Clinical Trial Protocol
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Title: If Hepatitis C Virus (HCV) in an Opportunistic Infection, Why has HAART not Lead to Dramatic Improvements in Liver Disease?

Abbreviated Title: If HCV is an OI why has HAART not improved liver disease?

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INTRODUCTION

Since the advent of highly active anti-retroviral therapy (HAART) there have been dramatic reductions of morbidity and mortality from virtually all causes of illness among HIV infected persons. One of the glaring exceptions to this trend is death from endstage liver disease (ESLD). Several studies indicate that the rates of liver-related deaths increased from 1.3-13% before HAART to 10-50% in the post-HAART era while other AIDS related mortality has fallen significantly. In many settings liver disease now represents the leading cause of death among HIV-infected individuals. Much of this excess has been driven by the growing epidemic of hepatitis C virus (HCV) co-infection. Due to shared routes of transmission, more than 30% of HIV infected patients are co-infected with HCV in developed countries including Canada. While there is little data on specific causes of mortality in HIV infected persons in the post-HAART era in Canada, there is reason to believe that the increasing trend in liver deaths is also being observed here. A study from Southern Alberta recently reported that the proportion of non-AIDS related deaths has increased from 7% before HAART to 32% in the post-HAART era. The leading causes of non-AIDS deaths were: accidental, including drug overdose (29%), hepatic disease (19%), non-AIDS related malignancies (19%), and cardiovascular diseases (16%).

In Montreal, we found HCV co-infected persons were at increased risk for death and hospitalisation in the post-HAART era even after taking into account antiretroviral use and its associated immunologic and HIV virologic benefits.

HCV has been shown to progress more rapidly in the context of HIV. The reasons for this altered natural history are not completely understood but include immune dysfunction from HIV infection as liver fibrosis has been correlated to lower CD4 cell counts. For this reason, HCV has been called an opportunistic infection. Consequently, it would be expected that HCV related disease should improve with the initiation HAART. Paradoxically, this has not been clearly demonstrated. While some studies have suggested that HAART, especially containing protease inhibitors (PIs) is associated with improved fibrosis rates others have seen no benefit. Several potential factors may be at play including chronic hepatotoxicity related to antiretrovirals, possible irreversibility of hepatic damage, incomplete immune recovery, alcohol use, and problems with access and/or adherence to HAART in a population with high rates of substance use. As yet no study has adequately explained why HCV co-infected persons have not realized the substantial health benefits associated with HAART. Recent Canadian and international consensus statements have identified the study of the natural history of HCV disease in patients treated with HAART as a top research priority.

Given the increasing numbers of co-infected patients presenting for care and the complexity of this condition, a full understanding of the interaction between these two chronic viral infections is essential to managing co-infection. As no centre alone has adequate numbers, we plan to use a Canada-wide prospective cohort as a platform to study how HAART impacts liver disease progression in HIV-HCV co-infection. The cohort will comprise a diverse population that will consist of persons from all populations affected by the epidemic. We will collect detailed information on important potential confounders such as drug and alcohol use, access to care and co-morbidities in addition to creating a biologic specimen bank. Our research team brings together expertise in HIV, hepatology, immunology, public health and epidemiology in a translational research program that can address the multifaceted nature of co-infection.
only will our study provide important information on the evolution of co-infection in Canada, but will also broaden the understanding of the immunopathogenesis of HCV infection and the roles HIV and HAART play through the conduct of specific sub-studies.

HYPOTHESES

Liver disease appears to be an increasing cause of morbidity and mortality in HIV-HCV co-infection despite the use of HAART. We hypothesize that while the immune benefits of HAART should lead to improved outcomes, HCV-related immune restoration is incomplete and that antiretroviral toxicities and ongoing substance use are foremost among factors thwarting the full benefits of HAART. Furthermore, liver disease progression will differ according to type of antiretroviral regimen.

BACKGROUND AND SIGNIFICANCE

Epidemiology of HIV-HCV Co-Infection

HCV infection is recognised as one of the fastest growing health problems facing industrialised countries with an estimated 170 million persons \(^{28}\) and 240,000 Canadians infected (0.8-1.8% of the population).\(^ {29}\) An estimated 10 million persons are co-infected worldwide.\(^ {30}\) As of 1999, 11,194 persons were estimated to be co-infected in Canada \(^ {31}\) and this number has likely increased substantially since. Currently, injection drug use (IDU) is the main mode of HCV transmission and is a growing risk for HIV infection in Canada accounting for an estimated 800-1,600 new HIV infections in 2002 (30%).\(^ {10}\) Women, youth and aboriginal IDU are particularly at risk for co-infection because of shared vulnerabilities. In 2002, 47% of new HIV infections in women and 63% in aboriginals were attributable to IDU.\(^ {10}\) In a recent Health Canada survey, prevalence rates of HCV co-infection among active IDU ranged from 54% in Toronto to 79% in Victoria.\(^ {32}\) The prevalence of HCV infection in other risk groups is lower but not negligible, e.g. from 4-11.7% in homosexual men.\(^ {33,34}\)

The effect of HIV on natural history of HCV

HIV infection exerts a negative impact on the course of HCV infection. Co-infected individuals progress more rapidly to liver fibrosis, cirrhosis and ESLD compared to those infected with HCV alone.\(^ {35-38}\) In studies from the pre-HAART era, the mean time to develop HCV-related cirrhosis in HIV+ individuals was as little as 7 years compared with > 20 years in HIV- individuals parentally infected with HCV.\(^ {39}\) Other studies have suggested the rates may be substantially less. For example, a small study estimated the time from HCV infection to cirrhosis to be 23 and 32 years in co-infected (n=55) and HCV mono-infected patients (n=158) respectively.\(^ {40}\) In a meta-analysis, Graham et al found the relative risk (RR) for ESLD was 2.92 (95% CI, 1.70-5.01) compared to HCV mono-infected persons.\(^ {41}\) Once cirrhosis develops, there is also a dramatic acceleration to decompensation and death (combined RR 6.14; 95% CI, 2.86-13.20).\(^ {41}\)
**Immunopathogenesis**

HCV is generally not considered directly cytopathic to hepatocytes as studies of acute HCV infection show that high levels of viremia are detectable for several weeks before the onset of hepatitis. Clinical hepatitis coincides with the appearance of virus-specific T cells in the blood and liver.\(^{42,43}\) Thus, the host immune response plays a major role both in controlling HCV infection and causing hepatocellular damage. HCV virologic control correlates most strongly with a virus specific CD8\(^{+}\) T cell response. Chronic persistent HCV infection is characterized by an ongoing weak but detectable T cell immune response that is unable to outpace viral replication.\(^{32,44}\) Intrahepatic cellular immune responses play a central role in mediating hepatocellular injury and fibrosis directly through the Fas Ligand pathway or by release of cytokines. Of these, TNF-\(\alpha\) and TGF-\(\beta\) have been shown to activate hepatocyte stellate cells, which then produce extracellular matrix that leads to fibrosis.\(^{45-47}\) In contrast, IFN-\(\gamma\) and IL-10 have been shown to be anti-fibrogenic.

