ASSESSING THE IMPACT OF ANTIRETROVIRAL TREATMENT INTERRUPTION ON PROGRESSION OF LIVER FIBROSIS IN ADULTS CO-INFECTED WITH HIV AND HEPATITIS C

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**ABSTRACT**

**Objective:** Despite the evidence that antiretroviral treatment (ART) interruption increases the risk of various negative outcomes, it is still likely that HIV/hepatitis C (HCV) co-infected patients will discontinue ART for several reasons. The impact of interruption on liver fibrosis progression in co-infected adults was examined, using the aspartate aminotransferase-to-platelet ratio index (APRI) as a surrogate marker of liver fibrosis.

**Method:** Time-dependent Cox regression, as well as inverse probability-of-treatment weighting (IPTW) in a marginal structural model, were used to evaluate the association of baseline and time-varying covariates with developing significant fibrosis.

**Results:** After accounting for potential confounders, such as HIV viral load and CD4+ T cell count, the hazard ratio for ART interruption was 2.52 (1.20-5.28). Use of IPTW resulted in a similar estimate of effect, suggesting that mediation by time-varying confounders was negligible.

**Conclusions:** ART interruption was independently associated with an increased risk of liver fibrosis progression in HIV/HCV co-infected patients.
**RÉSUMÉ**

**Objectif:** Certains patients coinfectés par le VIH et l’hépatite C interrompent leur traitement antirétroviral (TRT) pour des raisons variées, bien que cela augmente les risques de plusieurs effets indésirables. L’impact de l’interruption du traitement sur la progression de fibrose du foie chez des adultes coinfectés a donc été évalué grâce à l’utilisation du score APRI comme marqueur de fibrose du foie.

**Méthode:** Un modèle Cox ainsi qu’un modèle marginal avec pondération par l’inverse de la probabilité de traitement ont été utilisés.

**Résultats:** Après ajustement, le rapport de risque pour l’interruption du TRT était de 2.52 (1.20-5.28). Un effet similaire a été mesuré lorsque la pondération par l’inverse de la probabilité de traitement a été utilisée, ce qui suggère que l’effet des variables variant avec le temps était négligeable.

**Conclusions:** L’interruption du TRT est indépendamment associée avec un risque accru de la progression de fibrose du foie chez les patients coinfectés avec le VIH et l’hépatite C.
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DEDICATION

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<tbody>
<tr>
<td>APRI</td>
<td>aspartate aminotransferase-to-platelet ratio index</td>
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<tr>
<td>ART</td>
<td>antiretroviral treatment</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
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<tr>
<td>CCC</td>
<td>Canadian co-infection cohort</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>ETA</td>
<td>experimental treatment assignment</td>
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<td>HA</td>
<td>hyaluronic acid</td>
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<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
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<td>HCV</td>
<td>hepatitis C virus</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>hsCRP</td>
<td>high sensitivity C-reactive protein</td>
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<tr>
<td>IDU</td>
<td>injection drug use</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>IPTW</td>
<td>inverse probability-of-treatment weighting</td>
</tr>
<tr>
<td>IQR</td>
<td>inter-quartile range</td>
</tr>
<tr>
<td>MSM</td>
<td>men who have sex with men</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>SMART</td>
<td>Strategies for Management of Antiretroviral Therapy</td>
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INTRODUCTION

Due to shared routes of transmission and decreased immune function, individuals infected with HIV are at an increased risk of co-infection with hepatitis C (HCV). Among HIV-infected individuals with a history of injection drug use (IDU), it is estimated that between 50 and 90% are HCV co-infected. This is of concern due to the fact that morbidity can be worse, disease progression more rapid and treatment more complex for co-infected patients.

The introduction of antiretroviral treatment (ART) has lead to a substantial reduction in morbidity and mortality from nearly all illnesses among those with HIV. An exception to this trend is death from liver disease, which has emerged as a significant cause of mortality, especially in the co-infected population. Given the fact that HCV progresses at a higher rate in those experiencing immune dysfunction due to HIV infection, it would be expected that HCV-related liver disease should improve in patients treated with ART. However, this has not been demonstrated. The lack of full benefit of ART in co-infected patients may be due to several causes such as, irreversibility of hepatic damage, toxicity related to ART, incomplete immune recovery, alcohol use and inconsistent access or adherence to ART in a population with high rates of substance abuse.

In order to minimize HIV disease progression, a high level of adherence to ART is required, although is difficult to maintain. Structured treatment interruptions have been proposed in patients with well suppressed HIV to reduce toxicities, drug-related adverse events and costs, enhance adherence or to prevent resistance. However, large, randomized, controlled trials have established that interruption of ART is
potentially quite harmful, leading to viral rebound, reduced immune reconstitution and the emergence of resistance.\textsuperscript{26-31} In addition, patients who interrupt ART appear to be at an increased risk of developing non-opportunistic hepatic, renal and cardiovascular complications.\textsuperscript{26, 32-34} These findings were rather unexpected due to the hypothesis that treatment interruption would lead to a reduction in adverse outcomes related to ART use, but suggest that interruption affects multiple pathological processes. Results from several cohort studies also revealed that ART interruption is associated with a significant immunological disadvantage\textsuperscript{35, 36} and an increased risk of HIV-related disease progression compared to continuous treatment.\textsuperscript{37}

The Strategies for Management of Antiretroviral Therapy (SMART) study was a randomized controlled trial that included patients co-infected with HCV and demonstrated that treatment interruption is particularly unsafe in this population.\textsuperscript{38} Co-infected subjects had a much greater underlying risk of mortality due to a variety of non-opportunistic disease related events compared to those with only HIV. Therefore the absolute risk of non-AIDS related death by being randomized to the treatment interruption group was greater among co-infected subjects than in those mono-infected with HIV.

Despite the evidence that interruptions increase the risk of disease progression, in the clinical setting it is still likely that co-infected patients will discontinue ART for a number of reasons. The high frequency of active substance abuse in this population may lead to problems with access or adherence to ART.\textsuperscript{39, 40} Also, HIV patients co-infected with HCV are at an increased risk of liver injury related to antiretroviral use.\textsuperscript{41-44} Combined, these factors may contribute to the higher rates of ART discontinuation observed in the co-infected population compared to those mono-infected with HIV.\textsuperscript{45-48}
We hypothesized that liver disease progression in ART-treated co-infected patients may be due, in part, to the consequences of repeated treatment interruptions. The SMART study was the first randomized controlled trial to examine the impact of ART interruption in HIV/HCV co-infected patients and it appears that this has not been studied further in the clinical setting. Therefore, the main objective of this study was to determine the impact of ART interruption on fibrosis progression in HIV/HCV co-infected adults, using the aspartate aminotransferase (AST)-to-platelet ratio index (APRI) as a surrogate marker for liver fibrosis. We chose to use a surrogate marker for liver fibrosis in order to avoid the limitations associated with the use of liver biopsy as an outcome measure. It is invasive and difficult to repeat, often causing studies that use liver biopsy to measure fibrosis to be limited by sample size and patient selection bias. Liver biopsy results are also affected by tissue sampling and interpretation error. In addition, the more objective nature of the APRI does not require the need for blinding of those assessing the outcome, as this measurement is not subject to information bias.

**Literature Review**

*Epidemiology of HIV and Hepatitis C in Canada*

The emergence of the HIV/AIDS pandemic has been acknowledged as one of the most extraordinary events in global health over the past 30 years. The number of people living with HIV infection is estimated to be 33 million worldwide and approximately 58,000 in Canada. HIV is transmitted through sexual intercourse (anal or vaginal), sharing of contaminated needles, transfusion of contaminated blood and between mother and child during pregnancy, labour or breastfeeding. Men who have sex with men (MSM) continue to account for the largest number of new HIV
infections in Canada, however a significant proportion are attributable to IDU and heterosexual activity. The proportion of incident HIV cases due to IDU and heterosexual contact in 2007 were 17% and 31% respectively.\textsuperscript{54}

The social and economic conditions that contribute to the HIV/AIDS epidemic such as poverty, gender inequality, marginalization, violence, substance abuse and limited access to or use of health care services increase the vulnerabilities of youth, females, Aboriginals and IDU to HIV infection.\textsuperscript{53} Aboriginals form 3.3% of the Canadian population, yet represent 7.5% of prevalent HIV cases and have an HIV infection rate 2.8 times greater than among non-Aboriginals.\textsuperscript{53} Trends observed in surveillance data show that IDU is an important risk factor for HIV transmission in Aboriginal populations. In 2005, 53% of new HIV infections in Aboriginals were attributable to IDU.\textsuperscript{53} Also in contrast to HIV/AIDS cases in the non-Aboriginal population, females make up a comparatively large part of the Aboriginal HIV epidemic.\textsuperscript{53} Females represent 48.1% of all positive HIV test reports from 1998 to 2006 among Aboriginals, as compared to 20.7% of reports among non-Aboriginals.\textsuperscript{53} The VIDUS study in Vancouver found that among young injection drug users (13-24 years old), HIV prevalence was associated with being female, a history of sexual abuse, engaging in survival sex, injecting heroin and/or speedballs daily, and having numerous lifetime sexual partners.\textsuperscript{55} It is still important to note that among women and individuals from HIV-endemic countries, heterosexual contact remains to be the main mode of HIV transmission. Taken together, these surveillance trends underline the complexity and uniqueness of the HIV epidemic in Canada.

