still unclear whether one class is more harmful than the other in terms of clinical outcomes such as progression of fibrosis. In addition, the populations in which most studies have been conducted consisted in heterogeneous groups including persons with prior antiretroviral exposure, exposure to older or highly toxic drugs (such as dideoxynucleosides or full dose ritonavir) or inadequate HIV control, thus making inference difficult.

Chapter 3: Detailed methods

3.1. The Canadian Co-infection Cohort

3.1.1. Recruitment and inclusion in the cohort

The Canadian Co-infection Study started recruitment in 2003 in three clinics in Montreal for a pilot phase funded by the Fonds de recherche du Québec – Santé. Since then, the cohort has expanded with funding from the Canadian Institute for Health Research to include a total of 18 sites across Canada.

Recruitment takes place in four clinics in Vancouver, British Columbia: the Oak Tree Clinic, the Pender Clinic, the BC Centre for Excellence and the Vancouver Native Health Centre. Patients are recruited at the South Alberta Clinic in Calgary, Alberta and at the Saskatoon HIV/AIDS Research Endeavour, University of Saskatchewan in Saskatoon, Saskatchewan. In Ontario, participants come from the Ottawa General Hospital (Ottawa), the Toronto General Hospital (Toronto), the Sunnybrook & Women's College Health Sciences Centre (Toronto), the Sudbury Regional Hospital (Sudbury), McMaster University Medical Centre (Hamilton) and the Windsor Regional Hospital (Windsor). The Quebec participating clinics consist of Hôpital Notre-Dame (Montreal), Clinique médicale du Quartier Latin (Montreal), the Montreal General Hospital (Montreal), the Montreal Chest Institute (Montreal) and Centre hospitalier de l'Université Laval (Quebec City). Finally, the Queen Elizabeth Hospital (Halifax) contributes participants from Nova Scotia.

To be included in the study, participants must show evidence of co-infection with HIV and hepatitis C and be at least 16 or 18 years of age, according to provincial criteria. Potential participants are considered HIV-infected if they have a documented HIV infection measured by a positive enzyme-linked immunosorbent assay (ELISA) with western blot confirmation. Persons with either a chronic HVC infection or evidence of HCV exposure are considered HCV-infected. This is measured by a positive ELISA with recombinant immunoblot assay II or enzyme imunoassay confirmation, or if serologically false negative, HCV–RNA-positive.

3.1.2. Data collection

Demographic, behavioural and clinical information is collected at each study visit, spaced approximately six months apart. Blood is also collected at each study visit to obtain additional clinical information. All questions regarding behavioural information refer to the time since the last interview. Therefore, self-reported behaviours such as drug use refer roughly to the past six months, resulting in coarse data. Most of the outcomes investigated in this dissertation are markers of liver disease that are measured and calculated at each study visit. When a participant reaches a pre-determined cut-off, we classify them as a case, but it is impossible to know precisely when the marker reached the cut-off in the interval since the last study visit.

3.1.3. Liver outcomes

In this dissertation, we chose the APRI score as a marker for liver disease. The choice of the APRI score as opposed to other measures of liver disease was dictated in part by the data available. Indeed, liver biopsy was not routinely performed and would have been unethical to perform repeatedly for a research study. In addition, false negatives can occur due to sampling variability. It was estimated that as many as 35% of liver biopsies 15mm in length were

incorrectly classified when assessing the presence of liver fibrosis in persons with chronic HCV infection.[50]

Transient elastography is a non-invasive and rapid marker of liver fibrosis with good test characteristics,[52,54] although characteristics of the patient and the person performing the test are known to affect its performance.[52,53] Transient elastography measures are available in the Canadian Co-infection Cohort only for a fraction of the participants and repeated measures are limited because *FibroScan* was licensed for use in Canada in 2009[55] and not all sites are equipped to perform it.

Many markers of liver fibrosis have been developed as composite scores based on serological levels of liver enzyme and other clinical information. The APRI score is one of those markers. As shown in table 3.1 for a subset of the existing composite scores, these markers are all imperfect and the APRI score performs similarly to most markers in HIV-HCV co-infected persons.[186]

Table 3. 1 Standardized area under the standard operating characteristic curve (AUROC) of non-invasive markers of liver fibrosis in HIV-HCV co-infected persons.

Score	Components of the score	AUROC
APRI	AST, platelet count	0.74
Fib-4	Age, AST, ALT, platelet count	0.77
Fibrometer	Age, AST, platelet count, prothrombin index, alpha2macroglobulin, hyaluronic acid, urea	0.86
Fibrotest	Age, sex, alpha2macroglobulin, haptoglobin, total bilirubin, apolipoprotein A1, gamma-glutamyl transpeptidase	0.78
Forn's index	Age, platelet count, gamma-glutamyl transpeptidase, cholesterol level	0.73
Hepascore	Age, sex, alpha2macroglobulin, hyaluronic acid, total bilirubin, gamma-glutamyl transpeptidase	0.84
SHASTA	AST, hyaluronic acid, albumin	0.75

Source: Cacoub et al., *Journal of Hepatology,* 2008.[186]

The dichotomous APRI score is a good predictor of mortality[187-189] and advanced liver disease,[190] despite a poor sensitivity.[60] Although rarely used continuously, accepted predictors of liver

fibrosis in HIV-infected persons such as HBV or HCV co-infection, CD4 cell count ≤200 cells/ml and HIV viral load ≥100 000 copies/ml have been shown to predict ln(APRI).[191] Moreover, increases in the continuous APRI score are also associated with an increased risk of all-cause mortality.[192-194] In this dissertation, the APRI score was dichotomized using the validated cut-offs of 1.5 for significant fibrosis and of 2 for cirrhosis in survival analyses.[60] When the APRI score was used as a continuous outcome in linear regression, a natural log-transformation was applied to normalize its distribution because the untransformed APRI score has a highly skewed distribution.

Clinically defined liver disease was used in this dissertation as the outcome in sensitivity analyses due to the small number of cases occurring during follow-up. The presence of liver cirrhosis was ascertained clinically through a radiological exam or liver biopsy. End-stage liver disease consists in the diagnosis of any of the following diseases: ascites, portal hypertension, spontaneous bacterial peritonitis, encephalopathy, oesophageal varices or hepatocellular carcinoma.[195] Research personnel fill out dedicated case report forms when a case of end-stage liver disease arises which is then validated centrally.

3.2. Manuscript 1: Marijuana smoking does not accelerate progression of liver disease in HIV-HCV co-infected individuals

3.2.1. Analytical sample

For this objective, we selected participants who fulfilled additional conditions from the eligibility criteria to participate in the cohort study. First, because it is generally accepted that active HCV infection is an important risk factor for progression to liver disease,[28] we decided to include in the analysis only participants in whom active replication of the HCV virus could be

demonstrated at baseline, thus excluding all those who had cleared the virus spontaneously or following treatment. Because we were interested in the rate of progression to liver fibrosis, cirrhosis and ESLD, we also excluded the participants who already had these outcomes at baseline. Finally, we censored individuals when they started treatment for their HCV infection for two reasons. First, treatment could lower the risk of developing liver diseases associated with an active infection. Second, and more importantly, HCV treatment affects the platelet ratio in the blood,[196] which is used to calculate the APRI score. Therefore, initiation of HCV treatment results in an unreliable measurement of outcomes of interest: liver fibrosis (APRI ≥ 1.5), liver cirrhosis (APRI ≥ 1.5) and changes in the continuous APRI score.

3.2.2. Marijuana exposure

At each study visit, participants were asked to report marijuana use since the last interview. Marijuana smokers also reported how often they smoke (*occasionally/not every week*, *regularly/1-2 days per week*, *regularly/3-6 days per week*, *every day*) and the number of joints consumed on the days they smoke. They were also asked for which reason they use marijuana (*to relieve symptoms*, *to increase appetite*, *for fun*).

3.2.3. Statistical analyses

We were interested in assessing the rate of progression to liver diseases and whether it could be accelerated due to the use of marijuana. Time-dependent Cox proportional hazards models were used to assess the presence of an association between marijuana smoking and progression to liver fibrosis, cirrhosis and ESLD.

To explore the presence of a dose-response relationship between the number of joints smoked per week and the amount of liver damage, we opted for a linear spline regression model. This type of model is a simple way of introducing some flexibility to linear regression by allowing the slope of the curve to change at pre-specified knots. The location of the knots was selected based on the distribution of the number of joints smoked per day as well as the meaningfulness of the knots. Therefore, we selected the first knot to be placed at 7 joints per week, which roughly represents smoking one joint per day or a regular low dose use of marijuana. The second knot was placed at 21 joints per week, roughly representing an average of 3 joints per day, a regular intermediate dose of marijuana. For this model the independent variable was a natural log transformation of the continuous APRI score, to control for the skewedness of the untransformed data.

We conducted the analyses by using either the self-reported marijuana use in the six months before outcome assessment, or lagging the marijuana smoking variable by one visit in order to explore the possibility of confounding due to 'self-medication' and remediate the problem discussed earlier.

The selection of a current or lagged exposure to marijuana smoking, as opposed to a cumulative measure, was based on preliminary work exploring several models of time-updated marijuana exposure. A total of 12 models were explored, six of which referred to the current visit, and the others being lagged exposures. We considered a binary indicator of smoking, intensity of smoking, frequency of smoking, duration of smoking since cohort entry, a cumulative intensity of smoking over follow-up, or a cumulative intensity of smoking over the past two years. Although the binary indicator for smoking marijuana in the past six months presented overall

the best fit to the data, the model for the intensity of smoking (the number of joints smoked per week), resulted in goodness of fit estimates that were close to those for the binary indicator and was therefore selected because it allowed for the assessment of a dose-response relationship.

3.3. Manuscript 2: Use of opioids in a Canadian HIV/hepatitis C co-infected population and its relation to liver fibrosis

3.3.1. Analytical samples

Two research questions were addressed with this objective and therefore, two analytical samples have been defined. First, the *prevalence cohort* was used to estimate the average change in the ln(APRI) associated with use of prescribed and/or illicit opioids. The prevalence cohort was created by excluding participants who had only responded to the baseline questionnaire, and those without active HCV infection at baseline. Participants who started HCV therapy were censored. The inclusion criteria were made as broad as possible in this cohort to allow the estimate of changes in APRI score without introducing selection bias by eliminating participants with a fast progression of liver fibrosis from the analytical sample.

The *incidence cohort* consisted of a subset of the participants included in the *prevalence cohort*, further excluding those with and APRI score ≥1.5 or with an hepatic event at cohort entry, which was defined as a diagnosis of ESLD (liver cirrhosis, ascites, portal hypertension, spontaneous bacterial peritonitis, encephalopathy, oesophageal varices or hepatocellular carcinoma). In order to perform a survival analysis of time to significant liver fibrosis, we could only include event-free participants in the analytical sample, explaining the more stringent exclusion criteria for the *incidence cohort*.

3.3.2. Opioid exposure

Illicit use of opioids was assessed through participant self-report at each study visit. Specifically, participants were asked if they have used injection drugs since the last interview. A list containing six opioids was provided and they were invited to select which drugs they had used among heroin, methadone, morphine (MS Contin), dilaudid, pecocet and oxycodone (Oxycontin). They were also asked about illicit substances used without injecting (sniffed, smoked, ate, drank, used a patch). They were provided a list containing eight specific opioids: demerol, dilaudid, percocet, heroin, methadone, morphine (MS Contin), tylenol with codeine and oxycodone (Oxycontin). Finally, participants were asked to add any additional substance they used.

Prescribed narcotic use was assessed through participant self-report and the information provided was confirmed with the patient's file at the clinic. No information on dosage was recorded, although drug name and date of change in medication were available. In addition, participants reported enrolment in a methadone program for drug addiction.

Only opioid receptor agonists and mixed agonist-antagonists were selected as potential exposures in our evaluation of the association between opioid use and APRI score. This decision was made due the opposite mechanism of action of receptors agonists and antagonists. In addition, opioid receptor antagonists have not been suggested to cause any liver damage, as agonists have been in some studies and therefore, including those types of drugs could have diluted the effect estimated.

3.3.3. Statistical analyses

Because the quality of the drugs, quantities administered and the clinical, social and behavioural context during which opioids are prescribed or consumed illicitly differ vastly and are likely to impact their effect on liver health, we chose to treat prescribed and illicit use as two different exposures, while adjusting for one another. Exposure was lagged to the previous study visit, in order to preserve temporality. A number of sensitivity analyses were conducted, varying modelling of the exposure.

The analysis of the association between opioid use and changes in the median APRI score in the prevalence cohort was done using linear regression with generalized estimating equations (GEE). This marginal model focuses on the average trend, estimating the average treatment effect of opioid use on APRI score while accounting for longitudinal dependence.[197] Moreover, GEE are robust to misspecification of the correlation structure.[197] The correlation structures considered were the exchangeable, independent, unstructured, autoregressive (first and second order), stationary and non-stationary structures. The quasilikelihood under the independence model criterion (QIC) was used to select the correlation structure.[198] The independent structure offered the smallest QIC statistic and was selected.

In the incidence cohort, we performed a survival analysis of opioid use and time to progression to significant liver fibrosis. A pooled logistic regression model was fit, incorporating flexibility in the impact of time on the hazard by using polynomials of the visit number (up to the fourth order).

Missing data was handled with multiple imputation implemented using chained equations, producing 10 imputed datasets. These datasets were analysed separately and the coefficients and standard errors obtained were then combined to produce a single point estimate and standard error using Rubin's formula.[199]

The literature on the subject suggests that the validity of self-reported prescribed opioid use is unsatisfactory.[200-206] Many methods for measurement error correction assume the true sensitivity and specificity of the measure are known, which is problematic because the smallest change in the bias parameters could result in largely different effect measures.[207] Probabilistic bias analysis, also called Monte Carlo sensitivity analysis or uncertainty analysis, allows for correction of systematic bias by specifying a probability distribution for the bias parameter informed by previous research,[208] without making any assumption regarding the effect measure before analysing the data.[209] Therefore, the sensitivity and specificity of the measure is treated as a random variable.[207]

The first step of probabilistic bias analysis is to sample a pair of sensitivity and specificity from the probability distribution specified. The second step is to calculate a corrected, or bias adjusted relative risk. These two steps are repeated a large number of times, i.e. with a Monte Carlo sampling approach, and a frequency distribution of the corrected effect estimate is obtained.[208,210] A distribution of bias-corrected values of the association is obtained. The correction is based on a distribution of errors developed before viewing the data.[209] The median represents the corrected point estimate and the 2.5th and 97.5th percentiles of this distribution represent the 95% credible interval.[210] However, this interval only takes into account the uncertainty due to the misclassification of the exposure. To account for random error, the

standard error of the conventional ln(RR) estimate (sd) is multiplied by a random draw from a Normal distribution with mean 0 and standard deviation sd. This product is then subtracted from the corrected ln(RR) estimate and this process is repeated for each corrected estimate.[208,211]

3.4. Manuscript 3: Progression of liver fibrosis, class of combination antiretroviral therapy anchor agent and backbone in HIV-hepatitis C co-infected persons

3.4.1. Analytical sample

For this objective, one of the strategies selected to limit potential for confounding was restriction of the study population to obtain a homogeneous sample. First, we restricted the sample to persons with active HCV replication because of the importance of active HCV infection as a risk factor for liver disease. Persons infected with hepatitis B were excluded from the sample because TDF, FTC and 3TC have anti-hepatitis B action and a combination of TDF/FTC or TDF/3TC is therefore recommended for hepatitis B co-infected persons, thus leading to favourable liver outcomes, such as normalization of liver enzyme levels, histologic liver improvements and reduced liver-related mortality.[139,212,213] Discontinuation of these agents can lead to reactivation of the hepatitis B virus, causing liver damage.[139,214] The number of hepatitis B infected persons was too small in the analytical sample to adjust for it and we therefore decided to exclude them from the sample altogether.

The number of persons using an anchor agent other than a PI or NNRTI is also very small in the cohort because they have only recently come into use. We therefore restricted the study population to those using either a PI or NNRTI. We also restricted the study population to new users of PIs or NNRTIs, that is to say, those who initiated cART with these agents and remained

on the same class of anchor agent since initiation in order to avoid any carry-over effect that could occur with a change in the class of drug used. cART initiation could have occurred either before cohort entry or during follow-up. To homogenize the population and avoid confounding by the type of NRTI used, we only included persons whose treatment backbone consisted of either TDF/FTC or ABC/3TC, which are the two combinations of NRTIs recommended as first line backbones in the commonly used Department of Health and Human Services guidelines.[139] Finally, the sample was restricted to those on a first line PI (atazanavir, lopinavir, darunavir) or NNRTI (efavirenz, nevirapine, rilpivirine), as defined in the guidelines.[139] Evaluating the progression of liver fibrosis among modern regimens users ensures that the results obtained are relevant to current clinical practise and could therefore inform guidelines. The study population was censored at the start of HCV treatment.

A propensity score matched sample was created to make the PI and NNRTI users more comparable by balancing baseline covariates. Propensity score matching performs as well as inverse probability weighting and better than stratification on the propensity score and the use of the propensity score as a covariate to remove systematic differences between treatment groups.[215] The decision to prescribe a PI can be driven by important patient characteristics that could also influence liver fibrosis risk. For example, poor adherence may lead to uncontrolled HIV viral replication, drug resistance and increased fibrosis. Indeed, when there is poor adherence to treatment, resistance to NNRTI is more frequent than resistance to PI.[216] Therefore, PI-based regimens are often preferred for persons who exhibit some degree of instability in their life or are considered less likely to adhere to treatment, such as persons who inject drugs.

