ABSTRACT

Objectives: To determine the prevalence of hepatitis C co-infection among previously antiretroviral naive HIV-infected individuals initiating antiretroviral treatment (ART) in a population-based program; to describe the effect of HCV co-infection on immunologic response to ART; adherence to ART; and the effect of HCV co-infection on non-accidental mortality among this population.

Methods: British Columbia's HIV/AIDS Drug Treatment Program (DTP) provides antiretroviral therapy to all eligible HIV positive persons in British Columbia free of charge. Data were drawn from a nested cohort within the DTP of previously ART naïve individuals whose first ever ART was a triple-combination and who began treatment between August 1996 – July 2000. Retrospective HCV testing was performed on stored plasma samples from before and, in some cases, after ART initiation.

Results: Of the 1136 individuals whose stored baseline plasma samples were tested, 606 (51%) tested HCV antibody-positive (Ab+) and 580 (49%) were HCV antibody-negative. At baseline, 179 (30%) of the antibody positive results were HCV RNA negative. Of these, 118 samples were re-tested for HCV RNA on a sample taken 6-12 months post-ART initiation; 24 (20%) were now found to be HCV RNA positive. In this study population, people with positive HCV antibodies have an attenuated absolute CD4 response to ART, but a preserved CD4 fraction response; increased biochemical markers of hepatic injury, and are independently less likely to adhere to antiretroviral medication, after controlling for biochemical markers of hepatic injury and injection drug use. Finally, HCV antibody positive adults are nearly three times more likely to die a non-accidental death, after controlling for age, gender, injection drug use, and adherence to ART, and are twice as likely to die an HIV-related death.

Conclusions: Due to shared routes of transmission and the infectiousness of the hepatitis C virus, there is a very high prevalence of HCV in this population-based cohort of HIV-infected individuals. HCV adversely affects the effectiveness of ART, including immunologic response and ability to adhere. This has significant implications for the survival of HIV/HCV co-infected patients.
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DEDICATION

This dissertation is dedicated to the memory of Glen Edward Hillson, my friend, brother, and teacher.

Of course.
CHAPTER 1: 
INTRODUCTION

1.1 THE EPIDEMIOLOGY OF HIV AND HEPATITIS C CO-INFECTION

The Acquired Immune Deficiency Syndrome (AIDS) was first recognized in 1981 and the Human Immunodeficiency Virus (HIV) first isolated in 1983. While HIV occurred in isolated individuals much earlier, even as long ago as the late 1950’s, it did not emerge in epidemic proportions until the 1980’s due to a complex interaction of social, geopolitical, and biological factors [1]. Now, however, the disease is present in over 200 countries world-wide and is continuing to spread. Since its recognition, over 20 million people have died from AIDS. In 2003, approximately five million people became newly infected with HIV and three million died from AIDS. Over 42 million people are currently living with HIV. The HIV epidemic disproportionately affects developing countries, women, youth, and marginalized populations such as aboriginal people, those who are incarcerated, and the poor [2].

Since the approval of the first drugs for treatment of HIV infection in 1987 by the US Food and Drug Administration (FDA), over twenty different drugs have been approved for use. In 1996, the use of triple-combination ‘Highly Active Antiretroviral Therapy’ (HAART) heralded a new era in HIV/AIDS. Since HAART’s inception, mortality and morbidity due to human immunodeficiency virus (HIV) have declined dramatically [3, 4].

It is estimated that as much as 3% of the world’s population is chronically infected with the hepatitis C virus (HCV) [5], including an estimated 2% of people from the United States [6], and 1% of Canadians [7]. Chronic hepatitis C is a major cause of chronic liver disease and death throughout the world [8]. As survival has increased for
patients with HIV infection with access to HAART, liver disease has emerged as a leading cause of morbidity and mortality among them [9-11].

Numerous published studies have established that the prevalence of HCV co-infection in HIV-infected populations is very high (see Table 1.1) [12-29]. It is estimated that on average, approximately 30% of HIV-positive people in high-income countries are co-infected with HCV [30, 31]. However, because in some populations including injection drug users, prison inmates, and hemophiliacs, the prevalence of HCV is so widespread, the prevalence of HIV/HCV co-infection ranges from 50-99% [13, 14, 21].

HCV is prevalent in 1-5% of the world population, with Africa having the highest prevalence at 5% [32]. In a meta-analysis of 25 African cohorts, the relative risk for HCV infection for persons with HIV was 1.52 [22]. Reports from a diverse array of countries including Greece [20], Thailand [19], India [33], Russia [34], and China [14], all indicate that HIV/HCV co-infection is both common and entrenched, particularly where the primary HIV risk factor in co-infected patients is injection drug use or blood transfusion.

1.2 CURRENT ISSUES

Treatment of Chronic Hepatitis C Infection

The current standard treatment for HCV infected patients is pegylated interferon (PEG-IFN) combined with ribavirin, a nucleoside analogue. The goal of HCV treatment is to induce viral clearance and reduce or reverse the progression of hepatic fibrosis [8]. Even if viral clearance cannot be achieved, interferon therapy has been shown to reverse or significantly slow the progression of fibrosis and limit the potential for development of hepatocellular carcinoma [35, 36].
In HCV mono-infected patients; sustained viral response (SVR) rates of up to 88% have been achieved with the use of pegylated interferon in combination with ribavirin [37]. SVR is defined as the absence of HCV-RNA in peripheral blood 24 weeks after the termination of therapy.

Despite the high prevalence of HIV-HCV co-infection, the safety and efficacy of peg-interferon combination treatments designed for mono-infected HCV patients have only recently been evaluated for use with co-infected patients in randomized clinical trials (RCT) (Table 1.2). Results from three multicentre RCT’s involving co-infected patients have recently shown that the efficacy of pegylated interferon is decreased to approximately 40% overall, in comparison to its efficacy in mono-infected patients, approximately 55% overall, although no head-to-head studies have been performed [8, 37, 38]. HCV genotype 1 significantly reduces the probability of a successful treatment outcome [8], and accounts for 70-75% of all HCV infections in the United States [39] and Canada [40]. Genotype 1 is also predominant in HIV/HCV co-infected populations [41, 42]. HCV treatment effectiveness in HIV-positive individuals may also be limited by the prohibitory cost of growth factors (used to enable continued full-dose ribavirin, a significant predictor of treatment success [38], and treatment guidelines which require people to abandon treatment by week twelve if their HCV RNA has not declined by a minimum of 2 logs (in spite of evidence of altered HCV viral dynamics post-treatment in the presence of HIV [43, 44]). Given these limitations, HCV treatment can be expected to have a favorable impact on HCV-related morbidity and mortality in perhaps 20% of HIV/HCV co-infected individuals [31, 41, 45].
Influence of HCV on HIV disease progression

The question of the impact of HCV on the natural history of HIV disease remains of considerable interest. Prior to the availability of combination antiretroviral therapy (pre-1996), increased rates of HIV disease progression largely obscured any effects of HCV disease on HIV [46]. Since then, Greub et al. (2000) reported from the Swiss Cohort Study that HCV infection was associated with a 70% increased probability in progressing to a new AIDS-defining event or death [47]. Some have confirmed his findings since [48-50], while others refute them [46, 51]. There are many ways that HCV could impact HIV disease progression that may not be captured by current studies. For example, liver disease may preclude the use of antiretroviral therapy because of hepatotoxicity. Many co-infected individuals, because of sociodemographics or uncontrolled addiction, simply never access antiretroviral therapy and die of AIDS [52]. It is also possible that HCV has a direct influence on HIV infection, although the mechanism of action is as yet unknown [30].

Influence of HIV on HCV disease progression

Numerous authors have demonstrated that co-infection with HIV increases the relative risk of developing cirrhosis and that the rate of hepatic fibrosis is more rapid in HIV co-infected patients [31, 53-56]. In a study comparing rates of fibrosis progression in HIV/HCV co-infected patients and HCV mono-infected patients, Benhamou et al. (1999) found higher fibrosis scores, higher necro-inflammatory activity and more rapid progression to cirrhosis in co-infected patients [53]. Two large studies comparing disease progression in co-infected and mono-infected patients showed that cirrhosis developed in 15-25% HCV/HIV co-infected patients within 10-15 years compared with 3-6% of those patients who were HIV negative [57, 58]. A meta-analysis by Graham et al. (2001) examined the results of 8 different cohort studies and concluded that there is a
two to three-fold increase in the rate of fibrosis progression in HIV/HCV infected patients [59]. Other predictors of fibrosis progression include age over 35 years, alcohol consumption of over 50 g/day, and a CD4+ T cell count of <500 cells/mm3 [31].

**Immune Suppression**

In 1999, the US Public Health Service/Infectious Diseases Society of American amended their HIV-guidelines to include HCV as an opportunistic pathogen among HIV-positive people. In spite of this, the mechanisms or determinants involved in the increased severity of HCV in co-infected persons are not well understood. It is now evident that the impact of HIV on the course of HCV related liver disease is particularly marked as CD4 cell counts decline and immunodeficiency increases. A low baseline CD4 cell count is correlated with increased progression of fibrosis, higher incidence of cirrhosis and hepatocellular carcinoma among HIV/HCV co-infected persons [53, 60, 61]. Typically a CD4 count less than 200 cells/mm³ is considered to be “low”; however, two independent studies have found the severity of liver fibrosis in chronic hepatitis C infection to be independently associated with a CD4 count lower than 500 cells/mm³, after controlling for HIV infection, age, duration of HCV infection, and alcohol abuse [55, 62]. Although the mechanism(s) for the increased pathogenicity of HCV in HIV co-infected patients are not entirely clear, it is generally accepted that HIV and hepatitis C both contribute to dysregulation of the cellular immune response, which is itself the mechanism that causes fibrosis [38, 63]. Thomas (2002) has suggested that HIV selectively infects the CD4+ lymphocytes that have been activated against the HCV infection - thus reducing the cellular response to HCV infection and leading to the persistence of HCV in hepatocytes [64].
1.3 Outstanding Questions and Study Justification

Although there are numerous investigations in the literature regarding the prevalence of HIV/HCV co-infection, there is no evidence in the literature of a population-based sample having been examined. Further, there is only very limited data about the prevalence of HIV/HCV co-infection in British Columbia [13, 29, 65]; all of this data is drawn from the same cohort of injection drug users.

There are also numerous unresolved questions regarding the accurate diagnosis of HCV in HIV co-infected individuals. For example, there are case reports of both spontaneous HCV RNA clearance [66, 67] and spontaneous HCV RNA emergence upon treatment initiation [68]. There are references in the literature to HIV-positive individuals testing false-antibody negative for HCV [69, 70], and while 15-25% of HCV mono-infected individuals will spontaneously clear HCV RNA without treatment, this issue has not been well characterized in HIV-infected populations [71, 72].

Of the 1388 individuals who first initiated antiretroviral treatment in British Columbia between August 1996 and July 2000, and whose first ever ART was a triple-combination therapy, at the outset of this project only 538 people (39%) had any documented HCV serology. Those for whom we had data were more likely to be male (41% vs. 30%, p=0.002), and were more likely to have an HIV-experienced physician (42% vs. 30%, p<0.001) [73]. It was also confirmed through a preliminary diagnostic study that approximately 5% of HIV-positive individuals who have no antibodies to HCV will have HCV RNA present [73]. Thus, a study regarding diagnostic issues in HIV-co-infected patients initiating antiretroviral therapy was conceived of, funded (by a CIHR Operating Grant, $93,000), and implemented (Appendix 2) as part of this doctoral project.
The majority of people co-infected with HIV/HCV will never fully benefit from current HCV therapy, pegylated interferon combined with ribavirin. In order to maximize the effectiveness of ART, and to mitigate HCV disease progression, tailored HIV and antiretroviral management is critical. Outstanding antiretroviral-related questions of concern to clinicians and patients in the setting of HIV/HCV co-infection include when to start antiretroviral therapy, and what to start with. Central to the antiretroviral management of HIV/HCV co-infected patients is being able to predict the immunologic response to ART, and immunologic response to ART in HIV/HCV co-infected patients is not well understood. The safety and tolerability of antiretroviral agents in HIV/HCV co-infected individuals has not been fully characterized, and requires further study. Adherence to medication in ART treated patients is a critical determinant of outcome, and factors associated with non-adherence in HIV/HCV co-infected populations need greater elucidation. The impact of HCV disease on mortality among individuals in the era of highly active antiretroviral therapy also needs further study.

1.4 STUDY OBJECTIVES AND THESIS ORGANIZATION

The overall aim of this thesis is to address some of the outstanding questions regarding HIV/HCV co-infected individuals initiating antiretroviral therapy from a population health perspective. This thesis will explore 4 primary objectives:

Objective 1: To measure the baseline prevalence of Hepatitis C infection among HIV-positive individuals initiating Highly Active Antiretroviral Therapy (HAART) in a population-based HIV treatment cohort. In Chapter 4, I describe the results of the retrospective testing of stored baseline samples from the HOMER Cohort. I describe the
baseline prevalence of hepatitis C co-infection among this population using antibody-data, and sociodemographic characteristics (age, gender, median income) associated with testing antibody positive for HCV. In addition, I describe the prevalence of discordant responses, including how sociodemographics and immune status are associated with antibody and RNA discordant responses. My primary hypotheses were 1) that there would be a Hepatitis C prevalence of 30% among this population (defined as having positive antibodies to HCV) at the time of ART initiation; 2) that at baseline, 10-15% of those with positive HCV antibodies would have no evidence of HCV RNA; and 3) that in 25% of these cases, HCV RNA would emerge 6-12 months following ART initiation.

**Objective 2:** To characterize the impact of the hepatitis C virus (HCV) on CD4 response to antiretroviral treatment (ART) over the first 48 weeks of therapy in previously ART-naïve HIV-infected individuals. Specifically, in this chapter I examine immunologic response to ART using both absolute and CD4 fraction responses. In addition, I describe the role of baseline absolute CD4 count on these responses. My primary hypotheses were that HCV-positive individuals would have a smaller CD4 recovery over the first 48 weeks of ART using both absolute and fraction outcomes, and that this blunted response would be most pronounced among those initiating treatment with ≤200 CD4 cells/mm³.

**Objective 3:** To describe the effect of hepatitis C (HCV) co-infection on adherence to antiretroviral treatment (ART) during the first year of therapy in a population-based HIV/AIDS drug treatment program. The primary hypothesis was that while HCV co-infected individuals would be less likely to adhere to their ART, there would be a strong and significant interaction between HCV seropositivity and any biochemical
marker of liver injury, indicating that increased hepatic injury in HCV-co-infected patients would be a significant factor in this non-adherence. I also sought to elucidate the relative effects of hepatitis C infection and any history of injection drug use on adherence.

Objective 4: To describe the effect of HCV serostatus on the risk of non-accidental mortality in a population-based treatment program of previously ART naïve patients, and to describe causes of death among this population. People who are co-infected with HIV and HCV can have multiple high risk factors for mortality, including drug use (associated with accidents, overdoses, suicides), HIV disease, or end-stage liver disease secondary to HCV infection. This chapter sought to describe whether HCV serostatus was an independent predictor of non-accidental mortality, and to describe causes of death in this population. Chapter 7's primary hypothesis was that people who are co-infected with HCV would have an independently higher risk of non-accidental mortality.

This thesis is divided into 8 chapters. This first chapter provides some preliminary background and context, and outlines the study’s justification and objectives. Chapter 2 is a review paper currently in press with the journal AIDS that provides a summary and analysis of the research to date in the area of antiretroviral use in the context of HIV/HCV co-infection. Chapter 3 provides an overview of the study setting, the methods employed, and the limitations of my approach. Chapters 4 through 7 are based on four research papers that are currently, or will be shortly, submitted for publication. Finally, Chapter 8 provides a discussion of the findings,
outlines the unique contribution of the work, makes recommendations, and outlines areas for future research.