HCV is transmitted largely through blood, and injection drug use is considered to be the primary mode of transmission in the developed world.\textsuperscript{1, 56, 57} Approximately 3-4 million people become infected with HCV globally each year. Chronic infection can be found in an estimated 123-170
million people, including 250,000 Canadians.\textsuperscript{56, 58} In Canada, chronic HCV has become the leading indication for liver transplantation.\textsuperscript{59}

Individuals infected with HCV are at an increased risk of co-infection with HIV because of shared routes of transmission. This is of concern due to the fact that morbidity can be worse, disease progression more rapid and treatment more complex for patients co-infected with HIV.\textsuperscript{3, 4} It is estimated that 16 to 30\% of HIV-infected individuals are co-infected with HCV in developed countries.\textsuperscript{56, 60} Approximately 11 000 Canadians were co-infected in 1999, and the prevalence has likely increased since.\textsuperscript{61}

In Canada, 70-80\% of new HCV infections are attributable to IDU\textsuperscript{58} and among HIV-infected individuals with a history of injecting drug use, it is estimated that between 50 and 90\% are co-infected with HCV.\textsuperscript{1, 2} For example, in a cohort study that recruited participants into low-threshold methadone programs at 2 sites in Ontario, the HIV prevalence at entry was 7\%. Among those with HIV, 77\% were co-infected with HCV and the prevalence of HCV in the entire cohort was 48\%.\textsuperscript{62} Data from I-Track, a surveillance system of IDU at sentinel sites across Canada, showed that 87.7\% of HIV-positive IDU participants were co-infected with HCV.\textsuperscript{2} After several years of decline, preliminary data suggest that since 2006 there has been an increase in the reported rate of acute HCV infection in Canada. Between 2006 and 2008, the highest increase in reported rates of acute HCV infection was among females aged 15-24 with an increase if 114\%. It was also found that the reported rate of acute HCV infection was 5.5 times greater in Aboriginal persons than in non-Aboriginals.\textsuperscript{58} This data highlights the vulnerability of these groups to HCV infection and indicates that they may be at an increased risk for HIV/HCV co-infection.
The Natural History of HIV

The human immunodeficiency virus is a retrovirus that targets the CD4+ cells of the immune system. During the early period after primary infection there is a high rate of replication of the virus and a sudden decrease in the number of CD4+ T lymphocytes in peripheral blood. This is followed by an immune response to HIV accompanied by a decrease in detectable viremia and a period of clinical latency that typically lasts approximately 10 years in untreated patients. The functional abnormalities and depletion of CD4+ T lymphocytes continues and causes profound immunosuppression eventually leading to opportunistic infections. A number of opportunistic infections have been identified and classified as AIDS-defining illnesses, including Pneumocystis carinii pneumonia and cytomegalovirus. Prior to the widespread use of antiretrovirals, the typical time from seroconversion to death was between 8 and 13 years, depending on age at infection.

The current treatment for HIV, ART, typically consists of a combination of two nucleoside reverse transcriptase inhibitors and either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. The introduction of this combination therapy in the mid-1990’s lead to a substantial reduction in morbidity and mortality among HIV-infected patients. A study conducted in British Columbia found that the life expectancy at the age of 20 has increased from approximately 29 years in the early 1990’s to 44 years of age in the years 2002 to 2004.

The Effect of Co-infection with Hepatitis C

The impact of HCV co-infection on disease progression of HIV remains uncertain. Longitudinal studies conducted prior to the widespread use of ART were unable to show that HCV co-infection had a significant impact on length of survival or time to an AIDS-defining event. Results obtained since the introduction of ART are conflicting. Several studies report shorter survival while others still did not find a
significant difference in mortality between co-infected and HIV mono-infected patients. The association between HCV co-infection and time to an AIDS-defining illness was not found to be significant after the introduction of ART in several studies. Taken together, a greater risk of mortality among co-infected patients and the lack of an effect of HCV co-infection on AIDS-defining events suggests that co-infection may not influence HIV disease progression but rather, other health complications.

Several longitudinal studies have shown that while there is no difference in virological response to ART, patients co-infected with HCV have diminished CD4+ T-cell recovery after ART initiation compared to HIV mono-infected patients. However, others were unable to demonstrate this difference. The effect of HCV co-infection may have been diluted in several of these studies due to the lack of HCV RNA testing, leading to numerous subjects being misclassified as HCV co-infected who actually did not have active HCV replication. Potter et al. recently found that both spontaneous HCV clearers and successfully treated HCV RNA negative patients had greater yearly rates of CD4+ T-cell change after commencing ART compared to patients with detectable HCV RNA. This suggests that active HCV RNA replication has a negative impact on HIV disease progression, which may partially be alleviated by successful HCV treatment. Identifying the impact of HCV co-infection on HIV disease progression, mortality and response to treatment could lead to improvements in the care of co-infected patients. Further studies may support initiating ART earlier or treating HCV in more patients in order to maximize immune recovery.

The Natural History of Hepatitis C

First described in 1989, HCV is an RNA virus of the Flaviviridae family and is acquired mainly through exposure to infected blood. In developed
countries, the primary source of new HCV infections is IDU.\textsuperscript{1,56,57} Acute HCV infection is often asymptomatic, but persists in approximately 60 to 85\% of those infected.\textsuperscript{89-91} HCV replicates primarily within hepatocytes, which leads to progressive damage to the liver and fibrosis.\textsuperscript{92} It is a major cause of end-stage liver disease, which may be expressed as cirrhosis, ascites, encephalopathy, esophageal varices or hepatocellular carcinoma.\textsuperscript{56,91,93} The mean time from infection to the development of cirrhosis varies considerably between studies, and has been shown to be anywhere from 10 to 30 years.\textsuperscript{89,91,93-95} The variation observed among different studies is likely due to differences in the populations studied, length of follow-up and competing risks. Factors that have been shown to be associated with accelerated disease progression include co-infection with HIV or hepatitis B (HBV), heavy alcohol consumption, male gender, older age at HCV acquisition, obesity and insulin resistance.\textsuperscript{56,91,95-99} However, unlike other chronic viral infections such as HIV, HCV viral load has not been consistently shown to be correlated with disease progression in mono-infected patients.\textsuperscript{91,96,97,100} The current treatment for chronic HCV infection is a combination of pegylated interferon and ribavirin.\textsuperscript{89} After completion of treatment, the virus is eradicated in approximately 54 to 63\% of patients.\textsuperscript{101-103}

\textit{The Effect of Co-infection with HIV}

Co-infection with HIV affects HCV disease progression in several ways, beginning with an increased rate of progression to chronic infection; 90\% of HIV co-infected individuals compared to 70 to 85\% of those not infected with HIV.\textsuperscript{91,104,105} HCV RNA levels have been shown to be higher in HIV co-infected compared to HCV mono-infected patients,\textsuperscript{106,107} and a correlation with lower CD4+ T-cell counts has been suggested.\textsuperscript{95,104} Higher HCV viral loads may then contribute to greater transmission rates among those with HIV.\textsuperscript{108,109} Individuals co-infected with HIV also have a reduced probability, approximately 40\% or less, of achieving a sustained virologic response to
treatment for HCV.\textsuperscript{110-112} It has been suggested that only 10\% of co-infected patients are considered to be candidates for therapy,\textsuperscript{113} while among members of the Canadian Co-infection Cohort (CCC) study, approximately 24\% have been exposed to treatment for HCV.\textsuperscript{114} Combined with a diminished probability for treatment success, the low level of treatment uptake leads to a reduced rate of HCV eradication in the co-infected population. Finally, HCV-related liver disease progresses more quickly in those co-infected with HIV and has been shown to correlate with the degree of immunosuppression.\textsuperscript{13, 14, 115, 116} A meta-analysis that examined the effect of HIV co-infection on the progression of liver disease in patients with HCV prior to the widespread use of ART, found the adjusted risk ratio for cirrhosis or decompensated liver disease to be 2.92 (95\% confidence interval (CI): 1.70-5.01), comparing co-infected to HCV-mono-infected patients.\textsuperscript{4}

**Liver Disease as a Leading Cause of Death**

As previously mentioned, the introduction of ART in the mid-1990's has lead to a dramatic decline in morbidity and mortality from nearly all illnesses among those with HIV.\textsuperscript{5-7} However, an exception to this trend is death from liver disease, which has emerged as a leading cause of mortality in the HIV-infected population.\textsuperscript{9-12} This is likely due to a decline in opportunistic infections and the fact that patients have a longer life expectancy while taking ART, allowing for other complications, such as liver disease to be observed.\textsuperscript{117} However, it is also likely due to the high prevalence of co-infection with HCV.\textsuperscript{56}

Given that HCV progresses at a higher rate in those experiencing immune dysfunction due to HIV infection\textsuperscript{13, 14}, it would be expected that HCV-related liver disease should improve in patients treated with ART. However, this has not been fully demonstrated.\textsuperscript{8, 12, 15, 118-120} A recent meta-analysis found
that the rate ratio of cirrhosis comparing co-infected to HCV mono-infected patients was 2.5 (95% CI: 1.8-3.4) among those not treated with ART, and was still significant even in patients who were ART-treated (rate ratio: 1.7, 95% CI: 1.1-2.8). Another meta-analysis by Chen et al. concluded that prior to the introduction of ART, HCV co-infection did not increase mortality among those with HIV, however after its introduction co-infection with HCV increased the risk of mortality, but not the risk of AIDS-defining events. Therefore, although ART has improved HIV disease progression, HCV-related outcomes still cause a significant burden among the co-infected population. The lack of full benefit of ART in the co-infected population may be due to several causes such as, irreversibility of hepatic damage, toxicity related to ART, incomplete immune recovery, alcohol use and interruptions of treatment caused by inconsistent access or adherence to ART in a population with high rates of substance abuse.