The propensity score for initiating cART with an NNRTI was created with a logistic regression. The model included baseline values of years since cART initiation, age, sex, HCV duration, CD4 cell count, undetectable VL, alcohol use, IDU, income under 1 500CAD per month and the backbone used (TDF/FTC vs. ABC/3TC). The propensity score was used to match each individual to another person with a similar propensity score, but using the other class of anchor agent. Matching with replacement was performed with the nearest neighbour approach.

3.4.2. Antiretroviral therapy exposure

At each study visit, research personnel recorded changes in antiretroviral medication that occurred since the last study visit. The name of the antiretroviral, whether it was started or stopped and on what date, as well as reasons for stopping a drug were collected. Study coordinators at each study site validated the data with patient medical or pharmacy records. Research coordinators also collected additional information on treatment history before cohort entry for 80% of the cohort participants through chart review. Each drug used before cohort entry was reported along with the date of initiation and discontinuation of each drug.

Initiation of a PI- or NNRTI-based regimen could have occurred either before or at cohort entry, or during the study follow-up. Therefore, baseline was defined as either cohort entry or initiation of a first PI- or NNRTI-based cART regimen during follow-up, depending on the situation.

3.4.3. Statistical analyses

Linear regression with GEE was performed to estimate the rate of change in ln(APRI) among NNRTI and PI users while accounting for the clustered structure of the data. The correlation

structure was selected based on the QIC. The exchangeable structure provided the smallest QIC statistic and was thus selected. Multiple imputation implemented using chained equations was used to address the issue of missing data by creating 10 imputed datasets.

Equation 1 represents the general model fit for this analysis. We modelled the exposure as an indicator variable for NNRTI use at initiation of cART, the number of years since initiation of cART and an interaction term between NNRTI use and the time variable in order to obtain the rate of change in ln(APRI) among NNRTI and PI users. Based on this equation, β_1 represents the expected value of ln(APRI) associated with initiating cART with a NNRTI as opposed to a PI; β_2 represents the change in ln(APRI) per year in PI users and (β_2+β_3) represents the change in ln(APRI) per year associated in NNRTI users.

Equation 1

$$E[\ln(APRI)] = \beta_0 + \beta_1 NNRTI + \beta_2 Years + \beta_3 NNRTI * Years + \sum_{j=4}^{J} \beta_j covariate_j$$

The coefficients of interest are therefore β_2 and β_3. However a one unit change in ln(APRI) is difficult to interpret clinically and the results would be more useful if they can be applied to the raw APRI score. Exponentiating β_2 and (β_2+β_3) results in a statistic that is interpretable as the median change on the multiplicative scale in APRI score per year among PI and NNRTI users, respectively.

Frequency weights were used in the analysis because matching with replacement was performed and some individuals were matched more than once. The weights thus ensured that the frequency at which each person appeared in the matched sample was represented in the

analysis. The use of GEE and robust standard errors ensured that the dependence of the sample was accounted for in the estimation of the standard errors. We opted not to use bootstrapping because bootstrap standard errors do not provide valid inference for matching estimators.[217]

We performed an intention-to-treat analysis, evaluating the impact of initiating cART with a PI- or NNRTI-based regimen on liver fibrosis progression. Therefore, the indicator for NNRTI use represented the class used at cART initiation and exposure to NNRTI was assumed to be constant throughout follow-up. This model was adjusted for baseline values for age, sex, duration of HCV infection and the backbone used, as well as time-updated values for alcohol use, IDU, CD4 cell count, undetectable viral load and the interaction term between IDU and duration of HCV infection. The rational for conducting an intention-to-treat analysis was to emulate a randomized control trial and estimate the changes in APRI score over time associated with the class of anchor agent selected for cART initiation in treatment naïve persons. The results obtained thus reflect the impact that the treatment decision can have on liver fibrosis progression. This approach reduces bias due to unmeasured characteristics leading to a change in regimen and to the carry-over effect of the previous regimen if changes in the class of anchor agents occurred in follow-up.

Although this analysis adjusted for the backbone use, it did not take into account the effect that the backbone itself could have on the rate of change in APRI score. A second analysis was therefore performed to assess whether the relationship observed in the first analysis was modified by the choice of backbone. The same analysis was performed, including another interaction term, between time since cART initiation and the indicator variable for TDF/FTC use as represented in Equation 2. The rates of APRI score change are therefore calculated as β_2,

($\beta_2+\beta_3$), ($\beta_2+\beta_5$) and ($\beta_2+\beta_3+\beta_5$) for PI-ABC/3TC users, NNRTI-ABC/3TC users, PI-TDF/FTC users and NNRTI-TDF/FTC users, respectively.

Equation 2

$$E[\ln(APRI)] = \beta_0 + \beta_1 NNRTI + \beta_2 Years + \beta_3 NNRTI * Years + \beta_4 TDF.FTC + \beta_5 TDF.FTC * Years + \sum_{j=6}^{J} \beta_j covariate_j$$

Chapter 4: Use of marijuana and progression of liver diseases

4.1 Preamble to Manuscript 1

Despite the very small number of epidemiological studies linking marijuana smoking to liver damage, it has been generally accepted in the medical community that marijuana could be harmful to the liver of patients infected with hepatitis C. Some physicians in the US hesitate to or abstain from prescribing marijuana to their HIV-HCV co-infected patients, for fear of increasing their likelihood of developing liver problems, even though they acknowledge that marijuana can be beneficial for pain management or appetite stimulation, for example.

Where medical marijuana is legal, physicians base their decision not to prescribe marijuana –or not to support their patients in an application for an authorization to possess marijuana for medical purposes under the Canadian Marihuana Medicinal Access Regulations – on three epidemiological studies.[89-91] However, these three cross-sectional studies were likely affected by selection and information bias. In all cases, recruitment took place among patients with an indication for liver biopsy, and therefore more likely to have fibrosis, but also who could have been candidates for a biopsy because of risk factors for fibrosis that are associated with marijuana smoking such as alcohol use. In addition, these studies looked at the association between the presence of fibrosis in the biopsy and current marijuana smoking, but did not account for history of marijuana smoking and it is therefore possible that some participants would have started smoking marijuana to alleviate symptoms due to the worsening of their health state.

We therefore conducted a study to add to the very limited body of literature on marijuana use and liver fibrosis. This is the second and largest longitudinal study of this question and the first conducted in the setting of HIV-HCV co-infection. Moreover, great attention was given to the issue of temporality, insuring that the exposure to marijuana truly preceded the development of liver fibrosis.

The results of this study were presented at the Conference on Retroviruses and Opportunistic Infections in March 2013 and received very positive attention from many physicians who were frustrated with the quality of the publications on this topic.

This manuscript was published in *Clinical Infectious Diseases* in September 2013 (Advance Access published July 4, 2013).[218]

After publication, the results of this study were featured in Infectious Disease News, MedicalResearch.com, Reuters Health Information and was the Editor's choice in Hepatitis Central. This manuscript was also cited in two clinical guideline: the World Health Organisation's *Guidelines for the screening, care and treatment of persons with hepatitis C infection*,[36] and the European Association for Study of Liver's *EASL Clinical Practise Guidelines: Management of hepatitis C virus infection*.[219]

4.2 Manuscript 1: Marijuana smoking does not accelerate progression of liver disease in HIV-hepatitis C co-infection: a longitudinal cohort analysis

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ABSTRACT

Background: Marijuana smoking is common and believed to relieve many symptoms, but daily use has been associated in cross-sectional studies with liver fibrosis. We aimed to estimate the effect of marijuana smoking on liver disease progression in a Canadian prospective multicentre cohort of HIV/HCV co-infected persons.

Methods: Data were analyzed for 717 HCV PCR+ individuals without fibrosis or end-stage liver disease (ESLD) at baseline. Time-updated Cox Proportional Hazards models were used to assess the association between the average number of joints smoked/week and progression to liver fibrosis, cirrhosis or ESLD

Results: At baseline, 53% had smoked marijuana in the past 6 months, consuming a median of 7 joints/week (IQR: 1-21); 40% smoked daily. There was no evidence that marijuana smoking accelerates progression to significant liver fibrosis or cirrhosis as measured by the standard APRI cut-offs (Hazard ratio (HR): 1.02 [0.93-1.12] and 0.99 [0.88-1.12], respectively). Each 10 additional joints/week smoked slightly increased the risk of progression to a clinical diagnosis of cirrhosis and ESLD combined (HR: 1.14 [1.01-1.29]). However, when exposure was lagged to 6-12 months before the diagnosis, marijuana was no longer associated with clinical disease progression.

Conclusions: In this prospective analysis we found no evidence for an association between marijuana smoking and liver fibrosis progression in HIV/HCV co-infection. A slight increase in the hazard of cirrhosis and ESLD with higher intensity of marijuana smoking was attenuated after

lagging marijuana exposure suggesting that reverse causation due to self-medication could explain previous results.

INTRODUCTION

In developed countries, over 30% of HIV infected persons are co-infected with hepatitis C virus (HCV) [5] and HCV has been shown to progress more rapidly in the presence of HIV infection.[4] Liver disease is an important and growing cause of morbidity and mortality in co-infected patients.[220,221] While impaired immunity due to HIV infection may partly explain more rapid fibrosis progression, co-infected patients experience a number of other potentially hepatotoxic exposures that could contribute to liver disease such as illicit drug use.

Marijuana is widely used in Canada: in a 2005 survey, 44% of Canadians reported cannabis use in their lifetime, and 14% reported use in the past year.[68] In a study of medication and alternative therapy use among 104 HIV patients in Ontario, 43% of patients self-reported marijuana use in the past year, and 29% reported a medicinal use.[69]

The literature regarding the effects of cannabis on liver diseases is conflicting. Cell culture and animal model studies support that cannabinoids could have a therapeutic effect on liver injury and fibrosis progression.[78-81,222,223] However, three cross-sectional studies in patients with chronic HCV suggest that daily cannabis use is associated with fibrosis and steatosis.[89-91] A small cohort study of 58 HIV/AIDS patients reported no statistically significant change in liver enzymes in dronabinol and/or marijuana users over the span of one year.[92] There have been no large prospective studies of the effect of cannabis on liver fibrosis progression. Despite this, there is a general acceptance that cannabis use negatively affects liver fibrosis.

This study aimed to estimate the effect of marijuana smoking on liver disease progression longitudinally in HIV-HCV co-infected individuals.

METHODS

Cohort design and study population

The Canadian Co-infection Cohort study is a multi-centre longitudinal study of HIV-HCV co-infected individuals from 17 HIV clinics across Canada. The eligibility criteria are: 1) over 16 years old; 2) documented HIV infection (HIV positive by enzyme-linked immunosorbant assay with western blot confirmation); and 3) evidence of HCV infection (HCV seropositive by enzyme-linked immunosorbant assay with recombinant immunoblot assay II or enzyme immunoassay confirmation, or if serologically false negative, HCV RNA positive).

After informed consent, each participant underwent an initial evaluation followed by study visits approximately every 6 months. Socio-demographic and behavioural information were self-reported in questionnaires; medical treatments and diagnoses were collected by research personnel and laboratory analyses were performed at each visit. Details of the cohort are presented elsewhere.[224] As of July 1, 2012, 1 118 patients had been recruited and followed for a median of 19.1 months (IQR: 6.0-36.9). A sub-cohort was defined for this study and included all co-infected patients with HCV replication (plasma HCV RNA RT PCR, Roche COBAS Amplicor), and without significant fibrosis (aspartate aminotransferase-to-platelet ratio (APRI) score less than 1.5) or clinically diagnosed cirrhosis or end-stage liver disease (ESLD) at baseline.

Marijuana use

At each study visit, participants were asked to report their marijuana use since the last interview. Marijuana smokers also reported how often they smoked (*occasionally/not every week*, *regularly/1-2 days per week*, *regularly/3-6 days per week*, *every day*) and the number of joints consumed on the days they smoked. The average number of joints smoked per week for each interval was calculated by multiplying the number of joints reported by the mean number of days in the frequency interval reported. Participants were also asked for what reason they smoked marijuana. No information was collected on ingestion of marijuana.

Liver fibrosis, cirrhosis and end-stage liver diseases

We used the APRI calculated at each study visit as: APRI = 100[AST/upper limit of normal]/platelet count (10^9/L) to predict fibrosis and cirrhosis. An APRI score greater than or equal to 1.5 was used to indicate significant liver fibrosis and a score greater than or equal to 2 was used to indicate cirrhosis.[59] Clinical cirrhosis was determined radiologically or by liver biopsy. ESLD was defined as a diagnosis of ascites, portal hypertension, spontaneous bacterial peritonitis, encephalopathy, oesophageal varices or hepatocellular carcinoma collected using dedicated case report forms and validated centrally.

Statistical analyses

Participants were censored at initiation of HCV treatment because treatment can affect the AST and platelet counts, thus impacting the measurement of APRI score, and when curative, reduces risk of fibrosis progression and ESLD. Time-dependent Cox proportional hazards models were

used to assess the presence of an association between the number of joints smoked per week and progression to liver fibrosis, cirrhosis or ESLD. The average number of joints smoked per week in the six months preceding the study visit and a binary indicator of marijuana smoking were updated at each study visit. All models were adjusted for baseline APRI score, age, duration of HCV infection, sex, and income and for time-updated CD4 cell count, HIV viral load and ART use at the preceding visit, and alcohol abuse and injection drug use in the 6 months preceding the visit. Duration of HCV infection was based on the date of HCV seroconversion, if known, or year of first IDU or blood product exposure as a proxy of HCV infection.[225] Income was treated as a dichotomous variable, using a yearly income of 24 000 CAD as the cut-off based on low-income cut-offs calculated by Statistics Canada.[226] Alcohol abuse was defined as drinking at least 3 alcoholic drinks on a typical day when drinking, and having six or more drinks on one occasion more than once per month. We repeated these analyses using marijuana smoking exposures 6 to 12 months before outcome assessment in order to reduce the possibility of reverse causation by ensuring temporality is preserved.

We investigated the presence of a dose-response relationship between the number of joints smoked per week and the natural logarithm of the APRI score with a linear spline regression model, introducing flexibility to linear regression by allowing the slope of the curve to change at the pre-specified knots. The location of the knots was selected *a priori* based on meaningful cut-off at 7 joints/week (e.g. regular, low dose use) and 21 joints/week. This model was adjusted for baseline age, sex and income, and for updated alcohol use, IDU, ART use, CD4 cell count, HIV RNA and duration of HCV infection.

RESULTS

A total of 690 participants conformed to the eligibility criteria of this study, contributing 1875.3 person-years of follow-up, with a median follow-up time of 2.7 years (IQR: 0.8-3.8). Selection of the study population is represented in Figure 4.1. The majority were male, with low income and the median age at baseline was 44 years. The majority of the participants had undetectable HIV viral loads; the median CD4 cell count was 400 and 38% used injection drugs at baseline. About half the participants used alcohol, among whom 152 (44%) were hazardous drinkers at baseline, based on the score obtained on the AUDIT-C (score ≥4 in men and ≥3 in women).[227] The baseline and updated characteristics of the study population are presented in Table 4.1.

Table 4.2 summarizes marijuana smoking behaviours in the study population at baseline and over follow-up. Over half of the participants reported smoking marijuana in the past 6 months (median 7 joints/week). About 40% of marijuana users reported smoking daily. A little over 40% of participants who reported marijuana smoking used it for symptom relief at baseline and this proportion increased to half during follow-up.

Progression to liver diseases

Over the course of follow-up, 132 persons (19.1%) reached an APRI score of 1.5, 102 (14.8%) reached a score of 2, 8 developed cirrhosis alone (1.2%) and another 11 (1.6%) developed end-stage liver disease. The incidence rate for progression to an APRI≥1.5 was 39.2 per 1 000 person-visit; to an APRI≥2, 29.2 per 1 000 person-visits; to a clinical diagnosis of cirrhosis, 2.1 per 1000; and to ESLD, 2.9 per 1 000. There were no differences in the crude rates between marijuana users and non-users for any of the outcomes assessed.

Tables 4.3 and 4.4 present the results of the multivariate models for the association between marijuana smoking and progression to liver diseases. No significant association could be found between marijuana use and development of fibrosis or cirrhosis measured with the APRI score. Lagging the exposure variable by one visit had no impact on these conclusions. Neither current nor lagged marijuana smoking accelerated progression to ESLD.

Smoking marijuana seemed to accelerate progression to a clinical diagnosis of cirrhosis (HR: 1.33 per 10 joints/week; 95% CI: 1.09-1.62). However, lagging the exposure attenuated this association (HR: 1.12, 95% CI: 0.94-1.34). Marijuana smoking was also associated with a slightly increased risk of progression to clinically diagnosed cirrhosis and ESLD combined: hazard ratio 1.13 (95% CI: 1.01-1.28). This association was no longer significant when marijuana exposure was lagged (HR: 1.10, 95% CI: 0.95-1.26). Baseline APRI score was a strong predictor of reaching an APRI score of 1.5 or 2, and of a clinical diagnosis of cirrhosis.

Dose-response relationship with APRI score

There was no evidence of a dose-response relationship between marijuana and APRI scores in follow-up. Figure 4.2 illustrates that the APRI score would not be expected to change significantly with increased smoking in any of the joints/week intervals evaluated. The linear spline regression model did not provide a better fit to the data over a simple linear regression.