1.5 Summary

In summary, HIV and hepatitis C co-infection is a relatively recent phenomenon with wide-ranging clinical and public health implications. Although great strides have been made in the clinical management of HIV disease, major challenges have emerged, including co-morbidities such as hepatitis C. Very high HCV prevalence in high HIV risk communities, faster HCV disease progression, poorer response to HCV treatment, complications related to HIV management, plus the frequent complexities of uncontrolled addictions, poverty, and psychiatric issues, all combine to make HIV/HCV co-infection a very complex domain for clinicians and other care providers. As HIV treatment and management is becoming increasingly accessible to communities most at risk of HIV-HCV co-infection, the issue of hepatitis C is causing HIV clinics and health care providers to integrate HCV issues into their practices. This dissertation addresses several key issues related to the HIV management of patients who are co-infected with HIV and HCV, and has implications for patients, physicians, and policy makers.
1.6 References


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Table 1.1. Prevalence of HCV, HIV, and HIV/HCV Co-infection in Selected Countries

<table>
<thead>
<tr>
<th>Location</th>
<th>HCV Prevalence (%) in Country</th>
<th>HIV Prevalence (%) in Country</th>
<th>Main HIV transmission routes</th>
<th>HCV Prevalence in HIV+ Study Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>4.07</td>
<td>0.06</td>
<td>IDU</td>
<td>99.3% HIV+ persons [14]</td>
</tr>
<tr>
<td>Thailand</td>
<td>5.60</td>
<td>0.90</td>
<td>IDU</td>
<td>51% HIV+ men identified at screening for potential HIV vaccine trial (verses 8.3% if HIV-) [74] 49.5% of HIV-infected young MSM and 2.2% among uninfected men [19].</td>
</tr>
<tr>
<td>India</td>
<td>1.85</td>
<td>0.47</td>
<td>Heterosexual sex, MSM, IDU</td>
<td>21.4% HIV+ persons in hospital (verses 3.5% if HIV-) [33]</td>
</tr>
<tr>
<td>Brazil</td>
<td>2.60</td>
<td>0.37</td>
<td>Heterosexual sex, MSM, IDU</td>
<td>36.2% HIV+ persons (84.8% HIV+ with IDU) [25]</td>
</tr>
<tr>
<td>Russia</td>
<td>2.00</td>
<td>0.60</td>
<td>IDU</td>
<td>91% HIV+ with IDU [34]</td>
</tr>
<tr>
<td>Spain</td>
<td>0.74</td>
<td>0.34</td>
<td>IDU</td>
<td>42% HIV+ persons [75]</td>
</tr>
<tr>
<td>Italy</td>
<td>0.48</td>
<td>0.24</td>
<td>Heterosexual sex, IDU</td>
<td>54% HIV+ (80% HIV+ with IDU) [21]</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0.24</td>
<td>0.18</td>
<td>Foreign citizens, heterosexual sex, IDU, MSM</td>
<td>37.2% HIV+ persons on ART (87.7% of cohort with IDU) [47]</td>
</tr>
<tr>
<td>Canada</td>
<td>0.15</td>
<td>0.18</td>
<td>IDU, MSM</td>
<td>62.4% HIV+ cohort (91% of cohort with IDU) [18] 95% HIV+ with IDU [13]</td>
</tr>
<tr>
<td>USA</td>
<td>1.80</td>
<td>0.32</td>
<td>MSM, IDU</td>
<td>16% HIV+persons (73% HIV+ with IDU or MSM) [15]</td>
</tr>
<tr>
<td>South Africa</td>
<td>1.70</td>
<td>11.7</td>
<td>Heterosexual sex</td>
<td>1% Hospitalized AIDS persons (2000) [76]</td>
</tr>
<tr>
<td>Malawi</td>
<td>3.9 [77]</td>
<td>7.30</td>
<td>Heterosexual sex</td>
<td>13% HIV+ male sugar workers (verses 10% HIV-) [77]</td>
</tr>
<tr>
<td>Cameroon</td>
<td>12.50</td>
<td>3.44</td>
<td>Heterosexual sex</td>
<td>9.0% HIV+ pregnant women (verses 8.0 % HIV-pregnant women) [78]</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>0.18</td>
<td>2.07</td>
<td>Heterosexual sex</td>
<td>4.5% HIV+ women in community survey [79]</td>
</tr>
</tbody>
</table>
ART – antiretroviral therapy
IDU – injection drug use
MSM – men who have sex with men
HIV country prevalence calculated by dividing total number of adults and children with HIV by country population; data from UNAIDS/WHO Global HIV/AIDS Online Database
HCV country prevalence from World Health Organization Weekly Epidemiological Record No. 46, 14 November.
Table 1.2. Summary of Results of Three Recent Randomized Clinical Trials of Pegylated Inteferon + Ribavirin Treatment in HIV/HCV Co-infected Patients [38, 80, 81].

<table>
<thead>
<tr>
<th>Baseline Parameters</th>
<th>APRICOT</th>
<th>ACTG 5071</th>
<th>RIBAVIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>40</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>81</td>
<td>85</td>
<td>74</td>
</tr>
<tr>
<td>White race (%)</td>
<td>79</td>
<td>47</td>
<td>n/a</td>
</tr>
<tr>
<td>HCV Viral load (log IU)</td>
<td>6.7</td>
<td>6.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Genotype 1 (%)</td>
<td>61</td>
<td>74</td>
<td>58</td>
</tr>
<tr>
<td>Mean CD4 cell count (cells/mm$^3$)</td>
<td>540</td>
<td>470</td>
<td>514</td>
</tr>
<tr>
<td>Bridging fibrosis/cirrhosis (%)</td>
<td>16</td>
<td>37</td>
<td>39</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Characteristics</th>
<th>APRICOT</th>
<th>ACTG 5071</th>
<th>RIBAVIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>860</td>
<td>133</td>
<td>412</td>
</tr>
<tr>
<td>Number on PEG-IFN + RBV</td>
<td>289</td>
<td>66</td>
<td>205</td>
</tr>
<tr>
<td>Used RBV dose escalation</td>
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<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Used haematologic growth factors</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Treatment Discontinuation (%)</td>
<td>31</td>
<td>12</td>
<td>42</td>
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</table>

<table>
<thead>
<tr>
<th>Virologic Response Rates</th>
<th>IFN+RBV</th>
<th>PEG-IFN</th>
<th>PEG-IFN+RBV</th>
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</thead>
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<tr>
<td>APRICOT overall %</td>
<td>12</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Genotype 1 %</td>
<td>7</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>Genotype 2/3 %</td>
<td>20</td>
<td>36</td>
<td>62</td>
</tr>
</tbody>
</table>

| ACTG 5071 overall %                       | 12      | -       | 27          |
| Genotype 1%                               | 6       | -       | 14          |
| Genotype 2/3%                             | 33      | -       | 73          |

| RIBAVIC overall %                         | 18      | -       | 26          |
| Genotype 1%                               | 5       | -       | 11          |
| Genotype 2/3%                             | -       | -       | 43          |
CHAPTER 2:
BACKGROUND

2.1 FORWARD

The literature review contained in this chapter has appeared in AIDS as:


This review was conducted by Paula Braitstein who also prepared the first draft. A number of experts were then asked to provide feedback and be co-authors on the manuscript.

Two other reviews related to the topic of HIV/HCV co-infection were conducted but are not included in this dissertation. They are:

Braitstein P. “HIV and Orthotopic Liver Transplantation”. British Columbia Medical Journal [1].

2.2 INTRODUCTION

The use of highly active antiretroviral therapy (HAART) has led to dramatic reductions in HIV-related morbidity and mortality in HIV-infected individuals [2-4]. However, parallel with this success has been the subsequent emergence of co-morbidities, such as viral hepatitis. The Hepatitis C Virus (HCV) in particular, because of its high prevalence in HIV-positive populations (up to 90% in some [5-8]) and its greatly increased pathogenicity in the setting of HIV [7, 9], is now a leading cause of death of HIV-positive individuals [10-12].

A number of reviews have recently been published regarding the care and treatment of HIV/HCV co-infected persons [7, 9, 13]. These reviews have primarily focussed on the treatment and management of hepatitis C in HIV-co-infected individuals, but have not generally examined issues related to HIV management in these patients. Unfortunately, a large proportion of people co-infected with HIV/HCV will never fully benefit from HCV therapy. Many individuals will not be able to access therapy, either because of contraindications to treatment, such as psychiatric co-morbidity (including depression) or anemia, or because they are otherwise considered ineligible (e.g. are considered ‘non-responders’ to previous treatment, continue to use illicit drugs or alcohol, etc.). Of the individuals who do access treatment, recent data suggest that the probability of a sustained virologic response (SVR) may be significantly less in HIV-co-infected individuals (SVR: 40% overall in HIV-positive, vs. 55% in HIV-negative, and among genotype 1, 29% in HIV-positive, vs. 45% in HIV-negative [14-16]). Genotype 1 accounts for 70-75% of all HCV infections in the United States [17] and Canada [18], and is also predominant in HIV/HCV co-infected populations [19, 20]. HCV treatment effectiveness is limited by a number of factors including ineligibility for treatment, the prohibitory cost of growth factors to enable continued full-dose ribavirin, treatment
guidelines which force people to abandon treatment by week twelve if their HCV RNA has not declined by a minimum of 2 logs (in spite of evidence of altered HCV viral dynamics post-treatment in the presence of HIV [21, 22]), the predominant prevalence of genotype 1, and the negative influence of HIV infection. Given the above limitations, HCV treatment can be expected to have a favorable impact on HCV-related morbidity and mortality in perhaps 20% of HIV/HCV co-infected individuals [9, 19, 23]. Therefore, optimum HIV and antiretroviral management are critical both for controlling HIV, and for mitigating HCV.

Key antiretroviral-related questions of concern to clinicians and patients in the setting of HIV/HCV co-infection include the best time to initiate antiretroviral therapy. Factors to consider are CD4 count, HIV viral load, hepatic inflammation and function, and the anticipated consequences of immune restoration in terms of auto-inflammatory responses and elevation of HCV viral loads. Other important questions relate to the safety and tolerability of these agents in HIV/HCV co-infected individuals, including liver enzyme elevations, as well as metabolic and mitochondrial toxicities, which are themselves linked to HCV infection.

The objectives of this review are therefore to summarize the available evidence regarding 1) the impact of hepatitis C on the virologic and immunologic response to antiretroviral therapy, including what is known regarding treatment interruptions; 2) the safety of antiretroviral agents in co-infected individuals; and 3) the relationship between immune suppression, immune restoration and hepatic injury.

This review involved computerized, English-language literature searches of MEDLINE and PubMed databases (January 1985 to May 2004) for published studies in humans.
that examined HIV and hepatitis C. Keywords for the search included HIV, AIDS, human immunodeficiency virus, acquired immune deficiency syndrome, HCV, hepatitis C, hepatitis, immune deficiency, immune restoration, toxicity, diabetes, mitochondria, and metabolism. The bibliographies of selected articles were also searched for pertinent studies.

2.3 Impact of HCV on the Virologic and Immunologic Response to Antiretroviral Therapy

A number of studies have examined the impact of HCV on the virologic and immunologic response to antiretroviral therapy. While most have found that there is no impact of HCV on the HIV virologic response to antiretroviral therapy [12, 24, 25], there are mixed reports regarding immunologic response. Table 1 summarizes the conflicting evidence.

Greub et al. (2000) defined an immunologic response to treatment as an increase of at least 50 CD4 cells/mm3, and found that HIV/HCV co-infection was associated with a smaller CD4 recovery [12]. Zala et al (2004) found that while 86% of HCV-negative individuals had a CD4 increase of at least 75 cells/mm3 at 48 weeks, only 64% of HCV-positives did. Further, while it took a median of 17 weeks for HCV-negative individuals to achieve an increase of at least 75 cells/mm3, HCV-positive individuals took a median of 29 weeks [26]. In a population-based cohort of previously treatment-naïve individuals, these data were confirmed using mixed effects models, showing that while HCV-negative individuals gained an average of 36 cells per year after adjustment for confounders, HIV/HCV co-infected individuals lost on average 5 cells over a 12 month period [27]. Although Klein et al. (2003) found no difference in the mean CD4
cell count 24 months post-initiation of antiretroviral therapy, the HCV-positive individuals had a significantly reduced probability of achieving a CD4 increase of at least 50 cells/mm³ (Hazard Ratio 0.48, 95% confidence interval 0.23-0.97, p=0.04), after adjustment for baseline CD4, viral load, previous nucleoside experience, and duration of HIV infection [24]. A number of reports have found no difference in CD4 increase by HCV serostatus [24, 25, 28, 29].

The lack of consistency in results regarding immunologic response among HIV/HCV co-infected persons on HAART may be due to a number of factors. It can take up to 24 months of treatment for a complete CD4 response to occur [30], beyond the time frame of many studies. How a CD4 increase is defined is also another key factor (e.g. 'time to' CD4 increase of 50 cells vs. 100 cells vs. using all intra-subject CD4 measures), and most of the reports which found no difference in CD4 response did not clearly define what actually constituted a CD4 response. A third issue may be that a blunted response may mostly occur in those HIV/HCV co-infected individuals with lower baseline CD4 counts. For example, in Klein’s study where no difference in mean CD4 response was found, the baseline CD4 was well above 200 among both HCV-positive and HCV-negative subjects [24], whereas the baseline HCV-positive CD4 count in Greub’s landmark study was 172 cells [12]. The lack of consistency may also due to inconsistencies in measures of, or accounting for, adherence to ART.

Antiretroviral treatment interruptions are frequent among persons co-infected with HIV/HCV, and injection drug use has been identified as an important predictor of treatment interruptions among HIV/HCV co-infected persons [12, 31, 32]. However, the majority of injection drug users are co-infected with hepatitis C. Therefore, it is difficult to know whether the reduced levels of adherence and increased rates of
treatment discontinuation seen in this population are due to lifestyle issues commonly associated with injection drug use, or whether they are related to the increased toxicity experienced by many co-infected patients.

Only a few studies have examined HIV/HCV co-infection as an independent factor in switching or interrupting HAART [25, 33, 34]. Melvin et al. reported antiretroviral discontinuation rates due to hepatic toxicity of more than two-fold in HCV-co-infected individuals compared to HIV mono-infected persons [25]. Among a population of 465 previously antiretroviral naïve individuals, HCV seropositivity was associated with an adjusted 40% increased risk of discontinuing or changing initial HAART regimens within the first year of treatment [33]. The authors indicate that whether this was due to histological damage, reduced adherence, or increased hepatotoxicity is not clear, because hepatic cirrhosis was also independently associated with HAART discontinuation (AOR 2.1, 95% CI: 1.1-3.8) [33]. Aceti (2002) reported that 83% of those who discontinued antiretrovirals because of hepatotoxicity were HCV co-infected [28], whereas D'Arminio Monforte et al. (2000) found no impact of HCV on treatment interruptions due to toxicity [34].

2.4 SAFETY OF ANTIRETROVIRAL AGENTS IN HIV/HCV CO-INFECTED INDIVIDUALS

Antiretroviral agents are associated with a diverse array of short- and long-term toxicities, ranging in severity from benign to life-threatening [30]. There have been a number of reviews published addressing hepatotoxicity [35-37], but few that specifically focus on the role of HIV/HCV co-infection [9, 37]. Although hepatotoxicity is most commonly defined as an increase in liver enzymes, there are several other types of liver-related toxicities which are both associated with antiretroviral therapy, and
independently the result of hepatitis C virus infection (see Figure 1). These include mitochondrial dysfunction, including peripheral neuropathy, metabolic changes such as insulin resistance and diabetes mellitus, and hepatic steatosis. Thus the potential for overlapping pathology is significant among co-infected individuals receiving antiretrovirals, and while these interactions have not been well characterized, some data are available and are described below.

**Elevated Liver Enzymes**

Hepatitis C in the absence of HIV infection independently causes elevated liver enzymes [16, 38]. There is an emerging consensus that HCV infection increases the risk of developing elevated liver enzymes by 2-3 fold in HIV-co-infected individuals receiving antiretrovirals [39-42], and HCV accounts for the majority (over 75%) of severe hepatotoxicity in most studies (albeit that the prevalence of HCV co-infection in a geographical area will have an impact on this figure) [40, 42-47] (see Table 2). HIV/HCV co-infected individuals may also be more likely to experience the clinical symptoms associated with highly elevated liver enzymes, such as malaise, asthenia, nausea/vomiting, fever, jaundice, and decompensation of previous ascites [41]. Nunez (2001) found that while most study participants who developed liver enzyme elevations experienced pure cytolysis (68/222), of the 6 participants who developed cytolysis and cholestasis, all but one were HCV-positive [47].

It is notable that others have found little or no association between elevated liver enzymes and hepatitis C among those co-infected with HIV [48-50]. A meta-analysis of this question would be helpful. Some of the relatively low rates of severely elevated ALT may be due to small numbers [50], or selection bias [29]. Recently, Becker (2004) compared liver toxicity across the ATHENA (Amsterdam), Collaborations in HIV
Outcomes Research US (CHORUS), Italian Cohort of Naïve for Antiretrovirals (ICONA), and Target Cohorts [35]. In spite of differences in the prevalence of viral hepatitis and definitions of hepatotoxicity across the cohorts, the authors concluded that there was a low overall prevalence of hepatotoxicity in these cohorts (<6.5%), but that in each cohort the risk of developing hepatotoxicity was associated with viral hepatitis, and in 3 of the 4 cohorts, also with an elevated baseline ALT. Although there was no consistent association found between hepatotoxicity and a particular drug or drug class, ritonavir and the recent use of nevirapine (during the first 12 weeks) were significantly associated with hepatotoxicity [35]. Furthermore, female sex was independently associated with hepatotoxicity in the ATHENA cohort [35, 51], while age over 60 years was a factor in the Target cohort [35]. Also, HCV genotype 3 has been specifically associated with the development of acute liver enzyme elevations in HIV/HCV co-infected populations [52, 53]. Genotype 3 is also strongly predictive of hepatic steatosis [54, 55], underscoring the importance of knowing patients' HCV genotype prior to initiating antiretroviral treatment.

HIV/HCV co-infected individuals are more susceptible to the hepatotoxicity associated with certain drugs, particularly nevirapine [30, 35, 40, 41] and full dose ritonavir [28, 35, 48, 56]. An important and as yet unanswered question is whether small doses of ritonavir produce less hepatotoxicity than full-dose ritonavir, and whether even low doses should be avoided in co-infected individuals. In a small, retrospective analysis of HCV-co-infected participants enrolled in the Abbott 863 trial comparing nelfinavir to lopinavir/ritonavir, there was a statistically significant increase in mean ALT levels in the nelfinavir, but not the lopinavir/ritonavir, arm at 24 weeks. By week 48, both groups mean ALT level was back to baseline [57]. In contrast, Aceti (2002) found that saquinavir boosted with ritonavir, but not saquinavir alone, was associated with both
overall and severe hepatotoxicity in co-infected patients [28]. A recent Canadian study also found that independent predictors of grade 3/4 elevations in ALT among 202 HBV and/or HCV co-infected individuals were older age and current use of lopinavir/ritonavir [58].