**Interruption of ART**

In order to minimize HIV disease progression, a high level of adherence to ART is required, but is often difficult to maintain. Interruption of ART has been proposed in three main settings with distinct aims. During primary infection, it has been suggested that treatment interruption may promote an HIV-specific immune response and allow better viral control without continued therapy. Among patients experiencing treatment failure due to drug-resistant HIV, interruption of ART was proposed to allow reversion to wild-type virus, thus leading to an improved virologic response upon treatment re-initiation. However, interruption has not been shown to be beneficial in this setting and may be associated with disease progression.

Structured treatment interruptions have been proposed in chronically infected patients with well suppressed HIV to reduce toxicities, drug-
related adverse events and costs, enhance adherence or to prevent resistance.\textsuperscript{21-25} Several randomized, controlled trials have concluded that interruption strategies are not inferior to continuous treatment with regards to virologic or immunologic endpoints, however these trials were not powered to detect differences in mortality or AIDS-defining clinical events.\textsuperscript{128-131} More recent, larger trials have established that interruption of ART is potentially quite harmful, leading to viral rebound, reduced immune reconstitution and the emergence of resistance.\textsuperscript{26-31} It is important to note that studies such as SMART and Trivacan, which concluded that ART interruption is harmful, instructed patients to interrupt ART until CD4+ T cell counts decreased to 250 cells/μL.\textsuperscript{26, 27} In contrast, those that reported no difference between interruption and continuous treatment instructed patients to resume ART when CD4+ T cell counts declined to no less than 350 cells/μL.\textsuperscript{128-130}

In addition, patients who interrupted ART were found to be at an increased risk of developing non-opportunistic hepatic, renal and cardiovascular complications\textsuperscript{26, 32-34} and did not experience an increase in quality of life.\textsuperscript{29, 132, 133} These findings were rather unexpected due to the hypothesis that ART interruption would lead to a reduction in adverse outcomes related to ART use, but suggest that it affects multiple pathological processes. This is consistent with reports that immunosuppression, in this case aggravated by ART interruption, impacts the risk of disease outcomes not traditionally classified as being opportunistic, such as liver-disease related events.\textsuperscript{9, 13, 14}

Evidence from cohort studies also demonstrate that treatment interruption is associated with a significant immunological disadvantage\textsuperscript{35, 36}, an increased risk of disease progression compared to those on continuous treatment\textsuperscript{37} and frequent mutations associated with drug resistance.\textsuperscript{134} Additionally, cohort data reveal that ART interruption occurs frequently (19 to 26\% of patients) in the clinical setting.\textsuperscript{36, 37, 135} Some suggest that it
requires close monitoring, but can be safe in patients who had a relatively high nadir CD4+ T-cell count and also greater CD4+ T-cell count at the start of the interruption.136-139

**Interruption of ART in Co-infected Patients**

Treatment of HIV in individuals co-infected with HCV can be problematic. The high frequency of active substance abuse in this population may lead to problems with access or adherence to ART.39, 40 Also, HIV patients co-infected with HCV are at an increased risk of liver injury related to antiretroviral use.41-44 Combined, these factors may contribute to the higher rates of ART discontinuation observed in the co-infected population compared to those mono-infected with HIV.45-48

The SMART study included patients co-infected with HCV and/or HBV and demonstrated that treatment interruption is particularly unsafe in this population. Co-infected subjects had a much greater underlying risk of mortality due to a variety of non-opportunistic disease related events compared to those with only HIV.38 Therefore the absolute risk of non-AIDS related death by being randomized to the treatment interruption group was greater among co-infected subjects than those mono-infected with HIV. In fact, even though only 17% of the participants in the SMART study were co-infected, nearly half of all non-AIDS related deaths occurred in this group. However, it is important to note that co-infected patients did not have a greater risk of opportunistic disease compared to HIV mono-infected subjects.

A variety of causes of death unrelated to opportunistic disease were observed in the group who interrupted treatment, many of which were unknown. Only one participant died of liver-related causes, but if the study had not been terminated prematurely, it is likely that more liver-related deaths would have occurred.38 Alternatively, the use of surrogate markers
of liver fibrosis to measure study outcomes may allow for a more in depth investigation of the influence of ART interruption on liver disease progression. A biomarker sub-study of SMART found that those who had elevated baseline levels of hyaluronic acid (HA), a marker of liver fibrosis, were at greater risk of non-AIDS related death if they were in the treatment interruption arm. The change in known markers of coagulation and inflammation, such as interleukin-6 (IL-6), D-dimer and high sensitivity C-reactive protein (hsCRP), were assessed according to baseline HA levels. It was found that baseline levels of IL-6 and D-dimer were significantly higher in co-infected patients with elevated HA. Interruption of ART led to greater changes in IL-6 during follow-up only in subjects who had elevated HA. The risk of non-AIDS related death in patients who had elevated levels of hsCRP, IL-6 or D-dimer was greater only if HA was found to be elevated as well. These results suggest that among co-infected patients, those with impaired liver function are in a pro-inflammatory state that is associated with an excess risk of non-opportunistic disease related death. Interruption of ART further intensifies this pro-inflammatory state. Supporting the hypothesis that inflammation may impact hepatic outcomes, Macias et al. have reported that increased inflammatory activity independently predicted fibrosis progression in subsequent liver biopsies in HIV/HCV co-infected patients. To date however, there has not been any published data focused on examining the effects of treatment interruption on liver disease progression using a surrogate marker of liver fibrosis.

Despite the evidence that interruptions increase the risk of disease progression, in the clinical setting, it is still likely that co-infected patients will discontinue ART for a number of reasons. The SMART study was the first randomized, controlled trial to examine the impact of ART interruption in HIV/HCV co-infected patients and it appears that this has not been studied any further in the clinical setting. Further research in this
area may reveal a potential factor involved in the lack of improvement of liver disease outcomes in this population since the introduction of ART.

**OBJECTIVES**

The general objective of this project was to determine whether ART interruption was associated with the development of liver fibrosis, as measured by the APRI, in a Canadian cohort of HIV/HCV co-infected patients while accounting for potential confounders, such as demographic, behavioural and clinical characteristics.

The specific objectives of this project were to:

1. Ascertain the proportion of patients in this cohort who interrupted ART and to describe baseline characteristics among those who did and did not interrupt treatment during follow-up.

2. Assess the association of ART interruption, as well as several baseline and time-varying covariates, with developing liver fibrosis using time-dependent Cox proportional hazards regression.

3. Evaluate the impact of mediation by time-varying confounders that are also affected by prior treatment use on the association between ART interruption and developing liver fibrosis through the use of inverse probability-of-treatment weighting in a marginal structural model.
**Method**

*Study Design and Setting*

Data was obtained from the Canadian HIV/HCV Co-infection Cohort (CCC), a multi-site prospective cohort of HIV-infected patients with chronic HCV infection or evidence of HCV exposure. Recruitment began in 2003 and as of December 2009, 912 patients were enrolled from 16 sites across Canada. In order to accurately reflect the Canadian epidemic, participants were recruited from a variety of HIV centres across the country including those that serve individuals who may be extremely marginalized, access various models of care and have diverse risk profiles. A complete list of participating centres can be found in Table 1.

**Table 1: Centres participating in the Canadian Co-infection Cohort study.** The name, province, number of participants recruited as of December 2009, and number included in the final analysis from each centre are presented.