DISCUSSION

In this first longitudinal study of marijuana smoking and risk of liver disease among HIV-HCV co-infected persons without significant fibrosis at baseline, we found no evidence that cannabis

smoking increases the risk of progression to significant liver fibrosis or cirrhosis as measured by the standard APRI cut-offs. Furthermore, there was no evidence of any dose-response relationship with increasing cannabis use on APRI score. In addition, we did not observe any effect of marijuana use on the development of ESLD. We did observe a slight increase in the risk of progression of clinically diagnosed cirrhosis or cirrhosis and ESLD combined with high levels of marijuana smoking (33% or 13% increase for each additional 10 joints smoked per week, respectively). However, this association disappeared after lagging the exposure, suggesting that previous cross-sectional studies reporting an association between marijuana smoking and liver fibrosis may be biased by reverse causation due to self-medication.

The two principal studies implicating marijuana as an independent risk factor for liver fibrosis were cross-sectional in design. Hézode et al[89] estimated fibrosis progression rates among 270 HCV mono-infected patients undergoing biopsy in a single centre, which were correlated with history of marijuana use obtained contemporaneously with performance of the liver biopsy. They found daily cannabis use was associated with values of fibrosis progression rates greater than 0.074 and with severe fibrosis (≥F3); OR 3.4 [1.5-7.4] and 2.3 [1.1-4.8] respectively. Ishida et al[91] studied 204 consecutive HCV chronically infected patients recruited from community-based clinics who had undergone a liver biopsy; 21% were HIV co-infected. They found a strong association between daily cannabis use and moderate to severe fibrosis (OR=6.78, 95% CI 1.89-24.3) compared to mild fibrosis, but little association was apparent between cannabis use and the presence of mild fibrosis compared to no fibrosis. They concluded that cannabis may have little or no influence on the initiation of fibrosis, but once fibrosis is present, it may be an important cofactor in fibrosis progression.

To our knowledge, the only longitudinal study published described a small cohort of 58 HIV/AIDS patients recruited from an outpatient clinic and followed for 12 months and reported a non-statistically significant decrease in liver enzymes (ALT and AST) among dronabinol and/or marijuana users over the span of one year.[92] Finally, Hézode et al[90] estimated the association between cannabis use and the presence of marked steatosis (≥30% of hepatocytes containing cytoplasmic fat vacuoles) in a cross-sectional study of 315 HCV mono-infected patients undergoing biopsy. They found an OR of 0.5 [0.1-1.8] for occasional cannabis use and of 2.1 [1.01– 4.5] for daily cannabis use compared no use.

Reported use for symptom relief was very prevalent suggesting that the association of daily cannabis use and more advanced fibrosis may, in fact, be related to an increased use for symptom management as disease advances. Interestingly, Ishida et al[91] found that daily cannabis users had lower BMI and were much more likely to have medically prescribed cannabis (57% vs. 9%), suggesting they may have been experiencing more symptoms.

The cannabinoid system consists of two receptors (CB1 and CB2) to which cannabinoids can bind[222]. Depending on which receptor is expressed, cannabinoids could have opposite effects on the liver. Anti-fibrogenic and anti-inflammatory effects of CB2 receptor have been observed in mice.[81,85,86,228] Cannabinoids have been shown to decrease oxidative/nitrative stress and cell death[79], normalize liver enzymes in mice[80] and present anti-inflammatory properties such as: 1) suppression of macrophage function, of antigen presentation and of chemokine production by human B cells; 2) inhibition of macrophage nitric oxide production, of cytotoxic T cell activity and of T lymphocytes proliferative responses; and 3) regulation of tumour necrosis factor, interleukin I and interferon gamma production by human peripheral blood mononuclear

cells[66,67,79]. However, expression of the CB1 receptor seems to have pro-fibrogenic properties.[81,84] In cell cultures, cannabidiol induces death of hepatic stellate cells, activation of which contributes to development of fibrosis.[78] The role of CB1/CB2 receptors expression and ratio is unclear in HCV progression. However, levels of CB1 are 6 times higher in chronic HCV patients than in controls, and twice higher in cirrhotic patients than in those at a low fibrosis stage.[87,88,229] In this study, we selected a population with evidence of chronic HCV infection, thus more likely to express high levels of profibrogenic CB1 receptors. It is also possible that we favoured the inclusion of those with higher CB2 expression by selecting a population free of fibrosis and ESLD. However, this is unlikely to have biased our results since we were interested in studying progression to liver disease.

Our study has several strengths. It is a large, prospective, cohort study that is broadly representative of HIV-HCV co-infected persons in care in Canada. In previous studies, patients were only selected based on having undergone liver biopsy, which potentially introduces selection bias. Indeed, excluded patients in the Ishida study were significantly less likely to use marijuana. We assessed marijuana use and other potential confounders such as alcohol use and HIV disease stage concurrently at each study visit and exposures were updated longitudinally, thus limiting the potential for reverse causality. In addition to using a non-invasive surrogate for fibrosis and cirrhosis, we corroborated our results with clinical outcomes.

There are several limitations worth noting. We used APRI as a non-invasive surrogate for significant liver fibrosis, which may underestimate the degree of fibrosis present and may be influenced by factors other than fibrosis that affect AST and platelet values. The area under the receiver operating characteristic (AUROC) curve of APRI is 0.77 for significant fibrosis and 0.83

for cirrhosis without significant change in accuracy in HIV-HCV co-infected as compared to HCV mono-infected patients.[60] APRI is highly predictive of liver related and all-cause mortality in HIV-HCV co-infection.[185,187,225] However, the reference standard for the diagnosis of hepatic fibrosis, liver biopsy, is invasive, costly and prone to sampling error and therefore not amenable to be used repeatedly[50] and many studies have used the APRI score and showed its value in predicting liver fibrosis and mortality.[187,230,231] Given the 18 years median duration of HCV infection, it is expected that many participants would have some degree of fibrosis at baseline. For this reason we also adjusted for baseline APRI in multivariate models, itself a strong predictor of liver fibrosis progression.

Clinical outcomes were relatively rare over the time frame of this study so it remains possible that we have missed a true effect (type II error) that may have been present if follow-up were extended so as to capture more events. However, the upper bounds of the 95% confidence intervals we observed are not consistent with an effect anywhere near as large as those previously reported (ORs of 3.4 and 6.78) –our study was sufficiently powered to have detected such a large effect for fibrosis progression. Thus if there is any effect of marijuana exposure it is likely to be quite small and only in more advanced disease. Indeed, our estimates are in line with lower bounds of the 95% confidence intervals reported from previous studies (i.e. 1.01 and 1.89), and are much less than those for known important risk factors such as alcohol use.

We lacked detailed information on marijuana use history before cohort entry, and therefore were only able to lag marijuana exposure by 12 months. However, in analyses when exposures were lagged by this amount, risks were attenuated. It is therefore unlikely that even more remote use would be expected to have had an effect. Moreover, exploratory analyses showed

that current exposure provided the best fit to the data. We were also unable to investigate the effect of prescribed cannabinoid use such as marinol, nabilone or sativex. However, use of these drugs was limited in our population.

It remains possible that the risk associated with cannabis exposure differs among HIV co-infected persons for whom there may be other more important predictors of liver disease. However our analyses were controlled for antiretroviral use, and time updated CD4 cell count and HIV virologic control in addition to alcohol and drug use which might be more common in our population. Finally, we could not assess the role of hepatic steatosis and insulin resistance, both important predictors of fibrosis progression.[232,233] As marijuana use has been associated with the presence of steatosis, failure to account for steatosis would likely have biased our results away from the null rather than masking an effect of marijuana on fibrosis progression. Including BMI in the models did not change results (not shown).

To conclude, in this first prospective evaluation of liver disease progression among HIV-HCV infected persons, we could not demonstrate any important effect of cannabis on liver disease outcomes. Marijuana did not meet any criteria for causality: hazard ratios were weak, and most importantly were attenuated when accounting for temporality in the exposure-disease relationship and there was no dose-response effect. It is likely that previous studies have been biased by reverse causality as patients use more marijuana to relieve symptoms as liver disease progresses.

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TABLES

Table 4. 1 Baseline and updated characteristics of the study population

Characteristics	Baseline	Follow-up
N (persons or person-years)	690	1875.3
Follow-up time (months), median (IQR)	-	32.2 (10.1-45.6)
Age (years), median (IQR)	43.9 (38.4-49.2)	-
Male, n (%)	503 (72.9)	-
Yearly income less than 24,000CAD, n (%)	583 (84.5)	-
Homeless, n (%)	33 (4.8)	-
Alcohol use in past 6 months, n (%)	348 (50.4)	1,724 (55.4)
Alcohol abuse in past 6 months (among alcohol users), n (%)	53 (15.2)	253 (14.7)
Used injection drugs in past 6 months, n (%)	263 (38.1)	1,064 (34.2)
Duration of HCV infection (years), median (IQR)	18.0 (10.4-24.5)	-
Duration of HIV infection (years), median (IQR)	10.3 (5.6-15.6)	-
CD4 cell count, median (IQR)	400 (270-570)	420 (280-598)
CD4 cell count < 200 cells/mm^3, n (%)	99 (14.3)	394 (12.7)
Undetectable HIV viral load (<50), n (%)	375 (54.3)	2,018 (64.8)
BMI, median (IQR)	24.0 (21.2-26.8)	23.5 (21.0-26.8)
Non-fasting glucose (mmol/L), median (IQR)	5.1 (4.6-5.8)	5.1 (4.6-5.8)
Diabetes, n (%)	24 (3.5)	39 (5.6)
APRI score, median (IQR)	0.52 (0.37-0.79)	0.55 (0.37-0.90)
First occurrence of APRI ≥ 1.5, n (%)	-	132 (19.1)
First occurrence of APRI ≥ 2, n (%)	-	102 (14.8)
First occurrence of cirrhosis, n (%)	-	8 (1.2)
First occurrence of end-stage liver disease, n (%)	-	11 (1.6)
First occurrence of cirrhosis or end-stage liver disease, n (%)	-	16 (2.3)

Table 4. 2 Marijuana smoking behaviours of the study population

Marijuana smoking characteristic	Baseline (n= 690 persons)	Follow-up (n= 3 112 person-visits)
Smoked in past 6 months/since last interview, n (%)	367 (53.2)	1,654 (53.1)
Smoking frequency, n (%)		
Occasionally, not every week	119 (32.4)	513 (31.0)
Regularly, 1-2 days/week	50 (13.6)	203 (12.3)
Regularly, 3-6 days/week	50 (13.6)	232 (14.0)
Everyday	145 (39.5)	685 (41.4)
Missing	3 (0.8)	21 (1.3)
Number of joints/week, median (IQR)	7 (1-21)	7 (1-27)
Main reason for smoking[a], n (%)		
To relieve symptoms	150 (40.9)	838 (50.7)
To increase appetite	152 (41.4)	827 (50.0)
Recreational purposes	167 (45.5)	776 (46.9)
Sleep	1 (0.2)	1 (0.06)

[a] Categories are not mutually exclusive.

Table 4. 3 Effect of marijuana smoking on progression of liver diseases

Outcome	Model	Hazard ratio (95% CI)
APRI ≥ 1.5	10 joints/week, current[a]	1.02 (0.93, 1.12)
	Lagged exposure[b]	0.95 (0.85, 1.07)
APRI ≥ 2	Current exposure[a]	0.99 (0.88, 1.12)
	Lagged exposure[b]	0.96 (0.85, 1.10)
Cirrhosis	Current exposure[a]	1.33 (1.09, 1.62)
	Lagged exposure[b]	1.12 (0.94, 1.34)
ESLD	Current exposure[a]	1.08 (0.90, 1.28)
	Lagged exposure[b]	1.07 (0.85, 1.34)
Cirrhosis or ESLD	10 joints/week, current[a]	1.13 (1.01, 1.28)
	Lagged exposure[b]	1.10 (0.95, 1.26)

[a] Current exposure models report on the effect associated with an increase of 10 joints per week in the past 6 months. Models are adjusted for baseline: age, duration of HCV infection, sex, and income, time-updated alcohol and injection drug use in the 6 months preceding the visit, CD4 cell count, HIV viral load and ART use at the preceding visit and binary indicator of marijuana smoking.

[b] Lagged exposure models report on the effect associated with an increase of 10 joints per week in the past 6-12 months prior to the current follow-up visit. Models adjusted for same variables, with the exception of binary indicator of marijuana smoking relating to 6-12 months before outcome assessment.

Table 4. 4 Full models for the effect of current marijuana smoking on progression to liver fibrosis (APRI score ≥ 1.5) and cirrhosis or ESLD

Outcome	Model	Hazard ratio (95% CI)
APRI ≥ 1.5	10 joints/week, current	1.02 (0.93, 1.12)
	Marijuana use, current	0.78 (0.51-1.20)
	Baseline	
	APRI score	6.12 (3.46-10.83)
	Age (per 5 years)	1.00 (0.87-1.14)
	Duration of HCV infection (per 5 years)	1.06 (0.95-1.18)
	Female	1.50 (0.99-2.25)
	Income < 24,000CAD	0.92 (0.49-1.75)
	Time-updated	
	Alcohol abuse	1.49 (0.84-1.75)
	Injection drug use	1.12 (0.75-1.69)
	ART use	1.00 (0.56-1.80)
	CD4 count (per 100 cell)	0.98 (0.91-1.06)
	HIV RNA (log copies/ml)	1.03 (0.94-1.12)
Cirrhosis or ESLD	10 joints/week, current	1.13 (1.01, 1.28)
	Marijuana use, current	0.72 (0.20-2.55)
	Baseline	
	APRI score	2.41 (0.43-13.41)
	Age (per 5 years)	1.34 (0.91-1.98)
	Duration of HCV infection (per 5 years)	1.01 (0.77-1.34)
	Female	1.06 (0.27-4.15)
	Income < 24,000CAD	0.49 (0.12-2.03)
	Time-updated	
	Alcohol abuse	1.08 (0.13-8.65)
	Injection drug use	0.42 (0.09-2.04)
	ART use	-
	CD4 count (per 100 cell)	0.93 (0.72-1.19)

Abbreviations: APRI - Aspartate aminotransferase-to-platelet ratio; ART - Antiretroviral therapy.

FIGURES

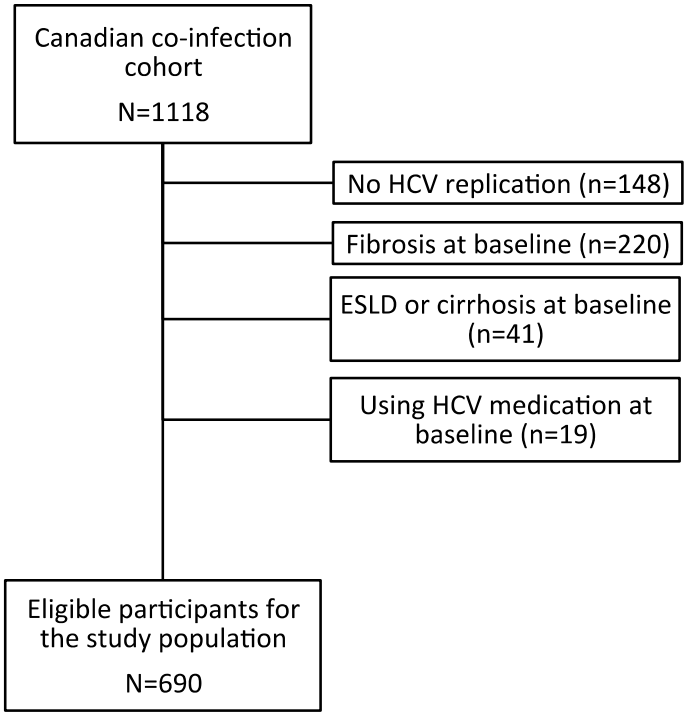

Figure 4. 1 Study population flow chart

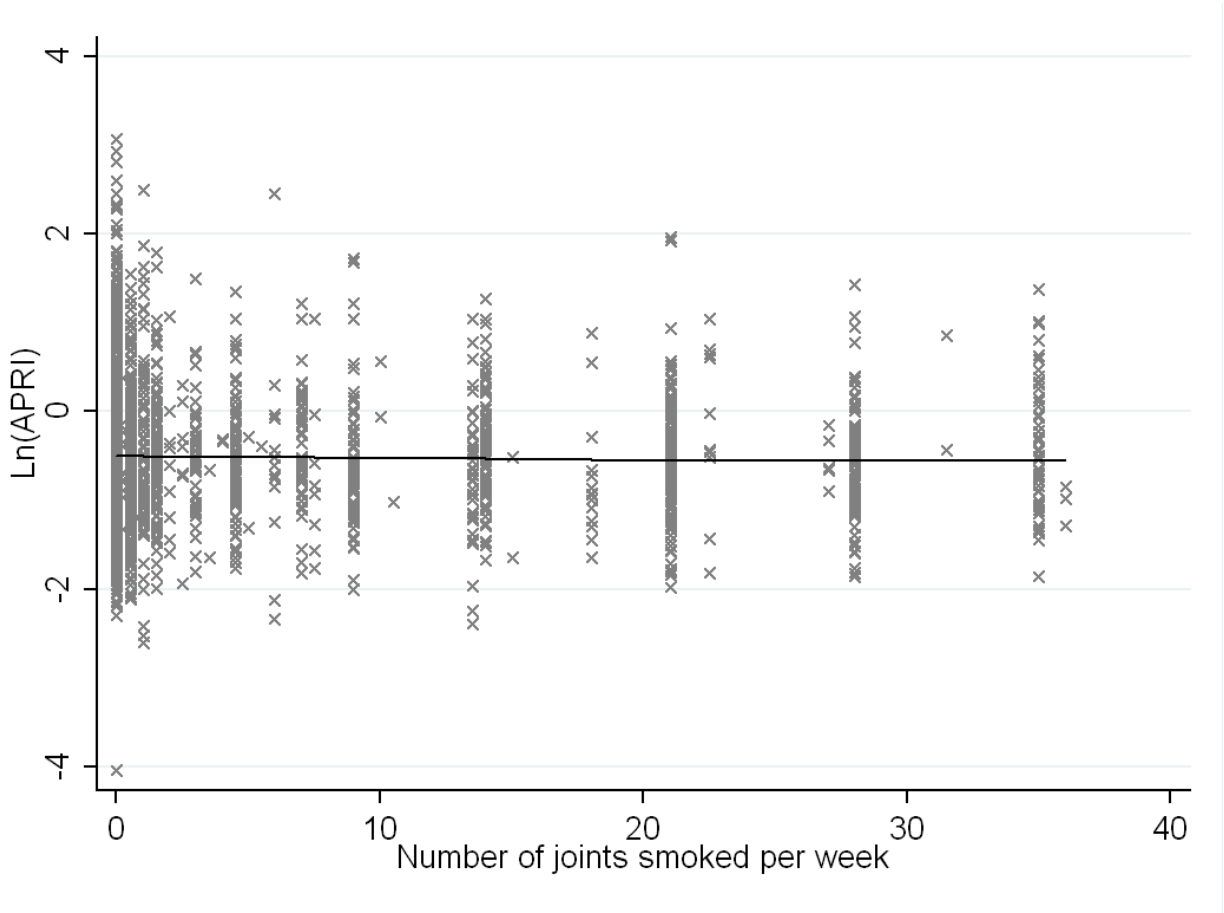

Figure 4. 2 Relationship between the number of joints smoked per week and the APRI score, linear spline regression model