**HCV as an opportunistic infection**

Given that cell mediated immunity is the primary defence against HCV infection, it has been hypothesised that immune impairment from HIV permits more rapid evolution to ESLD. In acute HCV infection, HIV co-infected persons were 70% less likely to clear HCV especially with CD4 cell counts <200 cells/ul.\(^{48}\) Furthermore, as HIV infection progresses and CD4 cell counts decline, HCV replication intensifies,\(^{49}\) suggesting that early control of HIV infection using antiretrovirals should be important in slowing the progression of liver disease. Although HAART can successfully control HIV replication, at least in the short term, HCV replication continues unabated such that liver disease may progress despite good HIV control.\(^{50,51}\) The pathogenic mechanism of the more aggressive course of HCV in the presence of HIV is still debated. A significant association of CD4 counts <50 with minimal hepatitis, and >200 with mild to moderate hepatitis, has also been reported and suggests that HIV co-infection may actually aggravate the course of hepatitis in the phase of immunocompetence.\(^{52}\) If this is the case, restoring immune function partially through administration of HAART without addressing completely the defects in cell mediated immunity may paradoxically worsen HCV-related disease. Furthermore, HCV+ patients appear to have blunted CD4 recovery on HAART (33 cells/ul lower on average).\(^{53}\) The lack of clear improvement in HCV-related disease with HAART stands in marked contrast to virtually all other opportunistic infections. Even disease from viral infections such as CMV is eliminated once CD4 cells are restored with HAART such that it is safe to discontinue secondary prophylaxis in most. Tuberculosis may be more analogous to HCV, as it affects hosts with intact immune systems. Unlike HCV however, the incidence of tuberculosis has diminished by 80% in HAART treated cohorts, although it remains more elevated than in HIV negative persons.\(^{54}\)

**HAART and liver disease**

Complicating matters, therapy for HIV in the context of HCV co-infection can be problematic. HAART itself may adversely affect HCV-related liver disease. Several reports have noted increased HCV replication associated with elevation of transaminases following the introduction of HIV therapy.\(^{55-57}\) In some cases, hepatic necrosis and inflammation and even
rapidly evolving cirrhosis with clinical decompensation, have been observed.\textsuperscript{55,56} Hepatic dysfunction following treatment with PIs has been attributed in some cases to restored anti-HCV responses.\textsuperscript{58} Although HCV levels and transaminases return to baseline after several months of therapy, they still remain abnormally high. Direct hepatotoxicity of antiretrovirals may also play a role. HAART and HCV have been shown to act synergistically in causing fulminant hepatic failure doubling its incidence. Furthermore, the risk of hepatic failure during the HAART era was several-fold higher than that in the pre-HAART era.\textsuperscript{59}

**HAART and fibrosis progression**

The true rate of hepatic fibrosis secondary to co-infection and the complex role of HAART on mitigating it are not known. This is particularly the case with more modern HAART regimens that have yet to be studied in co-infection. Several cross-sectional studies reported lower fibrosis scores among patients receiving HAART, especially those treated for longer durations.\textsuperscript{20-22} These observations have led to the recommendation that co-infected patients initiate HAART early. Other studies have highlighted the association of specific antiretrovirals, such as nevirapine, with more advanced fibrosis \textsuperscript{60} or have shown no clear benefit of HAART.\textsuperscript{23,24} In these later studies, it is possible that the short duration of HAART evaluated was insufficient to have resulted in improved histology. Intriguingly, Mehta et al reported that greater effectiveness and longer duration of HAART were independently associated with lower necroinflammatory scores, which might eventually translate into reduced fibrosis.\textsuperscript{23} Another potential reason for the failure to observe significant improvements in fibrosis is that long-term antiretroviral therapy has been associated with important metabolic complications, such as insulin resistance and hepatic steatosis. *Steatosis alone can lead to cirrhosis. Furthermore, chronic HCV infection itself (particularly genotype 3) is associated with steatosis leading to more severe inflammation and hepatic fibrosis.*\textsuperscript{61} In this setting, the expression of intrahepatic core protein was independently associated with steatosis suggesting HCV itself may play a role.\textsuperscript{62} Finally, nucleoside analogs, particularly stavudine and didanosine, have effects on hepatic mitochondrial function, in some cases leading to steatosis and fulminant hepatomegaly.\textsuperscript{63-65} The John’s Hopkins Cohort recently reported hepatic steatosis in 40% of their co-infected patients. Taken together, acute and chronic toxicities related to antiretrovirals may be negating potential immunologic benefits of HAART.

**Comorbidities**

Several other factors need to be considered when studying liver disease in co-infection. Despite the clear demonstration that alcohol plays an important role in liver disease progression and appears to be synergistic with low CD4 cell counts in increasing the rate of progression to cirrhosis,\textsuperscript{66} few studies have taken alcohol use into account when determining progression rates. Even less well studied are the effects of lifestyle and illicit drug use patterns. For example, in one study chronic marijuana use was linked to fibrosis in HCV mono-infection.\textsuperscript{67} Whether this simply represents self-medication or is exerting a pathogenic role is unclear. There is essentially no data on the impact of injection drugs, such as cocaine and heroin, which are metabolized by the liver on inflammation and hepatic fibrosis. One small study compared liver biopsies from active heroin users to ex-addicts and intriguingly suggested that heroin is responsible for an early and progressive centrolobular liver fibrosis.\textsuperscript{68} Unfortunately there was no information with respect to HCV infection in their subjects. In addition to direct effects of drugs on hepatic
function, high risk behaviours may further result in multiple exposures to HCV and other infections which could impact the natural history of co-infection.

**HCV Treatment**

Given the potential limitations of HAART, specific treatment of HCV would seem the obvious solution to prevent long-term consequences of co-infection. Unfortunately however, the current standard therapy of pegylated interferon and ribavirin results in relatively poor sustained virologic response rates (overall < 40%).\(^{27,69}\) We and others have shown that only a minority of co-infected patients have been treated for HCV in Canada (<10%) despite the existence of a universal health care system.\(^{70,71}\) If HCV therapy becomes more broadly used, there is the potential for a substantial impact on HCV related morbidity. Interestingly, a recent French study suggests ongoing liver mortality despite 40% uptake of HCV treatment in co-infection.\(^3\)

**Summary**

HAART may have both beneficial and detrimental effects on liver disease progression (see hypothesized trajectories, Fig.1). In order to better evaluate its impact, it is essential to longitudinally evaluate not only the type and duration of treatment received but also assess the contributions of substance use, sociodemographics and immunologic recovery which may unite in a complex interplay to affect outcomes in co-infection.

**LIMITATIONS OF THE LITERATURE**

The failure of the literature to provide conclusive evidence regarding the impact of HAART on HCV progression is likely due to the multitude and complexity of factors that influence the evolution of HCV and its evaluation. The most important of these are summarised below.

**Problems with evaluating liver fibrosis**

The development of fibrosis in chronic HCV infection usually follows a progressive course from portal to bridging fibrosis and eventually to cirrhosis. Liver enzymes are poor predictors of the degree of fibrosis present in chronic hepatitis. Similarly, unlike HIV viral loads, HCV viremia has no prognostic value. Liver biopsies are therefore the gold standard for staging liver disease for which several semiquantitative scores have been developed (Appendix A). In addition to the risk and expense of liver biopsy, sampling variability is a notable limitation.\(^{72}\) Also, the evolution of fibrosis in HCV is probably not linear. In reality, the course may be accelerated initially, slow down and then progress rapidly in the terminal phase. The standard way of modeling fibrosis progression divides the stage from liver biopsy performed at single time points by the time from HCV acquisition. This estimation has been shown to yield biased estimates particularly if no consideration is made for different rates of progression at different levels of fibrosis. Krahn *et al* have developed a Markov maximum likelihood model to estimate stage specific transition rates from single biopsies. In mono-infection, this method predicted true progression rates more accurately than traditional methods.\(^{73}\)
**Study Design**

The majority of studies of fibrosis in co-infection have been cross-sectional,74 relied on estimated liver fibrosis progression rates and failed to consider cumulative exposure to specific drugs included in HAART. Studies involving paired liver biopsies have been of very small sample size.75 Although some observational studies suggest HAART has had a beneficial effect on liver-related mortality, 76 they have been flawed by failing to take into account survival bias, competing risks and confounding by indication for HAART. 77 Finally, co-infected persons are often excluded from clinical trials. There have been no prospective studies evaluating the effects of HAART on hepatic endpoints.