<table>
<thead>
<tr>
<th>Site</th>
<th>Province</th>
<th>Number recruited as of December 2009</th>
<th>Number included in final analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montreal Chest Institute</td>
<td>QC</td>
<td>143</td>
<td>93</td>
</tr>
<tr>
<td>Centre Hospitalier de l'Université de Montréal</td>
<td>QC</td>
<td>167</td>
<td>92</td>
</tr>
<tr>
<td>Montreal General Hospital</td>
<td>QC</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>Clinique du Quartier Latin</td>
<td>QC</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Pender Clinic</td>
<td>BC</td>
<td>76</td>
<td>41</td>
</tr>
<tr>
<td>St. Paul's Hospital</td>
<td>BC</td>
<td>51</td>
<td>29</td>
</tr>
<tr>
<td>Native Health Clinic</td>
<td>BC</td>
<td>59</td>
<td>34</td>
</tr>
<tr>
<td>Oak Tree Clinic</td>
<td>BC</td>
<td>59</td>
<td>28</td>
</tr>
<tr>
<td>Southern Alberta HIV Clinic</td>
<td>AB</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>Sudbury Regional Hospital</td>
<td>ON</td>
<td>59</td>
<td>39</td>
</tr>
<tr>
<td>Hamilton Health Sciences</td>
<td>ON</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Toronto General Hospital</td>
<td>ON</td>
<td>59</td>
<td>32</td>
</tr>
<tr>
<td>Windsor Regional Hospital</td>
<td>ON</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>The Ottawa Hospital General Campus</td>
<td>ON</td>
<td>61</td>
<td>41</td>
</tr>
<tr>
<td>Sunnybrook Hospital</td>
<td>ON</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Capital District Health Authority</td>
<td>NS</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>912</td>
<td>541</td>
</tr>
</tbody>
</table>
**Study Population**

A detailed description of the CCC has been published elsewhere. In brief, patients were identified to participate in the CCC study from existing clinic populations at participating centres across Canada. These centres routinely screen all HIV infected patients for HCV infection. Eligible patients were adults over 16 years old with documented HIV infection (HIV-seropositive by enzyme-linked immunosorbant assay with western blot confirmation) and with chronic HCV infection or evidence of HCV exposure (HCV-seropositive by enzyme-linked immunosorbant assay with recombinant immunoblot assay II or enzyme immunoassay confirmation, or if serologically false negative, HCV RNA positive). All eligible patients were approached to participate and a refusal log was kept in order to assess response bias. Patients received $15 per visit to compensate for out-of-pocket expenses. The study was approved by the research ethics boards of all participating institutions. As of December 2009, 912 patients had been recruited and only 3% have been lost to follow-up.

All patients included in this analysis were HIV-seropositive adults with virologic evidence of active HCV infection (HCV RNA positive), who attended one of the participating centres for at least one visit between April 2003 and December 2009. Only patients who had been treated with ART were included. Because successful HCV treatment may alter progression of liver fibrosis, patients receiving HCV treatment at baseline were excluded and those who commenced HCV therapy in follow-up were censored at that point. For participants who began ART during follow-up, the baseline visit was considered to be the one at which ART was commenced. Patients with significant fibrosis, defined as having an APRI score $\geq 1.5$, at baseline were excluded. Patients were censored when an outcome occurred or at their
last clinic visit prior to December 2009. The analyzed cohort was comprised of 541 subjects (see Figure 2).

Data Collection

After providing informed consent, participants underwent an initial evaluation and then follow-up visits were to take place approximately every 6 months. During each visit, patients completed a questionnaire alone or with aid from a research co-ordinator. The questionnaires collected sociodemographic, medical and behavioural information using multiple choice and short answer questions. In addition, results from routine blood tests including measurements of plasma HIV and HCV RNA, lymphocyte subsets, AST and platelets were extracted from laboratory reports by research personnel and included in each questionnaire. Questionnaires were mailed to the co-ordinating office and scanned into a password protected computerized database. All data were handled confidentially.

Exposure and Covariate Assessment

ART was defined as taking at least three antiretrovirals concurrently for at least 30 days. The exposure of interest, ART interruption, was defined as the cessation of all antiretrovirals for at least 14 days and was included as a time-updated variable. Information about antiretrovirals taken prior to baseline and during follow-up was extracted from questionnaire responses provided by each participant and the occurrence of an interruption was assessed for each 6 month interval.

The following variables were assessed as confounders of the relationship between ART interruption and liver fibrosis: injection drug use or alcohol consumption in the previous 6 months, time-updated CD4+ T-cell count
(cells/μL) and HIV RNA (log copies/ml), age, gender, prior treatment for HCV, HBV co-infection, baseline APRI score, duration of HIV infection and duration of HCV infection. The date of HIV infection was taken to be the date which the subject believed they were infected or the date of their first positive test if this occurred first. If the subject was an IDU, the date of HCV infection was assumed to be either the date of their first injection drug use or the date they believed they were infected, depending on which was further in the past. If the date of first injection drug use was not available or applicable, then the date of HCV infection was taken to be when they believed they were infected or the date of their first positive test if this occurred first. In addition, nadir (lowest) CD4+ T-cell count and the highest recorded HIV viral load were ascertained from patients’ records at baseline and included as time-independent variables. CD4+ T cell count and HIV viral load were missing for 2% (n=27 visits) and 3% (n=39 visits) of visits respectively. For these visits, the value from the next closest visit was carried forward or backward. The analysis was also carried out replacing missing values with the median CD4+ T cell count or HIV viral load, with similar results.

**Outcome Assessment**

The outcome of interest was the presence of significant liver fibrosis, defined as an APRI score ≥ 1.5. An APRI score ≥ 1.5 has been shown to be predictive of significant fibrosis with an area under the receiver operating characteristic curve of 0.76-0.85. Therefore, this cut-off was used to ascertain the outcome and also to exclude those with significant fibrosis at baseline. A sensitivity analysis was also performed using clinical liver disease diagnoses or an APRI score ≥ 2.0, which is predictive of cirrhosis, to classify the outcome. The presence of any one of the following was considered to be a clinical liver disease diagnosis: cirrhosis, ascites, esophageal varices, spontaneous bacterial peritonitis, portal hypertension,
encephalopathy or hepatocellular carcinoma. The presence of significant fibrosis was chosen as the main outcome of interest rather than the more advanced endpoint of a clinical liver disease diagnosis or an APRI score ≥ 2.0 because few participants were expected to experience such an outcome during the relatively short follow-up period. Also, the impact of ART interruption on the development of cirrhosis or liver disease is likely to be delayed. Therefore we examined the effect of interruption on the development of fibrosis, an event that eventually leads to cirrhosis. The greater number of subjects who experienced this outcome allowed for the relationship between interruption and liver disease progression to be studied in greater detail.

Concurrent measures of AST and platelets were used to calculate APRI scores using the following formula: APRI = 100 x [AST (U/L)/upper limit of normal] / platelet count (10⁹/L).¹⁴⁵ Using the APRI score from each visit, the presence or absence of significant fibrosis was assessed. Participants were censored when an outcome occurred or at their last visit. Those who did not have an AST or platelet count at baseline, and therefore, did not have a baseline APRI score were excluded (n=14). An APRI score could not be calculated due to missing AST or platelet count at 2% (n=26 visits) of follow-up visits. For these visits, it was assumed that an outcome did not occur.

Statistical Methods

Data were analysed using R statistical software version 2.9.2. Baseline and follow-up characteristics were compared between those who interrupted ART during follow-up and those who did not using the chi-squared or Fisher’s exact test for categorical variables and the Wilcoxon rank sum test for continuous variables. Significance tests were carried out assuming two-sided alternative hypotheses and 0.05 level of significance.
**Time-Dependent Cox Proportional Hazards Models**

Univariate and multivariate time-dependent Cox proportional hazards models were used to evaluate the association of baseline and time-updated covariates with the outcome of interest, development of significant liver fibrosis. Time-varying variables included CD4+ T-cell count, HIV viral load, any active IDU or alcohol consumption, age, duration of HIV and HCV infections, ART interruption and development of significant liver fibrosis. Time-independent variables included gender, HBV status, HCV treatment prior to baseline, baseline and nadir CD4+ T-cell count, baseline and highest recorded HIV viral load and baseline APRI score. Baseline APRI scores were divided into three categories: APRI < 0.5, which is indicative of the absence of fibrosis and was used as the reference category, 0.5 ≤ APRI < 1.00, and 1.00 ≤ APRI < 1.5. Quadratic and natural logarithm transformations of baseline APRI scores were considered, however a categorized version of the variable was used to avoid making strong assumptions about the functional relationship between baseline APRI and the primary outcome. Several models were created that each included a different form of baseline APRI and when it was divided into categories the results were similar to that obtained using a linear form of the variable.

Univariate Cox regression models were carried out to estimate the crude hazard ratios (HRs) of developing significant liver fibrosis. A multivariate model was constructed that included covariates that had statistically significant HRs in the univariate analyses as well as variables that were determined a priori to be clinically significant. The final multivariate model included ART interruption, time-updated CD4+ T-cell count and HIV viral load, baseline APRI, age and gender. Robust variance estimation was used for all Cox regression analyses to account for the correlation of data contributed by the same participant at multiple visits.
**Marginal Structural Model**

In order to estimate the effect of ART interruption on liver disease, one must adjust for time-varying confounders such as CD4+ T-cell count and HIV viral load since these biomarkers are associated with liver disease progression as well as with decisions about future ART use. However, these time-varying confounders may also be intermediate variables because previous ART use will affect the CD4+ T-cell count and HIV viral load in the following time interval, which in turn may affect liver disease progression. Adjusting for the time-varying confounders that are also intermediates in a standard Cox proportional hazards model could result in a biased estimate for the net effect of ART interruption on development of significant fibrosis.\textsuperscript{147} Figure 1 depicts the relationship between time-varying confounders, ART interruption and APRI score.

![Causal Model Diagram](image)

**Figure 1: Causal Model.** The relationship between ART use and APRI score over time is shown, with X representing potential confounders such as CD4+ T cell count or HIV viral load. Due to the fact that ART use affects X in the following time interval, which then affects APRI, these confounders are also considered to be intermediate variables.
In order to appropriately adjust for time-varying confounders that may also be affected by prior ART interruption, inverse probability-of-treatment weighting (IPTW) was used in a marginal structural model estimated via pooled logistic regression. The purpose of IPTW was to remove the association between prior confounding variables and ART interruption while maintaining the relationship between ART interruption and liver disease.