**Mitochondrial Toxicity**

The pathogenesis of HCV is not fully understood [16], but is believed to be at least in part due to intra-hepatic mitochondrial damage [59-61]. Mitochondrial toxicity has also been widely associated with nucleoside analog use, particularly stavudine (d4T), didanosine (ddI), zidovudine (AZT) and the combination of ribavirin plus ddI [37, 62-64].

Inhibition of mitochondrial DNA synthesis can lead to hyperlactatemia, lactic acidosis and even death [65-68]. It is now well established that using ddI or d4T simultaneously with ribavirin is contraindicated because of the risk of fatal lactic acidosis [9]. Independent risk factors for the development of symptomatic lactic acidosis are HCV or HBV co-infection, being female, liver disease, pregnancy, and obesity [37].

Mitochondrial damage is also associated with fatty liver [37, 69], and is known to greatly accelerate fibrosis [37, 70], particularly among people infected with HCV genotype 3 [54, 55].

Although not life-threatening, peripheral neuropathy is a painful and often progressive symptom with substantial impact on quality of life. This is a well-documented side effect of d4T, ddI, and ddC [30], and is attributed to nucleoside-induced mitochondrial toxicity [71]. What is less known is that peripheral neuropathy is independently related
to HCV infection, possibly through its effects on mitochondria [72-74]. HCV infection is often associated with cryoglobulinaemia and peripheral neuropathy is a comparatively common complication of cryoglobulinaemia associated with HCV infection and is thought to be attributable to nerve ischaemia [74]. HCV RNA has been detected in nerve tissue suggesting a possible direct role of HCV [72]. A nerve conduction study showed abnormal findings in 77% of investigated HCV-positive patients [73].

Metabolic Changes

Hepatic steatosis is linked to insulin resistance, which itself can be caused by both HIV protease inhibitors and HCV infection [37, 65, 70, 75, 76]. Duong et al. (2001) found more insulin resistance in a group of 29 HIV/HCV co-infected people compared to 76 HIV mono-infected controls, but had rates comparable to a group of 121 HCV mono-infected controls [77]. Of note, all the HIV-infected persons were on HAART [77].

Similarly, HCV is linked with the development of type 2 diabetes [78-80]. In a large retrospective cohort of over 40,000 US HIV-positive veterans, HCV co-infected people had a nearly two-fold increased risk of developing diabetes mellitus, controlling for age, race, and history of drug/alcohol use [81]. Mehta et al. (2003) found that both HCV co-infection and protease inhibitor use were independently associated with developing hyperglycemia among 1230 individuals initiating antiretroviral therapy, adjusting for age and baseline glucose level [82].

Liver fat content may be higher in those with lipodystrophy, although the relationship to HCV infection has not been examined [75]. There is some evidence to suggest that co-infected patients may be more vulnerable to the symptoms associated with lipodystrophy syndrome, including lipoatrophy [71]. For example, Duong et al. (2001)
observed more frequent lipoatrophy in HIV/HCV co-infected patients (41% vs. 14%, p=0.003), and found that peripheral fat wasting was independently associated with HIV/HCV co-infection [77].

Surprisingly, a number of reports indicate that HIV/HCV co-infected individuals have more favorable profiles in terms of total cholesterol, low-density lipoprotein cholesterol (LDL) and triglyceride plasma levels, compared to HIV mono-infected people [77, 83, 84]). The underlying mechanism remains unknown but may represent impaired synthesis of cholesterol in the liver [84].

In summary, there are several potential adverse interactions between antiretroviral toxicities and hepatitis C infection. More research is needed to understand these interactions; to know whether their combined effects are additive or synergistic; to improve our understanding of the pathogenesis of HCV infection and the mechanisms of drug toxicities; and to optimize the clinical management of co-infected patients receiving antiretrovirals. More research is also needed to determine the safety and tolerability of specific antiretrovirals including low doses of ritonavir in HIV/HCV co-infected individuals, using either existing observational data or through clinical trials.

2.5 IMMUNE SUPPRESSION, IMMUNE RESTORATION, AND HEPATIC INJURY

The relationship between antiretroviral use, immune suppression, immune restoration, and hepatic injury is complex and not well characterized.

With or without antiretroviral use, low baseline CD4 count is strongly associated with progression of fibrosis, cirrhosis, and hepatocellular carcinoma among HIV/HCV co-
infected persons [85-88]. While most of these studies consider a 'low' CD4 count to be below 200 cells/mm³, Puoti et al. (2001) found that the severity of liver fibrosis in chronic hepatitis C infection was independently associated with a CD4 count of less than 500 cells/mm³ at time of biopsy, after controlling for HIV infection, age, duration of HCV infection, and alcohol abuse [89]. There are virtually no studies that contradict the finding that immunodeficiency is associated with the progression of hepatic disease.

Perhaps because of their role in preserving functional immunity, a number of studies have found a favorable impact of antiretrovirals in HIV/HCV co-infected persons in terms of fibrosis/cirrhosis progression and liver-related mortality. Not using protease inhibitor therapy was strongly associated with faster progression to cirrhosis among 182 HIV/HCV co-infected patients [90]. It is important to note that these findings may be limited by a possible selection bias whereby those individuals with more advanced liver disease may have been less likely to have been prescribed protease inhibitors because of their known hepatotoxicity. [90]. Qurishi et al. (2003) concluded that antiretroviral use significantly reduced long-term liver-related mortality among 285 HIV/HCV co-infected patients [91]. However, although the investigators showed decreasing rates of liver-related mortality between 1990 and 2002, their study population consisted predominantly of hemophiliacs infected during the early to mid-1980's. Thus it is likely that the majority of patients in their cohort died of other causes (e.g. their blood disorders, HIV infection, etc.) before dying of liver disease, since effective antiretroviral therapy only became available in the mid to late 1990's. A third study [92] concluded that antiretrovirals did not influence the progression of fibrosis, but the study was cross-sectional, small (n=42), and based on only one biopsy, the timing of which was not clear.
Individuals who are more immunocompromised when they initiate HAART are at risk for experiencing Immune Reconstitution and Inflammatory Syndrome (IRIS) [30, 93]. In 1999, the US Public Health Service/Infectious Diseases Society of American amended their HIV-guidelines to include HCV as an opportunistic pathogen among HIV-positive people. Chronic HCV is not the consequence of the direct destruction of hepatic cells by the virus. Rather, it results from an intermediate immune response that is large enough to induce hepatic cell destruction and fibrosis but not enough to eradicate the virus from its reservoirs [15]. It is believed that hepatic injury resulting from immune recovery is the result of a similar mechanism [94]. As the immune system recovers, the cytotoxic T-cell response becomes more efficient at lysing infected hepatocytes, releasing HCV RNA from the hepatocytes killed and increasing plasma levels of HCV RNA [94]. Increased lysis of infected hepatocytes upon immune reconstitution may also cause an increase in ALT levels [28]. The increased HCV replication then further damages liver cells through apoptosis and other means such as cytokine disruption [89, 95]. Hepatic fibrosis itself is a wound healing response to ongoing liver injury [89], and HCV is considered an immune-mediated disease [96].

The potential for immune restoration to enhance liver disease progression is supported by data regarding both increased viral replication, and fibrosis progression. Perhaps because of immune dysregulation, HIV/HCV co-infected individuals have higher baseline HCV viral loads compared to HCV mono-infected individuals [8, 20, 97, 98] and, with one exception [50], antiretroviral initiation has been found to significantly enhance HCV replication [29, 46, 89, 94, 99-101].

Numerous studies [29, 57, 98] have found a relationship between baseline immune function, as measured by CD4 counts, and the magnitude of the increase in HCV RNA.
In patients starting from a more immunocompromised state (baseline CD4 <350 cells/mm3), HCV RNA levels increased within the first 16 weeks following antiretroviral therapy initiation and remained high throughout the 48 weeks of therapy followed. In individuals with baseline CD4's >350 cells, significant increases in HCV RNA were found within the first 16 weeks, but these increases were transient and the HCV viral load returned to baseline levels within the following 16 week period [29]. Sherman (2004) found that only patients with baseline CD4 count below 100 cells/mm3 had significant increases in their HCV RNA (>0.5 log) at week 24 and 48 [57].

There are several case series and reports in the literature regarding the impact of antiretroviral-related immune reconstitution on the development of hepatic fibrosis, cirrhosis, and end-stage liver disease [99, 101-104]. Although this is a difficult question to study because of the need for repeat liver biopsies in patients prior to and following antiretroviral initiation, these reports suggest significant potential for severe hepatic deterioration parallel to an immune response among those individuals who are more immunocompromised on treatment initiation [102, 104]. Benhamou (2001) found that a baseline CD4 count of less than 200 cells/mm3 was independently associated with a nearly three-fold increase in the risk of developing cirrhosis following initiation of antiretroviral therapy [105].

Elevated ALT/AST levels are common in HIV/HCV co-infected individuals, and may be related to the increased destruction of infected hepatocytes that accompanies immune restoration. Sulkowski et al. found that a CD4 increase of more than 50 cells was associated with severe hepatotoxicity (defined as a grade 3 or 4 change in AST/ALT) [40, 106]. However, others have found only weak or no associations between baseline CD4 counts, CD4 recovery, and the development of elevated liver
enzymes, in spite of high levels of the latter among co-infected patients and evidence of other types of immune reconstitution syndrome [28, 47, 94].

While immune recovery may have deleterious consequences in many cases, it is noteworthy that there have been two case reports of spontaneous clearance of HCV RNA following HAART initiation, presumably related to immune reconstitution [8, 107]. Younger age at time of HAART initiation (<20 years of age) may improve the possibility of this outcome [8].

In spite of the widespread use of HAART, HCV is a leading cause of death among HIV-infected individuals [10-12]. The use of antiretroviral therapy and the subsequent preservation of functional immunity may have protective effects in preventing or slowing the progression of hepatic injury, especially among those individuals who initiate treatment while their immune system is still intact. However, the data suggest that immune restoration due to antiretroviral therapy among those individuals with immunodeficiency may have a deleterious impact on the progression of HCV disease. The issue has not been well studied. However, current data indicate that those co-infected individuals who are not eligible for HCV treatment might consider initiating HAART at higher CD4 levels than their HIV mono-infected counterparts to mitigate the potential immune reconstitutive effects of antiretroviral therapy. Treating HCV early in both the HIV and HCV disease process may have the dual beneficial effect of maximizing the chance of achieving a sustained HIV virologic response [9, 14], and maximize the benefits of future antiretroviral therapy. More prospective research is needed to quantify the impact of antiretroviral-related immune restoration on progressive histologic hepatic injury, and to elucidate the immune-mediated components of the two viruses combined. As there is also evidence that antiretroviral
use is associated with HCV RNA replication in previously HCV RNA undetectable individuals, and the development of HCV antibodies following antiretroviral initiation [108], patients should be tested for the presence of HCV RNA and antibodies before and after initiating treatment [102, 108].

2.6 CONCLUSIONS & RECOMMENDATIONS

Currently available data convey two key messages related specifically to the use of antiretroviral therapy in HIV/HCV co-infected individuals: 1) that both immune suppression and restoration can contribute to the onset and acceleration of HCV-related liver disease; and 2) that the morbidity associated with HCV infection, such as insulin resistance, diabetes, mitochondrial dysfunction, and liver inflammation are all also associated toxicities of antiretroviral therapy which together may be at least additive. Whether the apparent aggravation of liver injury and HCV disease progression among those co-infected is due to immune restoration, antiretroviral-related hepatotoxicity, or enhanced HCV replication is not clear, although it is likely due to interactions between the three. Specific recommendations based on the available evidence for the initiation and management of antiretroviral therapy in HIV/HCV co-infected individuals are summarized in Table 3.

Hepatitis C treatment has evolved to a degree where sustained virologic clearance is possible for some patients who are eligible for treatment, including up to 70% of those HIV-co-infected with genotypes 2/3, but fewer than 30% of those HIV co-infected with genotype 1, the dominant genotype among HIV/HCV co-infected populations [14, 16]. Thus while HCV treatment should be considered for most HIV/HCV co-infected individuals, only a relatively small proportion of them can expect to eliminate their
HCV infection. If HCV treatment is not an option, then HIV treatment becomes central to the health management of HIV/HCV co-infected patients. Unfortunately, insufficient research has thus far been conducted to indicate when the most appropriate time is to initiate ART in dually infected patients. Similarly, the relative effectiveness of various available regimens in HIV/HCV co-infected patients is not fully established.

The data regarding baseline CD4, CD4 response, and progression of hepatic injury, especially liver fibrosis/cirrhosis are intriguing. Clearly this is an area that requires more prospective research to confirm the data to date, and to elucidate the mechanisms by which immune deterioration and restoration are linked to the progression of fibrosis and cirrhosis. For example, if the pathogenetic mechanism of hepatitis C is immunologic cytotoxicity, why do people with the lowest immunity have the worst hepatic disease? The 2002 International AIDS Society treatment guidelines indicate that HAART treatment in people with CD4 counts over 200 cells/mm3 should be individualized, considering the rate of decline of the CD4 count, HIV viral load, patient interest and potential to adhere, and individual risks of toxicity and drug-drug interactions [30]. The data presented in this review suggest that HCV status should also be considered a factor in that decision. This may be particularly important in men, and in those who acquired HCV at a later age, since both are important predictors of liver fibrosis progression in HIV-co-infected individuals [20, 90, 109].

There is significant potential for overlap between HCV-attributable morbidity and antiretroviral-associated toxicity, and the clinical research to-date suggests this is resulting in elevated rates of mitochondrial and metabolic adverse events. More research is needed to understand whether these interactions are additive or synergistic, to elucidate the pathogenesis of HCV infection, and to develop better clinical
monitoring and responses to these emerging issues. Further research is also needed to clarify the role that this increased morbidity has on antiretroviral treatment discontinuation, and its subsequent impact on mortality.

In conclusion, there are numerous burgeoning and maturing epidemics of HIV/HCV co-infection around the world, and if epidemiological co-infection projections are accurate, both HIV and HCV diseases can be expected to progress, in the absence of treatment, within seven to fifteen years following infection [110]. A significant proportion of these individuals are current drug users and HCV treatment may never be an option for many. HIV treatment, however, is becoming ever more accessible to drug-using populations and others and may be the only treatment option available. This review suggests that managing the HIV aspects of HIV/HCV co-infection is a complex therapeutic domain, and one in which the ultimate outcome for patients is held in the balance.
2.7 References


10. Cacoub, P., et al., *Mortality among human immunodeficiency virus-infected patients with cirrhosis or hepatocellular carcinoma due to hepatitis C virus in French departments*


Table 2.1. Summary of Evidence For and Against Controversial Hypotheses

<table>
<thead>
<tr>
<th>Hypothesis: HCV Causes a Blunted Immune Response</th>
<th>For</th>
<th>Against</th>
</tr>
</thead>
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<tr>
<td>Zala et al. (2004): Smaller proportion of HCV+ had ≥ 75 cell increase, and took longer to achieve in those who did [26]</td>
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<td>Braitstein et al. (2003): Multivariate mixed effects models suggest smaller average gain of CD4's over 18 months [27]</td>
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<td>Klein et al. (2003): HCV+ had reduced hazard of achieving ≥ 50 cells/mm3 [24]</td>
<td></td>
<td>Aceti et al. (2002): no significant differences in CD4 cell count increases after 6, 12, or 24 months of therapy [28]</td>
</tr>
<tr>
<td>Greub et al. (2000): HCV+ had longer time to CD4 increase of ≥ 50 cells/mm3 [12]</td>
<td></td>
<td>Melvin et al. (2000): no significant difference in change from baseline of CD4 lymphocyte count [25]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypothesis: HCV co-infection leads to higher toxicity-related antiretroviral discontinuation</th>
<th>For</th>
<th>Against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melvin et al. (2000): HCV-positive twice as likely to discontinue due to severe hepatotoxicity [25]</td>
<td></td>
<td>D’Arminio Monforte et al. (2000): no impact of HCV on treatment interruptions due to toxicity [34]</td>
</tr>
<tr>
<td>Ripamonti et al. (2004): HCV-positive had 40% increased risk of discontinuing or changing ART in first year of treatment [33]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aceti et al. (2002): 83% of those who discontinued ART because of hepatotoxicity were HCV-positive [28]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypothesis: HCV-seropositivity leads to increased hepatotoxicity as measured by AST and/or ALT (also see Table 1)</th>
<th>For (HCV+ vs. HCV-)</th>
<th>Against (HCV+ vs. HCV-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livry et al. (2003): HCV+ odds ratio: 3.4 (vs. HCV- OR: 1.0) [52]</td>
<td></td>
<td>Palmon et al. (2002): 0% vs. 2.3% [49]</td>
</tr>
<tr>
<td>Aceti et al. (2002): 5% vs. 1% [28]</td>
<td></td>
<td>Sulkowski et al. (2000): 30% vs. 30% (ritonavir combinations) and 8% vs. 5% (non-ritonavir combinations) [106]</td>
</tr>
<tr>
<td>Sulkowski et al. (2002): 17% vs. 7% [40]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wit et al. (2002): 34% vs. 23% [51]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nunez et al. (2001): 16% vs. 5% [47]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monforte et al. (2001): 11% vs. 7% [111]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Den Brinker et al. (2000): 33% vs. 12% [42]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Comparison</td>
<td>Odds Ratio/Change</td>
</tr>
<tr>
<td>-------------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Saves et al. (2000)</td>
<td>8.0 vs. 1.0</td>
<td>[43]</td>
</tr>
<tr>
<td>Michelet et al. (1999)</td>
<td>57% vs. 11%</td>
<td>[46]</td>
</tr>
<tr>
<td>Saves et al. (1999)</td>
<td>10% vs. &lt;1%</td>
<td>[44]</td>
</tr>
<tr>
<td>Rodriguez-Rosaldo et al. (1998)</td>
<td>21% vs. 7.4%</td>
<td>[45]</td>
</tr>
</tbody>
</table>