**Characteristics of the populations studied**

Progression rates to cirrhosis in HCV mono-infection vary considerably depending on the type of cohort used for estimation. In a systematic review, Freeman et al concluded that community-based cohorts rather than liver clinic or transfusion cohorts are likely to give the most accurate estimates in HCV mono-infected patients.78 However, community-based cohorts may also not be representative due to selection biases or characteristics of participants. For example, the John’s Hopkins HIV cohort, which has arguably contributed the most to the understanding of the impact of antiretroviral therapy in HCV, is a large community based cohort but its population differs significantly from the Canadian context: 86% of patients are African Americans, 50% are heavy alcohol users and >90% HCV Genotype 1. In contrast, the Canadian epidemic has disproportionately affected women, aboriginals, intravenous cocaine users and has a high proportion of genotype 2,3. Gender, ethnicity and risk behaviours are all potentially important in driving liver disease progression and require study. Other HIV cohorts, such as the Swiss Cohort Study, only enrol subjects initiating HAART and who are therefore, by definition, a more stable group of individuals who are able to access care.

**Confounding factors not considered**

Several factors have repeatedly been shown to be associated with progression of liver disease in HCV infection, in particular age at HCV infection > 40 years, male gender, excessive alcohol use and HBV infection. Additional pathogenic factors include the necroinflammatory response and insulin resistance.79 These factors have been analysed in co-infection to varying extent. In particular, accurate alcohol and drug use information is usually lacking from retrospective studies and there is little published on pathophysiologic correlations with fibrosis progression. Furthermore, there has not been a systematic consideration of other potential confounders which may be frequent in the IDU population. For example, HCV re-infection and bacterial infections resulting from the use of contaminated injection materials both may affect immune restoration.
RATIONALE FOR A CANADIAN COHORT STUDY

Given the relatively slow course of HCV-related liver disease and the multiple potential factors that may affect its development, it is clear that long term follow-up in large numbers of patients will be necessary to truly assess the impact of HAART on clinical hepatic outcomes. This impact cannot be adequately evaluated by randomized clinical trials given their short duration of follow-up and general exclusion of individuals with hepatic co-infection or vulnerable populations. The Canadian Co-infection Cohort Study proposed will be uniquely positioned to assess this epidemic in novel ways. We will recruit from a variety of centres across the country in an attempt to reflect the Canadian epidemic. We have specifically sought to include individuals who may be extremely marginalized (L’Equipe Mobile), access various models of care (specialty clinics, directly observed therapy programs, outreach programmes) and have diverse risk profiles (e.g. active and ex-IDUs, women, aboriginals, hemophiliac populations; Appendix B). The data collected will be comprehensive and our study design and analysis plan attempts to minimize biases associated with observational studies. The total sample size and duration of follow-up will be sufficient and greater than previously reported studies to ensure enough power to detect small relative hazards and to allow for subgroup analyses (see details below). Finally, the study team brings together a unique blend of expertise that will permit new and important contributions to the understanding of co-infection from fundamental pathophysiology to sociobiology in Canada and beyond which will have direct implications for the health of co-infected persons. For example, many of our co-investigators participate in the co-infection working group at the Canadian HIV Trials Network. Discoveries made through this cohort may thus be translated into the development of clinical trials evaluating novel therapeutic interventions for co-infected persons.

OBJECTIVES

The primary objective of this study is to determine the effect of HAART on liver disease progression in HCV-HIV co-infection. We will evaluate the contributions of important social factors, toxicities and immunologic factors that may modify fibrosis progression rates. Because of the need to easily follow patients and intervene prior to the onset of ESLD, we will also focus on the role of non-invasive markers of hepatic fibrosis in predicting disease progression.

Specific Aims:

1) Establish a Canadian multicentre prospective cohort study of HCV-HIV co-infected persons and follow the cohort for at least 5 years

2) Estimate the effect of HAART on progression to ESLD in our cohort
   a. Measure the rates of liver disease according to receipt of HAART
   b. Compare rates of liver disease according to types and duration of HAART.

3) Identify factors that contribute to liver disease progression in HIV-HCV co-infection
   a. Describe factors associated with the access/receipt of HAART and its patterns of use
   b. Examine effects of alcohol and drug use on liver disease progression
   c. Examine the rates of chronic toxicities, specifically hepatic steatosis and insulin resistance, according to HAART use
d. Perform a detailed immunopathogenesis substudy to assess peripheral blood and liver HCV- and HIV-specific immune responses in HAART treated vs. untreated individuals

4) Adjust rates of ESLD for covariates that may play a role in liver disease progression.

**Secondary Aims:**

1) Develop methods for evaluating fibrosis progression rates in co-infection
   a. Estimate fibrosis progression rates using Markov maximum likelihood modeling according to duration of HAART exposure and accounting for covariates
   b. Validate non-invasive markers for predicting fibrosis progression comparing their performance to rates obtained from single and serial liver biopsies/clinical outcomes.

2) Establish a tissue bank of peripheral blood mononuclear cells (PBMC), plasma and liver tissue for additional research questions concerned with immune function, viral dynamics, and mechanisms of fibrosis.

**Long-term aims:**

We will focus on investigating means of slowing liver disease progression rates in co-infection. In particular, we will evaluate the role of HCV treatment in the evolution of liver disease with a particular emphasis on evaluating access to treatment, predictors of response and comparing responders vs. non-responders. The cohort will serve as a research network for additional questions important to understanding co-infection and related health outcomes. A cross-disciplinary approach will be encouraged by engaging a variety of researchers (i.e. virology, psychiatry, health services etc.).

**PRELIMINARY WORK**

**Prospective Clinical Cohort of HIV+/HCV Co-infected Patients**

A pilot project, prospective clinical cohort of HIV+/HCV co-infected patients, supported by the FRSQ Réseau SIDA-MI was established. Questionnaires were developed and tested in English and French (Appendix C). Recruitment began in 2002 at the Montreal Chest Institute (M. Klein) and Hôpital Notre-Dame (D. Rouleau). The Montreal General Hospital IDTC (J. Cox) began enrolment in April 2004. Participants undergo evaluation as indicated below. To date, 158 patients have been recruited, and 840 plasma and 968 PBMC specimens have been banked. A relational database using Teleform scanning software has been developed. The average follow-up is 2 years. Baseline data on the full cohort and 84 (53%) patients with follow-up have been analyzed. There have been 8 deaths and 1 withdrawal from study. Despite the social barriers faced by our participants, only 3 (2%) have been lost to follow-up. Baseline characteristics of the cohort are shown in Table 1. Of note, 77% were taking antiretrovirals (mean duration 1.7 years) and 10% were antiretroviral naïve. Among naïve patients with available follow-up, 37% began HAART. A comparison of baseline characteristics of naïve and HAART-treated patients is shown in Table 2. The mean time since HCV exposure is 17.9 years. A diagnosis consistent with ESLD had occurred in 14 subjects (10%) before their baseline visit. Four (4.8%) developed a
first outcome consistent with ESLD during follow-up including one death. Interestingly, 11/14 people diagnosed with liver disease at baseline, and all of those developing ESLD in follow-up were receiving HAART. Only, 21(12%) have received HCV treatment. Thus we have clearly demonstrated the feasibility of maintaining a cohort study with a population of patients that is traditionally considered difficult to follow. In addition, we obtained valuable information which can be used to estimate expected rates of exposures and outcomes for power calculations, as well as for, planning and anticipating the logistics required to conduct a larger Canadian study.

Examples of some of the work done to date include a study on health indicators of the first 62 patients enrolled.25 Our findings highlighted that HIV-HCV co-infected individuals face numerous barriers that may act synergistically to impact health. Commonly observed factors included poor socioeconomic status, active IDU, alcohol, smoking and psychiatric illness. Recently, we evaluated the contribution of various addictions, lifestyle and physiologic factors on baseline hepatic fibrosis in HIV-HCV co-infection in a cross-sectional analysis.80 Multivariate analyses indicated that IDU, duration of HCV infection, cigarette and marijuana smoking were not associated with hepatic fibrosis. Conversely, hepatic fibrosis was associated with CD4 count (OR 0.43 per 100 cell increase, p=0.03), alcohol use (OR 9.0, p=0.05), and BMI (OR 1.3, p=0.07). No contribution of other addictions or lifestyle factors was observed. Samples collected from subjects who are persistently HCV PCR negative are being used to study immunologic factors associated with HCV clearance in Dr. Nicole Bernard’s laboratory. In collaboration with Dr. Brian Ward, we are using plasma samples obtained from the cohort to analyze protein expression patterns according to fibrosis levels using SELDI-TOF.