To calculate the part of the stabilized weights arising from the treatment process, the probability of receiving the treatment actually received (interrupted or not) in each interval as a function of previous ART use (interrupted previously or not) and baseline covariates was found (probability of treatment numerator (PTN)). This was then divided by the probability of receiving the treatment actually received in each interval as a function of previous ART use, baseline and time-varying covariates (probability of treatment denominator (PTD)). To calculate the part of the stabilized weights arising from the censoring process, the probability of being observed in each interval as a function of ART use and baseline covariates was found (probability of being observed numerator (PON)). This was then divided by the probability of being observed in each interval as a function of ART use, baseline and time-varying covariates (probability of being observed denominator (POD)). For each subject, interval specific weights were calculated \([(PTN/PTD) \times (PON/POD)]\) and then multiplied by the weights from previous intervals.

Time-updated CD4+ T-cell count, HIV viral load and age were accounted for through the IPTW process. The marginal structural model included ART interruption as well as gender and baseline APRI score as regressors. Odds ratios (ORs) were then estimated using weighted pooled logistic regression. Pooled logistic regression approximates Cox regression when
the risk of an event is less than 10% per person-time interval. In this case, the risk of achieving an APRI score ≥ 1.5 was 7% per person-year of follow-up (or 3.5% per six-month interval), therefore the ORs obtained from the marginal structural model approximately estimate the HRs that would be calculated in a marginal structural Cox model. Nonparametric percentile-based bootstrap confidence intervals (CIs) were calculated by re-sampling 541 subjects with replacement from the observed data.

Use of IPTW relies on the assumptions that the models to determine the probabilities of being treated and censored were correctly specified and that there was no unmeasured confounding, however violations of these assumptions can not be tested for. In addition, it must be assumed that there was experimentation in the assignment of treatment (ETA assumption). This means that all subjects must have some positive probability of receiving the treatment or intervention (in this case, interrupting ART) regardless of their past. The ETA assumption may be violated theoretically if the presence of a certain characteristic is an absolute contraindication for treatment, causing members of the population to have zero probability of receiving the intervention. Practical violations of the ETA assumption occur by chance and are more likely to occur when the sample size is small. For example, as the sample size decreases, the probability of receiving the treatment may approach zero for members of a certain strata simply because none of them actually received the intervention.

Violations of the ETA assumption may lead to very large weights as well as unstable and possibly biased effect estimates. However, use of stabilized weights, as was performed in the current analysis, allows for a weaker form of the ETA assumption. In order to assess for violations, side-by-side boxplots were created to compare the distribution of probabilities of interrupting ART during a specific time interval given the current
covariates, between those who actually interrupted and those who did not. A large number of probabilities near to zero or one would signify violations of the ETA assumption.

**RESULTS**

**Description of the Cohort**

Of the 912 HIV-infected individuals enrolled in the CCC at the time of analysis, 732 had virologic evidence of HCV infection and were treated with ART. After censoring patients who began HCV treatment and excluding those who had an APRI score ≥ 1.5 at baseline, 541 participants remained. Figure 2 illustrates how subjects were selected for inclusion into the analysed cohort.

Descriptive statistics were carried out among the 541 analysed participants. The median duration of follow-up and age were 1.02 (Inter-quartile range (IQR): 0.5-1.78) and 44 (IQR: 40-49) years respectively. The majority of participants were male (n=401, 74%) and reported a history of IDU (n=449, 83%), while 40% (n=219) reported active IDU and 62% (n=335) reported any alcohol consumption during follow-up. A level of education greater than high school was achieved by 25% (n=136) of participants. The median durations of HCV and HIV infections were 19 (IQR: 11-26) and 11 (IQR: 7-16) years respectively. Approximately 3% (n=17) were infected with HBV. Baseline CD4+ T cell count, HIV viral load and APRI score were 389 (IQR: 252-543) cells/µl, less than 50 (IQR: <50-112) copies/ml and 0.525 (IQR: 0.362-0.814) respectively. During 760 person-years of follow-up, 10% (n=53) of subjects achieved an APRI score ≥ 1.5 and 10% (n=53) interrupted ART.
In order to be eligible for inclusion in this analysis, participants must have been HIV-seropositive, HCV RNA positive, had a history of ART and an APRI score < 1.5 at baseline. Once subjects began treatment for HCV during follow-up, they were censored (n=46). At baseline, 17 patients were receiving HCV treatment and therefore were excluded.

Table 2 presents characteristics among those who interrupted ART during follow-up and those who did not. The median duration of follow-up, nadir CD4+ T cell count and baseline HIV viral load were significantly greater among those who interrupted ART. Also, those who interrupted had significantly lower baseline CD4+ T cell count and platelet counts. The differences in CD4+ T cell count and HIV viral load at baseline may be explained by the fact that 34 of the 53 subjects who reported interrupting ART during
follow-up were on an interruption at the time of their baseline visit. It is also important to note that the difference in median platelet count at baseline was found to be statistically significant between those who interrupted and those who did not, however, in both groups the median platelet count is still well above the clinically normal cut-off of 150 x 10^9/L. In addition, the baseline APRI scores did not differ between those who interrupted and those who did not and were well below the cut-off of 1.5 used to identify the presence of significant fibrosis.

The total number of interruptions that occurred was 55. Two subjects interrupted twice. The median duration of treatment interruption during follow-up was 180 days (IQR: 65-310). However, 26 interruptions either began before baseline or were ongoing at the last clinic visit and therefore the entire duration could not be determined. The proportion of visits at which patients reported interrupting ART peaked in 2004 (15% of visits). The proportion of visits at which an interruption was reported were 8, 9, 8, 4, 5, and 5% for the calendar years 2003, 2005, 2006, 2007, 2008 and 2009 respectively. Among 21 subjects who had a measurement of platelets and AST performed before and during the interruption, the median change in platelets was -2 (IQR: -34 - 24) x 10^9/L and the median change in AST was 21 (IQR: 1-38) U/L.
Table 2: Characteristics of 541 study subjects at baseline and during follow-up. Values shown are count (%) or median (inter-quartile range).
Continuous variables were compared using the Wilcoxon-rank sum test. Categorical variables were compared using the chi-squared test or Fisher’s exact test if the count was less than 7. P-values < 0.05 were considered to be evidence for a statistically significant difference. *HbsAg+: Hepatitis B surface antigen positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Interrupted (53)</th>
<th>Did not interrupt (488)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of follow-up (years)</td>
<td>1.45 (0.86, 1.98)</td>
<td>1.00 (0.5, 1.73)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 (38, 46)</td>
<td>45 (40, 49)</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>35 (66)</td>
<td>366 (75)</td>
<td>NS</td>
</tr>
<tr>
<td>Greater than high school education</td>
<td>10 (19)</td>
<td>126 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>History of IDU</td>
<td>49 (92)</td>
<td>400 (82)</td>
<td>NS</td>
</tr>
<tr>
<td>Active IDU during follow-up</td>
<td>21 (40)</td>
<td>198 (41)</td>
<td>NS</td>
</tr>
<tr>
<td>Active alcohol use during follow-up</td>
<td>36 (68)</td>
<td>299 (61)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of HCV infection (years)</td>
<td>17 (11, 22)</td>
<td>19 (11, 26)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of HIV infection (years)</td>
<td>12 (8, 16)</td>
<td>11 (7, 16)</td>
<td>NS</td>
</tr>
<tr>
<td>Previously treated for HCV</td>
<td>7 (13)</td>
<td>57 (12)</td>
<td>NS</td>
</tr>
<tr>
<td>Nadir CD4+ T cell count (cells/ul)</td>
<td>213 (100, 353)</td>
<td>150 (60, 250)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Highest HIV viral load (copies/ml)</td>
<td>65416 (19879, 175662)</td>
<td>96878 (27742, 254669)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline CD4+ T cell Count (cells/ul)</td>
<td>330 (160, 471)</td>
<td>399 (260, 550)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Baseline HIV Viral Load (copies/ml)</td>
<td>4260 (80, 52150)</td>
<td>&lt;50 (&lt;50, 57)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Achieved APRI≥1.5 during follow-up</td>
<td>9 (17)</td>
<td>44 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline APRI score</td>
<td>0.558 (0.422, 0.857)</td>
<td>0.521 (0.357, 0.799)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline platelet count (10⁹/L)</td>
<td>199 (170, 239)</td>
<td>225 (182, 264)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Baseline AST (U/L)</td>
<td>39 (33, 52)</td>
<td>40 (29, 54)</td>
<td>NS</td>
</tr>
<tr>
<td>HbsAg+*</td>
<td>1 (2)</td>
<td>16 (3)</td>
<td>NS</td>
</tr>
</tbody>
</table>
The reason for 31 of the 55 interruptions was unknown. Among the 24 interruptions that had a known reason, 88% (n = 21) were due to the patient’s decision to discontinue treatment. Among those subjects who decided to interrupt ART, undesirable symptoms believed to be caused by ART were named as the primary reason by 38% (n = 8). Only one of 24 patients who decided to interrupt treatment reported a drug relapse as the cause. One patient chose to interrupt because of HIV viral rebound, two patients interrupted relatively briefly because they were delayed in refilling their prescriptions and the remaining nine patients who chose to interrupt ART did not specify a reason.