**Hypothesis: Antiretroviral therapy increases HCV replication**

<table>
<thead>
<tr>
<th>For</th>
<th>Against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chung et al. (2002): Among baseline CD4 &lt;350 cells/mm³, HCV RNA increased 0.43 log at week 16 and 0.59 log at week 48; among baseline CD4 &gt;350, HCV RNA increased 0.26 log at week 16 and 0.10 log at week 48</td>
<td>Zylberberg et al. (1998): no significant change in HCV RNA after 9 months of ART [50]</td>
</tr>
<tr>
<td>Martin-Carbonero et al. (2002): Increase in HCV RNA from baseline 5.71 logs to 5.88 logs (p=0.01)</td>
<td>[94]</td>
</tr>
<tr>
<td>Puoti et al. (2000): from baseline of 7 log, HCV RNA increased to 7.33 log at day 14 and 7.29 log at day 21</td>
<td>[112]</td>
</tr>
<tr>
<td>Michelet et al. (1999): HCV RNA increases between 0.22 log and 0.61 log depending on treatment group</td>
<td>[46]</td>
</tr>
<tr>
<td>Ragni et al. (1999): HCV RNA increased from 141 X 10^5 Eq/ml at baseline to 198 X 10^5 Eq/ml at week 48 and 227 X 10^5 Eq/ml at week 96 post-ART initiation</td>
<td>[101]</td>
</tr>
</tbody>
</table>

**Hypothesis: That immune restoration has a deleterious impact on progression of HCV-related hepatic fibrosis**

<table>
<thead>
<tr>
<th>For</th>
<th>Against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients initiating ART from a more immunocompromised state have greater increases in HCV RNA [29, 57, 98]</td>
<td>Finding that that not using protease inhibitors was associated with faster progression to cirrhosis [90]</td>
</tr>
<tr>
<td>Case reports and case series indicating rapid evolution of fibrosis, cirrhosis, and end-stage liver disease upon ART initiation [99, 101, 103, 104, 113]</td>
<td>Finding that antiretroviral use reduced long-term liver-related mortality among co-infected hemophiliac patients [91]</td>
</tr>
<tr>
<td>Low baseline CD4 count is strongly associated with progression of fibrosis, cirrhosis, and hepatocellular carcinoma [85-88, 90]</td>
<td>Case reports of spontaneous clearance of HCV RNA following antiretroviral initiation [8, 107]</td>
</tr>
<tr>
<td>Finding that CD4 increase of ≥ 50 cells was associated with a grade</td>
<td>Other findings suggesting weak or no association between baseline CD4, CD4</td>
</tr>
<tr>
<td>3/4 change in AST/ALT [40, 106]</td>
<td>recovery, and the development of elevated liver enzymes [28, 47, 94]</td>
</tr>
</tbody>
</table>
Table 2.2. Summary of available literature regarding hepatotoxicity of antiretroviral agents in HIV/HCV co-infected individuals.

<table>
<thead>
<tr>
<th>Reference &amp; Sample Size</th>
<th>Hepato-toxicity Defined</th>
<th>Overall Rate</th>
<th>HCV+ Rate</th>
<th>HCV- Rate</th>
<th>Predictive Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livry 2003 [52] n=239</td>
<td>ALT &gt;2.5X ULN</td>
<td>21%</td>
<td>Odds ratio: 3.4</td>
<td>Odds ratio: 1.0</td>
<td>HCV, HBV, antiretroviral therapy, CDC Stage C, baseline AST</td>
</tr>
<tr>
<td>Aceti 2002 [28] n=1325</td>
<td>ALT &gt;5X ULN</td>
<td>3.2%</td>
<td>5%</td>
<td>1%</td>
<td>HCV, ritonavir, among co-infected only, with or without saquinavir, alcohol, no CD4 response to HAART</td>
</tr>
<tr>
<td>Martin-Carbonero 2002 [94] n=42</td>
<td>Severe &gt;=5XULN</td>
<td>14%</td>
<td>14%</td>
<td>-</td>
<td>NNRTI's (undifferentiated)</td>
</tr>
<tr>
<td>Sulkowski 2002 [40] n=568</td>
<td>Grade 3 or 4 change from baseline</td>
<td>15.6% (nevirapine) 8% (efavirenz)</td>
<td>17% for EFV + PI's 7% for EFV + PI's</td>
<td>HCV, HBV, nevirapine, protease inhibitors</td>
<td></td>
</tr>
<tr>
<td>Palmon 2002 [49] n=272</td>
<td>Grade 3 or 4 change from baseline</td>
<td>1.1%</td>
<td>0%</td>
<td>2.3%</td>
<td>-</td>
</tr>
<tr>
<td>Wit 2002 [51] n=560</td>
<td>Grade 4 (10XULN or &gt;200U above baseline)</td>
<td>6.3%</td>
<td>34%</td>
<td>23%</td>
<td>Baseline ALT, ritonavir, nevirapine, saquinavir, HCV, HBV, female sex, recent discontinuation of 3TC, first ever HAART</td>
</tr>
<tr>
<td>Nunez 2001 [47] n=222</td>
<td>ALT &gt;5X ULN or &gt;=3.5X</td>
<td>9%</td>
<td>16%</td>
<td>5%</td>
<td>Alcohol abuse, older age, HCV</td>
</tr>
<tr>
<td>Study</td>
<td>Comparator</td>
<td>ALTrise</td>
<td>OR1</td>
<td>OR2</td>
<td>Infection, ddl, ritonavir</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>---------</td>
<td>-----</td>
<td>-----</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Monforte 2001 [111]</td>
<td>ALT &gt;=200 IU/L</td>
<td>4.5%</td>
<td>11%</td>
<td>7%</td>
<td>Baseline ALT, HCV, HbsAg, previous non-HAART therapy, AZT</td>
</tr>
<tr>
<td>Den Brinker 2000 [42]</td>
<td>ALT or AST &gt;=5XULN and absolute increase of &gt;100U/L</td>
<td>18%</td>
<td>33% (45% HbsAg+)</td>
<td>12%</td>
<td>Baseline ALT, HBV, HCV</td>
</tr>
<tr>
<td>Sulkowski 2000 [106]</td>
<td>Severe (grade 3/4 AST/ALT)</td>
<td>10%</td>
<td>Ritonavir combo: 30% Non-ritonavir combo: 8%</td>
<td>Ritonavir combo: 30% Non-ritonavir combo: 5%</td>
<td>Ritonavir, CD4 increase &gt;50 cells</td>
</tr>
<tr>
<td>Saves 2000 [43]</td>
<td>Severe: ALT &gt;=5XULN</td>
<td>5%</td>
<td>OR: 8.0</td>
<td>OR: 1.0</td>
<td>HBV, HCV</td>
</tr>
<tr>
<td>Michelet 1999 [46]</td>
<td>Mean ALT rise</td>
<td>-</td>
<td>57% ritonavir, 10% indinavir</td>
<td>11%</td>
<td>ritonavir</td>
</tr>
<tr>
<td>Saves 1999 [44]</td>
<td>ALT &gt;=5XULN</td>
<td>2%</td>
<td>10%</td>
<td>&lt;1%</td>
<td>Baseline ALT, HBV, HCV infection</td>
</tr>
<tr>
<td>Rodriguez-Rosado 1998 [45]</td>
<td>AST or ALT &gt;2-fold from baseline</td>
<td>14%</td>
<td>21%</td>
<td>7.4%</td>
<td>HCV</td>
</tr>
</tbody>
</table>
Table 2.3. Special Considerations and their rationale regarding the initiation and management of antiretroviral therapy in HIV/HCV co-infected individuals.

<table>
<thead>
<tr>
<th>Special Consideration</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before Starting Antiretroviral Therapy:</strong></td>
<td></td>
</tr>
</tbody>
</table>
| Treat the HCV if possible, even without indicators of HCV disease progression, prior to initiating antiretroviral therapy and at high CD4 counts. | • reduce toxicity of antiretroviral therapy  
• reduce probability of liver disease progression  
• maximize probability of successful HCV treatment outcome |
| Genotype the HCV | • genotype 3 is prone to severely elevated liver enzymes and development of fatty liver  
• genotype 3 has favorable HCV treatment profile |
| **When to Start Antiretroviral Therapy:** | |
| Consider initiating antiretroviral therapy at higher CD4 counts (e.g. >350 cells/mm³), particularly among men and those who acquired HCV at an older age. | • even mild immune suppression may enhance HCV replication and accelerate liver disease  
• moderate and severe immune suppression are strongly associated with accelerated liver disease  
• immune reconstitution may cause enhanced HCV replication and histologic damage through an auto-inflammatory response  
• evidence may suggest a blunted immune response among co-infected initiating HAART |
| **When Starting Antiretroviral Therapy:** | |
| Avoid using antiretroviral drugs or combinations of drugs known to cause mitochondrial toxicity (i.e. ddI, d4T), and monitor closely for signs of lactic acidemia/acidosis, particularly among women and older individuals. | • hepatitis C independently causes mitochondrial damage;  
• clinical manifestations of mitochondrial damage include fatty liver (especially in those with genotype 3 and/or lipodystrophy), peripheral neuropathy, lipoatrophy, lactic acidemia |
| Avoid concomitant use of AZT and ribavirin, and concomitant use of ddI or d4T and ribavirin | • both AZT and ribavirin can cause profound anemia  
• ddI, d4T, and ribavirin can all cause lactic acidosis, especially when combined |
| Avoid using nevirapine and full dose ritonavir and be aware of the | • both agents are strongly associated with severe hepatic toxicity |
| lack of data regarding booster doses of ritonavir in co-infected patients. |
| Avoid concomitant use of hepatitis C treatment. | • overlapping and additive toxicities are very difficult to tolerate |
| Consider baseline liver biopsy. | • to know whether liver disease post-HAART initiation is stable or progressing |

| **After Starting Antiretroviral Therapy:** |
| If patient tested HCV antibody negative prior to antiretroviral therapy initiation, retest within 6-12 months; if patient had a negative HCV RNA test prior to HAART initiation, retest within 6-12 months. | • Antibodies to HCV may only appear with an immune response • Limited evidence suggests the re-emergence of HCV RNA upon HAART initiation |
| Rely on a variety of markers of liver inflammation and disease, including ultrasound and biopsy, to assess HCV disease progression. | • ALT levels are a poor predictor of liver disease |
| Monitor co-infected patients closely for fatty liver, particularly among those with HCV genotype 3. | • associated with mitochondrial damage, insulin resistance, and lipodystrophy |
| Monitor co-infected patients closely for development of insulin resistance and diabetes, and encourage proactive preventive measures (diet and exercise). | • insulin resistance and diabetes are linked to both HCV infection and antiretroviral use |
Figure 2.1. Overlap Between Morbidity Associated with Hepatitis C and Toxicity Associated with Antiretrovirals

- **Antiretrovirals**
  - Metabolic Abnormalities
    - dislipidemia
    - insulin resistance
    - diabetes
    - lipoatrophy, lipodystrophy
  - Mitochondrial toxicity
    - hyperlactatemia
    - lactic acidosis/acidosis
    - peripheral neuropathy
    - lipoatrophy, lipodystrophy

- **Hepatitis C**
  - Hepatic Injury
    - transaminitis
    - fatty liver
    - fibrosis/cirrhosis
    - end stage liver disease

- **Age & Biological Sex**
CHAPTER 3:
STUDY SETTING, OVERVIEW OF METHODS, AND LIMITATIONS

3.1 STUDY SETTING: THE BRITISH COLUMBIA HIV/AIDS DRUG TREATMENT PROGRAM AND THE HOMER COHORT

Antiretroviral medications have been centrally distributed at no cost to eligible HIV-infected individuals since 1986. In October 1992, the distribution of antiretroviral agents became the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Centre for Excellence in HIV/AIDS. This antiretroviral drug distribution program remains the only free source of antiretroviral medication in this Canadian province (and is a unique program in Canada). The Centre's HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul's Hospital site, and the program conforms with the province's Freedom of Information and Protection of Privacy Act.

The Centre distributes antiretroviral drugs based on specific guidelines generated by the Therapeutic Guidelines Committee [1]. These guidelines have been and continue to be consistent with those treatment guidelines published by the International AIDS Society [2-5]. The Centre's guidelines recommend that CD4 and plasma HIV-1 RNA levels be monitored at baseline, at 4 weeks after starting antiretroviral therapy and every three months thereafter. Plasma viral loads are measured using the Amplicor HIV-1 Monitor™ (Roche Diagnostics Branchburg, NJ).
All classes of federally licensed antiretroviral drugs are currently available through the program, including all nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). Tenofovir, atazanavir, and enfuvirtide are also available. Eligibility for antiretroviral medication has remained consistent with current international recommendations [5].

Individuals are automatically entered into the DTP when they are first prescribed any antiretroviral agent. At DTP entry, sociodemographic information is captured, and the participant’s complete history (if any) of antiretroviral use, CD4 cell count, plasma viral load, and disease stage are recorded at entry and with each subsequent physician visit. Typically, patients are followed-up at 3-month intervals, at which time prescriptions are renewed or altered based on treatment success and other clinical factors. Blood drawn for the purposes of CD4 and viral load testing is stored for each individual at baseline and at each follow-up visit for future research activities related to HIV disease.

3.2 STUDY POPULATION

The HAART Observational Medical Evaluation and Research (HOMER) study is a nested cohort within the BC Centre for Excellence’s HIV/AIDS Drug Treatment Program. It includes all previously ART naïve individuals who initiated antiretroviral therapy with a triple-drug regimen consisting of either two nucleoside reverse transcriptase inhibitors (NRTI) plus either a protease inhibitor
(PI), or a non-nucleoside reverse transcriptase inhibitors (NNRTI), between August, 1996 and July, 2000. The data used in these analyses are based on individuals from the HOMER Cohort, for whom there was HCV serological data.

HOMER consists of 1388 participants. There were available samples with sufficient volume for testing for 1257 of them, and unambiguous HCV antibody results were obtained for 1186. Therefore, there were 1186 individuals available for analysis.

3.3 Other Data Sources

Laboratory Data: HCV antibody and HCV RNA testing were conducted on samples stored at baseline (0 to –6 months pre-ART initiation) as part of treatment recipients participation in the HIV/AIDS Drug Treatment Program. All antibody and RNA testing was conducted at the UBC Virology Laboratory, located in St. Paul’s Hospital (Appendix 3.1).

CD4 and plasma HIV viral load data are collected routinely as patients receive their regular monitoring, as part of the approved data collection component of the HIV/AIDS Drug Treatment Program. The biochemical markers of hepatic injury (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, and International Normalized Ratio (INR)) were obtained through a UBC Ethics approved linkage with the Providence Health Laboratory (Appendix 3.2).
Mortality: All deaths from HIV/AIDS in the province were determined through a BC Centre for Excellence in HIV/AIDS's initiated linkage with the province’s death registry housed at the British Columbia Ministry of Health’s Vital Statistics Agency in Victoria. A file was acquired from the British Columbia Ministry of Health's Vital Statistics Agency, which queried their database to extract records for all deaths that contained an ICD-9 (1995–1999) or ICD-10 (2000–2001) code indicating that HIV met the criteria of an underlying or associated cause of death. Vital Statistics defines a death as "HIV underlying" if the attending physician and/or coroner determines that the death was directly due to HIV infection. Alternatively, Vital Statistics defines a death as "HIV associated" if the attending physician and/or coroner determines that HIV infection was only a condition that contributed to the individual's underlying cause of death. Persons who died of accidental causes—such as illicit drug overdoses, suicides, and accidents—are not routinely tested for HIV in the province, and unless an individual is experiencing morbidity due to confirmed HIV infection, their death should not be coded as associated with HIV [19].

Adherence to Antiretrovirals: The measure of adherence used by the HIV/AIDS Drug Treatment Program is a continuous variable based on the ratio of time that medication dispensed would last as a proportion of follow-up time. This calculation is restricted to each patient’s first year on therapy to avoid reverse causation that could occur among patients who cease antiretroviral therapy after they have become too sick to take medication [6]. This measure is a strong predictor of HIV virologic response [7] and survival [8, 9]. For the purposes of the present analyses, adherence was a binary variable where 1 was being greater
than 95% adherent (meaning having refilled at least 95% of their prescriptions during the year), and 0 less than 95% adherent. This is based on the rationale that maximal virologic suppression requires greater than 95% adherence to antiretroviral medication [9, 10].