**Validation and application of the APRI to study liver fibrosis in hepatitis co-infection**

Non-invasive markers have been validated as predictors of significant liver fibrosis in HCV mono-infection. Most of the models developed involve measurement of non-routine laboratory tests or use complex or proprietary formulas.81 Using data from our cohort, we demonstrated that a simple model, the AST to platelet ratio index (APRI)82 is highly predictive of significant fibrosis in HIV/HCV co-infection.83 Forty-six co-infected patients who underwent liver biopsy and had concomitant laboratory measurements were studied. For significant fibrosis, the area under the ROC for the APRI was 0.847±0.057 (Fig. 2). APRI scores > 1.5 (higher cut-off) were 100% specific and 52% sensitive; PPV 100% and NPV 45%. In a retrospective cohort study, we subsequently evaluated the evolution of the APRI, determined its predictive value for hepatic outcomes in HIV+ patients with chronic HBV and HCV co-infection, and assessed the effect of HAART on the progression rate of liver fibrosis (84 and submitted). 708 HIV+ patients without liver complications at baseline (520 HIV+ only, 133 HCV+, 55 HBV+) were followed over 14 years (median 4.7 years). The trend in APRI over time was estimated using repeated measures multiple linear regression. Natural log of APRI (lnAPRI) was modeled adjusted for age, sex, time HIV seropositive, HAART, CD4 cell count, and HIV RNA. At baseline, both HCV+ and HBV+ had higher APRI compared with HIV+ (1.01, 0.95, respectively, vs. 0.52, p < 0.0001). APRI changed significantly over time, rising an average 0.07 units/year in HCV co-infection. This translated to an estimated time to develop significant fibrosis from baseline in HCV+ of 7.5 years. Baseline lnAPRI was a strong independent predictor of liver complications (adjusted HR: 3.45, 95% CI 1.99 -6.12) as was HCV (adjusted HR: 4.19, 95% CI 1.19-14.87). Cumulative time on HAART did not protect against liver complications and protease inhibitor-
based HAART was actually significantly associated with progression of APRI in viral co-infection and HIV alone (Fig. 3). Thus, APRI has the potential to be a marker for progression of liver disease and may be useful in predicting hepatic complications. In order to validate our findings and tease out independent effects of various HAART regimens, including the nucleosides, studies in larger populations are necessary.

**Evaluating immunopathogenic mechanisms of disease progression in HIV/HCV co-infection**

Dr. Ostrowski’s laboratory has been successful in characterizing ex vivo intra-hepatic T cells taken from biopsies of HCV mono-infected and HCV/HIV co-infected individuals without having to non-specifically expand them in tissue culture. Using peptide mega-pools containing peptides spanning the entire genomes of HIV (clade B) and HCV (subtype 1a or 1b) to identify HIV or HCV specific T cells they found that significant numbers of HIV-specific T cells are being deposited in the liver of co-infected individuals, and thus are likely contributing to bystander activation. Representative experiments are shown in Figs. 4-5. Compared to HCV mono-infected individuals, there are about 10-fold more virus specific TNF-α producing T cells within livers of co-infected patients, indicating more intense inflammatory activity. The individuals studied were not receiving antiretrovirals. It will be important to determine whether HAART affects the immune milieu of the liver in co-infection.

Similar analyses on PBMCs have been performed. Generally, and in keeping with previous work, IFN-γ immune responses to HCV are weak to undetectable in peripheral blood whereas HIV specific responses are stronger. However, TNF-α specific T cell responses against both viruses were substantially more intense. Since TNF-α is a fibrogenic cytokine, it will be important to study the effect of TNF-α viral-specific responses on disease progression in this cohort and also to examine whether specific immune responses in peripheral blood correlate with intra-hepatic immune responses and how HAART affects these responses. Recent data also suggest the potent HCV specific CD4 immune responses particularly those producing IL-2 correlate with HCV control. The large collaborative group proposed here will be in a position to ask whether IL-2 specific immune responses can predict control of HCV disease and whether HAART modifies this.

**METHODS**

**General Overview of Study Design—Establishment of the Cohort**

Patients will be identified from existing clinic populations by their primary care physicians or when referred for evaluation. Most clinics routinely screen all HIV infected patients for HCV. All eligible patients will be approached. Inclusion criteria are broad to avoid limiting future research questions.

**Eligibility criteria:**
1. Age >16 years old (i.e. adults, may vary according to provincial criteria)
2. HIV seropositive (ELISA with western blot confirmation)
3. HCV infected or evidence of exposure (HCV-seropositive by ELISA with RIBA II or EIA confirmation, or if serologically false negative, HCV RNA positive).*
4. Able to provide informed consent

Patient follow-up:

Subjects will undergo an initial evaluation and then follow-up visits every 6 months (± one month). Visits may be scheduled specifically for the study or incorporated into routine follow-up. Medical information will be collected using questionnaires (Appendix C). All patients will provide informed consent prior to enrolment into the cohort.

Initial evaluation will be aimed at:
1. Establishing diagnosis and reviewing other medical conditions that may impact of liver function (i.e. HBV infection, alcohol, iron overload, autoimmune disease, medications etc.)
2. Review of past medical history with specific attention to hepatic and HIV-related diagnoses, HIV-and HCV-specific treatments and vaccinations, nadir CD4, highest HIV viral load, BMI
3. Review of past and present substance abuse and risk behaviour and assessment of quality of life

Tests to be performed at each visit (except where indicated by †):
1. Liver profile (ALT, AST, GGT, alkaline phosphatase, total bilirubin, albumin, PT/PTT/INR)
2. Complete blood count, biochemistry and fasting lipid profile
3. Fasting glucose and insulin
4. Serum alpha-feto protein, cryoglobulin levels and c-reactive protein†
5. Autoimmune markers (i.e. immunoglobulins)†
6. Hepatitis A and B serology (if not documented within 1 year of study entry)
7. Serum ferritin †
8. Plasma HIV RNA
9. Lymphocyte subsets (i.e. absolute and relative CD4, CD8 cell counts, and CD4/CD8 Ratio)
10. Plasma HCV RNA (qualitative and if positive, quantitative†)
11. HCV genotype†
12. Plasma, serum and cell sample for storage
13. TSH, and when TSH is abnormal evaluate T3, T4

Additional information collected will include:

1. Detailed information on sociodemographics, drug and alcohol use and injection behaviours.
2. EQ5D (quality of life measure) and SMAC adherence questionnaire
3. Detailed information on causes of death are to be collected using the Coding of Death in HIV (CoDe) system. Sites are also asked to link to provincial death registries/ vital statistics to determine precise causes of deaths from death certificates in patients known to have died and to determine whether patients lost to follow-up are deceased.

* Serologic anti-HCV positive but HCV RNA negative will also be included in the study
† Tests that are only performed once, at baseline visit
4. Endstage liver disease are similarly verified using a specific data abstraction form. An endpoints committee will be in charge of reviewing endpoints and adjudicating in cases where an outcome is uncertain.
5. Weight, height, waist circumference and blood pressure.

Site descriptions/Data source:
ADD NEW SITES
McGill University Health Centre (Montreal Chest Institute and Montreal General Hospital), Centre Hospitalier de l’Université de Montréal (Hopital Notre-Dame, L’Equipe Mobile), Clinique du Quartier Latin (Montreal, QC), The Ottawa Hospital-General Campus (Ottawa, ON), Toronto General Hospital/University Health Network (Toronto, ON), Sunny Brooke Hospital (Toronto, ON), Hamilton Health Science (Hamilton, ON), The Haven Program (Sudbury, ON), Windsor Regional Hospital Metropolitan Campus (Windsor, ON), Capital District Health Authority (Halifax, NS), Southern Alberta Clinic (Calgary, AB), Downtown Infectious Disease Clinic/Pender Clinic (Vancouver, BC), BC Centre for Excellence/ St. Paul’s Hospital (Vancouver, BC) and Oak Tree Clinic (Vancouver, BC). These sites comprise diverse patient populations and modes of practice. Together the sites follow approximately 1705 co-infected patients of whom 950 are expected to be recruited in the study time frame (Appendix B).