**Impact of ART Interruption on Developing Liver Fibrosis**

Univariate time-dependent Cox regression analyses revealed a significant harmful effect of ART interruption (HR: 2.75, 95% CI: 1.26-5.98), higher HIV viral load (HR: 1.22, 95% CI: 1.02-1.47 per log copies/ml) and baseline APRI ≥ 0.5 (HR comparing 0.5 ≤ APRI < 1.00 to APRI < 0.5: 2.89, 95% CI: 1.40-5.94; HR comparing 1.00 ≤ APRI <1.5 to APRI < 0.5: 7.86, 95% CI: 3.73-16.6) on the development of liver fibrosis. Higher CD4+ T cell counts (HR: 0.92 95% CI: 0.86- 0.995 per 50 cells/μl increase) were protective against achieving an APRI ≥ 1.5. The effect of age, gender, active IDU, alcohol use, duration of HIV and HCV infections, nadir CD4+ T cell count, highest recorded HIV viral load and co-infection with HBV on the outcome were not statistically significant. Hazard ratios for the effect of each variable on the development of liver fibrosis are presented in Table 3.

Covariates that had statistically significant HRs in the univariate analyses as well as variables that were determined a priori to be clinically significant were included in the multivariate model. In the multivariate analysis, higher CD4+ T cell count (HR: 0.95, 95% CI: 0.88-1.00 per 50 cells/μl increase) was protective against developing an APRI score ≥ 1.5, but the
95% confidence interval included the null value of 1.00. A baseline APRI score ≥ 0.5 (HR comparing 0.5 ≤ APRI < 1.00 to APRI < 0.5: 3.02, 95% CI: 1.49-6.12; HR comparing 1.00 ≤ APRI <1.5 to APRI < 0.5: 7.80, 95% CI: 3.75, 16.2) was also associated with the development of an APRI score ≥ 1.5.

After adjustment, the effect of ART interruption on liver fibrosis was slightly attenuated but still statistically significant (HR: 2.52, 95% CI: 1.20-5.28). The effect of HIV viral load, age and gender were not statistically significant. The hazard ratios for each variable included in the multivariate Cox regression model are shown in Table 3.

Table 3: Univariate and multivariate Cox proportional hazards regression to assess the effect of ART interruption and other covariates on developing liver fibrosis. Hazard ratios (HRs) and 95% confidence intervals (CI) are shown for each variable analysed in univariate Cox proportional hazards regression as well as for the variables included in the multivariate analysis. *HbsAg+: Hepatitis B surface antigen positive.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate HR (95% CI)</th>
<th>Multivariate HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interruption of ART during previous time interval</td>
<td>2.75 (1.26, 5.98)</td>
<td>2.52 (1.20, 5.28)</td>
</tr>
<tr>
<td>Age (per 5 year increase)</td>
<td>1.07 (0.91, 1.24)</td>
<td>1.07 (0.95, 1.21)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.06 (0.58, 1.95)</td>
<td>1.28 (0.71, 2.30)</td>
</tr>
<tr>
<td>Active IDU</td>
<td>0.83 (0.46, 1.49)</td>
<td>n/a</td>
</tr>
<tr>
<td>Active alcohol consumption</td>
<td>1.37 (0.79, 2.39)</td>
<td>n/a</td>
</tr>
<tr>
<td>Duration of HIV infection (per 5 year increase)</td>
<td>1.09 (0.92, 1.31)</td>
<td>n/a</td>
</tr>
<tr>
<td>Duration of HCV infection (per 5 year increase)</td>
<td>1.03 (0.91, 1.15)</td>
<td>n/a</td>
</tr>
<tr>
<td>Previously treated for HCV</td>
<td>1.53 (0.74, 3.16)</td>
<td>n/a</td>
</tr>
<tr>
<td>Nadir CD4+ T cell count (per 50 cell/uL increase)</td>
<td>0.98 (0.91, 1.05)</td>
<td>n/a</td>
</tr>
<tr>
<td>Highest HIV viral load (log copies/ml)</td>
<td>1.09 (0.87, 1.38)</td>
<td>n/a</td>
</tr>
<tr>
<td>Time-updated CD4+ T cell count (per 50 cells/uL increase)</td>
<td>0.92 (0.86, 0.995)</td>
<td>0.95 (0.88, 1.00)</td>
</tr>
<tr>
<td>Time-updated HIV viral load (log copies/ml)</td>
<td>1.22 (1.02, 1.47)</td>
<td>1.13 (0.91, 1.41)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>2.89 (1.40, 5.94)</td>
<td>3.02 (1.49, 6.12)</td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>7.86 (3.73, 16.6)</td>
<td>7.80 (3.75, 16.2)</td>
</tr>
<tr>
<td>HbsAg+*</td>
<td>0.71 (0.10, 5.21)</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Adjustment for Time-varying Confounders Affected by Prior Interruption

In order to appropriately adjust for potential mediation by time-varying confounders that are also affected by treatment, the effect of ART interruption on achieving an APRI score ≥ 1.5 was estimated using IPTW in a marginal structural model. Time-updated CD4+ T cell count, HIV viral load and age were accounted for through the IPTW process. Gender and baseline APRI score were included in the marginal structural model as regressors. The OR for the effect of ART interruption on experiencing an outcome was similar to the HR obtained from the multivariate Cox proportional hazards model, but it did not reach statistical significance (OR: 2.60, 95% CI: 0.61-6.18). The effect of gender (OR: 1.26, 95% CI: 0.51-2.51) was also not found to be significant, however baseline APRI score ≥ 0.5 (OR comparing 0.5 ≤ APRI < 1.00 to APRI < 0.5: 3.28, 95% CI: 1.45-10.1; OR comparing 1.00 ≤ APRI <1.5 to APRI < 0.5: 9.84, 95% CI: 4.42-30.6) was significantly associated with progression to an APRI score ≥ 1.5, as seen in Table 4. This was comparable to the results obtained from the Cox proportional hazards analysis, suggesting that mediation by time-varying confounders that are also affected by treatment was negligible in the Cox proportional hazards regression model.
Table 4: Accounting for time-varying confounders that are also affected by prior ART interruption in a marginal structural model. The odds ratio (OR) and 95% confidence intervals (CI) for the effect of ART interruption, as well as the time-independent covariates, gender and baseline APRI, on fibrosis progression are presented. Time-updated CD4+ T cell count, HIV viral load and age were accounted for through the inverse-probability-of-treatment process.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Interruption</td>
<td>2.60 (0.61, 6.18)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.26 (0.51, 2.51)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>1</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>3.28 (1.45, 10.1)</td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>9.84 (4.42, 30.6)</td>
</tr>
</tbody>
</table>

Theoretically, the ETA assumption was met because all subjects had the potential to interrupt or to be on treatment. However, in the actual sample it was still possible that each exposure category (interrupted or not) was not represented among every strata of the covariates included. Therefore, the ETA assumption was tested. In order to do so, side-by-side boxplots were created (see Figure 3) to compare the distribution of probabilities of interrupting ART during a specific time interval given the current covariates between those who actually interrupted and those who did not. Among those who interrupted, the probabilities of interruption given the current covariates ranged from zero to one, with the median being less than 0.8, supporting an agreement with the ETA assumption. Among those who did not actually interrupt, the majority of probabilities of interruption in each interval given the current covariates were very close to zero. However, given the small number of interruptions that actually occurred (6% of visits), the probability of interruption given the current covariates in this group was expected to be small. In addition, the median of the stabilized weights was 0.985 (IQR: 0.913-1.06), which illustrates that any violations of the ETA assumption did not results in extreme weights.
Figure 3: Assessing for violations of the ETA assumption. Boxplots of the distribution of probabilities of interrupting ART during each time interval given the current covariates are shown for those who did not actually interrupt ART (left) and those who actually did interrupt (right).

Sensitivity Analyses

Sensitivity analyses were performed in order to assess the robustness of the modelling choices that were made and in turn, the primary findings. These analyses included evaluating various specifications of the baseline APRI variable, assessing the impact of interruption on developing liver disease rather than significant liver fibrosis and further evaluation of the ETA assumption.

Alternate Specifications of Baseline APRI Score

As previously mentioned, in the final models baseline APRI scores were divided into three categories: APRI < 0.5, which is indicative of the absence of fibrosis and was used as the reference category, 0.5 ≤ APRI < 1.00, and 1.00 ≤ APRI < 1.5. Quadratic and natural logarithm transformations of baseline APRI scores were also considered and several models were
created that each included a different form of the baseline APRI variable (see Tables 5 and 6). When baseline APRI was divided into categories, the results were similar to that obtained using a linear form of the variable, however a categorized version was used because it appeared to be the most stable and to avoid making strong assumptions about its functional relationship with the primary outcome.