Chapter 5: Use of opioids and progression of liver fibrosis

5.1 Preamble to Manuscript 2

In Chapter 4, we concluded that marijuana, one of the drugs most frequently used by HIV-HCV co-infected persons, was unlikely to accelerate progression to significant liver fibrosis. A recent study showed that the presence of medical marijuana laws was associated with a 25% reduction in rates of fatal opioid overdoses in the United States. The authors have speculated that availability of medical marijuana could lead to a reduction in the use or dosage of opioids for analgesia.[234]

Opioids are often prescribed to HIV-HCV co-infected persons, not only for pain management, but also as a substitution therapy in the context of addiction treatment. Opioids are also often used recreationally. Persistent heroin use was associated with a non-significant increase in CD4 cell count and intermittent use was associated with a non-significant decrease in CD4 cell count compared to no heroin use in a prospective pilot study of 77 ART-naïve persons living with HIV in Russia, where opioids are not prescribed for pain or addiction management.[235] It is unclear whether heroin could have an impact on the liver through its effect on HIV disease progression, a known predictor of liver fibrosis. Heroin, although frequently used, is far from being the only opioid used illicitly. Prescription opioids are available in the street and increasingly popular, resulting in an important rise in overdose in 2014 in Montreal.[236]

Although many studies on the relationship between opioids and the liver have been published, very few high quality longitudinal studies are available. Moreover, they generally focus on illicit drug use, not taking into account the growing use of prescription opioids in North America.

Some studies that explored prescribed opioid use did not include illicit opioid drug use in their analyses.

We therefore aimed to assess the association between both prescribed and illicit opioid use and progression of liver fibrosis in a longitudinal setting of HIV-HCV co-infection. A close examination of the data brought to our attention the potential for misclassification of prescribed opioids use. We noticed that several participants had reported dates at which they had stopped taking prescribed opioids, but we had no records of them ever being prescribed these opioids. This prompted us to find a solution to correct for this measurement error. We did not have access to high quality external data to validate the self-reported opioids use, which would have allowed us to apply methods such as regression calibration or multiple imputation for measurement error. After thoughtful consideration, we selected the Monte Carlo sensitivity analysis approach to quantify the bias potentially introduced by the misclassification of the exposure while incorporating uncertainty with regards to the accuracy of the self-reported measure.

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5.2 Manuscript 2: Opioid use and risk of liver fibrosis in HIV/hepatitis C virus-coinfected patients in Canada

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ABSTRACT

Objectives: Opioid use and opioid-related mortality have increased dramatically since the 1990s in North America. The effect of opioids on the liver is incompletely understood. Some studies suggest opioids cause liver damage and others fail to show any harm. HIV-hepatitis C co-infected persons may be particularly vulnerable to factors enhancing liver fibrosis. We aimed to describe opioid use in a HIV-hepatitis C co-infected population in Canada and to estimate the association between opioid use and liver fibrosis.

Methods: We conducted a cross-sectional descriptive analysis of the Canadian Co-infection Cohort study to characterize opioid use. We then conducted a longitudinal analysis to assess the average change in APRI score associated with opioid use using a generalized estimating equation with linear regression. We assessed the progression to significant liver fibrosis (APRI≥1.5) associated with opioid use with pooled logistic regression.

Results: In the 6 months preceding cohort entry, 32% of the participants had received an opioid prescription, 28% used opioids illicitly and 18% had both received a prescription and used opioids illicitly. Neither prescribed nor illicit opioid use were associated with a change in the median APRI scores (exp(β): 0.99 [95% CI: 0.82, 1.12] and exp(β): 0.95 [95% CI: 0.81, 1.10], respectively) or with faster progression to liver fibrosis (hazard odds ratio (HOR): 1.20 [95% CI: 0.73, 1.67] and HOR: 1.09 [95% CI: 0.63, 1.55], respectively).

Conclusions: Although opioids were commonly used both legally and illegally in our cohort, we were unable to demonstrate a negative impact on liver fibrosis progression.

INTRODUCTION

Since the 1990s, opioid use has increased dramatically in North America and opioid-related overdose and mortality have increased in parallel.[95] Many behavioural and drug-related factors such as the type and doses of opioids prescribed and poly-substance use appear to contribute to the observed increase in opioid-related deaths.[238] Liver-related deaths are frequent among opioid users. In Australia, among 841 cases of fatal opioid toxicity, significant liver damage was the most frequent pathology revealed at autopsy.[112] Opioid-dependent individuals were reported to be about 17 times more likely to die of any liver cause and far more likely to die of chronic liver disease and liver cancer compared to the general population.[239,240] The increased risk of liver disease in opioid users is largely explained by chronic hepatitis C (HCV) infection, which is common in this population.[33] It is however unclear whether opioids directly contribute to liver fibrosis progression, which is a prerequisite for cirrhosis and is associated morbidity and mortality.

Opioid analgesics and methadone substitution therapy are frequently prescribed both to HIV- and HCV-infected persons[241] who might be at greater risk of opioid-related liver morbidity because co-infection is associated with an accelerated progression of liver fibrosis.[27] Morphine has also been shown to enhance expression of HCV mRNA in cell culture,[104] suggesting the potential for a direct effect of opioids on liver morbidity in a co-infected population.

Current literature on the effect of opioids on the liver is conflicting. In experimental studies, administration of opioid-receptor agonists to animals or in cell cultures leads to increased levels of transaminase,[97,99] hepatic glutathione s-transferase,[101] histopathological abnormalities,[97] liver

inflammation, fatty accumulation, and fibrosis.[102] Supporting evidence from observational studies is weak. Cross-sectional studies demonstrate that heroin users have higher transaminase levels compared to users of other drugs or alcohol[242] and increased collagen deposition compared to non-drug users.[243] Other experimental studies have identified only a moderate effect of opioids on elevation of transaminase levels,[98] glutathione levels[105] and fibrosis.[107] A cross-sectional study of HCV-infected male veterans failed to show an association between methadone use and liver fibrosis.[113] Although studies have suggested that opioid receptor antagonists may prevent opioid-related liver damage,[115-117,122,123] two randomized controlled trials comparing use of methadone (an agonist) to buprenorphine (a mixed agonist-antagonist) demonstrated similar transaminase levels in the two groups.[124,125] The effect of opioid use and abuse on liver-related outcomes in HIV-HCV co-infected persons remains unknown.

The first objective of this study was to evaluate both the use of prescribed and illicit opioids by the participants of the Canadian Co-infection Cohort Study. As a second objective, we assessed the role of prescribed and/or illicit opioid use on the development of liver fibrosis in HIV-HCV co-infected persons.

METHODS

The Canadian Co-infection Cohort study

The Canadian Co-infection Cohort study is a multi-centre longitudinal study of HIV-HCV co-infected persons from 18 HIV clinics across Canada. The cohort's eligibility criteria are the following: (a) to be 16 years or older; (b) to have documented HIV infection (HIV positive by

enzyme-linked immunosorbant assay with western blot confirmation); and (c) to show evidence of HCV infection (HCV seropositive by enzyme-linked immunosorbent assay with recombinant immunoblot assay II or enzyme immunoassay confirmation, or if serologically false negative, HCV RNA positive). After providing informed consent, participants are followed every six months, completing questionnaires regarding sociodemographic factors and drug exposures, and also providing blood samples. Clinical events are recorded by research coordinators through chart review. The cohort design and protocol have previously been described.[224] As of October 1, 2013, 1238 patients had been recruited.

Approval has been obtained from the relevant ethics committee for each study site and the Canadian Co-infection Cohort Study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Exposure to opioids

Prescribed opioid use (natural, semi-synthetic or synthetic opioid receptor agonists or mixed agonist-antagonist) was assessed through participant self-report and the information supplemented with the patient's clinical file. Illicit opioid use and route of administration (injection or not) was assessed through participant self-report at each study visit.

APRI score and liver-related morbidity and mortality

At each visit, we calculated the AST-to-platelet ratio index (APRI) score as: 100[AST/upper limit of normal]/platelet count (10^9/L). An APRI score ≥ 1.5 indicates significant liver fibrosis (equivalent to a score ≥2 on the Metavir scale).[60] We chose to use this validated biomarker

because while liver biopsies are considered the gold standard to detect fibrosis, they are expensive, prone to sampling error, unethical to perform every 6 months for research purposes, and often unacceptable for patients unless a treatment decision is being considered.

End-stage liver disease (ESLD) was defined as a diagnosis of liver cirrhosis, ascites, portal hypertension, spontaneous bacterial peritonitis, encephalopathy, oesophageal varices or hepatocellular carcinoma. Participants were categorized as having experienced a hepatic event if they had a diagnosis of ESLD or experienced ESLD-related death. Study site coordinators completed dedicated case report forms in the event of death or ESLD diagnosis. Two investigators independently classified causes of death using the Coding of Death in HIV (CoDe) system.[244] Participants in British Columbia, Alberta and Quebec (74% of the cohort) were linked to provincial vital statistics to capture deaths among patients lost to follow-up.

Statistical analyses

Two analytical cohorts were selected. The *prevalence cohort* consisted of all participants with at least two study visits and with active HCV viral replication at baseline. This cohort allowed us to evaluate the effect of opioid use regardless of the baseline disease stage. We created the *incidence cohort* by restricting the population to patients without significant fibrosis or ESLD events at baseline in order to study the effect of opioid use on progression to these outcomes in follow-up. All participants were censored at initiation of HCV treatment because it can affect the APRI measure. Missing data were handled using multiple imputation with chained equations.

We performed descriptive statistics for prescribed and illicit opioid use at cohort entry in both cohorts. In the prevalence cohort, we estimated the average response in the natural log of the

continuous APRI score corresponding with prescribed and/or illicit opioid use by fitting a linear regression with generalised estimating equations (GEE). Different correlation structures were used to find the best fitting model using the quasi-likelihood under the independence model criterion (QIC). In the incidence cohort, we performed a survival analysis using pooled logistic regression with a polynomial representation of time to assess the association of prescribed and/or illicit opioid use compared to no use with progression to significant fibrosis (defined as APRI≥1.5).

All statistical models were adjusted for baseline age, sex and estimated time since HCV infection in addition to the previous visit's time updated alcohol use, other illicit drugs use, antiretroviral therapy, CD4 cell count and presence of detectable HIV viral load. The linear regression model was further adjusted for years of follow-up. Two indicators of opioid use during the previous study interval were included in each model: an indicator for prescribed opioid use and one for illicit opioid use. Stata, version 13 (StataCorp, College Station, Texas) was used to perform all analyses.

Sensitivity analyses

Four planned sensitivity analyses were performed with both the prevalence and the incidence cohort by replacing the exposure variables in the models described above. The first sensitivity analysis consisted of replacing the indicators for prescribed and illicit opioid use by an indicator for any opioid use. With the second, we explored the effect of the number of different opioids prescribed or taken illicitly during a study interval. Third, we used the cumulative number of intervals during which opioids were used with or without a prescription. Finally, we introduced

an indicator for opioid injection and one for other routes of opioid administration instead of the indicator for illicit use in general.

Two further sensitivity analyses were performed with the incidence cohort data only. First, we repeated the survival analysis using hepatic events as the outcome. We also performed a Monte Carlo sensitivity analysis with record-level correction of the measurement error in prescribed opioid use to assess the progression to liver fibrosis while accounting for potential measurement error of the exposure to prescribed opioids.[211] We used trapezoidal probability distributions for sensitivity (0.45, 0.5, 0.6, 0.99) and specificity (0.7, 0.8, 0.9, 1) selected based on published validation studies.[204-206]

RESULTS

Prevalence cohort: opioid use and changes in median APRI score

Selection of participants in the prevalence and incidence cohorts is described in Figure 1. The prevalence cohort comprised 800 participants who contributed a median of 39 months of follow-up for a total of 3058 person-years. The majority were male with low income. Approximately 50% had been using alcohol and the majority were on antiretroviral therapy with controlled HIV infection. Further details regarding baseline demographic and clinical characteristics are summarized in Table 1.

Prescribed and illicit opioid use is presented in Table 2. At cohort entry, 43% of the participants in the prevalence cohort were using prescribed and/or illicit opioids. Almost a third had received at least one prescription for opioids in the previous 6 months and the majority were prescribed

only one type of opioid. Opioids were used illicitly by 28% of the participants, among whom 29% injected only, 45% used other routes of administration only and 26% used opioids by both injection and alternate routes.

Figure 2A shows that methadone was the type of opioid most often prescribed at baseline (62% of users). The most frequently injected opioids reported were heroin (84%), morphine (64%), speedball (heroin and cocaine injected together) (60%) and hydromorphone (56%) as presented in Figure 2B. Methadone (42%) and codeine combined with acetaminophen (33%) were the illicit opioids most often used without injection (Figure 2C).

At cohort entry, participants in the prevalence cohort had a median APRI score of 0.7, 24% had already reached the cut-off for significant fibrosis and 11% had experienced an ESLD event (Table 1). In follow-up, ESLD caused or contributed to the death of 19 persons (21% of deaths). Figure 3 presents the types of opioids reported at the visit prior to their last by persons who finished follow-up without liver fibrosis, those who developed liver fibrosis and those who were diagnosed or died of end-stage liver disease. After accounting for age, sex, time since HCV infection, alcohol use, ART use, CD4 cell count, undetectable HIV viral load and years of follow-up, neither prescribed nor illicit opioids were associated with a statistically significant change in the median APRI score, as presented in Table 3. Changes in the median APRI score were modest. For example, use of prescribed opioids was associated with a 1% decrease in the median APRI score (exp(β): 0.99, 95% CI: 0.85, 1.12) and illicit use was associated with a 5% decrease in the median APRI score (exp(β): 0.95, 95% CI: 0.81, 1.10). Alcohol use however was associated with a 20% increase in the median APRI score and a higher CD4 cell count was protective.

Incidence cohort: opioid use and progression to significant liver fibrosis

The incidence cohort consisted of 582 participants, contributing a median of 42 months of follow-up for a total of 2293 person-years as shown in Figure 1. There were no important differences between the prevalence and the incidence cohorts either with respect to baseline demographic and clinical characteristics or pertaining to opioid use (Tables 1 and 2).

At cohort entry, participants in the incidence cohort had a median APRI score of 0.5. Over the course of follow-up, 163 participants (28%) developed significant fibrosis and 27 (5%) experienced a clinical hepatic event. There was no ESLD-related death during follow-up in the incidence cohort. Neither prescribed (hazard odds ratio (HOR): 1.20, 95% CI: 0.73, 1.67) nor illicit opioid use (HOR: 1.09, 95% CI: 0.63, 1.55) were significantly associated with faster progression to significant liver fibrosis. Similar associations between alcohol use or CD4 cell count and progression to liver fibrosis were observed as in the prevalence cohort, however, 95% CI included 1.00. Results are summarized in Table 3.

Sensitivity analyses

Using different exposure definitions (any, intensity, consistency of use or separating injected/not injected illicit use), the results obtained were not appreciably different from those presented in Table 3 (data not shown). Using hepatic events as the outcome in the incidence cohort produced results comparable to those shown above for prescribed opioids but were far more imprecise (HOR: 2.26, 95% CI: 0.78, 6.50). The results for illicit use pointed in the opposite direction although the confidence interval was also very wide (HOR: 0.81, 95% CI: 0.22, 2.90).

Accounting for potential measurement error in prescribed opioid use with the Monte Carlo sensitivity analysis did not change the results appreciably for the progression to significant liver fibrosis associated with prescribed opioid use (HOR: 1.11, 95% simulation interval: 0.62, 1.98).