3.4 Statistical Methods

Standard statistical techniques were used in each study. In Chapters 4, 5, 6 and 7, parametric and non-parametric tests were used, some in cross-sectional analysis and some in both cross-sectional and longitudinal analyses. For the cross-sectional analyses, such as baseline characteristics, categorical explanatory variables were analyzed using Pearson’s Chi-square test and continuous variables were analyzed using the Wilcoxon rank sum test. Fisher’s exact test was used for 2x2 contingency tables in which any of the expected cell frequencies was less than 5.

Multivariate methods were used in Chapters 4-7 to account for potential confounding effects. Chapters 4 and 6 used multivariate logistic regression; chapter 5 used a combination of mixed effects models and Cox Proportional Hazards methods to describe the impact of HCV co-infection on CD4 response to ART. Chapter 7 used standard survival analysis techniques. Cumulative event rates were derived using Kaplan-Meier methods, and survival curves were compared between groups with the log-rank test. Cox proportional hazards regression was used to calculate univariate and adjusted relative hazards and
95% confidence intervals (CIs). In each case, the assumption of proportional hazards was validated by inspection of \( \log(-\log [\text{survival function}]) \) estimates against \( \log \) time plots.

All statistical analyses were performed using SAS software version 6.2 (SAS, Cary, NC). All tests of significance were two-sided, with a p-value of less than 0.05 indicating that an association was statistically significant. A more detailed description of the specific statistical methods employed in each manuscript can be found in the relevant chapters.

3.5 **Strengths and Limitations**

Each study that makes up this thesis has specific strengths and limitations that will be addressed in detail in each chapter. Nevertheless, some of these issues are common through many or all of the studies, and should be discussed at the outset.

A key first strength is that the data are from a setting where all HIV/AIDS care, antiretrovirals, and laboratory monitoring are available free of charge, and where previous studies have shown that virtually all patients acquire antiretrovirals through a centralized source [10]. As such, the interpretability of the findings presented here are less compromised by selection factors that may bias other cohort studies. The HOMER cohort may be considered representative of those individuals coming forward for HIV treatment, but will not be representative of all HIV-infected individuals in British Columbia [11]. This is especially pertinent in the context of HIV/HCV co-infection because the people
most likely to have died of HIV in BC without accessing antiretrovirals, may also have been more likely to have been co-infected with HCV [11].

The measure of adherence used in these analyses has been validated as a predictor of virologic suppression and mortality [7, 9, 12], and the fact that we were able to account for adherence to ART in chapters 5-7 significantly strengthens the interpretability of our findings. However, it should be noted that this is quite a crude measure, and is a proxy for actually swallowing pills. Further, though generally referred to as ‘adherence’, conceptually it includes people who don’t adhere because of lifestyle or other, and those people who can’t adhere, because of toxicity or co-morbidity.

Having HCV serology data for nearly the entire HOMER cohort for chapters 5-7 enabled these analyses to have sufficient power, and to be conducted in a population-based setting. These data are nonetheless limited by the fact that the testing was conducted on baseline samples, and may not reflect a patient’s current HCV status. For example, they may have acquired HCV infection since they initiated ART, or they may have been effectively treated for HCV since the time of testing. A related strength of this study was that we were able to re-test most antibody positive/RNA negative discordant samples following treatment initiation.

Objectives 5, 6, and 7 all use HCV serological data to define HCV co-infection. This could potentially lead to a misclassification bias by counting as ‘HCV-negative’ those 5% who have no positive antibodies but do have detectable HCV RNA [13, 14]. Misclassification could also occur in counting as ‘HCV-
positive' those individuals with positive antibodies, but no RNA. At the outset
of this project, reports in the literature indicated that a smaller proportion of HIV
co-infected spontaneously clear HCV (resulting in positive antibodies but no
RNA) compared to HIV-uninfected, although good studies examining this
question are still lacking [15, 16]. As will be seen in Chapter 4, a very high
proportion of individuals with positive HCV antibodies in this cohort had no
detectable HCV RNA at baseline (30%) or 6-12 months following HIV treatment
initiation (25%), resulting in a concern that estimates based on HCV serology
would be biased.

PCR testing was conducted on all baseline antibody-positive samples, and
10% of the antibody-negatives. Therefore in order to assess if there was a
possible misclassification bias because of the high proportion of antibody-
positive/PCR negative discordant results in the study, among individuals with
positive HCV antibodies, I performed a survival analysis comparing those with
detectable and undetectable HCV RNA. In unadjusted and adjusted Cox
models, there were no differences detected, suggesting less of a potential for
bias.

There is justification to define 'HCV co-infected' based primarily on HCV
serology. The absence of HCV RNA could be due to several factors, one of
which is spontaneous clearance. Most of the samples tested were quite old (7-9
years), and had already been repeatedly frozen and thawed. Further,
undetectability does not indicate that there is no virus present: the
AmpliPrep/COBAS system used in this study for qualitatively detecting HCV
RNA has a lower limit of detection of 100 copies/ml [17]. There is literature to
suggest that in HIV co-infected patients, there is poor correlation between levels of HCV RNA in the liver and in the plasma, showing low levels in the plasma but high levels in the liver [18]. HIV has recently been found to facilitate HCV infection of macrophages [19], possibly creating a reservoir for HCV. In sum, lack of detectability of the virus may not mean the virus is not there. Therefore, basing analyses on HCV serological data is considered an acceptable approach.

3.6 SUMMARY

In summary, the methods employed in this thesis are diverse and rely on a number of data sources. Through a retrospective diagnostic testing study and the statistical evaluation of quantitative data, these studies have endeavoured to describe some of the impact of HCV in HIV-infected individuals initiating antiretroviral treatment. While each of the approaches applied in the various sub-studies has individual strengths and weaknesses, together the combined studies provide insight into some of the possible consequences of HIV/HCV co-infection in the HAART era. The unique contribution that these studies have made will be described in detail in Chapter 8.
3.7 REFERENCES


CHAPTER 4

DIAGNOSTIC ISSUES AMONG HIV/HCV CO-INFECTED INDIVIDUALS

4.1 FORWARD

This chapter presents the findings of the CIHR-funded study, “Establishing the True Prevalence of Hepatitis C Among HIV-Infected Individuals Initiating Antiretroviral Therapy” (Appendix 2). It is under review with Hepatology as:


73
It is estimated that as much as 3% of the world's population is infected with the Hepatitis C virus (HCV) [1], including an estimated 2% of people from the United States [2], and 1% of Canadians [3]. As of December, 2002, the United Nations had estimated that there were 42 million people living with HIV/AIDS in the world [4], including approximately 40,000 in Canada [5]. Due to shared routes of transmission such as receiving contaminated blood products and injection drug use, the prevalence of co-infection by both HIV and Hepatitis C is considerable, particularly in some populations. In Canada and elsewhere in the Developed World including the United States, it is estimated that as many as 30% of all individuals who have HIV also have Hepatitis C [6-8]. However, these estimates are drawn from clinic-based, clinical trial based, or population-specific (e.g. injection drug users) samples. In British Columbia, all estimates of HIV/HCV co-infection are drawn from an urban cohort of injection drug users [7, 9, 10]. End-stage liver disease has become a leading cause of death in HIV-infected individuals and is threatening gains made by highly active antiretroviral therapy, particularly among HCV/HIV co-infected individuals [11-13].

The accurate diagnosis of HCV in HIV-infected individuals is complicated by several factors. Due to a variety of factors including immune suppression, approximately 5% of HIV-infected patients will test false antibody-negative for HCV [14, 15]. Further, among those not infected with HIV, approximately 15-20% can expect to spontaneously clear HCV RNA through a host immune response [16]. However, the probability of spontaneous HCV RNA clearance among HIV co-infected individuals has been reported to be much lower, at 5-10%, particularly among those with lower CD4 counts.
Factors found to be associated with spontaneous clearance include significant alcohol use, race, and HIV co-infection [19].

Immune reconstitution may play an additional complicating factor in the diagnosis of hepatitis C among HIV-infected patients. There have been two case reports of spontaneous clearance of HCV RNA following HAART initiation, presumably related to immune reconstitution [20, 21]. And of much greater concern, a recent but very small study found that of 50 HIV and HCV seropositive individuals assessed, HCV RNA was not detectable at baseline in 10 of them. However following antiretroviral initiation, 4 (25%) of these subsequently became HCV RNA positive [22].

Hepatitis C treatments are evolving and offer hope to many HIV/HCV co-infected patients [23]. For those individuals for whom HCV treatment will not be effective, there are several issues related to their HIV management and lifestyle that can be modified to reduce the probability of disease progression and maximize their therapeutic options [24]. The timely and accurate diagnosis of HCV in HIV-positive individuals is of the utmost clinical importance, and understanding the impact of antiretroviral therapy on that diagnosis is imperative.

Therefore, the objectives of the present study were to measure the baseline (pre-ART) prevalence of Hepatitis C antibodies among HIV-positive individuals initiating antiretroviral therapy in a population-based HIV treatment cohort, the prevalence of discordant positive antibody/negative RNA results at baseline, and to describe factors associated with each. Secondly, we sought to measure the impact of antiretroviral therapy on the detectability of HCV RNA among those individuals who were HCV RNA negative but antibody positive at baseline using a sample taken six to twelve
months following treatment initiation, and to describe factors associated with newly detectable HCV RNA.

4.3 METHODS

The HIV/AIDS Drug Treatment Program

Antiretroviral medications have been centrally distributed at no cost to eligible HIV-infected individuals since 1986. In October 1992, the distribution of antiretroviral agents became the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Center for Excellence in HIV/AIDS. This antiretroviral drug distribution program remains the only free source of antiretroviral medication in the province. The Centre's HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul's Hospital site, and the program conforms with the province's Freedom of Information and Protection of Privacy Act.

The Centre distributes antiretroviral drugs based on specific guidelines generated by the therapeutic guidelines committee, and have remained consistent with international standards [25-28]. Plasma viral loads were measured using the Amplicor HIV-1 Monitor™ (Roche Diagnostics Branchburg, NJ). All three classes of federally licensed antiretroviral drugs are currently available through the program, including all nucleoside and nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors.

Individuals are automatically entered into the DTP when they are first prescribed any antiretroviral agent. At DTP entry and with each subsequent physician visit, the
participant's complete history (if any) of antiretroviral use, CD4 cell count, plasma viral load, and disease stage are recorded. Typically, patients are followed-up at 3-month intervals, at which time prescriptions are renewed or altered based on treatment success and other clinical factors. Blood drawn for the purposes of CD4 and viral load testing is stored for each individual at each follow-up visit for future research activities related to HIV disease.

Study Population:

The HAART Observational Medical Evaluation and Research (HOMER) study is a nested cohort within the BC Centre for Excellence's HIV/AIDS Drug Treatment Program. It includes all previously ART naive individuals who initiated antiretroviral therapy with a triple-drug regimen consisting of either two nucleoside reverse transcriptase inhibitors (NRTI) plus either a protease inhibitor (PI), or a non-nucleoside reverse transcriptase inhibitors (NNRTI), between August, 1996 and July, 2000. The data used in these analyses are based on individuals from the HOMER Cohort.

Study Design:

Frozen, archived samples of blood plasma are available to the Drug Treatment Program. The first sample taken, that from within 6 months prior to HAART initiation, was tested for HCV antibodies (Ab). All individuals with positive antibodies were tested for the presence of HCV RNA, and due to the prohibitive cost of PCR testing, only a random sample of 10% of those with negative HCV antibodies were tested using PCR methods. Individuals with positive-antibody and negative PCR results at baseline
were re-tested using a sample from 6 - 12 months post-ART initiation to determine HCV RNA detectability following treatment initiation.

HCV Screening:

Blood samples are collected and stored frozen at -20°C until time of processing. Plasma is separated within 6 hours of collection by centrifuging at 800-1600xg for 15 minutes at room temperature. Some 0.025 µL of sample was aliquoted from the stored sample. Certified negative human plasma was added to the aliquot, and then used for testing.

Blood samples were tested for HCV antibody using the Abbott primary screen. This assay has a sensitivity greater than 99% and a specificity of 99%, in immune-competent patients [29]. HCV RNA was detected using the Roche Cobas HCV AmpliPrep/COBAS Amplicor test. The AmpliPrep/COBAS Amplicor Hepatitis C Virus test (v.2.0) is a qualitative nucleic acid amplification test for the detection of HCV in clinical specimens of human plasma. Sample preparation is automated using the COBAS AmpliPrep Instrument, and amplification and detection are automated using the COBAS Amplicor Analyzer. This is a qualitative assay with a lower level of detection of HCV RNA of 100 copies per ml. The specificity for this assay exceeds 98% [29].

All testing was completed at the University of British Columbia Virology Laboratory at St Paul's Hospital, and ethics approval was obtained prior to testing.

Statistical Methods:

Baseline sociodemographic and clinical factors associated with HCV antibody-positive prevalence and antibody-positive/PCR-negative results at baseline were performed using parametric and non-parametric methods. Student’s t-test was used for normally
distributed continuous variables, and the Wilcoxon Rank Sum test for non-normal continuous variables. Categorical data were analyzed using Pearson’s Chi-Square test. Fisher’s Exact Test was used for contingency tables in which 25% or more of the expected cell frequencies were less than 5. As this was a descriptive analysis, factors independently associated with each outcome were assessed using forward stepwise multivariate logistic regression. The multivariate models were constructed by entering those variables which were bivariately statistically significant (p<0.05) or if they were considered potential confounders.

Independent variables measured were gender, age at baseline, whether they had any history of injection drug use, CD4 count at baseline, HIV log$_{10}$ plasma RNA at baseline, whether they had an AIDS diagnosis at baseline, and type of ART initiated (protease inhibitor based or non-nucleoside reverse transcriptase inhibitor based). Ethnicity was not examined because ethnicity data is not routinely collected in the DTP.

4.4 Results

Of 1388 eligible individuals in the HOMER Cohort, 1257 samples were both available and had sufficient quantity for testing. Of these, 1186 samples had clear antibody results, including 606 (51%) antibody positive, and 580 (49%) antibody negative (Figure 1). Factors associated with HCV positive antibody prevalence are summarized in Table 1 and were male sex (78% vs. 93%, p<0.001), any history of injection drug use (47% vs. 6%, p<0.001), an AIDS diagnosis at baseline (11% vs. 15%, p=0.028), and CD4 fraction at baseline (19% vs. 16%, p<0.001). Not associated with HCV antibody prevalence were
median age at baseline, absolute baseline CD4 count, baseline HIV RNA, or type of ART initiated.

Among those with positive antibody test results, 605 samples were tested for HCV RNA at baseline (one clotted), and 425 (70%) were positive, while 179 (30%) were negative. Among those with negative antibody results, 58 were tested for HCV RNA. One clotted and was therefore not included; 56 (98%) were RNA negative, while 1 (2%) was HCV RNA positive. Factors associated with antibody positive/RNA negative discordancy at baseline were any history of injection drug use (32% vs. 53%, p<0.001), having an AIDS diagnosis at baseline (17% vs. 8%, p=0.002), median baseline CD4 (230 vs. 290, p=0.001), but not male sex, median age at baseline, or median baseline log10 HIV RNA. In multivariate logistic regression (Table 2), factors independently associated with baseline antibody-positive/RNA-negative discordancy were any IDU (Adjusted Odds Ratio, AOR 0.45, 95% confidence interval, CI: 0.31 – 0.65, p<0.001), having an AIDS diagnosis at baseline (AOR 1.86, 95% CI: 1.05 – 3.28, p=0.033), and absolute baseline CD4 count (per 100 cells) (AOR 0.89, 95% CI: 0.81 – 0.98, p=0.018).

There were 118/179 discordant patients at baseline for whom a sample taken 6-12 months post-ART initiation was available. Of these, 94 (80%) remained HCV RNA negative, while 24 (20%) became HCV RNA positive. Compared to patients whose HCV RNA remained undetectable, factors associated with new HCV RNA detectability following ART-initiation were median baseline CD4 (70 vs. 230, p=0.040) and median log10 HIV RNA at baseline (5.5 vs. 5.0, p=0.018), but not male sex (67% vs. 78%, p=0.226), any history of IDU (42% vs. 29%, p=0.235), having an AIDS diagnosis at baseline (13% vs. 18%, p=0.761), or median age at baseline (35.8 vs. 38.0, p=0.124). As summarized in Table 3, in multivariate logistic regression, the only factor
independently associated with newly detectable HCV RNA in those undetectable at baseline was baseline plasma log$_{10}$ HIV RNA (AOR 2.80, 95% CI: 1.08 – 7.24, p=0.034).

4.5 DISCUSSION

Our data indicate a very high prevalence of HCV antibodies at baseline in this population-based cohort, at 51%. These participants were less likely to be male, more likely to have any history of injection drug use, and had a higher CD4 fraction at baseline. Our study also found a very high proportion of discordant antibody positive/PCR negative results at baseline (30%). Factors independently associated with this baseline discordancy include injection drug use, baseline CD4 count, and having an AIDS diagnosis at baseline. Our data further suggest that HCV RNA may become detectable in 20% of individuals who at baseline were HCV RNA negative, following ART initiation, and that baseline HIV RNA may play an important role in this change.