Table 5: Alternate specifications of baseline APRI used in the Cox proportional hazards regression model. Hazard ratios (HRs) and 95% confidence intervals (CI) are shown for each variable analysed in multivariate Cox proportional hazards regression models using different variations of the baseline APRI variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categorized baseline APRI HR (95% CI)</th>
<th>ln(baseline APRI) HR (95% CI)</th>
<th>[ln(baseline APRI)]² HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interruption of ART during previous time interval</td>
<td>2.52 (1.20, 5.28)</td>
<td>2.82 (1.14, 6.71)</td>
<td>2.44 (1.12, 5.30)</td>
</tr>
<tr>
<td>Age (per 5 year increase)</td>
<td>1.07 (0.95, 1.21)</td>
<td>1.08 (0.96, 1.22)</td>
<td>1.06 (0.94, 1.19)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.28 (0.71, 2.30)</td>
<td>1.37 (0.74, 2.57)</td>
<td>1.35 (0.75, 2.44)</td>
</tr>
<tr>
<td>Time-updated CD4+ T cell count (per 50 cells/uL increase)</td>
<td>0.95 (0.88, 1.00)</td>
<td>0.94 (0.87, 1.00)</td>
<td>0.94 (0.88, 1.00)</td>
</tr>
<tr>
<td>Time-updated HIV viral load (log copies/ml)</td>
<td>1.13 (0.91, 1.41)</td>
<td>1.09 (0.86, 1.38)</td>
<td>1.12 (0.89, 1.40)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>ln(APRI) 1</td>
<td>ln(APRI) 5.01 (2.76, 9.09)</td>
<td>ln(APRI) 8.38 (3.32, 21.2)</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>3.02 (1.49, 6.12)</td>
<td></td>
<td>[ln(APRI)]² 1.71 (0.72, 4.03)</td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>7.80 (3.75, 16.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Alternate specifications of baseline APRI used in the marginal structural model. The odds ratio (OR) and 95% confidence intervals (CI) for the effect of ART interruption, as well as the time-independent covariates on fibrosis progression are presented using different variations of the baseline APRI variable.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Categorized baseline APRI OR (95% CI)</th>
<th>ln(baseline APRI) OR (95% CI)</th>
<th>[ln(baseline APRI)]^2 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Interruption</td>
<td>2.60 (0.61, 6.18)</td>
<td>2.67 (0.62, 6.73)</td>
<td>2.80 (0.59, 7.41)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.26 (0.51, 2.51)</td>
<td>1.36 (0.63, 2.91)</td>
<td>1.37 (0.60, 2.66)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>3.28 (1.45, 10.1)</td>
<td>ln(APRI) 6.00 (3.11, 14.2)</td>
<td>ln(APRI) [ln(APRI)]^2 10.7 (2.97, 33.9)</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>9.84 (4.42, 30.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>1.92 (0.36, 4.60)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Impact of ART Interruption on Developing Liver Disease

The impact of ART interruption on the development of an APRI score ≥ 2.0 or a clinical liver disease diagnosis was assessed using the same multivariate Cox proportional hazards model as above. After excluding those classified as having an outcome at baseline, the analysed cohort for this analysis was comprised of 554 subjects. An APRI score ≥ 2.0 or a liver disease diagnosis was achieved by 9% (n=48) of participants.

As shown in Table 7, the effect of interruption on the outcome was similar to that seen when an APRI score ≥ 1.5 was used as the endpoint, however the HR did not reach statistical significance in the multivariate model (HR: 2.12, 95% CI: 0.87-5.16). In addition, this more advanced endpoint was evaluated using the same marginal structural model (see Table 8). The OR for interruption was slightly less than the OR obtained using the less advanced endpoint and again, was not found to be statistically significant (HR: 2.34, 95% CI: 0.54-10.2).
Table 7: Univariate and multivariate Cox proportional hazards regression to assess the effect of ART interruption and other covariates on developing cirrhosis or a clinical liver disease diagnosis.

Hazard ratios (HRs) and 95% confidence intervals (CI) are shown for each variable analysed in univariate Cox proportional hazards regression as well as for the variables included in the multivariate analysis, using an APRI score ≥ 2.0 or a clinical liver disease diagnosis and the endpoint. *HbsAg+: Hepatitis B surface antigen positive.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate HR (95% CI)</th>
<th>Multivariate HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interruption of ART during previous time interval</td>
<td>2.19 (1.02, 4.69)</td>
<td>2.12 (0.87, 5.16)</td>
</tr>
<tr>
<td>Age (per 5 year increase)</td>
<td>1.09 (0.93, 1.28)</td>
<td>1.14 (0.99, 1.31)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.01 (0.51, 1.97)</td>
<td>1.55 (0.77, 3.13)</td>
</tr>
<tr>
<td>Active IDU</td>
<td>1.22 (0.67, 2.20)</td>
<td>n/a</td>
</tr>
<tr>
<td>Active alcohol consumption</td>
<td>1.44 (0.80, 2.60)</td>
<td>n/a</td>
</tr>
<tr>
<td>Duration of HIV infection (per 5 year increase)</td>
<td>1.02 (0.86, 1.20)</td>
<td>n/a</td>
</tr>
<tr>
<td>Duration of HCV infection (per 5 year increase)</td>
<td>1.04 (0.91, 1.18)</td>
<td>n/a</td>
</tr>
<tr>
<td>Previously treated for HCV</td>
<td>1.16 (0.50, 2.69)</td>
<td>n/a</td>
</tr>
<tr>
<td>Nadir CD4+ T cell count (per 50 cell/uL increase)</td>
<td>0.95 (0.87, 1.05)</td>
<td>n/a</td>
</tr>
<tr>
<td>Highest HIV viral load (log copies/ml)</td>
<td>1.32 (1.06, 1.66)</td>
<td>n/a</td>
</tr>
<tr>
<td>Time-updated CD4+ T cell count (per 50 cells/uL increase)</td>
<td>0.90 0.83, 0.98)</td>
<td>0.92 (0.84, 1.00)</td>
</tr>
<tr>
<td>Time-updated HIV viral load (log copies/ml)</td>
<td>1.21 (1.00, 1.46)</td>
<td>1.09 (0.85, 1.40)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>1.88 (0.78, 4.54)</td>
<td>2.01 (0.85, 4.79)</td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>8.84 (3.65, 21.4)</td>
<td>9.04 (3.81, 21.4)</td>
</tr>
<tr>
<td>Baseline APRI 1.50 to 1.99</td>
<td>11.7 (4.64, 29.3)</td>
<td>13.0 (5.32, 31.5)</td>
</tr>
<tr>
<td>HbsAg+*</td>
<td>0.79 (0.11, 5.82)</td>
<td>n/a</td>
</tr>
</tbody>
</table>
Table 8: Assessing the effect of ART interruption on developing cirrhosis or a clinical liver disease diagnosis in a marginal structural model. The odds ratio (OR) and 95% confidence intervals (CI) for the effect of ART interruption, as well as the time-independent covariates, gender and baseline APRI, on development of an APRI score ≥ 2.0 or a clinical liver disease diagnosis are presented. Time-updated CD4+ T cell count, HIV viral load and age were accounted for through the inverse-probability-of-treatment process.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Interruption</td>
<td>2.34 (0.54, 10.2)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.44 (0.67, 3.09)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>1</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>1.90 (0.5, 7.22)</td>
</tr>
<tr>
<td>Baseline APRI 1.00 to 1.49</td>
<td>10.6 (2.73, 25.7)</td>
</tr>
<tr>
<td>Baseline APRI 1.50 to 1.99</td>
<td>12.7 (2.92, 55.2)</td>
</tr>
</tbody>
</table>

Exploring the ETA Assumption

As shown in Figure 3, in order to assess for violations of the ETA assumption, side-by-side boxplots were created to compare the distribution of probabilities of interrupting ART during a specific time interval given the current covariates, between those who actually interrupted and those who did not. Among those who interrupted, the distribution of probabilities supported that there were no violations of the ETA assumption. However, among those who did not interrupt, the majority of probabilities were very close to zero and so this was further investigated. It was found that when the variable that specified whether or not each subject interrupted treatment in the previous time interval was excluded from the marginal structural model, the distribution of probabilities of interruption among those who did not interrupt became less clustered around zero (see Figure 4). The results from this smaller
model were compared to what was initially found in order to ensure that this variable did not cause the results from the initial model to be biased. As shown in Table 9, the effect of ART interruption on developing significant fibrosis was attenuated when the variable indicating whether or not the subject interrupted during the previous time interval was omitted. However, the point estimate still suggests an association.