DISCUSSION

This study is the first description of illicit and prescribed opioid use and assessment of their association with liver fibrosis in an HIV-HCV co-infected population. A high proportion of the Canadian co-infection cohort participants reported opioid use. Over 40% of the participants received opioid prescriptions, almost 30% used opioids illicitly and close to 20% who were prescribed opioids also used them illicitly during the same period. The rate of prescribed opioid use is similar to that recently described among HIV infected Veterans[241] and among patients being treated for non-cancer pain conditions covered by two American commercial health plans.[245] The high rates of concurrent prescribed and illicit opioid use as well as the large proportion of prescription opioids used illicitly however suggest that opioid misuse is common in our population and similar to misuse rates recently reported among indigent HIV infected persons.[246] A study of chronic non-cancer pain opioid users also revealed that between 24 and 31% exhibited prescription opioid abuse behaviours.[247]

Methadone was the most frequently prescribed opioid in the co-infection cohort. Opiate substitution therapy with methadone is an important component of harm reduction strategies. In addition to reducing injection drug use, it decreases the risk of HIV transmission[248] and is associated with favourable health outcomes including longer survival.[249] However, methadone has been associated with a substantial proportion of opioid-related deaths.[238] Therefore, it is

important to investigate potential harmful clinical effects of prescribing methadone and other opioids in this population, such as liver outcomes.

The high prevalence of non-methadone opioid use in this co-infection cohort and other cohorts of HIV-infected individuals could partly be explained by the need for pain management. Pain is a common problem for HIV-infected individuals.[250,251] HCV infected persons may also experience pain due to mixed cryoglobulinemia, HCV-associated arthritis, peripheral neuropathy, or fibromyalgia.[34] A high prevalence of pain could have explained some opioid misuse if patients attempt to control inadequately managed pain on their own.

In our co-infected population, neither prescribed opioids nor illicitly used opioids were associated with an increase in median APRI score or with a faster progression to advanced liver fibrosis. Our results were consistent between the prevalence and the incidence cohorts, and across a range of sensitivity analyses using different approaches to model opioid exposure (any use, intensity or consistency of use, separating injection and other illicit use) or correcting for measurement error.

The majority of studies suggesting a potential harmful effect of opioids on the liver are derived from experiments conducted in cell culture or animal models and may not accurately reflect the reality of people regularly using opioids. Often, the doses used in animal experiments are much higher than those prescribed and the purity of the opioids used is generally greater than that of the opioids that can be purchased on the street. The observational studies suggesting a link between opioid use and liver diseases are predominantly case studies[243,252,253] or cross-sectional studies.[112,113,242] Results from these types of studies should be interpreted carefully because it is

not possible to ascribe temporality of exposure and events. There was no evidence of an association between methadone use and advanced fibrosis (OR: 1.29, 95% CI: 0.56, 3.01) in a cross-sectional analysis of 571 male veterans with HCV.[113] Another cross-sectional study concluded that opioids had only a reversible effect on the liver, because current heroin users without HIV or HBV (but uncertain HCV status) exhibited microvasular alterations that were not present in liver biopsies of ex-heroin users.[110] The participant selection in cross-sectional studies of opioid use and liver disease may also introduce potential bias. For example, one study only included information on people who died due to opioid overdose, among whom 37% of the cases had steatosis, 11% had fibrosis and 7% had cirrhosis.[112] Another study grouped users of various drugs together and did not attempt to isolate the effect of opioids from the effect of other types of drugs in their analyses.[242] Randomized controlled trials have shown that groups who received an opioid agonist did not experience greater elevations in liver enzymes than groups who received a mixed agonist-antagonist,[124,125] or groups who received no opioids.[119] In fact, it seems that use of opioids is not a determining factor for elevated liver enzymes or other liver outcomes, as opposed to a diagnosis of viral hepatitis.[118,125]

This study was conducted with data from a large, multi-centre Canadian cohort. The population studied is representative of Canadians with co-infection who access care and particular efforts have been made to reach vulnerable populations such as Aboriginal people, women and people who inject drugs. Multiple sensitivity analyses were conducted to confirm the robustness of our findings. The quality of exposure measurement can have a substantial impact on the effects estimated. The validity of self-reported use of prescribed opioids, narcotics or pain killers has been assessed in various populations and showed low to moderate sensitivity but high

specificity compared to administrative database, urine toxicology or medical records.[204-206] Considering the likelihood that the validity of the prescribed opioids measure is poor, we conducted a sensitivity analysis to account for this potential misclassification and confirmed the results obtained without correction. However, the Monte Carlo sensitivity analysis could not be performed for the analysis of continuous APRI score due to the complexity of applying this method of bias correction to a continuous outcome. We did not attempt to correct potential misclassification of illicit opioid use because validation studies suggest that self-report of these types of drugs is overall a valid measure.[254] We were unable to perform a dose-response analysis because information on prescribed doses and quantification of amount of illicit opioids used was not available. However, we were able to perform sensitivity analyses investigating the effect of the intensity and consistency of use.

It is possible that we lacked the power to detect an effect in the incidence cohort, as suggested by the wide confidence intervals around the estimates for the effects of alcohol use or lower CD4 cell counts, which are both established risk factors for progression to significant liver fibrosis. However, sufficient statistical power was present to identify an association between alcohol use or CD4 cell count and change in median APRI score in the prevalence cohort. This suggests that sufficient power was present to detect a clinically meaningful effect of opioids in the prevalence cohort.

Liver diseases progress slowly and the duration of follow-up in this cohort was relatively short, which limits the possibility of observing liver disease related clinical outcomes. It is for this reason that we selected liver fibrosis progression as the outcome for the main analyses, as liver

fibrosis is a precursor of liver disease. Longer follow-up would however be useful to confirm the results of this study and assess whether a longer exposure to opioids could be harmful.

We chose to use the APRI score as a marker for liver fibrosis because the invasiveness of liver biopsy precludes its use in a longitudinal research setting. Moreover, transient elastography was not performed in all study sites and repeated measures are currently limited in our cohort. Although the specificity of the APRI score cut-off for significant fibrosis is excellent, it has a low sensitivity,[60] which could lead to some degree of outcome misclassification. However, most non-invasive markers of liver fibrosis available have been shown to perform with similar accuracy.[62] The APRI score has been validated in HIV-HCV co-infected populations.[60] This marker has been shown to predict all-cause mortality[230] and occurrence of liver complications.[185] The results reported here are therefore clinically pertinent despite the lack of power to study ESLD as an outcome.

In conclusion, opioids are widely used in this Canadian HIV-HCV co-infected population. A large proportion received one or more prescription for an opioid and many also used opioids without a prescription in the same period of time. However, opioid users were not at increased risk of developing liver fibrosis compared to non-users. While opioid use may have other negative consequences, aggravating liver disease does not seem to be a major concern when managing addiction or pain in HIV-HCV co-infected patients.

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The CCC cohort is comprised of 18 sites across Canada who recruit and follow HIV-HCV co-infected patients. The following co-investigators have all taken part in the data collection pertaining to this manuscript, and have reviewed its content. However, they did not partake in the analysis, writing or editing of this paper nor has anyone received compensation for any contributions.

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Hospital & Westside Community Clinic, University of Saskatchewan, Saskatoon, SK; and David Wong, University Health Network, Toronto, ON.

TABLES

Table 5. 1 Baseline characteristics of the study populations

Characteristics	Prevalence cohort	Incidence cohort
Number of participants	800	582
Person-visits	4865	3652
Number of study visits, median (IQR)	7 (4-10)	8 (4-11)
Months of follow-up, median (IQR)	39 (19-56)	42 (19-58)
Age (years), median (IQR)	44 (39-50)	43.8 (38-49)
Male, n (%)	582 (73)	408 (70)
Monthly income of $1,500 CAD or less, n (%)	615 (77)	455 (78)
Homeless, n (%)	101 (13)	70 (12)
Alcohol use in past 6 months, n (%)	415 (52)	293 (50)
Used injection drugs in past 6 months, n (%)	294 (37)	228 (39)
Other drug use (not injected) in past 6 months, n(%)	364 (46)	269 (46)
Duration of HCV infection (years), median (IQR)	18 (10-25)	18 (10-25)
Time since HIV diagnosis (years), median (IQR)	11 (6-17)	11 (6-16)
CD4 cell count, median (IQR)	374 (240-540)	388 (258-551)
Undetectable HIV viral load (<50 copies/ml), n (%)	438 (55)	319 (55)
HIV viral load if detectable (copies/ml), median (IQR)	2047 (111-30065)	2344 (117-30231)
Baseline APRI score, median (IQR)	0.70 (0.43-1.46)	0.53 (0.38-0.80)
Prevalent cases of APRI ≥ 1.5, n (%)	189 (24)	NA
Prevalent cases of ESLD, n (%)	84 (11)	NA

Abbreviations: IQR: Inter-quartile range, APRI: Aspartate to platelet ratio index, ESLD: End-stage liver disease.

Table 5. 2 Prescribed and illicit opioid use in the study population at cohort entry

Opioid use	Prevalence cohort (N=800) n (%)	Incidence cohort (N=582) n (%)
Any opioid use	344 (43)	260 (45)
Prescribed opioid use	255 (32)	198 (34)
Number of different opioids prescribed*		
0	545 (68)	384 (66)
1	234 (29)	180 (31)
2	19 (2)	16 (3)
3	2 (0)	2 (0)
Illicit opioid use	228 (28)	176 (30)
Injected only (among illicit opioid users)	67 (29)	50 (28)
Not injected only (among illicit opioid users)	102 (45)	79 (45)
Both (among illicit opioid users)	59 (26)	47 (27)
Both prescribed and illicit opioid use	144 (18)	116 (20)

* Prescribed opioids included the following: codeine, morphine, hydromorphone, oxycodone, diphenoxylate, fentanyl, meperedine, methadone or tramadol.

Table 5. 3 Relationship between opioid use and change in median aspartate aminotransferase-to-platelet ratio index score in the prevalence cohort or development significant liver fibrosis in the incidence cohort

	Prevalence cohort GEE Ln (APRI) score* Exp(β) (95% CI)	**Incidence cohort Pooled logistic regression Significant fibrosis† HOR (95% CI)**
Prescribed opioid use‡	0.99 (0.85, 1.12)	1.20 (0.73, 1.67)
Illicit opioid use‡	0.95 (0.81, 1.10)	1.09 (0.63, 1.55)
Baseline		
Age (5 years increments)	1.03 (0.99, 1.07)	1.01 (0.90, 1.12)
HCV duration (5 years increments)	1.02 (0.99, 1.05)	1.00 (0.91, 1.09)
Female	0.98 (0.84, 1.11)	1.40 (0.89, 1.94)
Updated		
Alcohol use‡	1.20 (1.08, 1.32)	1.42 (0.89, 1.94)
Other illicit drug use‡	0.91 (0.81, 1.00)	1.22 (0.71, 1.74)
Antiretroviral use‡	0.94 (0.81, 1.07)	0.84 (0.42, 1.26)
CD4 cell count (per 100 cell/μl)‡	0.96 (0.94, 0.98)	0.99 (0.92, 1.06)
Undetectable HIV RNA‡	0.97 (0.87, 1.08)	0.94 (0.52, 1.37)
Time since cohort entry (years)	1.02 (1.00, 1.05)	NA¶
Intercept	0.62 (0.47, 0.95)	0.06 (0.01, 0.31)

*Ln(APRI) was used as the outcome. We show exp(β), which represents the change in the median APRI score associated with a one unit increase in the continuous dependent variables, or the presence of the characteristic recorded by dichotomous variables.

†Significant fibrosis: APRI >=1.5

‡Last visit before outcome assessment

§The time component (the study visit) is entered in the model as a polynomial function of the study visit. If visit number is denoted by v, the first order polynomial is v, the second order polynomial is v^2, the third order is v^3 and the forth order is v^4.

¶ Models were adjusted for time using a polynomial function of the visit number (visit, $visit^2$, $visit^3$, $visit^4$).

FIGURES

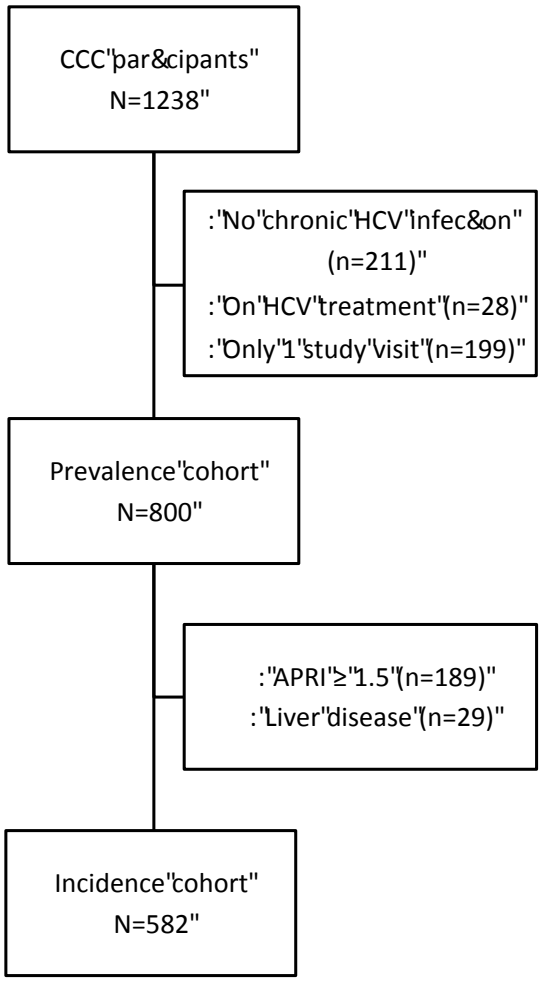

Figure 5. 1: Study population flowchart

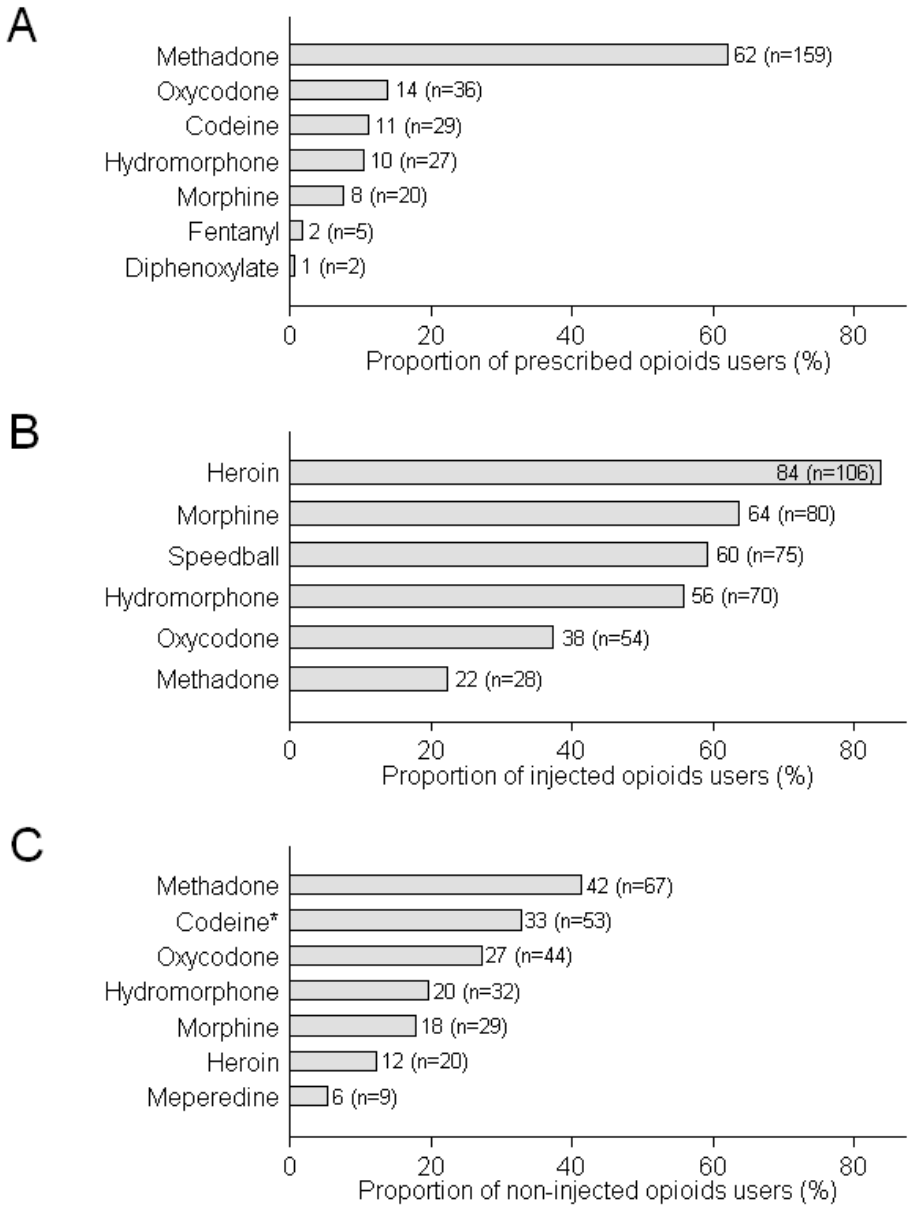

Figure 5. 2 Types of opioids used in the prevalence cohort.
A: Prescribed opioids; B: Illicit opioids –injected; C: Illicit opioids –Other routes of administration.
* Codeine in combination with acetaminophen.