Data collection/sample storage:
A unique identifier will be assigned to each participant. The identifier encodes information that can be used to non-nominally identify subjects who may have moved to access care at one of the other sites. Patients may complete questionnaires alone or with the aid of the research nurse. Supplementary information will be abstracted from medical records and laboratory reports. Data will be scanned and verified into a password protected computerised database (Teleform® software). All data will be handled confidentially. Plasma, serum and PBMCs will be collected using standard isolation techniques. Individuals undergoing liver biopsy as part of routine care will be asked to consent to have a second sample procured for tissue banking at centres with capacity to perform and store these samples. Plasma and cells will be aliquoted and frozen. All samples will be logged using non-nominal identifiers and will be destroyed after 15 years. The purpose of sample collection is to perform tests that will enable the study of biologic variables associated with clinical outcomes and responses to therapy in dual infection. These may include, but are not limited to: isolation of HCV and HIV virus (e.g. for quantification, genotyping, evaluation of viral quasispecies), measurement of immune responses to both viruses (e.g. cytotoxic T-lymphocyte assays, cytokines), and evaluation of host factors associated with clinical course (e.g. HLA typing). Additional testing on these samples may occur as new technologies are developed for the study of chronic immune and viral diseases. The results of these tests will not be made available to the subjects or their physicians as they are considered experimental at present.

Definitions for specific aims
Population: Only patients that have documented chronic infection (i.e. HCV RNA positive) will be included in the primary analyses. Subjects with triple infection with Hepatitis B will be excluded (i.e. HBSAg positive) as will subjects who have received HCV therapy. Exploratory
subgroup analyses will be performed however to examine rates of liver outcomes in HCV-exposed individuals who are persistently HCV RNA negative and in triply infected patients.

**Duration of HCV infection:** We will use age at first injection or parenteral exposure to estimate duration of HCV infection in those with these risk factors. It has been shown that IDU and blood product recipients who acquire HCV do so within the first 5 years of exposure, with a weighted average of 3.4 years.\(^\text{86,87}\) For subjects with other risk factors, we will use the date of presumed infection provided by the patient as a proxy.

**Exposure measures:** HAART will be broadly defined as at least 3 antiretrovirals taken concurrently for at least 30 days. Types of HAART will be classified for sub-analyses as PI-based, NNRTI-based, combined PI and NNRTI, or triple nucleosides. As the effects of HAART are likely to depend on duration of exposure we will also examine cumulative time on treatment in various ways (see below).

**Outcome measures:** The primary outcome measure will be the development of a diagnosis consistent with ESLD defined as any one of the following: cirrhosis, portal hypertension, esophageal varices, hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, hepatorenal or hepatopulmonary syndrome, hepatocellular carcinoma or death due to liver cirrhosis.

**Rationale for choice of control group:** While the superiority of randomized controlled trials for evaluating efficacy of treatments is not debated, the sample sizes required, duration of follow-up and ethics of randomizing individuals to delay treatment are significant limitations in addressing our objectives. The question is not whether or not to treat co-infected patients with HAART at advanced levels of immune impairment (i.e. CD4<200) where the risk of HIV disease progression clearly warrants it, but rather: what is the best course of action during relative immunocompetence (i.e.CD4 cell counts between 300-500) where many individuals elect to defer therapy as per current guidelines (perhaps even more the case in co-infection where there are concerns re adherence, resistance and toxicities). We could choose to study only individuals initiating HAART but this would require enrolling significantly greater numbers than presently proposed as we estimate only 10% initiate HAART per year. Thus, we have elected to use untreated patients as a control for HAART exposed. While there are obviously differences with respect to the stage of HIV at the time of HAART initiation between the groups, in other respects they are more similar than dissimilar (see table 2). Importantly, they share the same duration of HCV infection one of the strongest driving factors for HCV progression and CD4 cell counts at cohort entry are very similar. Naïve patients who subsequently initiate HAART will contribute observation time both to the untreated group then to the treated group.

**Time zero:** For the primary analysis time zero will be time at cohort entry.

**Hepatotoxicity:** Defined according to ACTG criteria for grade 3 or 4 hepatotoxicity using serum ALT and AST levels and direct bilirubin.

**Insulin resistance:** Estimated using the homeostasis model assessment model (HOMA-IR= Fasting Insulin (\(\mu\text{U/mL}\)) \(\times\) Fasting Glucose (mmol/L)/22.5).\(^\text{88}\)
Liver histopathology: Scored by local pathologists using standard semiquantitative scores which grade inflammation and fibrosis separately (Appendix A). We will request quantification of percent of hepatocytes exhibiting steatosis. The results from all biopsies performed will be converted to a single scoring system for the purposes of modeling progression. A subset of participants initiating HAART will be asked to undergo a second liver biopsy after 1 year of therapy to evaluate histology longitudinally. For the immunopathogenesis substudy however, a single pathologist experienced in liver histology will read all biopsies blinded to treatment status.

Non-invasive markers Initially we will focus on the APRI \( [100 \times (AST \text{ / upper limit of normal})/\text{platelet count (10}^9/L)] \) and the FIB-4 \( [\text{Age (years)} \times AST (U/L)/ [\text{platelet count (10}^9/L) \times \text{ALT}^{1/2}]] \) \(^8^9\). Significant fibrosis will be defined as F2-F4 using Batt and Ludwig scoring.\(^9^0\)
DATA MONITORING/VALIDATION

Liver outcomes and all deaths will be verified against source documents to ensure accurate capture of data. Because no standardized and objective diagnostic criteria for the major complications of liver disease exist (identified as a research priority by NIH), we will use literature guided definitions. A working group of the steering committee will be struck to review all outcomes in a blinded fashion and develop diagnostic criteria that may be applicable in other settings.

ANALYSES

Primary cohort analysis

The cumulative incidence of ESLD and liver deaths will be calculated using the Kaplan-Meier survival method, comparing subjects who have received HAART to those never treated. Cox proportional hazards models will be used to estimate the crude and adjusted relative hazards for reaching the primary endpoint. Multivariate analyses will include adjustments for potential confounders and study centre. Given that untreated patients are likely to be less advanced with respect to disease stage than those on HAART, we will pay particular attention to prognostic factors including baseline CD4+ cell count, nadir CD4 cell count, HIV viral load (baseline and set-point) and estimated time from HCV acquisition. We will likewise assess the contributions of traditional risk factors for fibrosis such as, age at HCV acquisition, gender and level of alcohol intake as well as other factors such as, risk for HIV/HCV acquisition, substance use, HCV genotype, level of HCV RNA, BMI, insulin resistance and adherence. Changes in CD4 cell counts and HIV RNA will be handled as time-dependent covariates. The percentage of time spent on mono, dual or triple therapy will be determined and also included as a time dependent covariates to permit the evaluation of patients who interrupt treatment for various periods of time. Additional analyses include stratification by type of HAART (PI vs. NNRTI).