Our data have several critical implications. One, because the study population is a population-based sample, the prevalence of HCV we have found is the most accurate indication of the prevalence of HCV/HIV co-infection in British Columbia, Canada, and is, to our knowledge, the only population-based measure reported in the literature. Our finding is higher than other reported averages [30, 31], and may in part reflect the large injection drug using population in British Columbia. However, previous work done by our center suggests that 30% of people dying HIV-related deaths never access antiretroviral therapy and are therefore not captured in our cohort. These individuals are more likely to have sociodemographic profiles consistent with BC’s injection drug using population and therefore may be more likely to be co-infected with HCV [32].
Therefore, it may be expected that our estimate of 51% is a conservative reflection of the true prevalence of HCV among HIV-infected patients in this province.

Two, although factors associated with HCV antibody-positive prevalence are consistent with others reported in the literature [6, 33, 34], the majority of individuals with positive HCV antibodies in our study have no reported history of injection drug use. This may indicate that HCV is a problem that goes well beyond the traditional populations considered at high risk of acquiring HCV (i.e. injection drug users). HCV is approximately 10 times more infectious than HIV [30], and can be spread through minute amounts of blood that may be present in intranasal drug-using equipment, improperly sterilized tattoo equipment, or certain types of sexual behavior. Our study underlines the importance of HCV testing in all HIV-infected patients.

The third major implication is that having undetectable HCV RNA may not mean that the virus is cleared from these patients. Being undetectable may be due to a variety of factors, including the age of our samples, or the fact that some of them were frozen and thawed a number of times prior to this study. The lower limit of detectability for the assay used in this study is 100 copies/ml [29].

However, the prevalence of discordant positive antibodies/undetectable RNA at baseline in our study (30%) is significantly higher than other reports which suggest that only 5-10% of HIV co-infected may spontaneously clear HCV RNA [6, 17, 18]. If these results are really due to spontaneous clearance, they may be so high because of the high prevalence of non-IDU in our study. Many individuals, particularly men who have sex with men over the age of 40 or 50, may have experimented with injection drugs in the
1970's or 1980's, contracted HCV infection prior to contracting HIV infection, and were thus more likely to have spontaneously cleared the HCV infection.

A previous very small report indicated that 4/10 baseline HCV RNA negative HIV-infected patients developed detectable HCV RNA following ART initiation [22]. Our study confirms that at least 20% of HIV-infected individuals may develop detectable HCV RNA following ART initiation, and further suggests that this may be related to plasma HIV RNA levels. This may be an indication of direct viral-viral interactions, or it may be related to the presence of HCV RNA reservoirs, particularly in HIV co-infected patients [35]. Laskus et al. (2004) recently found that HCV was more likely to replicate in monocytes/macrophages in HIV co-infected patients [36]. The association in our data with baseline HIV RNA may be related to the enhanced expression of HCV RNA from monocytes following treatment initiation. Other data indicate several mechanisms by which suppression of HIV replication and a reconstituted immune system influence HCV viral diversity in HIV co-infected patients [37]. Controlled clinical trials prospectively examining some of these questions would be very useful.

This investigation has several important strengths. The study population is a population-based cohort, and therefore has the advantage of being broadly representative of the population of people living with HIV and Hepatitis C who have initiated antiretroviral therapy in the HAART era, in the Developed World (in spite of the limitation referred to earlier). Two, because stored samples were available from both before and after treatment initiation, we were able to examine the effect of ART on the prevalence of HCV in this HIV-infected population, and to investigate factors associated with antibody positive/RNA negative discordancy and post-treatment HCV RNA detectability. Three, effective HCV treatment only became available very recently,
and all samples tested were from a time period where the effect of HCV treatment, particularly in HIV-co-infected patients, would be negligible, and is therefore not a significant potential confounder in this analysis. Fourth, all the testing was done using the same assays, in the same laboratory, eliminating any bias due to differences in assays or laboratories.

There may also be limitations to this analysis. Due to cost constraints, we were not able to test the entire group of nearly 4000 individuals currently receiving antiretroviral therapy in the province of British Columbia. We hoped that selecting previously antiretroviral naive individuals who had initiated treatment with a triple-combination would enable us to control for the potential confounding effects of extensive pre-treatment. Also due to cost constraints, we were only able to test 10% of antibody-negative results. However, the probability of false-antibody negative results has been well established elsewhere [14] and was not a primary objective of our analysis. Third, the age of the samples and the repeated thawing of them may have compromised the integrity of sample. However, samples that were not clearly antibody positive or negative were excluded from all future analysis, as were samples that were not clearly either PCR positive or negative. Four, our measure of injection drug use is a composite using data available either from physician-report or patient self-report. There is likely some under-reporting of injection drug use, particularly among men who have sex with men whose experience of using injection drugs may have been twenty or more years ago, and was only recreational in nature. Finally, the sub-study examining HCV RNA detectability post-treatment had a small number of people with the outcome of interest, limiting the interpretability of factors associated with experiencing it.
Although screening for hepatitis C among HIV-infected persons is considered standard of care in British Columbia, many physicians do not routinely test their patients because they perceive them to be at low-risk. Routine screening involves only antibody testing, with PCR testing only in exceptional circumstances. Our data suggest a much higher prevalence of HCV than previously expected, and underscore the importance of testing all HIV-infected individuals for the presence of HCV antibodies and RNA prior to initiating antiretroviral therapy. This would enable appropriate therapeutic and lifestyle management choices to be made, ultimately maximizing both HIV and HCV treatment options. Patients who have no evidence of HCV antibodies or RNA at baseline, should be re-tested following ART initiation so that the optimum medical management of these patients can occur.

In summary, HCV co-infection is an important and clinically very relevant co-morbidity in HIV. It is widely prevalent among HIV-infected populations, and may be higher than expected because of its infectiousness. There are numerous options for the HIV and HCV treatment and management of these patients, but in order to effect these choices, an accurate and timely diagnosis must be made, recognizing that antiretroviral therapy and its subsequent effects on immune reconstitution may alter that diagnosis.
4.6 REFERENCES


Table 4.1. Baseline factors associated with HCV seroprevalence among 1186 HIV-infected individuals initiating antiretroviral therapy

<table>
<thead>
<tr>
<th></th>
<th>HCV-positive n=606</th>
<th>HCV-negative n=580</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>473 (78%)</td>
<td>541 (93%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any IDU*</td>
<td>285 (47%)</td>
<td>36 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median age (IQR**)</td>
<td>37.8 (32.2 – 44.0)</td>
<td>36.8 (32.0 – 43.8)</td>
<td>0.505</td>
</tr>
<tr>
<td>AIDS diagnosis</td>
<td>65 (11%)</td>
<td>87 (15%)</td>
<td>0.028</td>
</tr>
<tr>
<td>Absolute CD4 (median, IQR)</td>
<td>280 (130 – 430)</td>
<td>270 (130 – 420)</td>
<td>0.562</td>
</tr>
<tr>
<td>CD4 Fraction (%)</td>
<td>19 (11 – 27)</td>
<td>16 (9 – 24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log₁₀ HIV RNA (median, IQR)</td>
<td>5.0 (4.6 – 5.0)</td>
<td>5.0 (4.6 – 5.0)</td>
<td>0.994</td>
</tr>
<tr>
<td>Started with a PI</td>
<td>417 (69%)</td>
<td>388 (67%)</td>
<td>0.480</td>
</tr>
</tbody>
</table>

* IDU = injection drug use
** IQR = interquartile range
Table 4.2. Logistic regression analysis of factors associated with positive antibody but negative HCV RNA results at baseline (n=179)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Odds (95% CI)</th>
<th>p-value</th>
<th>Adjusted Odds (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>0.88 (0.58 – 1.33)</td>
<td>0.876</td>
<td>0.75 (0.48 – 1.17)</td>
<td>0.201</td>
</tr>
<tr>
<td>Age (per 10 yrs)</td>
<td>1.13 (0.92 – 1.34)</td>
<td>0.257</td>
<td>1.10 (0.89 – 1.37)</td>
<td>0.385</td>
</tr>
<tr>
<td>Any IDU (yes vs. no)</td>
<td>0.43 (0.30 – 0.62)</td>
<td>&lt;0.001</td>
<td>0.45 (0.31 – 0.65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AIDS diagnosis (yes vs. no)</td>
<td>2.24 (1.33 – 3.79)</td>
<td>0.003</td>
<td>1.86 (1.05 – 3.28)</td>
<td>0.033</td>
</tr>
<tr>
<td>Absolute CD4 (per 100 cells)</td>
<td>0.87 (0.79 – 0.94)</td>
<td>0.001</td>
<td>0.89 (0.81 – 0.98)</td>
<td>0.018</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; HIV RNA</td>
<td>0.93 (0.72 – 1.20)</td>
<td>0.594</td>
<td>0.85 (0.65 – 1.10)</td>
<td>0.214</td>
</tr>
</tbody>
</table>
Table 4.3. Logistic regression analysis of factors associated with undetectable HCV RNA at baseline but detectable HCV RNA 6 – 12 months post-ART initiation among HIV-infected patients (n=118)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Odds (95% CI)</th>
<th>p-value</th>
<th>Adjusted Odds (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>0.55 (0.21 – 1.46)</td>
<td>0.239</td>
<td>0.50 (0.15 – 1.66)</td>
<td>0.246</td>
</tr>
<tr>
<td>Age (per 10 yrs)</td>
<td>0.62 (0.35 – 1.08)</td>
<td>0.093</td>
<td>0.60 (0.31 – 1.17)</td>
<td>0.132</td>
</tr>
<tr>
<td>Any IDU (yes vs.no)</td>
<td>0.55 (0.21 – 1.46)</td>
<td>0.239</td>
<td>1.89 (0.65 – 5.50)</td>
<td>0.246</td>
</tr>
<tr>
<td>AIDS diagnosis</td>
<td>0.64 (0.17 – 2.39)</td>
<td>0.505</td>
<td>0.26 (0.06 – 1.21)</td>
<td>0.086</td>
</tr>
<tr>
<td>Absolute CD4 (per 100 cells)</td>
<td>0.81 (0.62 – 1.07)</td>
<td>0.137</td>
<td>0.77 (0.54 – 1.09)</td>
<td>0.144</td>
</tr>
<tr>
<td>Log10 HIV RNA</td>
<td>2.44 (1.09 – 5.50)</td>
<td>0.031</td>
<td>2.80 (1.08 – 7.24)</td>
<td>0.034</td>
</tr>
</tbody>
</table>
Figure 4.1 Summary of Study Design and Results

Stored baseline plasma samples (HOMER) n=1388
Samples available and sufficient volume for testing n=1257

HCV antibody testing (Abbott primary screen) n=1257
Results available n=1186

HCV-Ab pos
N=606

HCV Ab neg
N=580

PCR testing on all available samples n= 605
PCR-pos: 425 (70%) PCR-neg: 179 (30%)

PCR testing on random 10% n=58
PCR-pos: 1 (2%) PCR-neg: 56 (97%)

6-12 months post-treatment:
N=118 Ab+/PCR at baseline with an available & sufficient sample
PCR-pos: 24 (20%) PCR-neg: 94 (80%)
CHAPTER 5:
RESPONSE TO ANTIRETROVIRAL THERAPY
AMONG HIV/HCV CO-INFECTED ADULTS

5.1 FORWARD

This chapter is currently under review with the Journal of Infectious Disease as:

Braitstein P, Zala C, Yip B, Moore D, Hogg RS, Montaner. "Attenuated Absolute but Preserved CD4 Fraction Response to Antiretroviral Therapy Among HIV/HCV Co-Infected Adults in a Population-Based Antiretroviral Treatment Program".
5.2 INTRODUCTION

The use of Highly Active Antiretroviral Therapy (HAART) has led to dramatic reductions in HIV-related morbidity and mortality in HIV-infected individuals [1-3]. However, parallel with this success has been the subsequent emergence of co-morbidities, such as viral hepatitis. The hepatitis C virus (HCV) is present in approximately 30% of HIV-positive people in the Developed World, and in some populations such as injection drug users and hemophiliacs, nearly all those who are HIV infected are co-infected with HCV [4-7].

Immunologic and virologic response to antiretroviral therapy in HCV co-infected populations have been previously examined. Most studies have found that there is no impact of HCV on the HIV virologic response to antiretrovirals [8-11]. However, there are conflicting reports in the literature regarding immunologic response. Greub et al. found that HCV infection independently predicted a poorer immune response to antiretroviral therapy, defined as time to an increase of at least 50 cells/mm3 [8]. In contrast, several others have found little or no difference in CD4 increase by HCV serostatus [9, 11, 12].

The data regarding immunologic response to antiretroviral treatment may be inconsistent for several reasons. It can take up to 24 months of treatment for a complete CD4 response to occur [13], beyond the time frame of many studies. How a CD4 increase is measured and defined is another key factor. Indeed, T-cells may become sequestered secondary to HCV infection, potentially impacting on the consistency between, and interpretability of, absolute and fraction T-cell populations [14, 15]. Immune dysregulation from both HIV and HCV infections may also lead to an impaired immunologic response primarily among those with a lower baseline CD4
count. For example, in two studies that found no difference in CD4 response, the baseline CD4 was well above 200 among both HCV-positive and HCV-negative subjects [9, 11], whereas the baseline HCV-positive CD4 count in the Swiss Cohort was 172 cells [8].

The question of immunologic response to antiretrovirals among those HIV/HCV co-infected is critical because of its implications for when to start antiretroviral treatment in these patients. HCV-related hepatic fibrosis progression in HIV-infected individuals is strongly associated with a weakened immune system [16], and delaying antiretrovirals for too long may result in more rapid progression of HCV disease – particularly if patients' immunologic recovery will only be partial. Although treatments for hepatitis C are becoming more effective, unfavorable genotype [17, 18], and concomitant anemia, depression [16, 19], and HIV infection [20-22] mean that only between 20-30% of HIV/HCV co-infected patients can expect to successfully complete HCV treatments. Antiretroviral therapy is thus paramount to the health management of co-infected individuals.

Therefore the primary objective of this study was to examine immunologic response to the first 48 weeks of antiretroviral treatment among previously antiretroviral naïve HIV/HCV co-infected and HIV mono-infected individuals in a population-based HIV/AIDS drug treatment program, examining both absolute CD4 and CD4 fraction outcomes. Our secondary objective was to describe the contribution of baseline CD4 count to this response.
5.3 Methods

Data Source: The British Columbia HIV/AIDS Drug Treatment Program

Antiretroviral medications have been centrally distributed at no cost to eligible HIV-infected individuals since 1986. In October 1992, the distribution of antiretroviral agents became the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Center for Excellence in HIV/AIDS. This antiretroviral drug distribution program remains the only free source of antiretroviral medication in this Canadian province (and is a unique program in Canada). The Centre's HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul's Hospital site, and the program conforms with the province's Freedom of Information and Protection of Privacy Act.

The Center distributes antiretroviral drugs based on specific guidelines generated by the Therapeutic Guidelines Committee [23]. These guidelines have been and continue to be consistent with those treatment guidelines published by the International AIDS Society [13, 24-26]. The Centre's guidelines recommend that CD4 and plasma HIV-1 RNA levels be monitored at baseline, at 4 weeks after starting antiretroviral therapy and every three months thereafter. Plasma viral loads are measured using the Amplicor HIV-1 Monitor™ (Roche Diagnostics Branchburg, NJ).

All classes of federally licensed antiretroviral drugs are currently available through the program, including all nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). Tenofovir, Atazanavir, and Enfuvirtide are also available. Eligibility for antiretroviral medication has remained consistent with current international recommendations [13].
Individuals are automatically entered into the DTP when they are first prescribed any antiretroviral agent. At DTP entry and with each subsequent physician visit, the participant’s history (if any) of antiretroviral use, CD4 cell count, and plasma viral load are recorded. Typically, patients are followed-up at 3-month intervals, at which time prescriptions are renewed or altered based on treatment success and other clinical factors. Blood drawn for the purposes of CD4 and viral load testing is stored for each individual at each follow-up visit for future research activities related to HIV disease.

**Study Population**

The HAART Observational Medical Evaluation and Research (HOMER) study is a nested cohort within the BC Center for Excellence’s HIV/AIDS Drug Treatment Program. It includes all previously ART naive individuals who initiated antiretroviral therapy with a triple-drug regimen consisting of either two nucleoside reverse transcriptase inhibitors (NRTI) plus either a protease inhibitor (PI), or a non-nucleoside reverse transcriptase inhibitors (NNRTI), between August, 1996 and July, 2000. The data used in these analyses are based on individuals from the HOMER Cohort, for whom there was HCV serological data.

**Outcome Measures**

The primary outcome measure for this analysis was CD4 response, measured using both absolute CD4 counts and CD4’s as a percentage of the total lymphocyte count (referred to throughout this manuscript as CD4 fraction), comparing HIV mono-infected individuals to HIV/HCV co-infected individuals. Events were defined, respectively, as achieving an absolute CD4 increase of ≥75 cells or a CD4 fraction increase of at least 10% within the first 48 weeks of treatment. Both events were
defined *a priori* as clinically significant changes. Secondly, we examined both absolute and CD4 fraction measures as continuous outcomes throughout the first 48 weeks of ART. Participants were only included in the repeated measures analysis if they had at least three CD4 (absolute and fraction) and plasma viral load results during the 48 weeks, including a baseline measure.