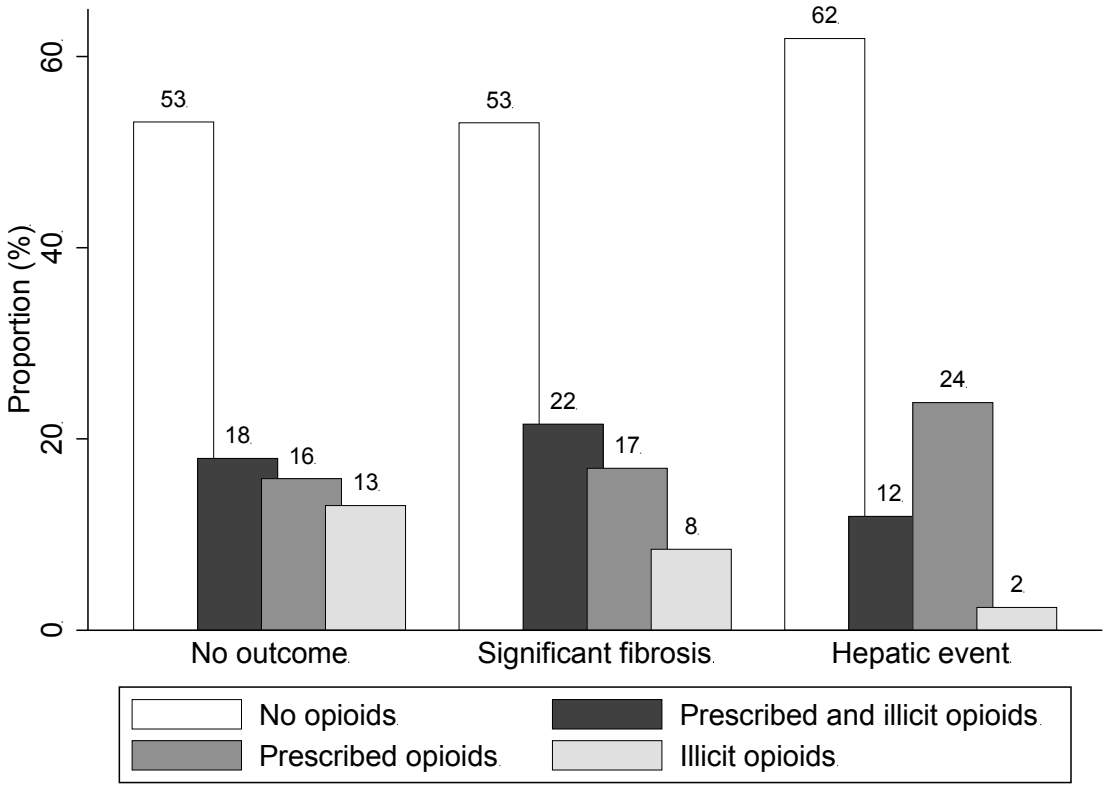

Figure 5. 3 Opioid use by liver outcome at the visit before last (if no outcome) or at the visit before a liver outcome was reported in the prevalence cohort.
Significant fibrosis: APRI ≥ 1.5, Hepatic event: diagnosis of ESLD or ESLD-related death. The same participant can contribute to more than one category if the different liver outcomes occurred at different study visits. If outcomes were reported at the same visit, then they were attributed to the most severe category.

Chapter 6: Combination antiretroviral therapy regimens and progression of liver fibrosis

6.1 Preamble to Manuscript 3

In previous chapters of this thesis, we saw that neither marijuana nor opioid use appeared to be associated with progression of liver disease in the Canadian Co-infection Cohort. Opioids are widely used to manage addiction and pain in HIV-HCV co-infected populations. However, the type of treatment most commonly used in this population is without a doubt antiretroviral therapy. In a study including data from U.S. clinic-based cohort studies participating in the North American AIDS Cohort Collaboration on Research and Design in 2008, it was estimated that of 23 941 HIV-infected persons, 83% were using cART, 10% were treatment-naïve and the rest were either on a treatment other than cART or had stopped treatment. Among those receiving treatment, 51% were prescribed PI-based cART regimens, 37% were prescribed NNRTI-based cART regimens and the remaining 12% used both a PI and a NNRTI, a NRTI only or a new agent.[255]

Because most persons who initiate cART remain on treatment for the remainder of their life, it is essential to understand the long-term effects of this therapy. However, the majority of studies addressing the hepatic effects of cART have focused on short-term hepatotoxicity. The data available on long-term outcomes such as liver fibrosis, steatosis, cirrhosis and end-stage liver disease is scarce and conflicting. Moreover, the populations in which clinical liver outcomes associated with cART use were studied were often very heterogeneous with persons on modern cART regimens being compared to naïve or mono/dual-therapy users. It is therefore difficult to

know whether the effects found are a reflection of the specific drug or drug class studied or rather the result of HIV infection progression, which is known to affect liver disease.

Conducting observational studies of cART use and liver disease progression is challenging because PI and NNRTI users can be inherently different. For example, PIs are often favoured for persons who are believed to have an unstable lifestyle and may be more likely to miss doses, such as injection drug users. In these situations, a PI-based regimen can be selected because resistance to PIs is less likely than resistance to NNRTIs in situations of poor adherence.[216] If more PI users have unstable lives, resulting in poorer adherence to treatment and exposure to potential risk factors for liver injury (such as alcohol use and uncontrolled HIV replication), observational studies are susceptible to confounding by indication. NNRTI users are also more likely to use a backbone consisting of TDF/FTC than PI users due to the availability of a fixed dose co-formulation of efavirenz with TDF/FTC.[139]

In this context, we sought to isolate the effect of PI and NNRTI use on rates of liver fibrosis progression, using changes in the APRI score as a marker of liver fibrosis progression. The role of the backbone used in this association was also investigated. The analytic approach was inspired by randomized controlled trial analytical methods. We used strict restriction criteria to include new PI or NNRTI users only, avoiding carry-over effects. However, most participants in the Canadian Co-infection Cohort Study initiated cART before cohort entry and no information on progression of liver fibrosis is available between cART initiation and entry in the cohort. Propensity score matching was performed to balance population characteristics between NNRTI and PI users and eliminate systematic differences to reduce confounding bias. We performed an

intention-to-treat analysis to evaluate how the initial choice of therapy can affect the marker of liver fibrosis, regardless of future modifications.

6.2 Manuscript 3: Progression of liver fibrosis and modern combination antiretroviral therapy regimens in HIV-hepatitis C co-infected persons

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ABSTRACT

Background: Liver diseases progress faster in HIV-hepatitis C (HCV) co-infected persons than HIV-mono-infected persons. The aim of this study was to compare rates of liver fibrosis progression (measured by the aspartate-to-platelet ratio index, APRI) among HIV-HCV co-infected users of modern protease inhibitor (PI)- and non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens with a backbone of tenofovir/emtricitabine (TDF/FTC) or abacavir/lamivudine (ABC/3TC).

Methods: Data from a Canadian multicentre cohort study were analyzed, including 315 HCV PCR+ persons who initiated antiretroviral therapy with a PI or NNRTI and a backbone containing either TDF/FTC or ABC/3TC. Multivariate linear regression analyses with generalized estimating equations were performed after propensity score matching to balance covariates across classes of anchor agent.

Results: Of 71% matched participants treated with a PI-based regimen, 67% received a backbone of TDF/FTC. Among NNRTI users, 69% received a backbone of TDF/FTC. Both PI and NNRTI use were associated with increases in APRI over time when paired with a backbone of ABC/3TC: 16% (95% CI: 4%, 29%) per 5 years and 11% (95% CI: 2%, 20%) per 5 years, respectively, whereas with TDF/FTC use, no clear association was found among PI users (8% per 5 years, 95% CI: -3%, 19%) or NNRTI users (3% per 5 years, 95% CI: -7%, 12%).

Conclusion: Liver fibrosis progression was more influenced by the backbone than by the class of anchor agent in HIV-HCV co-infected persons. Only ABC/3TC containing regimens were

associated with an increase of APRI score over time, regardless of the class of anchor agent used.

INTRODUCTION

With improvements in combination antiretroviral therapy (cART), life expectancy of HIV-infected persons approaches that of the general population,[256] resulting in long-term cART exposure and the potential for cART-related liver damage. HIV-hepatitis C (HCV) co-infected persons experience more rapid progression of liver disease than HIV mono-infected persons,[4] but to date only a small proportion of co-infected persons have undergone HCV treatment and liver damage persists despite a cure. It is therefore essential to understand whether specific classes of cART agents are harmful to minimize the risk of additional liver disease in this population.

Both acute and long-term hepatotoxicities have been associated with protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). Metabolic effects of PIs, including increases in lipids, insulin resistance and deposition of free fatty acids in the liver, could lead to steatosis and inflammation.[177] These metabolic changes are not commonly associated with NNRTI use although efavirenz can cause lipid changes.[257] PI-associated acute hepatotoxicity has been reported in several studies of HIV-infected persons, with or without HCV co-infection.[57,161,172,178-181] In co-infected persons, cumulative exposure to PIs was associated with liver steatosis.[159] However, lower risks of fibrosis,[170,183] cirrhosis,[184] and slower fibrosis progression rates[170,183,184] were reported when comparing PI-based regimens to either absence

of treatment or mono/dual-therapy with nucleoside reverse transcriptase inhibitors (NRTI). Elevated risks of long-term hepatotoxicity have also been reported with NNRTI use.[170,171]

The inclusion of dideoxynucleoside-containing backbones complicates the interpretation of the results of early studies as these NRTIs are highly disruptive of mitochondrial function and are associated with steatosis.[143] Currently, the recommended NRTI backbone combinations are tenofovir (TDF)/emtricitabine (FTC) or abacavir (ABC)/lamivudine (3TC).[139] While these modern backbones have low levels of mitochondrial toxicity[143] and are not generally considered hepatotoxic, there is limited information on their long-term impact on liver fibrosis.[139]

No long-term studies of modern cART regimens and liver disease in co-infected persons are available to indicate the long-term hepatic safety of PIs and NNRTIs. The objective of this study was to assess the progression of liver damage among HIV-HCV co-infected users of modern PI-based and NNRTI-based cART regimens, taking into account the backbone used. We sought to determine if either class of anchor agent is associated with accelerating liver fibrosis.

METHODS

Study population and analytical sample

The Canadian Co-infection Cohort study is a multi-centre cohort of HIV-HCV co-infected persons followed every six months. As of July 1st 2014, 1 321 persons had been enrolled from 18 clinics in Canada. All participants are adults, have documented HIV infection, evidence of HCV infection, and provide informed consent. The cohort has been described in greater detail elsewhere.[224]

We included in the analyses all HCV PCR positive persons who initiated cART with either a first line PI or NNRTI as the anchor agent with a TDF/FTC or ABC/3TC backbone.[139] Although initiation of cART prior to cohort entry was allowed, changes in the class of anchor agent before cohort entry were not. We excluded those with chronic hepatitis B infection because it increases the risk of liver-related outcomes and they are preferentially prescribed TDF/FTC.[139] None of the included individuals had a history of dideoxynucleoside use. Individuals were censored at the start of HCV therapy, which can affect the platelet count and influence our liver fibrosis measure.

We created a propensity score matched sample to minimize pre-existing imbalances in selected covariates between PI and NNRTI users, thus reducing confounding. For example, the choice of anchor agent is closely related to the choice of backbone and to certain risk factors for liver disease. Propensity score matching can alleviate this concern by making the PI and NNRTI users more similar with respect to these characteristics. This score is obtained with logistic regression by calculating the predicted probability of initiating cART with an NNRTI vs. a PI. The model included baseline values for age, sex, HCV duration, alcohol and injection drug use (IDU), income under CAD 1 500, CD4 cell count, HIV RNA <50 copies/ml, years since cART initiation and the backbone used. Each individual was matched with replacement based on their propensity score using the nearest neighbour approach.[258]

Antiretroviral use and aspartate aminotransferase-to-platelet ratio index (APRI) measurement

Information on current and past antiretroviral drugs was collected at the first study visit. At each follow-up visit, study coordinators recorded regimen changes. This information was

validated with medical or pharmacy records. Chart reviews were conducted to collect additional information on the initiation and discontinuation date for each drug used before cohort entry.

Liver fibrosis was measured at each study visit with the APRI score, calculated using the aspartate aminotransferase (AST) levels and platelet count as APRI = 100[AST/upper limit of normal]/platelet count (10^9/L). The natural logarithm of this score was used as a continuous outcome to normalize its distribution.[185,259]

Statistical analysis

We estimated the rate of change in ln(APRI) among those who initiated cART with a PI or NNRTI, using the anchor class at initiation as the exposure. This intention-to-treat analysis was selected to obtain the effect of initiating a new regimen.

We performed multivariate linear regression with generalized estimating equations (GEE) to account for the correlated nature of the longitudinal measures. Frequency weights corresponding to the number of times each individual was matched to another were included in the model to account for certain individuals being selected more than once.[258] Years since cART initiation and the interaction term between time and NNRTI vs. PI use served to estimate the average rates of change in ln(APRI) among PI and NNRTI users. The model was adjusted for the backbone used, age, sex and years since HCV infection at cohort entry. We further adjusted for time-updated alcohol use in the previous six months, HIV RNA <50 copies/ml and CD4 cell count at the previous visit.

NNRTI users are more likely to use TDF/FTC than PI users due to the availability of an efavirenz-TDF/FTC co-formulation. We therefore explored the potential role of the backbone in fibrosis progression by adding an interaction term between time and TDF/FTC use to the model described above.

For both models, the correlation structure was selected based on the model fit, measured by the quasi-likelihood under the independence model criterion (QIC).[198] To handle missing data, multiple imputation implemented with chained equations was used to create 10 imputed datasets, using Rubin's rule to combine standard errors.[199] Because changes in ln(APRI) are difficult to interpret clinically, the coefficients obtained were exponentiated to represent the median change in APRI score on the multiplicative scale (percent change).

RESULTS

Study population characteristics

After matching, the sample consisted of the equivalent of 628 persons divided equally between NNRTI and PI users; see Figure 1. Demographic and clinical characteristics at cohort entry are detailed in Table 1 for PI and NNRTI users before and after matching. Before matching, baseline imbalances were observed between PI and NNRTI users, notably in IDU, alcohol use, HIV RNA <50 copies/ml and TDF/FTC use. These imbalances were reduced after matching on the propensity score. Figure 2 shows the proportion of users of individual anchor agents in the matched sample. The majority (92%) of NNRTI users initiated cART with efavirenz. The PIs most frequently used were atazanavir/ritonavir (47%) and lopinavir/ritonavir (29%).

Progression of fibrosis over time

In the first analysis, PI users experienced a significant median increase in APRI score of 11% per 5 years (95% CI: 1%, 21%). The median increase was slower in NNRTI users: 7% per 5 years (95% CI: -1%, 14%); see Table 2. The overall median APRI score was 32% higher (95% CI: 14%, 50%) among TDF/FTC backbone users compared with ABC/3TC backbone users, although it remained below the cut-off for significant fibrosis (median APRI: 0.79 and 0.61 among TDF/FTC and ABC/3TC users, respectively).

After including an interaction term between the backbone and time, rates of change in APRI score appeared driven by ABC/3TC use with a 16% median increase in APRI score per five years (95% CI: 4%, 29%) when ABC/3TC was used in combination with a PI and an 11% increase per five years (95% CI: 2%, 20%) when used with an NNRTI. However, TDF/FTC users did not experience a statistically significant change in APRI score over time with PI-based (8% increase per 5 years, 95% CI: -3%, 19%) nor with NNRTI-based cART (3% increase per 5 years, 95% CI: -7%, 12%). Table 2 presents the back-transformed results for the full models. Although APRI scores are higher overall with TDF/FTC use than with ABC/3TC, the rate of increase in APRI score is greater over time with PI use; see Figure 3.

DISCUSSION

To our knowledge, this is the first study exploring the rates of liver fibrosis progression according to the class of anchor agent and the backbone used in HIV-HCV co-infected persons on modern cART regimens, with results applicable to current clinical practice. In this longitudinal study, we attempted to isolate the effect of modern cART regimens on long-term liver outcomes

by emulating a randomized controlled trial using propensity score matching and an intention-to-treat analysis of new users of the anchor agent class. Initiation of cART with a PI appeared to be associated with increases in APRI score over time whereas NNRTI use was not. This study was not designed to explain the role of the backbone on liver fibrosis progression. However, when the backbone was accounted for, ABC/3TC use with either a PI or NNRTI was associated with changes in APRI over time regardless of the anchor class. In contrast, use of TDF/FTC did not result in significant changes in the APRI score over time. It remains possible that PI use itself could contribute to fibrosis progression given the estimate among PI-TDF/FTC users was 1.08 (95% CI: 0.97, 1.19) per 5 years, although we lacked the power to confirm this.

Several studies including HIV-HCV co-infected persons have shown an association between hepatotoxicity, fibrosis or clinical liver outcomes and nevirapine use, but not efavirenz,[155,161,163,166] which represented 92% of NNRTI use in this cohort. The results of a sensitivity analysis including only efavirenz users were not appreciably different from those reported here.

There have been several reports of acute PI-associated hepatotoxicity in cohorts of HIV-infected persons, in which some participants were co-infected with HCV [57,161,172,178,179,181] and in one co-infection cohort.[180] Only one study reported an increased risk of steatosis with PI use in co-infected persons,[159] but others showed a decreased risk of liver fibrosis,[170,183] cirrhosis[184] and slower rates of fibrosis progression.[170,183,184] These studies compared PI users to untreated persons or mono/dual-NRTI therapy users. These protective effects may have been driven by a better control of HIV infection among cART users on a PI. Ignoring the backbone used, including dideoxynucleoside-containing regimens and selecting improper comparison groups biased the

conclusions of previous studies. Our group has investigated the relationship between PI and NNRTI use and changes in APRI score in two previous studies, with conflicting results.[185,259] Unlike the present study, these were not restricted to co-infected persons, allowed for switches in the class of anchor agent and failed to account for the backbone used.