To address potential biases inherent in these methods when applied to time-varying covariates and competing risks we will also apply modern statistical techniques. Marginal structural models (MSM) were originally introduced by Robbins and colleagues and provide a powerful tool for estimating the causal effects of a treatment. They are useful for the analysis of longitudinal data in which each subject's treatment and covariate history are measured over time, and an outcome is recorded at a final time point. A modification of these techniques has been proposed by van der Laan and Peterson termed history-adjusted MSM (HA-MSM) which further allow adjustment by time-dependent covariates, thus permitting estimation of time-dependent effect modification. HA-MSM have been applied to the study of antiretroviral therapy. Our parameter of interest will be difference in rates of ESLD in those treated with HAART compared to those not treated (see Fig. 7-8). To deal with time dependent confounding we will employ the inverse probability of treatment weights estimator in a pooled logistic regression model to estimate the causal effect of treatment on outcomes. We will also employ HA-MSM to evaluate the causal effect of treatment given past history of treatment, and use double robust estimators, to evaluate the effect of dynamic treatment regimens. Structural Cox models will be used to evaluate the causal treatment effect. MSM will be analysed using R programming language. Another potential pitfall in studying liver outcomes in HIV-HCV co-infection is that non-liver disease mortality rates in this population are high (i.e. violent deaths,
suicides and overdoses etc.). Failure to take into account competing risks could lead to biased estimates in the rates of liver disease progression. Competing risks can be handled to some extent by using an incidence density measure instead of cumulative incidence, thus we will also determine incidence density ratios for ESLD.

**Secondary Analyses**

We will describe rates of hepatoxieties, insulin resistance and other toxicities over time according to HAART exposure and class. These rates will be correlated to inflammation and fibrosis stage in individuals with liver biopsies and to long-term clinical outcomes.

**Prospective, longitudinal assessment of HCV and HIV specific T cell immunity**

Cryopreserved PBMC, obtained at 6 month intervals over at least one year in 50 individuals initiating HAART, will be used to quantify viral specific T cells producing IL-2, TNF-α and IFN-γ using a flow cytometrically based intracellular cytokine staining methodology as described. These assays will be novel in that IL-2, which is a good measure of CD4 T cell proliferative function, and TNF-α, which may reflect fibrogenic potential, have not been well characterized in such a cohort. Peripheral immune responses in subjects receiving HAART (PI or NNRTI) for varying periods of time will be compared to those never treated accounting for important prognostic factors. In conjunction with a CANFAR grant, we will also do a liver biopsy sub-study in patients recruited from Toronto as fresh samples are required. These studies aim to systematically characterize HCV and HIV-1 specific T cell immune responses and correlate them with markers of hepatic disease (inflammation and fibrosis scores). Individuals who are fully suppressed (plasma HIV RNA<50 copies/ml) on HAART will be compared to untreated patients.

**Fibrosis progression**

Rates of fibrosis progression will be modeled from liver biopsies using Markov maximum likelihood models for liver fibrosis developed by Krahn and then correlated to non-invasive measures made at time of biopsy. Briefly, this method represents HCV prognosis using a Markov model as a series of health states F1 through F4. Transition rates (time taken to pass from one state to next) between states are estimated using maximum likelihood procedure to obtain the most likely parameters for stage transition probabilities, given the observed data (stage distribution and time elapsed). Dr. Krahn has offered to collaborate in providing detailed methods and advising on their use (see letter of support). To validate non-invasive markers, we will use SAS PROC LOGISTIC to calculate the area under the ROC curve (AUC) with standard errors according to methods of Hanley and McNeil. Sensitivities, specificities, positive predictive values and negative predictive values will be compared using cut-offs previously identified in the literature (i.e. APRI>1.5 for significant fibrosis, >2 for cirrhosis and <0.5 for ruling out significant fibrosis). Because current indices have been limited in their ability to discriminate between middle grades of fibrosis, we will also attempt to develop a more sensitive non-invasive formula that relies on clinical and biochemical data collected through this cohort. Univariate correlations of biopsy fibrosis stage with clinical and laboratory markers will be assessed using Spearman correlation. We will then use multiple logistic regressions to assess the
independent discriminative value of selected variables and construct indices that combine the most discriminatory variables. We will validate the diagnostic value of each formula by ROC AUC as above against categories of fibrosis.

**SAMPLE SIZE/POWER BASE**

The event rate for the primary outcome will primarily be affected by the time to develop the outcome from cohort entry, the rate of accrual and duration of subsequent follow-up in the cohort and the number of controls per treated subject. We have based our sample size calculations on the event rate of 4.7% in median follow-up of 2 years that we have observed in the pilot project yielding an overall expected event rate of 10-15% in 5 years. The anticipated proportion of subjects who will be exposed to HAART is 70%. These rates are consistent with data we have observed in our retrospective cohort study where ESLD occurred in 1.0/100 person years in HCV infected (Fig. 8) and with other projections from the literature. We hypothesize that net effect of HAART should be positive, but will not reduce progression rates to the level seen in mono-infection. With a sample size of 950 patients (650 exposed to HAART) we can detect a relative risk (RR) of ESLD among HAART treated patients relative to untreated controls of 0.48 with 80% power and a type I error probability of 0.05 (see Table 3). In other words, the corresponding RR of ESLD in untreated patients compared to HAART exposed is 1.65 (as a point of reference HIV-HCV infection has a RR of 3 for progression to ESLD compared with HCV mono-infection). The impact of changing the event rate on detectable RR is illustrated in Table 3, as are detectable RR if the net effect of HAART turns out to be hazardous. From a survey of the participating centres it is estimated that 70% of patients treated with HAART are on PI based regimens. Our sample size thus provides 80% power to detect a RR of 0.37 comparing PI to NNRTI based regimens. The large sample size should likewise afford the possibility of exploring intra-class differences (such as nevirapine vs. efavirenz or stavudine vs. other nucleosides).

If HAART restores HCV immunity, very low (<1%) peripheral HCV responses should increase towards levels seen in HCV mono-infection (up to 12%). To detect a modest increase of 2% (σ = ±5%) in HCV specific CD8s from pre-HAART baseline at one year, 50 individuals initiating HAART will be studied (paired t-test). For the fibrosis progression studies, we anticipate from the pilot study and from participating centres, that 30-40% will undergo biopsies in the course of the study thus we will be able to evaluate fibrosis and steatosis scores in liver biopsies from 300 individuals.

**STUDY TEAM AND MODE OF FUNCTIONING**

Dr. Klein will have overall responsibility for conduct of the project and will preside over the study steering committee. She has broad training (MSc Epidemiology and a CTN Clinical Associateship), and has significant experience in the conduct of multicentre clinical trials in HIV and HIV-HCV co-infection and observational research. The co-applicants represent a wide variety of health professionals with expertise in the fields of HIV/HCV, hepatology, public health and epidemiology. Specifically, Drs. Conway, Cooper, Côte, Haidar, Rouleau, and Walmsley are experts in the management of HIV and HCV co-infected patients. Dr. Cox conducts HIV and HCV surveillance studies at the Montreal Public Health Department; he is
interested in psychosocial determinants of bloodborne virus transmission risk behaviours in IDU and MSM populations. Dr. Wong is a hepatologist treating co-infected patients. Dr. Ostrowski has trained in immunology in Dr. Fauci’s laboratory and has recently been awarded a grant from CANFAR to study intrahepatic immune responses in HCV and HIV/HCV infection. Dr. Suissa, a pharmacoepidemiologist with an extensive track record studying medication adverse effects, will guide statistical analyses. Dr. Nitika Pai, currently completing her PhD in Epidemiology at the University of California, Berkeley, has pursued specific training in HA-MSMs and will be joining Dr. Klein as a post-doc in 2006. In addition to the above, the study steering committee will include a member from the HIV-HCV infected community. The committee will be responsible for overseeing the progress of the study, defining the research agenda, forming working/writing groups to prepare data for presentation or publication and approving new study questions and entering into collaborations with other Canadian or international researchers. All manuscripts will acknowledge that the data were collected through the Canadian Co-infection Cohort Study and credit all collaborating institutions and financial support received. Authorship will include each person participating on a writing committee who makes substantive contributions to the conception of the work, design of the analysis, interpretation and content of the data. Investigators participating in the study remain free to request data from their respective sites to use as they wish.

TIMELINES

Recruitment to the cohort is targeted over the first 2 years of the study with follow-up continuing thereafter. In the first 2 years, methodology and modeling will be developed and tested on data already acquired in the pilot phase. In the third year we will describe baseline characteristics, covariates, patterns of HAART use and short term clinical outcomes. Studies on fibrosis progression will take place in year 3-4. In the final year the primary analyses will be conducted, reported and submitted for publication.