Baseline was defined as the most recent CD4 test taken within 180 days prior to starting antiretrovirals. Baseline characteristics examined were gender, age, baseline CD4 (absolute and percent) and HIV log\(_{10}\) plasma viral load (pVL), and whether patients had an AIDS diagnosis. These were analyzed comparing HCV-antibody positive individuals to HCV antibody-negative individuals.

Our definition of adherence to antiretroviral medication is based on the proportion of time that medication dispensed would last over the first year of follow-up. This calculation was restricted to each patient’s first year on therapy to avoid the reverse causation that could occur among patients who cease antiretroviral therapy after they have become too sick to take medication. We have previously demonstrated how this estimate strongly predicts virologic response [27] and survival [28, 29]. For the purposes of our analyses, adherence was a binary variable where 1 was being greater than 95% adherent (meaning prescriptions dispensed would last throughout the first year of follow-up), and 0 being less than 95% adherent.

**Statistical Analysis**
HCV positive and HCV negative participants were compared using both parametric and distribution-free methods. Categorical data were analyzed using Pearson’s $\chi^2$ test. Fisher’s exact test was used for contingency tables in which 25% or more of the expected cell frequencies were less than 5. Continuous variables were analyzed using the Wilcoxon rank-sum test. Kaplan-Meier analysis was used to calculate cumulative rates of a CD4 increase of ≥75 cells or a CD4 fraction increase of at least 10%. In secondary analyses, we stratified the population by baseline CD4 count (≤200 cells, <350 cells, ≥350 cells). Cox proportional hazards regression was used to calculate univariate and adjusted relative hazards and 95% confidence intervals (CI’s). Possible confounders adjusted for were gender (male vs. female), age at baseline (continuous), AIDS diagnosis at baseline (yes vs. no), baseline CD4 and log10-transformed plasma HIV-1 RNA levels (continuous), and adherence to antiretroviral therapy in the first year of treatment (≥95% vs. <95%). The assumption of proportional hazards was validated by inspection of log(-log(survival function)) estimates against log time plots.

Multivariate mixed effects models were constructed modeling CD4 slope (absolute and percent) over the first 48 weeks of ART, primarily for the whole population and secondarily stratified by baseline CD4 count (≤200 cells, <350 cells, ≥350 cells). Independent fixed effects examined included HCV antibody status (positive vs. negative), time (months), gender (male vs. female), age at baseline, and log10-transformed plasma HIV-1 RNA levels (continuous) over time. The only random effect was patient. Correlation between repeated measures was assumed constant. The data were analyzed using SAS software, Version 6.12. All reported $p$ values are two-tailed.

5.4 RESULTS
There were 1186 patients available for this analysis, including 606 (51%) who were HCV antibody positive, and 580 (49%) who were HCV antibody negative. Baseline characteristics comparing HCV-positive versus HCV-negative individuals are summarized in Table 4.1. (page 90). There were no statistical differences at baseline in their gender, median age, absolute CD4 count, log plasma HIV RNA, or type of ART initiated. However, HCV-positive individuals were less likely to have had an AIDS diagnosis at baseline (11% vs. 15%, p=0.028), and in spite of the absolute CD4 count, had a significantly higher baseline CD4 percent (19% vs. 16%, p<0.001).

Figure 5.1 displays the results of the Kaplan-Meier analysis examining the effect of HCV on time to CD4 absolute (p<0.001) and fraction events (p=0.021). When stratified by baseline CD4 count, consistent associations were found with each respective measure, (Absolute event: baseline CD4 ≤200 cells/mm3: p<0.001, <350: p<0.001, and ≥350: p=0.006; Fraction event: baseline CD4 ≤200: p=0.408, CD4 <350: p=0.703, CD4 ≥350: p=0.001).

Adherence, as expected, had a significant on the outcomes. After restricting the Kaplan-Meier analysis to those ≥95% adherent, HCV has a much lesser but nonetheless significant effect on absolute CD4 response (p=0.024), but no longer had an effect on CD4 fraction response (p=0.729). These results again remained consistent across the baseline CD4 strata (absolute CD4 response: baseline CD4 ≤200: p=0.301, CD4 <350: p=0.037, CD4 ≥350: p=0.341; fraction CD4 response: baseline CD4 ≤200: p=0.613, CD4 <350: p=0.306, CD4 ≥350: p=0.070).

As summarized in Tables 5.1.1. and 5.1.2., in multivariate Cox Regression models, controlling for age at baseline, gender, having an AIDS diagnosis at baseline, baseline
CD4 and plasma log viral load, as well as adherence over the first 12 months of ART, HCV seropositivity remained predictive of being less likely to achieve ≥75 cells (overall Adjusted Hazard Ratio, AHR: 0.84, 95%CI: 0.72-0.97, p=0.021), but there was no effect of HCV on the probability of achieving a CD4 fraction increase of ≥10% (AHR: 0.89, 95%CI: 0.70-1.14, p=0.369). The effect of baseline CD4 on these estimates was limited (absolute outcome: baseline CD4 ≤200 AHR: 0.81, 95%CI: 0.65-1.00; CD4 <350 AHR: 0.82, 95%CI: 0.67-0.99, p=0.036; CD4 ≥350 AHR: 0.86, 95%CI: 0.67-1.10, p=0.248; fraction outcome: baseline CD4 ≤200 AHR: 0.68, 95%CI: 0.46-1.01), p=0.129; CD4 <350 AHR: 0.77, 95%CI: 0.56-1.05, p=0.056; CD4 ≥350 AHR: 0.97, 95%CI: 0.62-1.50, p=0.874).

The effects of examining both absolute and fraction outcomes as continuous measures are summarized in Table 5.2 and 5.3, and Figure 5.2. The median (IQR, interquartile range), unadjusted, absolute CD4 change among the HCV co-infected was 60 (-10, 170) compared to 140 (50, 230) among the non-co-infected (p<0.001). This significant contrast remained across the three baseline CD4 strata (Table 5.3). Similarly, while the HCV co-infected gained a median CD4 fraction of 4% (-1, 8) over 48 weeks, the HCV-negative group gained a median of 6% (2, 9) (p<0.001), and this difference was consistent regardless of baseline CD4 (Table 5.3).

Figures 5.2 displays the effect of HCV serostatus on adjusted CD4 change over 48 weeks of ART using the mixed effects analyses, with the actual model results provided in Table 5.2. In the absolute CD4 analysis, controlling for time (months), age at baseline, gender, having an AIDS diagnosis at baseline, and plasma log viral load response (as a repeated measure) over the 48 week period, although both HCV-positive and HCV-negative individuals had statistically significant increases over time, the HCV co-infected individuals only gained an average of 17 cells, compared to the 85
cells that the non-co-infected gained over 48 weeks of treatment. Even after restricting the analysis to those individuals who were greater than 95% adherent, those who were HCV positive on average gained 40 cells, compared to the 105 cells among the HCV negative. This strong difference remained across the baseline CD4 strata (Table 5.3).

The multivariate mixed effect CD4 fraction analysis shows similar results, whereby the HCV co-infected saw an average increase of only 0.96%, compared to the HCV-negatives in whom their average CD4 percent increased by 2.5%. Both increases were statistically significant. After restricting to those who were at least 95% adherent, where the HCV-positive individuals gained on average 2.5%, the HCV-negative gained 3.4%. However, the HCV positive individuals have a statistically significant higher baseline CD4 fraction, while there was no difference in the baseline absolute CD4 between the two groups.

5.5 DISCUSSION AND CONCLUSIONS

Our results suggest that HCV has a strong influence on the CD4 response to antiretroviral therapy over the first 48 weeks of treatment. Baseline CD4 count does not significantly alter the magnitude of response in either absolute or fraction analysis. However, it must be noted that, as can be seen from Table 4.1, the baseline fraction of HCV co-infected individuals in our cohort is significantly higher than the non-co-infected (p<0.001). This results in a much higher starting point in the mixed effects analyses, and in spite of the smaller average gain, the HCV co-infected still have a higher average CD4 fraction than the HCV-negative by the end of 48 weeks of treatment. This is also probably why in adjusted Cox analysis, HCV had no effect on
the time to a CD4 fraction increase of at least 10% of the CD4 percent, but did negatively affect the time to an absolute CD4 event (≥75 cells).

Our findings do support those of others [8, 30]. An Italian cohort used linear regression and found that HCV-positive (HbsAg-) patients had 34 cells fewer than HCV-negative patients, and in Cox multiple regression found a reduced time to achieving a CD4 increase of 100 cells or else reaching the 500 cell/mm3 threshold (p=0.01) [30]. These results are quite similar to Greub et al.‘s, although they defined their event as an increase of at least 50 cells [8]. Zala et al. (2004) have also reported a blunted CD4 response using mixed effects measures in a nested cohort of several clinical trials populations [32]. Our data are in contrast to those studies that found little or no impact of HCV on CD4 response to treatment [9-12, 33]. In a university-based cohort at Johns Hopkins Hospital HIV Clinic, Sulkowski et al. (2002) found no difference in the proportion of HCV co-infected vs. non- in gaining either 50 or 100 cells, after 1, 2, and 3 years of ART. Their cohort also found that HCV co-infected individuals had a statistically much higher CD4 fraction at baseline than the HCV-negative patients, but found no evidence of altered effects of HCV on either absolute CD4 or the fraction [11]. A recent study from the Thai HIV-NAT Cohort found that mean increases were significantly lower at week 4, but by week 48 the difference disappeared. They also found that HCV co-infection was not associated with a hazard rate increase of at least 100 cells [34].

Our analyses have several advantages and strengths. First, our sample is drawn from a substantially sized population-based program, making it more generalizable than clinic-based studies, cohort data, or clinical trials populations. Secondly, we were able to account for the confounding effect of adherence, both through our adherence measure,
as well as controlling for log viral load response over time in mixed effects analysis. Third, by using both repeated measures and survival analyses, and both absolute and fraction outcomes, we were able to elucidate some of the complexities in analyzing immunologic response to treatment in HIV/HCV co-infection.

The results of this study could be affected by several potential limitations. One, our measure of adherence is a proxy measure; however, we have previously shown this measure to be highly predictive of both virologic response [27] and survival [28, 29]. Two, the number of analyses conducted may predispose to a type 1 error occurring. However, the data are relatively consistent across baseline CD4 strata, and absolute/fraction outcomes respectively, so we feel confident the effects of this occurring are limited. Three, our center has previously shown that individuals who are not treated for HIV infection in spite of treatment eligibility are substantially different sociodemographically from those who receive treatment, and they are more likely to be co-infected with HCV [35]. However, this bias may only be diluting the effect because increasing numbers of HCV co-infected patients would increase our power to detect differences.

The damage caused by chronic HCV infection is not believed to be the consequence of direct destruction of hepatic cells by the virus, but from an intermediate immune response that is large enough to induce hepatic cell destruction and fibrosis but not enough to eradicate the virus from its reservoirs [21]. A blunted immune response in HIV/HCV co-infected individuals may be due to the non-specific immune stimulation driven by chronic HCV infection, or it may be that infection of immune cells by HCV could favour CD4 T-cell depletion [36]. Our data support the hypothesis that HCV is related to a smaller CD4 recovery in both absolute and fraction terms. Our data also
raise the potentially significant issue of CD4 sequestration among HCV-co-infected patients. They indicate the importance for physicians to measure both absolute and fraction CD4 measures among their HIV/HCV co-infected patients, and suggest that all future analyses related to hepatitis C and immunologic function should examine both absolute and CD4 fraction outcomes.

In conclusion, immunologic response to ART among HIV/HCV co-infected adults is a complex issue. Our data suggest that immunologic indicators, in this case absolute CD4 and CD4 fraction, may be strongly affected by the presence of HCV. In our population, while there was no difference between baseline absolute CD4 counts, there was a marked difference in absolute CD4 response to ART. In contrast, while there was a significant difference at baseline in CD4 fraction depending on HCV serostatus, the effect of HCV on fraction immune response was much more limited. Baseline CD4 count appears, in our study, to have had minimal effect.
5.6 REFERENCES


Table 5.1.1. Unadjusted and adjusted factors associated with an absolute CD4 increase of at least 75 cells/mm³

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Hazard (95% CI)</th>
<th>P-Value</th>
<th>Adjusted Hazard (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% Adherent (yes vs. no)</td>
<td>2.15 (1.84-2.51)</td>
<td>&lt;0.001</td>
<td>1.99 (1.69-2.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline log HIV viral load (per log)</td>
<td>1.26 (1.07-1.49)</td>
<td>0.006</td>
<td>1.27 (1.07-1.50)</td>
<td>0.006</td>
</tr>
<tr>
<td>HCV Serostatus (Positive vs. Negative)</td>
<td>0.68 (0.59-0.78)</td>
<td>&lt;0.001</td>
<td>0.84 (0.72-0.97)</td>
<td>0.021</td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>1.70 (1.34-2.17)</td>
<td>0.594</td>
<td>0.88 (0.70-1.10)</td>
<td>0.248</td>
</tr>
<tr>
<td>Age (per 10 yr increase)</td>
<td>1.06 (0.98-1.15)</td>
<td>0.129</td>
<td>0.99 (0.91-1.07)</td>
<td>0.744</td>
</tr>
<tr>
<td>Baseline CD4 (per 100 cells)</td>
<td>0.98 (0.95-1.01)</td>
<td>0.266</td>
<td>0.99 (0.96-1.02)</td>
<td>0.476</td>
</tr>
<tr>
<td>AIDS Diagnosis at Baseline (yes vs. no)</td>
<td>1.06 (0.86-1.31)</td>
<td>0.594</td>
<td>0.88 (0.70-1.10)</td>
<td>0.248</td>
</tr>
</tbody>
</table>
Table 5.1.2. Unadjusted and adjusted factors associated with a CD4% increase of at least 10%

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Hazard (95% CI)</th>
<th>P-Value</th>
<th>Adjusted Hazard (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline log HIV viral load (per log)</td>
<td>1.72 (1.26-2.34)</td>
<td>&lt;0.001</td>
<td>1.81 (1.32-2.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>95% Adherent (yes vs. no)</td>
<td>2.00 (1.53-2.58)</td>
<td>&lt;0.001</td>
<td>2.00 (1.51-2.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV Serostatus (Positive vs. Negative)</td>
<td>0.76 (0.61-0.96)</td>
<td>0.021</td>
<td>0.89 (0.70-1.14)</td>
<td>0.369</td>
</tr>
<tr>
<td>Baseline CD4 (per 100 cells)</td>
<td>1.03 (0.98-1.09)</td>
<td>0.194</td>
<td>1.05 (0.99-1.10)</td>
<td>0.099</td>
</tr>
<tr>
<td>AIDS Diagnosis at Baseline (yes vs. no)</td>
<td>0.85 (0.60-1.22)</td>
<td>0.379</td>
<td>0.81 (0.56-1.18)</td>
<td>0.280</td>
</tr>
<tr>
<td>Gender (male vs. female)</td>
<td>1.16 (0.80-1.70)</td>
<td>0.442</td>
<td>0.91 (0.61-1.36)</td>
<td>0.648</td>
</tr>
<tr>
<td>Age (per 10 yr increase)</td>
<td>0.95 (0.84-1.08)</td>
<td>0.437</td>
<td>0.92 (0.81-1.05)</td>
<td>0.217</td>
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</table>
Table 5.2. Mixed Effects Model Results of Impact of HCV Co-infection on CD4 increase Over 48 Weeks of ART in Both Cohorts, Overall and Among Those ≥95% Adherent

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std Error</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Absolute CD4 Increase Overall (n=893)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>577.31</td>
<td>39.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV status at time 0</td>
<td>3.31</td>
<td>15.27</td>
<td>0.828</td>
</tr>
<tr>
<td>HCV- CD4 increase per 4 wks</td>
<td>6.63</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV+ CD4 increase per 4 wks</td>
<td>-4.81</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-1.01</td>
<td>0.82</td>
<td>0.219</td>
</tr>
<tr>
<td>Male</td>
<td>31.16</td>
<td>23.68</td>
<td>0.188</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>-203.97</td>
<td>21.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log10 Viral load over time</td>
<td>-50.69</td>
<td>2.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B) Absolute CD4 Increase Among Those ≥95% Adherent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>543.91</td>
<td>49.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV status at time 0</td>
<td>-12.46</td>
<td>19.45</td>
<td>0.522</td>
</tr>
<tr>
<td>HCV- increase/4 wks</td>
<td>8.93</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV+ increase/4 wks</td>
<td>-3.91</td>
<td>0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.58</td>
<td>0.99</td>
<td>0.560</td>
</tr>
<tr>
<td>Male</td>
<td>22.57</td>
<td>34.56</td>
<td>0.514</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>-206.12</td>
<td>24.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log10 Viral load over time</td>
<td>-44.33</td>
<td>2.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C) CD4% Increase Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>33.03</td>
<td>1.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV status at time 0</td>
<td>2.16</td>
<td>0.69</td>
<td>0.002</td>
</tr>
<tr>
<td>HCV- increase/4 wks</td>
<td>0.22</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV+ increase/4 wks</td>
<td>-0.14</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.07</td>
<td>0.04</td>
<td>0.071</td>
</tr>
<tr>
<td>Male</td>
<td>-0.82</td>
<td>1.11</td>
<td>0.458</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>-9.56</td>
<td>0.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log10 Viral load over time</td>
<td>-2.39</td>
<td>0.08</td>
<td>&lt;0.001</td>
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<tr>
<td>D) CD4% Increase Among Those ≥95% Adherent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>31.78</td>
<td>2.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV status at time 0</td>
<td>2.09</td>
<td>0.87</td>
<td>0.017</td>
</tr>
<tr>
<td>HCV- increase/4 wks</td>
<td>0.28</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV+ increase/4 wks</td>
<td>-0.07</td>
<td>0.03</td>
<td>0.039</td>
</tr>
<tr>
<td>Age</td>
<td>-0.06</td>
<td>0.04</td>
<td>0.176</td>
</tr>
<tr>
<td>Male</td>
<td>-0.44</td>
<td>1.54</td>
<td>0.775</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>-10.01</td>
<td>1.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log10 Viral load over time</td>
<td>-2.27</td>
<td>0.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 5.3. Absolute and Fraction CD4 Responses to Initiation of Antiretroviral Therapy Among a Population-Based Program of HCV-positive and HCV-negative Individuals, Stratified by Baseline CD4 Count