Although TDF, FTC and 3TC are associated with low levels of mitochondrial toxicity, ABC can reduce hepatocyte proliferation and increase intracellular lipids and lactate levels.[143] ABC use can also cause hypersensitivity reactions, which are associated with transient and mild liver enzyme elevations,[148] but they usually occur within six weeks of initiation.[147] Because cART was initiated for a median of 3.5 years before cohort entry, hypersensitivity reactions are unlikely to have caused the elevated APRI scores observed. Finally, ABC is extensively metabolized by the liver. Bioactivation of ABC to a conjugated aldehyde has been recently identified as a potential trigger for ABC-induced toxic events.[260] Acetaldehyde is one of the principal mediators of fibrogenic and mutagenic effects of alcohol in the liver raising the possibility of additive effects in the setting of alcohol use, which is frequent in the co-infected population.[261]

Although there was no statistically significant increase in APRI score over time with TDF/FTC use, the use of a TDF/FTC backbone was associated with a higher median APRI score overall compared to ABC/3TC use. In the D:A:D cohort, chronic alanine aminotransferase (ALT) elevation was unexpectedly associated with current use of regimens containing TDF or FTC in HIV mono-infected persons particularly in the first two years.[156] A randomized controlled trial comparing TDF/FTC and ABC/3TC also found stable elevations in AST, ALT and alkaline phosphatase after 96 weeks of treatment in the TDF/FTC group only.[153] However, in treatment experienced adults, there was no difference in AST and ALT between TDF/FTC and ABC/3TC in a

combined analysis of two trials (BICOMBO trial in Spain and STEAL trial in Australia).[154] HCV status was not reported in these trials. In our study, while TDF/FTC users had higher APRI scores overall, they did not experience statistically significant changes in APRI score over time. The AST levels were statistically higher among TDF/FTC users compared to ABC/3TC users (p=0.01), but not the platelet counts (p=0.48), resulting in higher overall APRI scores, which suggests that the elevation does not reflect development of fibrosis.

While this study presents limitations, these were mitigated by careful design and analysis. One shortcoming was the impossibility of applying a strict new-user design, in which no changes of specific anchor agent would be tolerated, because of the small number of eligible persons. We therefore implemented a design of new users of the anchor class, deemed adequate because we were interested in a class effect rather than the effect of a specific drug. Modifications of the anchor agent within the same class are usually triggered by an adverse reaction in the first three months. The most frequently reported drug intolerances resulting in a modification of the regimen are gastrointestinal tract intolerance, hypersensitivity reactions and central nervous system adverse events.[262] These intolerances are generally acute and occur early after treatment initiation. The drugs provoking these reactions are therefore likely to have been discontinued close to the time of their initiation. With the exception of hypersensitivity reactions, these adverse events do not impact the liver and are unlikely to affect the relationship studied.

Another limitation is the presence of left truncation because APRI measurements were not available prior to cohort entry and follow-up started after cART initiation for most participants. However, the new user design implemented ensured that the class of anchor agent did not

change between treatment initiation and cohort entry, limiting the impact of left truncation on our analyses. At cohort entry, the median time since cART initiation was 3.5 years in the matched sample, but therapy had been initiated a year or less before cohort entry in 30% of the sample. It is possible that the changes in APRI score occurring early after treatment initiation would differ, but our aim was to study long-term fibrosis development, not acute toxicity, and early effects are likely to be moderate.

The gold standard for liver fibrosis assessment is liver biopsy, which was only performed in a limited number of participants during clinical care and could not be performed ethically every six months for research purposes. Transient elastography (Fibroscan) is replacing liver biopsy for the assessment of liver fibrosis, but all study sites did not perform this test, and the number of repeated measures is limited. The APRI score is a widely used alternative that performs similarly to other markers.[62] It has been validated in HIV-HCV co-infected populations,[60] and predicts occurrence of liver complications[185] and all-cause mortality.[192] It is usually employed as a dichotomous measure,[60] but the continuous score can be useful for research purposes as it predicts overall five-year survival in HCV infected persons (hazard ratio: 2.8, 95% CI: 1.6, 4.7).[192] Known predictors of liver disease also predict the continuous APRI score.[191]

Finally, this study is limited by the close relationship between anchor agents and backbones, which are hard to dissociate and are subject to confounding. PIs are often favoured for persons with poor adherence in order to lower risks of resistance.[216] Confounding by indication could bias the results if more PI users had unstable lives, resulting in poorer adherence to treatment and exposure to potential risk factors for liver injury such as alcohol use and uncontrolled HIV replication. NNRTI users are more likely to use a TDF/FTC backbone than PI users due to the

availability of a fixed dose co-formulation of efavirenz with TDF/FTC.[139] Pre-existing differences in demographic and clinical characteristics between treatment groups were reduced by the implementation of propensity score matching and adjustment for time updated alcohol use and HIV RNA, thus reducing confounding.

Despite these limitations, this study has several strengths. The Canadian Co-infection Cohort is a large prospective cohort broadly representative of the co-infected population accessing care in Canada (women, Aboriginal people, current and ex-injection drug users, men who have sex with men, etc.). The results obtained are also relevant to current clinical practice because all participants received modern cART regimens[139] and never received dideoxynucleosides, which are known to have high levels of mitochondrial toxicity.[143]

Another strength is the emulation of a randomized controlled trial. Propensity score matching balanced baseline differences in potential confounders between treatment groups, removing part of the confounding bias. The intention-to-treat analysis takes a clinician's perspective, investigating the effect of initiating a certain treatment, regardless of future changes in the class of anchor agent that could be caused by factors associated with liver fibrosis and would result in a carry-over effect of the previous regimen. Finally, several sensitivity analyses were conducted to test the robustness of our findings. No clear patterns were apparent when stratifying by year of cART initiation. Censoring when regimen changes occurred or including only boosted PI and efavirenz recipients produced results comparable to those presented here.

In conclusion, the rate of change in APRI score seemed more influenced by the backbone than by the class of anchor agent in co-infected persons. Both PI- and NNRTI-based regimens were

associated with increases in APRI over time when combined with ABC/3TC. However, the APRI score did not increase significantly over time when PI- and NNRTI-based regimens were used with a backbone of TDF/FTC. This study was designed to investigate the role of the class of anchor agent on progression of liver fibrosis, not the backbone. Therefore, further investigation is required to better understand how different backbone/anchor drug combinations can affect the liver of HIV-HCV co-infected persons in the long-term.

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The CCC cohort is comprised of 18 sites across Canada who recruit and follow HIV-HCV co-infected patients. The following co-investigators have all taken part in the data collection pertaining to this manuscript, and have reviewed its content. However, they did not partake in

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TABLES

Table 6. 1 Demographic and clinical characteristics and antiretroviral use of the participants at cohort entry, stratified by the class of anchor agent used in the unmatched and matched samples

	Unmatched sample		Matched sample	
Demographic characteristics	PI users	NNRTI users	PI users	NNRTI users
Participants, n	246	102	222	92
Participants (including repeats), n	NA	NA	314	314
Person-visits, n	1019	477	927	443
Person-visits (including repeats), n	NA	NA	1314	1409
Frequency in the sample, median (IQR), max	NA	NA	1 (1-2)	3 (1-4)
Male, n (%)	180 (73)	78 (76)	225 (72)	210 (67)
Age, median (IQR)	45 (39-50)	44 (38-49)	45 (38-49)	44 (39-50)
Monthly income of 1 500CAD or lower, n (%)	192 (78)	77 (75)	237 (75)	261 (83)
Homeless, n (%)	29 (12)	7 (7)	42 (13)	21 (7)
Alcohol use in the 6 months before cohort entry, n (%)	131 (53)	63 (62)	167 (53)	172 (55)
Injection drug use in the 6 months before cohort entry, n (%)	97 (39)	34 (33)	130 (41)	121 (38)
Clinical characteristics				
Years of HCV infection, median (IQR)	19 (11-27)	17 (7-24)	18 (11-25)	20 (9-25)
Years of HIV infection, median (IQR)	10 (5-16)	10 (5-17)	9 (5-16)	9 (5-16)
CD4 cell count, median (IQR)	379 (250-579)	430 (280-580)	380 (250-610)	420 (270-540)
Undetectable HIV viral load (<=50 copies/m), n (%)	156 (63)	76 (74)	213 (68)	207 (66)
HIV viral load if detectable, median (IQR)	350 (89-4147)	3048 (121-17000)	349 (82-4147)	3116 (285-17000)
APRI score, median (IQR)	0.56 (0.38-1.17)	0.71 (0.40-1.28)	0.54 (0.36-1.10)	0.70 (0.40-1.31)
Significant liver fibrosis (APRI≥1.5), n (%)	46 (19)	17 (17)	55 (18)	46 (15)
Liver cirrhosis (APRI≥2), n (%)	36 (15)	11 (11)	43 (14)	32 (10)
End-stage liver disease, n (%)	25 (10)	8 (8)	33 (10)	38 (12)
Antiretroviral use				
Years since initiation of cART, median (IQR)	3.3 (0.4-8.0)	2.3 (0.2-9.6)	3.3 (0.4-7.2)	2.3 (0.2-7.9)
Backbone : TDF/FTC, n (%)	155 (63)	73 (72)	211 (67)	218 (69)
Backbone : ABC/3TC, n (%)	90 (37)	29 (28)	103 (33)	96 (31)

Table 6. 2 Median changes in APRI score on the multiplicative scale associated with time on cART among protease inhibitors or non-nucleoside reverse transcriptase users estimated by linear regression with generalized estimating equations

	Model 1[a] exp(β) (95% CI)	**Model 2[b] exp(β) (95% CI)**
Age (5 years) at cohort entry	1.00 (0.96, 1.04)	1.00 (0.96, 1.04)
Female	0.90 (0.79, 1.02)	0.90 (0.79, 1.02)
Time since HCV infection at cohort (5 years)	1.04 (1.01, 1.07)	1.04 (1.01, 1.07)
CD4 cell count at the previous study visit (100 cells/ml)	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)
Undetectable viral load (<50 copies/ml)	0.98 (0.91, 1.06)	0.98 (0.91, 1.06)
Alcohol use in the past six months	1.03 (0.95, 1.11)	1.03 (0.95, 1.11)
TDF/FTC backbone at cohort entry	1.32 (1.14, 1.50)	1.44 (1.20, 1.68)
cART initiated with NNRTI	1.00 (0.84, 1.16)	1.00 (0.84, 1.16)
Time on cART among PI users (5 years)[c]	1.11 (1.01, 1.21)	NA
Time on cART among NNRTI users (5 years)[c]	1.07 (0.99, 1.14)	NA
Time on cART among PI-ABC/3TC users (5 years)[d]	NA	1.16 (1.04, 1.29)
Time on cART among PI-TDF/FTC users (5 years)[d]	NA	1.08 (0.97, 1.19)
Time on cART among NNRTI-ABC/3TC users (5 years)[d]	NA	1.11 (1.02, 1.20)
Time on cART among NNRTI-TDF/FTC users (5 years)[d]	NA	1.03 (0.93, 1.12)

[a] Model 1: $E[\ln(APRI)] = \beta_0 + \beta_i NNRTI + \beta_2 Years + \beta_3 NNRTI \times Years + \sum \beta_j covariates_j$

[b] Model 2: $E[\ln(APRI)] = \beta_0 + \beta_1 NNRTI + \beta_2 Years + \beta_3 NNRTI \times Years + \beta_4 TDF/FTC + \beta_5 TDF/FTC \times Years + \sum \beta_j covariates_j$

[c] Represents the rate of change in APRI score over five years. Obtained with the interaction term between NNRTI use and time since cART initiation

[d] Represents the rate of change in APRI score over five years. Obtained with the interaction terms between NNRTI use and time since cART initiation and between between TDF/FTC use and time since cART initiation from the equation

FIGURES

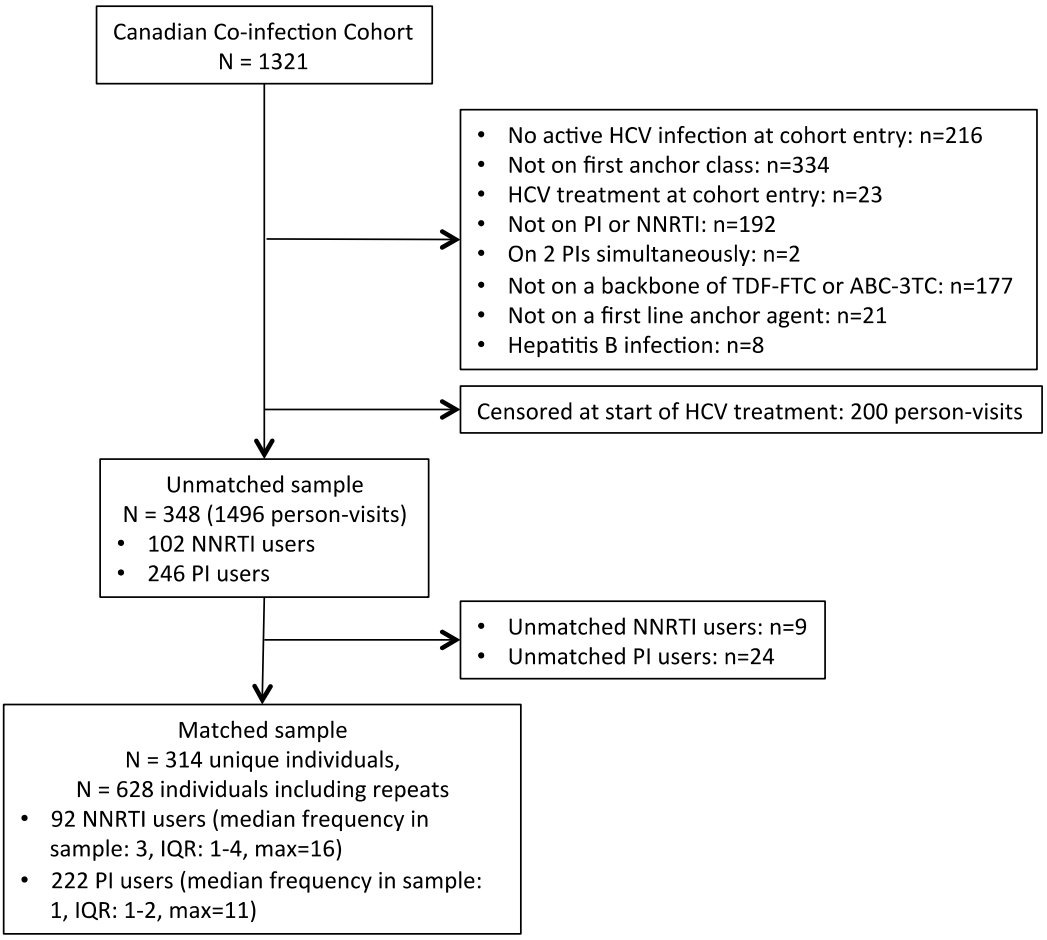

Figure 6. 1 Inclusion of participant in the study population

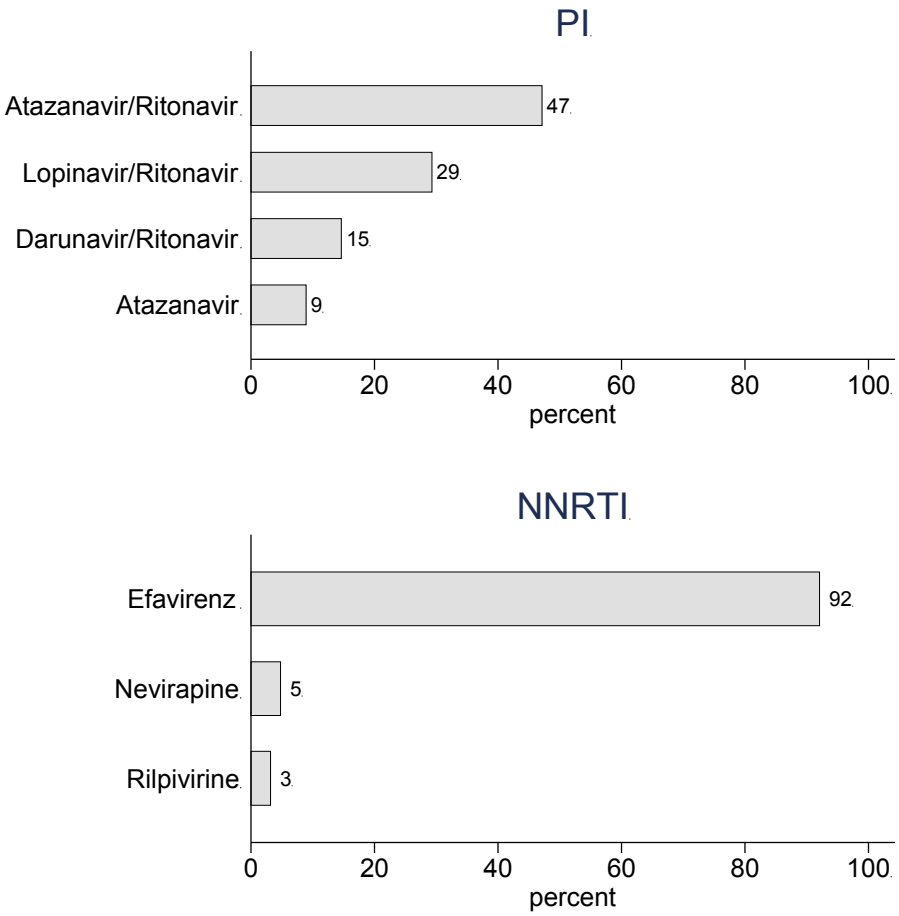

Figure 6. 2 Anchor agents used at baseline by class of drug

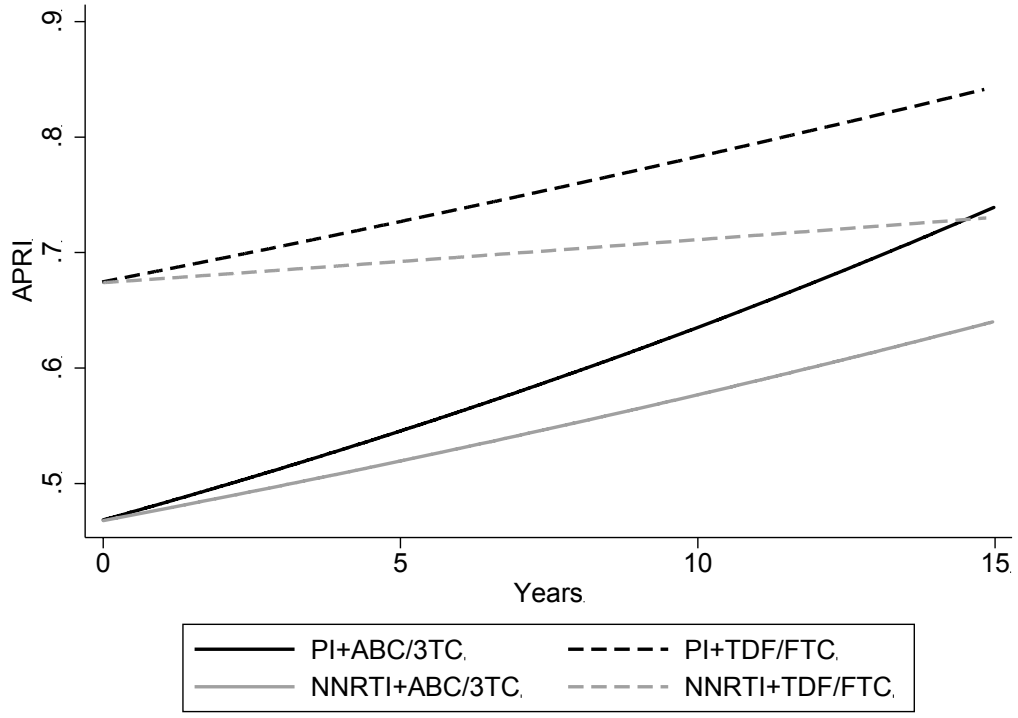

Figure 6. 3 Predicted APRI score over time since cART initiation, stratified by the regimen used