ETHICS

The final protocol, consent form and any subsequent protocol amendments or revised consent forms will be reviewed and approved by the Ethics Committee of each participating centre prior to implementation. All participants (or their legal guardian) will receive detailed oral and written information on the study and are required to sign an Informed Consent prior to entry into the cohort. Participation is entirely voluntary and subjects are free to withdraw at any time.

STRENGTHS AND WEAKNESSES

Our large sample size, comprehensive and long-term follow-up will enable us to determine with precision the rate of liver progression in HAART exposed versus unexposed patients. All observational research faces challenges in large part due to the non-random allocation of treatments. Although we will make careful ascertainment of covariates as appropriate to control for important potential confounders, try to be as inclusive as possible and will employ sophisticated analysis strategies to minimize biases, we may not be able to completely avoid all pitfalls. Certainly losses to follow-up and missing data will occur but we have demonstrated that this can be minimized even in this patient population. We have targeted
an ambitious recruitment over 2 years which we feel is feasible given demonstrated ability to recruit at this level in the pilot phase, the easy integration of the visits into routine clinical care for the majority of subjects and our positive experience with willingness of patient participation. Furthermore, more intensive studies target far fewer individuals. We anticipate the possibility that fewer outcomes will be observed than predicted despite our preliminary findings especially if over the course of the study new HAART regimens become more effective and less hepatotoxic or more patients are successfully treated for HCV. Although these interventions may be on the horizon, their incorporation into routine clinical practice will likely be longer than this initial study period. We will however monitor event rates and adjust our research plan if necessary. We feel confident that our data collection and design will allow us to redirect to study a myriad of important questions should this become a problem.

**IMPLICATIONS AND FUTURE DIRECTIONS**

In order to present a comprehensive and focused research plan we have chosen to limit the present proposal to one overriding research theme: the effects of HAART on progression of HCV related disease. We feel that this question is of immediate importance particularly for directing the timing and nature of HIV treatment in this population. If HAART is indeed associated with improved rates of fibrosis progression, then it becomes imperative to initiate HAART early in HCV co-infection to avoid the consequences of ESLD, a fatal condition for which there are few treatment options. If on the other hand, HAART fails to improve liver outcomes due to incomplete immune restoration or cumulative toxicities, this shifts emphasis to earlier HCV treatment and has implications for the development of newer safer HIV treatments. Furthermore our study should provide important insights into the relative contributions of other factors that may modify disease progression thus allowing targeted health interventions that may benefit co-infected persons. Clearly the richness of the data acquired and the expertise of the investigators assembled through this cohort will permit in depth studies of many additional aspects of co-infection. Our group has identified HCV treatment as an emerging priority for subsequent work. We will also be well positioned to address the social determinants of health outcomes, make comparisons between models of care, and evaluate quality of life issues and thus contribute knowledge that will be essential for managing this complex condition. We have therefore provided a mechanism whereby additional projects may be assessed and undertaken by members of the steering committee or outside collaborators interested in this growing epidemic with the goal of improving the care and treatment of co-infected persons.
TABLES AND FIGURES

Table 1. Baseline characteristics of the 158 subjects enrolled in the Quebec Co-infection Cohort Pilot Study

<table>
<thead>
<tr>
<th>Demographics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>122 (77%)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (20%)</td>
</tr>
<tr>
<td>Transsexual</td>
<td>4 (2.5%)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>43 ± 8 [19–64]</td>
</tr>
<tr>
<td><strong>BMI (n=106)</strong></td>
<td>24.4 ± 5.5 [15–55]</td>
</tr>
<tr>
<td><strong>Completed post-secondary education</strong> (CEGEP or university)</td>
<td>45 (29%)</td>
</tr>
<tr>
<td><strong>Welfare recipient</strong></td>
<td>109 (69%)</td>
</tr>
<tr>
<td><strong>No fixed address</strong></td>
<td>18 (8%)</td>
</tr>
<tr>
<td><strong>HIV/AIDS</strong></td>
<td></td>
</tr>
<tr>
<td>Probable route of HIV transmission (patient reported)</td>
<td></td>
</tr>
<tr>
<td>IDU</td>
<td>70 (44%)</td>
</tr>
<tr>
<td>Sex</td>
<td>58 (37%)</td>
</tr>
<tr>
<td>IDU/Sex</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Transfusion</td>
<td>17 (11%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (4%)</td>
</tr>
<tr>
<td><strong>Time since HIV Dx (yrs)</strong></td>
<td>9.6 ± 5.7 [0.2–22]</td>
</tr>
<tr>
<td><strong>Nadir CD4</strong></td>
<td>212 ± 170 [0–981]</td>
</tr>
<tr>
<td><strong>Baseline CD4</strong></td>
<td>395 ± 219 [20–985]</td>
</tr>
<tr>
<td><strong>Highest viral load (log10)</strong></td>
<td>4.6 ± 1.2 [1.7–7.9]</td>
</tr>
<tr>
<td><strong>Baseline viral load (log10)</strong></td>
<td>2.6 ± 1.3 [1.7–5.6]</td>
</tr>
<tr>
<td><strong>Past AIDS</strong></td>
<td>35 (23%)</td>
</tr>
<tr>
<td><strong>Baseline ARV</strong></td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>98 (63%)</td>
</tr>
<tr>
<td>Mono/Dual</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Treatment interruption</td>
<td>14 (9%)</td>
</tr>
<tr>
<td>Naïve</td>
<td>16 (10%)</td>
</tr>
<tr>
<td><strong>Hepatitis</strong></td>
<td></td>
</tr>
<tr>
<td>Probable route of HCV transmission (patient reported)</td>
<td></td>
</tr>
<tr>
<td>IDU</td>
<td>86 (54%)</td>
</tr>
<tr>
<td>Sex</td>
<td>23 (15%)</td>
</tr>
<tr>
<td>Sex/IDU/Tattoo</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Transfusion</td>
<td>19 (12%)</td>
</tr>
<tr>
<td>Other (needle stick, fighting, tattoo)</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>19 (12%)</td>
</tr>
<tr>
<td><strong>Time since HCV Dx (yrs)</strong></td>
<td>6.6 ± 5.6 [0–30]</td>
</tr>
<tr>
<td><strong>HCV genotype (1 or 4)</strong></td>
<td>66/86 (77%)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td><strong>HCV PCR</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>124 (78%)</td>
</tr>
<tr>
<td>Negative</td>
<td>22 (14%)</td>
</tr>
<tr>
<td>Not reported</td>
<td>12 (8%)</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>67 ± 83 [11–725]</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>75 ± 93 [10–701]</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>206 ± 100 [3–781]</td>
</tr>
<tr>
<td><strong>APRI</strong></td>
<td>1.68 ± 4.48 [0.16–50]</td>
</tr>
<tr>
<td><strong>Hepatitis B sAg+</strong></td>
<td>11/130 (8%)</td>
</tr>
<tr>
<td><strong>Advanced liver disease (yes)</strong></td>
<td>12 (8%)</td>
</tr>
</tbody>
</table>

**Substance use and other comorbidity**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>IDU (last 6M)</strong></td>
<td>51 (33%)</td>
</tr>
<tr>
<td><strong>IDU (ever)</strong></td>
<td>125 (79%)</td>
</tr>
<tr>
<td><strong>Time since 1st IDU (yrs; n=139 hmmm number problem)</strong></td>
<td>17 ± 8.7 [3–35]</td>
</tr>
<tr>
<td><strong>Current alcohol use</strong></td>
<td>78 (50%)</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>110 (72%)</td>
</tr>
<tr>
<td><strong>Pack-years</strong></td>
<td>22 ± 19 [0–131]</td>
</tr>
<tr>
<td><strong>Marijuana use (&gt;= once per week)</strong></td>
<td>44 (28%)</td>
</tr>
<tr>
<td><strong>Ever diagnosed with depression</strong></td>
<td>43 (29%)</td>
</tr>
<tr>
<td><strong>Ever admitted to psychiatric institution</strong></td>
<td>28 (19%)</td>
</tr>
</tbody>
</table>