Figure 4: Further exploration of the ETA assumption. Boxplots of the distribution of probabilities of interrupting ART during each time interval given the current covariates are shown for those who did not actually interrupt ART (left) and those who actually did interrupt (right) after omitting the variable that indicated an interruption in the previous time interval from the marginal structural model.
Table 9: Assessing the impact of omitting the variable that indicated if an interruption occurred in the previous time interval from the marginal structural model. The odds ratio (OR) and 95% confidence intervals (CI) for the effect of ART interruption, as well as the time-independent covariates, gender and baseline APRI, on development of significant fibrosis are shown. Time-updated CD4+ T cell count, HIV viral load and age were accounted for through the inverse-probability-of-treatment process, however the variable that indicated if an interruption occurred in the previous time interval was not included.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Interruption</td>
<td>1.92 (0.41, 9.05)</td>
</tr>
<tr>
<td>Female gender</td>
<td>1.67 (0.81, 3.44)</td>
</tr>
<tr>
<td>Baseline APRI &lt; 0.5</td>
<td>1</td>
</tr>
<tr>
<td>Baseline APRI 0.5 to 0.99</td>
<td>3.37 (1.34, 8.47)</td>
</tr>
<tr>
<td>Baseline APRI 1.0 to 1.49</td>
<td>8.94 (3.36, 23.8)</td>
</tr>
</tbody>
</table>

Summary of Sensitivity Analyses

Therefore, it was shown that the use of a categorical variation of the baseline APRI variable was stable and consistent with results obtained using different transformations. The primary findings that ART interruption was associated with developing significant fibrosis were also consistent with the results obtained using a more advanced endpoint of liver disease as the outcome. Finally, further exploration revealed that the removal of the variable indicating whether or not an interruption occurred in the previous time interval led to a greater agreement with the ETA assumption. Removing this variable from the marginal structural model caused the effect of ART interruption on outcome to be slightly attenuated, but still suggests an association. These sensitivity analyses illustrate the consistency of results across various modelling assumptions.
**DISCUSSION**

The results of this project revealed that over the course of six years, 10% of ART-treated, co-infected patients in this Canadian cohort reported interrupting ART for at least 14 days. The majority of interruptions continued for at least six months. A study conducted among HIV-infected individuals in Denmark between 1995 and 2004 found that 30% of HIV/HCV co-infected patients interrupted ART during the study period. The lower incidence of interruption found in the CCC may be explained by the fact that it was conducted more recently. Improvements in the tolerability of ART regimens as well as evidence from randomized, controlled trials that highlighted the potential harmful effects of interruptions, may have led to a decrease in the proportion of patients who interrupted treatment. This is also consistent with data from a cohort study in British Columbia that took place between the years 2000 and 2006, which showed that ART interruptions occurred less frequently in more recent years (29% in 2000 versus 19% in 2006). However, the proportion of participants who reported interrupting ART in this HIV-infected cohort was still greater than that seen in the CCC. This may be due to the fact that the CCC study population consisted of patients who were in care and therefore may be more stable than the co-infected population as a whole.

Time-dependent Cox proportional hazards regression revealed a significant, harmful effect of ART interruption and baseline APRI ≥ 0.5 on the development of liver fibrosis, independent of age, gender, time-updated CD4+ T cell count and HIV viral load. The effect of CD4+ T cell count nearly reached statistical significance, while HIV viral load, age and gender were not found to be associated with development of the outcome. Therefore, even after adjustment for the immunological consequences of ART.
discontinuation, interruption of treatment remained associated with developing liver fibrosis.

To evaluate the impact of mediation by time-varying confounders, IPTW was used in a marginal structural model. The association between baseline APRI and outcome was found to be statistically significant, while ART interruption was not. However, the point estimate for the effect of interruption on developing significant liver fibrosis was similar to that obtained using Cox regression. This suggests that mediation by time-varying confounders that are simultaneously intermediate variables was actually negligible using the more traditional method of survival analysis. The weighted model was highly variable compared to Cox regression however, its use is a strength of this study as it confirmed that the results obtained using Cox regression were properly adjusted for time-dependent variables.

In addition, an association between ART interruption and developing an APRI score ≥ 2.0 or a clinical liver disease diagnosis was suggested, however did not reach statistical significance. This finding is consistent with the fact that ART interruption was found to be associated with developing liver fibrosis. However, it is not surprising that the association between interruption and developing the more advanced endpoint of liver cirrhosis or a clinical diagnosis of liver disease was not found to be significant, given the smaller number of participants who reached this outcome during follow-up. Also, the exposure of interest was interruption of ART during the six months prior to a given visit. The impact of ART interruption on development of cirrhosis or liver disease is likely to have a more delayed effect.

The results of this study are consistent with the observation from the SMART study that HIV/HCV co-infected patients who were randomized to
interrupt ART experienced a greater risk of non-opportunistic disease related death.\textsuperscript{38} Even though only one participant in the arm that interrupted ART died of liver-related causes, the results from SMART and this study still suggest that ART interruption impacts the risk of disease outcomes not traditionally classified as being opportunistic, such as liver-disease related events. If the SMART study had not been terminated prematurely, it is likely that more liver-related deaths would have occurred. The current analysis supports this hypothesis and the use of significant liver fibrosis as the endpoint rather than the more advanced outcome of liver disease-related death, allowed for the relationship between interruption and liver disease progression to be studied in greater detail.

A possible explanation for the association of ART interruption with developing liver fibrosis even after accounting for changes in CD4+ T cell counts and HIV RNA may be that interruption increases inflammatory processes. A biomarker sub-study of SMART found that those who had elevated baseline levels of hyaluronic acid (HA), a marker of liver fibrosis, were at greater risk of non-AIDS related death if they were in the treatment interruption arm. Baseline levels of known markers of coagulation and inflammation, interleukin-6 (IL-6) and D-dimer, were significantly higher in co-infected patients with elevated HA. Interruption of ART led to greater changes in IL-6 during follow-up. The risk of non-AIDS related death in patients who had elevated levels of IL-6 or D-dimer was greater in subjects who had elevated HA as well. These results suggest that among co-infected patients, those with impaired liver function were in a pro-inflammatory state that is associated with excess risk of non-opportunistic disease related death. Interruption of ART further intensified this pro-inflammatory state.\textsuperscript{140} Supporting the hypothesis that inflammation promotes the development of liver disease, Macias \textit{et al.} have reported that
increased inflammatory activity independently predicted fibrosis progression in subsequent liver biopsies in HIV/HCV co-infected patients.\textsuperscript{15}

A strength of this study was the use of data from the CCC which specifically sought to recruit patients from a variety of centres in order to appropriately represent the co-infected population in care in Canada. In doing so, the cohort included participants who may be extremely marginalized, access various models of care and who have diverse risk profiles. Many of these groups, in particular IDU, are typically underrepresented in randomized, controlled trials, often limiting the generalizability of their results.\textsuperscript{153} In addition, this study included data about active IDU during follow-up which is an important factor to take into account, but has not been considered in previous cohort studies that investigated the consequences of interruption in HIV-infected patients.\textsuperscript{36, 37, 135}

The outcome of interest for this analysis was the development of significant liver fibrosis, as measured by a non-invasive, surrogate biomarker. The APRI has been validated in HIV/HCV co-infected patients\textsuperscript{143} and a score of 1.5 or greater has been shown to be predictive of significant liver fibrosis with a sensitivity and specificity of 52\% and 100\% respectively, using liver biopsy as the gold standard.\textsuperscript{118} However, liver biopsy itself has several limitations. It is invasive, difficult to repeat and therefore studies that use liver biopsy to measure fibrosis are often limited by sample size and patient selection bias. Liver biopsy results are also affected by tissue sampling and interpretation error.\textsuperscript{49, 50} The more objective nature of the APRI does not require the need for blinding of those assessing the outcome, as this measurement is not subject to information bias. However the APRI is not without limitations. It has been shown that interruption of ART may lead to a greater risk of thrombocytopenia.\textsuperscript{154} Due to the fact that APRI would be affected by fluctuations in platelets, it is possible that changes in
APRI in those who interrupt ART may be attributed to changes in platelets that are not necessarily due to the progression of liver fibrosis. Although, among 21 subjects who had a measurement of platelets performed before and during the interruption, the median change in platelets was a decrease of 2 (IQR: -34 - 24) x 10⁹/L, which is relatively small and the inter-quartile range suggests that the platelet count actually increased during an interruption in several participants.

A potential limitation of this analysis is the possibility of residual confounding, alcohol consumption being the most significant variable to consider. The crude HR of developing liver fibrosis was determined using a variable that only indicated whether or not participants reported consuming any alcohol during the previous six months. In the univariate Cox proportional hazards model, the HR for alcohol consumption was not found to be significant and therefore was not included in any of the multivariate models. However, it is possible that this relatively crude measurement would not have allowed for binge drinking or consistently heavy consumption to be distinguished from less harmful drinking habits. Heavy alcohol consumption has been shown to be associated with faster fibrosis progression⁹⁶, ¹¹⁵, ¹⁵⁵ and is also likely associated with decreased medication adherence.¹⁵⁶ Therefore, it has the potential to be a confounding variable and it would be beneficial to consider a more detailed measurement of the quantity and/or frequency of alcohol consumed when determining the association between ART interruption and developing significant fibrosis. Had this more detailed information been included, the estimated effect would have likely decreased.

One must also consider the fact that data regarding ART interruptions were based on patient recall so it is possible that the number of interruptions reported is less than the number that actually occurred. This may be attributed to poor recall, but also possibly to the fact that some patients
may have been hesitant to report interrupting treatment, knowing that it was likely against the advice of their doctor. In addition, the relatively low specificity of the APRI in detecting individuals with liver fibrosis may have resulted in fewer outcomes being documented than actually occurred. Had both of these factors been corrected for, these analyses may have been better powered to detect the association between ART interruption and the development of liver fibrosis. Therefore, the estimated HR may have been slightly under-estimated.

**CONCLUSION**

In conclusion, ART interruption was associated with the development of liver fibrosis in this Canadian cohort of HIV/HCV co-infected patients. This suggests that some of the liver disease progression observed in ART treated co-infected patients may in fact be due to negative consequences of treatment interruption. Even though interruption of ART has been shown to increase the risk of disease progression, in the clinical setting, it is still likely that patients will discontinue treatment for a number of reasons. Further studies to determine factors associated with ART interruption in co-infected patients would be beneficial and may assist clinicians in preventing treatment discontinuation.
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