Chapter 7: Discussion and Conclusions

7.1 Summary of findings

Liver disease is a growing cause of morbidity and the third cause of mortality among people living with HIV.[1] Up to 85% of persons chronically infected with HCV develop liver fibrosis after 10 years of infection[28] and HIV infection aggravates the natural history of HCV infection, leading to a faster progression to fibrosis and advanced liver disease.[4,5]

Some of the drugs to which co-infected persons are exposed could be predictors of advancing liver fibrosis and the development of liver disease. Marijuana, opioids and cART are all commonly used in the co-infected population. However, previous studies investigating the presence of a relationship between the use of these drugs and liver health have not provided satisfying answers to these questions. Evaluating the association between these drugs and liver disease is paramount to inform whether or not these exposures are particularly harmful to co-infected persons. Indeed, illicit and prescribed drug use can be intervened upon, thus potentially reducing the risk of liver damage. This doctoral thesis therefore aimed to address a gap in our understanding of the relationship between marijuana, opioids or cART and liver fibrosis progression in HIV-HCV co-infected persons.

In the first manuscript, we described marijuana use in the Canadian Co-infection Cohort. Over half of the participants reported smoking marijuana in the six months preceding cohort entry, among whom 40% smoked daily and 25% smoked regularly. We further estimated the association between marijuana smoking and progression to significant liver fibrosis, cirrhosis or

end-stage liver disease. Contrary to the cross-sectional studies published,[89-91] we found no association in our longitudinal study between an increased number of joints smoked per week and development of liver fibrosis or end-stage liver disease using Cox Proportional Hazards models to perform a survival analysis. These results were further confirmed by the absence of a dose-response relationship between the number of joints smoked per week and the continuous ln(APRI) score, using linear splines to introduce flexibility in the model. Another cohort study has failed to show an association between marijuana use and liver enzyme elevation over a year in HIV-infected persons.[92] Protopathic bias[263] could explain the discrepancy between the three cross-sectional studies showing increased odds of current marijuana smoking among persons with liver fibrosis or steatosis[89-91] and our findings. This hypothesized reason for the differing results is supported by the fact that the cross-sectional studies only included HCV-infected persons with an indication to undergo biopsy suggesting they had advanced liver disease.

In the second manuscript, we first described prescribed and illicit use of opioids in the Canadian Co-infection Cohort. About 43% of the participants were using opioids at cohort entry: 32% used opioids with a prescription, 28% used illicitly and 18% used opioids both with a prescription and illicitly. We then assessed the presence of an association between opioid use and changes in ln(APRI) score over time or progression to significant liver fibrosis. Using generalized estimating equations with linear regression, we found no relationship between prescribed and illicit opioid use and changes in ln(APRI); similarly, using pooled logistic regression, no relationship was found between opioid use and significant liver fibrosis. A series of sensitivity analyses were conducted, including a probabilistic bias analysis to control for

misclassification of prescribed opioid use. The results of these sensitivity analyses confirmed the absence of any evidence of an association in our population. Our results were concordant with those of randomized controlled trials and cross-sectional studies published,[110,113,118,119,125] although there exist case-reports and cross-sectional studies that contradict our results.[112,242,243,252,253]

In the third manuscript, we estimated the median change in APRI score over time among PI and NNRTI users in a propensity score matched sample of new users of the anchor agent class. We found a statistically significant increase in median APRI score over five years in PI users and a marginally significant increase in NNRTI users. We investigated the impact of the backbone used on these rates with the addition of an interaction term between time and the backbone used in the model. An increase in median APRI score over time was observed in users of ABC/3TC combined with either a PI or NNRTI. Users of a PI combined to a backbone of TDF/FTC might also experience, to a lesser extent, an increase in median APRI score over time, although the confidence intervals included the null. Several mechanisms have been proposed to explain the potential toxicity of ABC,[143,148,260,261] although no consensus has been reached. Our study is the first to restrict the comparison to modern cART regimens and to suggest that the choice of backbone may play a more important role in liver fibrosis progression than the class of anchor agent in HIV-HCV co-infected persons.

7.2 Strengths and limitations

The strengths of this dissertation include the use of data from the Canadian Co-infection Cohort study, a large prospective cohort of HIV-HCV co-infected persons across Canada. Participants

are recruited from 18 sites across Canada and represent all groups touched by HIV-HCV co-infection.[224] Follow-up of some participants (from pilot sites) dates from as early as 2003, thus providing extremely rich longitudinal data. It is a population-based observational study, allowing for the measurement of detailed information on illicit drug use and behaviours that are not routinely recorded in clinical or administrative databases. This not only allowed for the study of exposures such as marijuana smoking and illicit opioid use, but also for a better control of confounding. Detailed information on cART use before cohort entry allowed the implementation of a new user of anchor agent class design, limiting bias due to carry-over effect and confounding by indication in the study of liver fibrosis progression in PI or NNRTI users.

APRI is a valid marker of liver fibrosis that is often used for research purposes, but only rarely used for clinical decision-making. Therefore, changes in our study outcomes are unlikely to have affected our exposures, thus limiting the risk of confounding by indication. Several sensitivity analyses were conducted to test the robustness of our findings. Various specifications of the exposures, the analytical samples or the statistical analysis were explored and we attempted to correct for misclassification of the exposure to prescribed opioids with a Monte Carlo sensitivity analysis.

However, the work described in this dissertation also has some limitations. Among these is the fact that although all participants in the Canadian Co-infection Cohort show evidence of infection with HCV, not all were chronically infected at cohort entry. Since our interest focused on chronically infected persons, we had to exclude from the analytical samples an important number of persons who had cleared their HCV infection either spontaneously or after

undergoing treatment before cohort entry. This significantly reduced the sample size and thus the power to detect significant associations. This limitation was aggravated in the study of cART and liver fibrosis progression as we implemented a new user design, further reducing our sample size.

Despite the richness of the data available with the Canadian Co-infection Cohort study, no information on the dosage of prescribed medication was available, limiting the possibility to conduct dose-response analyses. Our analysis of prescribed opioids could not therefore differentiate between the impact of using high or low doses of opioids for a short, or a long period of time. Using street drug as the exposure presents an additional challenge. Indeed, the exact formulation and potency of the drugs used are unknown, making the exposure difficult to assess with precision. No information on the history of marijuana or opioid use was available beyond six months prior to cohort entry, thus limiting our ability to study long-term effects. Although detailed information was collected on the frequency and intensity of marijuana use, the quantity of marijuana in a joint can vary greatly between users. In terms of illicit use of opioids, information was available on the number of different opioids used and the frequency of drug use overall, but not the frequency or amount of specific opioids.

Additionally, an important limitation of the data was the presence of interval censoring. Information on socio-demographic characteristics and behaviours was obtained at each study visit, approximately six months apart and referred to the time since the last study visit. Clinical and laboratory results were obtained from the medical charts and from a blood draw performed on the day of the study visit. If the APRI score was higher than the cut-off of 1.5 for significant liver fibrosis, we know it could have reached the cut-off at any time between the

current visit and the last, but the exact timing is unknown. Similarly, the same person could have reported using the drug of interest at any time during that interval. They could have used it continuously, or only for a few days or weeks. However, it is impossible to know from the data available if the exposure preceded the moment when the APRI crossed over the cut-off for significant fibrosis or not. Using the exposure reported at the previous study visit ensured that the temporality of the exposure-outcome relationship was maintained. It is, however, an imperfect way of dealing with the problem of interval censoring because it does not preclude some level of measurement error in the exposure.

Because no information was available on the exposures or the outcomes before cohort entry, we were limited by left truncation. Because the exposure to marijuana, opioids and cART precedes cohort entry for a part of the study population, a complete picture of the progression of liver disease in association to drug use cannot be obtained. In survival studies, left truncation can result in a bias towards the null due to the inclusion of survivors in the sample.[264] In our analyses of the continuous APRI score using linear regression with GEE, it is possible that the effect of opioid use or cART combinations is different in the short-term than over a longer period of time. When studying opioid use, we had no information on the age at which opioids were first used and it is therefore difficult to assess the impact of left truncation on the results. However, we used a recent exposure model, limiting the impact that left truncation could have. When studying cART regimens, time since cART initiation was known and included in the model. In addition, half the population had started treatment less than 3.5 years before cohort entry and 30% had started less than a year before, reducing the impact that left truncation could have on the results.

In this dissertation, measurement of illicit drug exposures relied on self-report. Although most of the literature on this subject suggests that self-report of illicit drug use can be an accurate measure,[254,265-267] the presence of information bias cannot be ruled out. Some participants could have under reported the types of drugs, or the frequency of use due to social desirability bias. Moreover, the exposure to drug use was coarse as it referred to the entire six month period. Measurement error of prescribed opioid use was certainly a concern despite research personnel performing chart reviews to confirm the information in the database. Indeed, self-report of pain medication usually is not accurate[204-206] and opioids can be prescribed outside the HIV clinic. However, a probabilistic bias analysis was conducted to address this limitation and did not affect the results substantially. Measurement error of cART use is less of a concern because decisions to change cART regimens are only made at the HIV clinic, and can therefore all be captured by the chart reviews. Another potential source of measurement error is the use of a marker of liver fibrosis, the APRI score. However, all the markers available (including biopsies) could have introduced measurement error of the outcome. The implications of selecting this measure have been discussed extensively in the methods chapter of this thesis. The ideal outcome for the research questions posed here would have been a diagnosis of end-stage liver disease, although the small number of cases limited the use of this outcome to sensitivity analyses.

Some selection bias could be present, especially in the survival analyses because they were restricted to a population free of liver disease and with an APRI score <1.5 at baseline. This healthier population could comprise a higher proportion of survivors who are less likely to develop elevated APRI scores or end-stage liver disease than others. However, we made sure to

include those with APRI score ≥1.5 in the analyses of the continuous APRI score to avoid this potential selection bias and the results were always concordant with the survival analyses.

Finally, it is possible that residual or unmeasured confounding remains. For example, hepatitis B is an important risk factor for liver disease progression and its acquisition is associated with IDU. We did not adjust for hepatitis B infection because of the very small number of persons with the three infections in our analytical sample. However, hepatitis B infection is unlikely associated with marijuana and prescribed or non-injected illicit opioid use in an HIV-HCV co-infected population. The few persons with this infection were excluded for the analysis of cART regimens and liver fibrosis progression. Certain regimens are recommended in this population because they have an anti-hepatitis B action and lower the risk of liver damage.[212,213] Another example of unmeasured confounding is adherence to cART, as missed doses were not measured in the Canadian Co-infection Cohort. However, poor adherence usually results in high viral loads and low CD4 counts, which we have accounted for in our analyses. Residual confounding due to an imperfect measure of alcohol use is also likely. Hazardous drinking was measured at cohort entry with the AUDIT-C questionnaire, but not at follow-up. In the first manuscript, a non-validated measure of alcohol abuse was used. However, a bias analysis conducted after the publication of the first manuscript showed that a simple measure of alcohol use was a more accurate marker of hazardous drinking when studying liver fibrosis;[268] this measure was therefore used in the second and third manuscripts.

It is also very difficult to tease out the effect of individual drugs when studying combination therapy such as cART. There could be residual confounding by indication because certain drugs are preferentially prescribed to certain patients based on co-morbidities or life-style

characteristics. The availability of co-formulations further impacts the probability of being prescribed certain regimens, complicating the study of specific drugs or drug classes. For example, PIs are often preferred for patients with a potential for low adherence and ABC should be avoided in persons with a certain allele and with liver damage. Efavirenz, an NNRTI is offered in co-formulation only with TDF/FTC. Therefore, it is very difficult to isolate the effect of the class of anchor agent from the effect of the backbone combination.[139,216]

7.3 Implications of findings for HIV-HCV co-infected persons and their physicians and directions for future research

The first manuscript was the first large longitudinal analysis of the association between marijuana smoking and liver fibrosis, cirrhosis and end-stage liver disease. The results obtained refuted those of three cross-sectional studies that have had a great impact among physicians who are caring for HCV-infected persons using marijuana and have already been cited in the World Health Organisation guidelines.[36] Based on the results of these methodologically problematic studies, some physicians were hesitant to prescribe marijuana or counselled their patients to stop using it despite demonstrated benefits for HIV and HCV infected persons.[75-77] Our results, combined to those of the only other longitudinal analysis, have been reassuring to co-infected persons and many physicians who see a health benefit of marijuana. Our results do not, however, imply that marijuana smoking is safe as we only studied its association with liver disease and marijuana can have an impact on many other more or less extensively studied aspects of health. Medical use of marijuana is likely to increase over the years. Monitoring of its use and of occurrence of liver disease will therefore be important.

The findings of the second manuscript likely won't change the clinical practise when it comes to prescribing opioids for addiction or pain management and counselling co-infected persons on their illicit drug use. However, it adds some clarity to a conflicting body of literature. This study addressed some of the methodological problems of previously published research and applied appropriate methods to a large population, therefore contributing to a better understanding of the association between opioid use and liver fibrosis.

The study of liver fibrosis progression by cART regimen could potentially provide some clinical guidance for the treatment of HIV in patients with concomitant liver disease. ABC/3TC-containing regimens were associated with progression of liver fibrosis over time, regardless of the class of anchor agent used. However, this relationship needs to be explored in greater details in a study of new users of both the backbone and class of anchor agent. If confirmed, an acceleration of fibrosis progression associated with certain anchor class/backbone combinations could have important implications in future treatment guidelines. Indeed, these findings align with the World Health Organisation recommendations to initiate cART with a combination of efavirenz (an NNRTI), TDF, and either FTC or 3TC.[269] Future steps also include the study of newer drug classes such as fusion inhibitors and integrase inhibitors. The Canadian Co-infection Cohort currently includes too few persons using these classes of anchor agent to allow a thorough evaluation of the associated fibrosis progression.

For the three studies presented in this doctoral thesis, a longer follow-up allowing for the evaluation of the association between marijuana, opioids or cART use and end-stage liver disease would permit a confirmation of our results. Indeed, liver fibrosis is a precursor of advanced liver disease, but some persons develop end-stage liver disease despite showing no

evidence of fibrosis. A diagnosis of end-stage liver disease is also less prone to measurement error than any measure of liver fibrosis. However, these events are rare in the cohort and only develop over a long period of time. The next step would therefore be to repeat the studies presented in this doctoral thesis at a later stage, when follow-up has been sufficiently long for a greater number of long-term clinical outcomes to occur.

7.4 Conclusion

The high prevalence of liver-related morbidity and mortality in HIV-HCV co-infected persons prompted the evaluation of risk factors for liver fibrosis progression in this doctoral thesis. A large proportion of HIV-HCV co-infected persons are exposed to illicit drugs such as marijuana and opioids and to prescribed drugs such as opioids and cART.

The studies presented in this dissertation sought to guide clinical decision-making by investigating the presence of an association between exposure to marijuana, opioids or cART and progression of liver fibrosis among HIV-HCV co-infected persons. We found that neither marijuana nor opioid use was associated with progression of liver fibrosis. However, we found that ABC/3TC-containing regimens were associated with progression of liver fibrosis.

This doctoral thesis contributes to the understanding of predictors of liver disease progression in HIV-HCV co-infected persons in Canada and responds to a gap in our knowledge of the unintended effects of marijuana, opioid and cART on the liver. Comparing the safety of these drugs in a real world setting with longitudinal studies is essential, especially considering the high prevalence and long-term use of these drugs in the co-infected population.

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