Categorical variables are described by frequency (%) and continuous variables are described with mean ± standard deviation [range]. Unless otherwise stated, >=95% response rate.
### Table 2. Comparison of HAART treated and untreated patients at baseline in Quebec Pilot Co-infection Cohort study

<table>
<thead>
<tr>
<th></th>
<th>HAART n=98</th>
<th>Naïve n=14</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>22 (22%)</td>
<td>2 (14%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Age</td>
<td>42 ± 8 [21–61]</td>
<td>42 ± 8 [24–53]</td>
<td>0.90</td>
</tr>
<tr>
<td>HCV genotype (1 or 4)</td>
<td>48/60 (80%)</td>
<td>6/8 (75%)</td>
<td>0.72</td>
</tr>
<tr>
<td>IDU (ever)</td>
<td>75 (77%)</td>
<td>11 (79%)</td>
<td>0.87</td>
</tr>
<tr>
<td>IDU (last 6M)</td>
<td>30 (31%)</td>
<td>8 (57%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Past AIDS</td>
<td>24 (25%)</td>
<td>1 (7%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Liver disease (yes)</td>
<td>7 (7%)</td>
<td>1 (7%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Current alcohol use</td>
<td>50 (52%)</td>
<td>9 (64%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Current smoker</td>
<td>65 (70%)</td>
<td>10 (71%)</td>
<td>0.91</td>
</tr>
<tr>
<td>Welfare recipient</td>
<td>63 (64%)</td>
<td>12 (86%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Fixed address</td>
<td>87 (92%)</td>
<td>11 (85%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Nadir CD4</td>
<td>152 ± 113 [0–449]</td>
<td>315 ± 124 [146–590]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Highest viral load</td>
<td>4.5 ± 1.2 [1.7–6.5]</td>
<td>4.4 ± 1.0 [2.8–6.7]</td>
<td>0.75</td>
</tr>
<tr>
<td>Baseline viral load</td>
<td>2.2 ± 1.0 [1.7–5.3]</td>
<td>4.1 ± 1.0 [2.6–5.6]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3 ± 5.5 [15–55]</td>
<td>25.5 ± 3.0 [20–29]</td>
<td>0.55</td>
</tr>
<tr>
<td>Time since HIV Dx (yrs)</td>
<td>9.7 ± 5.7 [0.2–22]</td>
<td>3.0 ± 3.2 [0.2–12]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time since HCV Dx (yrs)</td>
<td>6.6 ± 5.6 [0–30]</td>
<td>3.0 ± 3.1 [0.2–11]</td>
<td>0.03</td>
</tr>
<tr>
<td>Time since 1st IDU (yrs)</td>
<td>17 ± 8.7 [3–35] (n=86)</td>
<td>16 ± 9.6 [1–31] (n=11)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

**Notes:**
- Includes 136 HCV PCR + (or unknown, n=12) at baseline.
- ARV use at baseline: 103 (76%) receiving ARV (98 on HAART); 14 naïve; 16 off ARVs, but experienced; and 3 unknown.

- **Differences:**
  - Naives less far long in HIV disease (more recent dx, higher nadir, fewer AIDS cases).
  - Naives may have less stable lifestyles more current/recent IDU and alcohol use, more welfare and fewer with fixed address.
- Naturally, naives have higher current viral load (but similar current CD4).
- Also no difference in education, current LFTs, current CD4 and CD8.
Table 3. Detectable rates and relative risk estimates given proposed sample size

<table>
<thead>
<tr>
<th>Event Rate in untreated (n=285)</th>
<th>Detectable Rate in Treated (n=665)</th>
<th>Detectable RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equal to or Less than: <strong>or</strong></td>
<td>Equal to or Greater than:</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
<td>10.1</td>
</tr>
<tr>
<td>7</td>
<td>2.6</td>
<td>12.8</td>
</tr>
<tr>
<td>10</td>
<td>4.7</td>
<td>16.6</td>
</tr>
<tr>
<td>12</td>
<td>6.2</td>
<td>19.1</td>
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<tr>
<td>15</td>
<td>8.5</td>
<td>22.6</td>
</tr>
<tr>
<td>20</td>
<td>12.6</td>
<td>28.4</td>
</tr>
</tbody>
</table>

Assumes 70% of participants will be on HAART. Detectable alternatives, two-sided p-values.
Figure 1. Liver Fibrosis Progression Schema—hypothesized trajectories under different treatment conditions and effects

Assumes HIV/ HCV acquired near same time

A. HAART worsens liver disease through acute and chronic toxicities
B. HAART initially worsens liver disease due to acute toxicity, but risk returns to baseline
C. Liver disease progression rate in HIV co-infected, untreated with HAART
D. HAART improves partially liver disease through immune reconstitution
E. HCV treatment results in marked improvements in liver disease
Figure 2. Area under the ROC of the APRI for predicting significant hepatic fibrosis (Batt and Ludwig score of F2-F4); HIV Medicine, in press.
Figure 3.
Patterns in the lnAPRI by infection group
Baseline distribution of lnAPRI;
(B-E) Modelled change in lnAPRI over time
using adjusted regression coefficients: B. In all
patients;
C. In patients naïve at baseline;
D. Attributable to time on PI (all patients);
E. Attributable to time on NNRTI (all patients).
HIV solid; HIV/HCV dashed; HIV/HBV dotted
Figure 4.

Legend for Figure 4: Characterization of intrahepatic virus-specific T cells from an HCV/HIV-1 co-infected individual (OM178). HIV-1 and HCV specific cells were identified by stimulating liver biopsy derived mononuclear cells in the presence of peptide pools spanning the entire genomes of HIV-1 (HIV pool), HCV (HCV pool) or control antigen (DMSO) x 6 hours and Brefeldin A and then assessed for intracellular cytokine (IFN-γ or TNF-α). Using multi-parameter flow cytometry, cells are gated in the lymphocyte gate, and CD4 or CD8 cells are examined for expression of cytokines. The frequencies of antigen specific cells producing IFN-γ or TNF-α are indicated as % of CD4 or CD8s after subtraction from control antigen stimulated conditions.
Figure 5.

Legend for Figure 2: Summary data of immune assays performed on intrahepatic mononuclear cells in two HCV mono-infected and three HIV/HCV co-infected individuals. Ex vivo cells were exposed to antigens or PHA for 6 hours as described in Figure 1 and then assessed using multiparameter flow cytometry for the frequency of cytokine producing cells in response to antigen. Frequencies of antigen specific cells were calculated after subtraction from background cytokine expression in control antigen stimulated conditions. In a) are shown the subpopulation composition of intra-hepatic lymphocytes. In b) and c) are antigen specific CD4 T cells and in d) and e) are antigen specific CD8 T cells. In f) the average absolute numbers of total viral (HCV plus HIV) specific T cells producing TNF-α / biopsy specimen are shown for the two cohorts of patients studied so far. Bar graphs are shown without error bars to show trends rather than significance, as this is preliminary data.
Figure 6. Graphical causal model:

Confounders
1. age, sex, race
2. CD4, VL, Treatment regimens (time dependent covariates)
3. IDU use
4. Co-morbidities
5. nadir CD4, pretreatment viral load
6. Stage of HIV infection
7. ART regimens/duration
8. Pre-existing liver disease

HAART (exposure)  →  ESLD (outcome)
Figure 7. Directed Acyclic Graphs to show relationship between Exposure and Disease

Direct and indirect interrelationships between various confounders and alternative pathways in the proposed causal model.
Figure 8. Cumulative incidence of ESLD in viral hepatitis co-infection in retrospective cohort study from the Montreal Chest Institute, stratified by infection group (HIV solid; HIV/HCV dashed; HIV/HBV dotted).
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