<table>
<thead>
<tr>
<th>CD4 Response</th>
<th>Overall</th>
<th>Baseline CD4 ≤200</th>
<th>Baseline CD4 &lt;350</th>
<th>Baseline CD4 ≥350</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSOLUTE CD4 CHANGE N=538</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (interquartile range) CD4 change over first 48 weeks (unadjusted)</td>
<td>N=222</td>
<td>N=341</td>
<td>N=197</td>
<td></td>
</tr>
<tr>
<td>HCV+</td>
<td>50 (-20, 160)</td>
<td>50 (0, 50)</td>
<td>60 (0, 160)</td>
<td>30 (-80, 170)</td>
</tr>
<tr>
<td>HCV-</td>
<td>140 (50, 240)</td>
<td>140 (50, 200)</td>
<td>140 (40, 210)</td>
<td>160 (60, 290)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>p=0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Ω Adjusted average number of CD4's gained over 48 weeks

| HCV+ | 17 cells (p<0.001) | 40 cells (p=0.001) | 37 cells (p<0.001) | -15 cells (p<0.001) |
| HCV- | 85 cells (p<0.001) | 80 cells (p<0.001) | 84 cells (p<0.001) | 88 cells (p<0.001) |

*Among >95% Adherent Only: Adjusted average number of CD4's gained over 48 weeks

| HCV+ | 40 cells (p<0.001) | 54 cells (p=0.022) | 56 cells (p=0.001) | 8 cells (p=0.002) |
| HCV- | 105 cells (p<0.001) | 91 cells (p<0.001) | 105 cells (p<0.001) | 108 cells (p<0.001) |

CD4 FRACTION CHANGE N=522 | N=222 | N=330 | N=197 |
| Median (interquartile range) CD4% change over first 48 weeks (unadjusted) |       |       |       |
| HCV+ | 2 (-1.7, 7) | 1 (-2, 7) | 2 (-1, 8) | 2 (-2, 6) |
| HCV- | 6 (2, 10) | 5 (3, 10) | 6 (2, 10) | 6 (2, 10) |
| p-value | <0.001 | p<0.001 | <0.001 | <0.001 |

*Adjusted average percent change over 48 weeks

| HCV+ | 0.36% (p<0.001) | 0.24% (p<0.001) | 0.72% (p=0.001) | -0.48% (p<0.001) |
| HCV- | 2.5% (p<0.001) | 2.6% (p<0.001) | 2.5% (p<0.001) | 2.6% (p<0.001) |

*Among ≥95% Adherent Only: Adjusted average number of CD4’s gained over 48 weeks

| HCV+ | 1.9% (p=0.017) | 1.6% (p=0.040) | 2.0% (p=0.094) | 1.6% (p=0.074) |
| HCV- | 3.2% (p<0.001) | 3.2% (p<0.001) | 3.1% (p<0.001) | 3.4% (p<0.001) |

* Adjusted for time (months), age, gender, AIDS diagnosis at baseline, plasma log viral load response over 48 week period.
‡ Adjusted for baseline age, gender, AIDS diagnosis, baseline CD4 and HIV viral load, and adherence over first 12 months of therapy.
Ω See Table 3 for the models from which these 'adjusted average number 'results are derived.
Figure 5.1. (A-D): Kaplan-Meier Analysis of the Effect of Hepatitis C on the:

**Figure 5.1.A**

Time to Absolute CD4 Increase of At Least 75 cells/mm3

![Kaplan-Meier plot for HCV-negative and HCV-positive groups](image)

Days from initiation of ART

**Figure 5.1.B.**

Time to Absolute CD4 Increase of At Least 75 cells/mm3 - Among >95% Adherent Only

![Kaplan-Meier plot for HCV-negative and HCV-positive groups](image)

Days from Start of ART

$p<0.001$

$p=0.024$
Figure 5.1.C.

Time to CD4 Percent Increase of At Least 10%

Time from Start of ART

Figure 5.1.D.

Time to CD4 Percent Increase of At Least 10% Among >95% Adherent Only

Days from Start of ART
Figure 5.2.A. Adjusted Average Number of Absolute CD4 Cells Gained Over 48 Weeks of Antiretroviral Treatment
Figure 5.2.B. Adjusted Average Number of Absolute CD4 Cells Gained Over 48 Weeks of Antiretroviral Treatment Among Individuals At Least 95% Adherent
Figure 5.2.C. Adjusted Average Increase of CD4 Fraction Gained Over 48 Weeks of Antiretroviral Treatment

![Graph showing the adjusted average increase of CD4 fraction over 48 weeks of antiretroviral treatment for HCV-positive and HCV-negative patients.]

- HCV+: +0.96%, p<0.001
- HCV-: +2.5%, p<0.001
Figure 5.2.D. Adjusted Average Increase of CD4 Fraction Gained Over 48 Weeks of Antiretroviral Treatment Among Individuals At Least 95% Adherent to Therapy

HCV+: +2.5%, p=0.017
HCV-: +3.4%, p<0.001
CHAPTER 6:

SAFETY AND TOLERABILITY OF ANTIRETROVIRAL THERAPY IN HIV/HCV CO-INFECTED PATIENTS

6.1 FORWARD

This chapter is under review with the Journal of Acquired Immune Deficiency Syndromes as:

6.2 INTRODUCTION

It is estimated that on average, 30% of HIV-positive people in North America and Europe are co-infected with the Hepatitis C Virus (HCV) [1, 2]. However, because in some populations including injection drug users, prison inmates, and hemophiliacs, the prevalence of HCV is so widespread, the prevalence of HCV co-infection among HIV-positive individuals in these populations ranges from 50-99% [3-6]. As survival has been increasing for patients with HIV infection due to the use of Highly Active Antiretroviral Therapy, liver disease has emerged as a leading cause of morbidity and mortality in HIV-infected populations [7-9]. The Hepatitis C Virus (HCV) in particular, because of its high prevalence in HIV-positive populations, and its greatly increased pathogenicity in the setting of HIV [2, 10], is now a leading cause of death of HIV-positive individuals [9, 11, 12].

Adherence to antiretroviral therapy is paramount to its effective use [13-15]. Factors previously found to be associated with adherence are physician experience [16], age [17], alcohol use and incarceration [17], toxicity [18], and injection drug use [17, 19]. Injection drug use has also been identified as an important predictor of treatment discontinuation [15, 20]. However, although the majority of HIV-positive injection drug users are also co-infected with hepatitis C, only a few studies have examined HIV/HCV co-infection as a factor in switching or interrupting HAART. While most have found that there is a strong relationship between treatment interruptions and HCV co-infection [21-23], none have looked explicitly at the question of adherence.

The primary objective of the present study was to describe the effect of hepatitis C (HCV) co-infection, controlling for biochemical markers of liver injury and injection
drug use (IDU), on adherence to antiretroviral treatment (ART) during the first year of therapy in a population-based HIV/AIDS drug treatment program. The primary hypothesis was that HCV serostatus would have an independent negative effect on adherence, and that this would be the result of more liver injury, as evidenced by an interaction between HCV serostatus and any biochemical marker of liver injury. The secondary hypothesis was that HCV would be more strongly negatively associated with adherence than a history of injection drug use.

### 6.3 METHODS

Data Source and Study Population:

*The British Columbia HIV/AIDS Drug Treatment Program (DTP)*

Antiretroviral medications have been centrally distributed at no cost to eligible HIV-infected individuals since 1986. In October 1992, the distribution of antiretroviral agents became the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Centre for Excellence in HIV/AIDS. This antiretroviral drug distribution program remains the only free source of antiretroviral medication in this Canadian province (and is a unique program in Canada). The Centre's HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul's Hospital site, and the program conforms with the province’s Freedom of Information and Protection of Privacy Act.

The Centre distributes antiretroviral drugs based on specific guidelines generated by the Therapeutic Guidelines Committee [24]. These guidelines have been and continue
to be consistent with those treatment guidelines published by the International AIDS Society [25-28]. The Centre’s guidelines recommend that CD4 and plasma HIV-1 RNA levels be monitored at baseline, at 4 weeks after starting antiretroviral therapy and every three months thereafter. Plasma viral loads are measured using the Amplicor HIV-1 Monitor™ (Roche Diagnostics Branchburg, NJ). All classes of federally licensed antiretroviral drugs are currently available through the program, including all nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). Tenofovir, Atazanavir, and Enfuvirtide are also available. Eligibility for antiretroviral medication has remained consistent with current international recommendations [28].

Individuals are automatically entered into the DTP when they are first prescribed any antiretroviral agent. At DTP entry and with each subsequent physician visit, the participant’s complete history (if any) of antiretroviral use, CD4 cell count, plasma viral load, and disease stage are recorded. Typically, patients are followed-up at 3-month intervals, at which time prescriptions are renewed or altered as necessary. Blood drawn for the purposes of CD4 and viral load testing is stored for each individual at each follow-up visit for future research activities related to HIV disease.

The HAART Observational Medical Evaluation and Research (HOMER) study is a nested cohort within the BC Center for Excellence’s HIV/AIDS Drug Treatment Program. It includes all previously ART naïve individuals who initiated antiretroviral therapy with a triple-drug regimen consisting of either two nucleoside reverse transcriptase inhibitors (NRTI) plus either a protease inhibitor (PI), or a non-nucleoside reverse transcriptase inhibitors (NNRTI), between August, 1996 and July, 2000. The data
used in these analyses are based on individuals from the HOMER Cohort, for whom there was HCV serological data. HCV serological data was obtained through retrospective testing of stored plasma samples taken just prior to treatment initiation.

The BC Centre for Excellence in HIV/AIDS is located within Providence Health Hospital in Vancouver. The markers of hepatic injury were obtained through a linkage with the Providence Laboratory, where a majority of HIV-infected patients in the province receive their HIV care.

Outcome Measures

The primary outcome measure for this analysis was adherence to antiretroviral therapy. Our definition of adherence is based on the ratio of the time that medication dispensed would last as a proportion of follow-up time. This calculation was restricted to each patient’s first year on therapy to avoid the reverse causation that could occur among patients who cease antiretroviral therapy after they have become too sick to take medication. We have previously demonstrated how this estimate strongly predicts virologic response [13] and survival [29, 30]. For the purposes of our analyses, adherence was a binary variable where 1 was being greater than 95% adherent (meaning having refilled at least 95% of their prescriptions during the year), and 0 less than 95% adherent.

Baseline characteristics examined were gender (male vs. female), age (continuous), absolute baseline CD4 (continuous) and HIV log_{10} plasma RNA (pVL) (continuous), whether patients had an AIDS diagnosis (yes vs. no) or any history of injection drug use (yes vs. no), and type of therapy initiated (PI-based vs. NNRTI-based).
Markers of liver injury were alanine aminotransferase (ALT) (International Units per liter (IU/L)), aspartate aminotransferase (AST) (IU/L), albumin (grams/L), and International Normalized Ratio (INR). The analysis was restricted to the patient's first year on ART, and if a patient had more than one test result in that year, the latest result was taken. These markers were analyzed as continuous variables to allow for the possibility that patients could be experiencing symptoms of liver injury without necessarily meeting a particular threshold of 'significant' liver injury (e.g. ALT ≥ 2.5 times the upper limit of normal). Markers of liver injury were also defined dichotomously accordingly to clinically significant thresholds: ALT ≥ 2.5Xuln, AST ≥ 2.5Xuln, albumin ≤30, and INR ≥ 1.3. A global measure of hepatic injury was created by defining a patient as having any liver injury if they had at least one of the four dichotomous outcome measures. The analysis was restricted to the patient's first year on ART, and if a patient had more than one test result in that year, the latest result was taken.

Statistical Analysis

HCV positive and HCV negative participants were compared using both parametric and distribution-free methods. Categorical data were analyzed using Pearson's χ² test. Fisher's exact test was used for contingency tables in which 25% or more of the expected cell frequencies were less than 5. Continuous variables were analyzed using the Wilcoxon rank-sum test. Adherent versus non-adherent individuals were analyzed in a similar fashion.

In unadjusted logistic regression, HCV antibody-status (positive vs. negative), any history of injection drug use (yes vs. no), gender (male vs. female), baseline CD4 (per
100 cells), age at baseline (per 10 years) and whether the patient had an AIDS diagnosis at baseline (yes vs no) were separately tested for their association with adherence, as were ALT, AST, albumin, and INR, as both continuous and dichotomous variables. Separate models were constructed to assess the relative effects of 1) HCV + biochemical markers of liver injury; 2) IDU + biochemical markers of liver injury; 3) HCV + IDU + biochemical markers of liver injury. The final adjusted model of factors independently associated with ≥95% adherence used a forward stepwise multivariate logistic regression model fit by including HCV + IDU + biochemical (continuous) markers of liver injury + baseline sociodemographic and clinical characteristics considered potential sources of confounding (gender, age, baseline CD4, and whether they had an AIDS diagnosis at baseline).

Separate modeling was conducted using the global measure of hepatic injury. This was done in order to create an interaction term between HCV co-infection and hepatic injury, based on the hypothesis that while those with HCV infection would be less likely to adhere to treatment, this would be most strongly associated with the combined effect of HCV infection and liver injury.

To elucidate the differential effects of injection drug use and hepatitis C, a four-level variable was constructed: 0 = no IDU/no HCV; 1 = IDU but no HCV; 2 = no IDU but HCV-positive; and 3 = both IDU and HCV-positive. This variable was described using Pearson's Chi Square statistic. These categories were also used to create separate variables, each with no IDU and no HCV as the reference, and entered into multivariate logistic regression models. Pearson's Correlation Coefficient was used to assess correlation between the HCV and injection drug use variables.
6.4 Results

There were 1186 patients eligible for analysis, including 606 (51%) HCV-antibody (Ab) positive, and 580 (49%) HCV-Ab negative. As summarized in Table 4.1 (page 90), HCV-positive individuals were less likely to be male (78% vs. 93%, p<0.001), and to have an AIDS diagnosis at baseline (11% vs. 15%, p=0.028), but were more likely to have any history of IDU (47% vs. 6%, p<0.001), and had a higher fraction CD4 at baseline (19% vs. 16%, p<0.001). There were no statistical differences (p>0.05) between HCV-positive and HCV-negative patients at baseline in terms of age, absolute CD4 count, log plasma HIV RNA, or type of therapy initiated.

In their first year of antiretroviral therapy (Table 6.1), HIV/HCV co-infected patients were significantly less likely to be at least 95% adherent to their treatment (42% vs. 72%, p<0.001). They had a higher median ALT (52 vs. 35, p<0.001), AST (46 vs. 29, p<0.001), INR (1.1 vs. 1.0, p=0.037), and lower albumin (39 vs. 41, p<0.001). The HCV co-infected were significantly more likely to have had an ALT ≥ 2.5X uln in their first year of treatment (26% vs. 8%, p<0.001), an ALT ≥ 5X uln (4% vs. 1%, p=0.049), an AST ≥ 2.5Xuln (15% vs. 2%, p<0.001), or an albumin ≤ 30 (20% vs. 11%, p=0.034). There was only a trend of a difference in the proportions of having an INR ≥ 1.3 (15% vs. 8%, p=0.136). Overall, the HCV co-infected were significantly more likely to have any biochemical marker of liver injury (26% vs. 8%, p<0.001).

As summarized in Table 6.2, adherent patients were more likely to be male (91% vs. 78%, p<0.001), to have had an AIDS diagnosis at baseline (16% vs. 8%, p<0.001), and to be slightly older at baseline (median 37.6 vs. 36.7, p=0.001). They were less likely to have any history of IDU (21% vs. 35%, p<0.001), to be HCV Ab-positive (38% vs. 69%, p<0.001), and to have any biochemical marker of liver injury (12% vs. 25%, p<0.001).
including ALT ≥ 5X uln (1% vs. 4%, p=0.019), and an albumin result of ≤ 30 (8% vs. 24%, p<0.001). Adherent patients had significantly lower median AST (31 vs. 37, p<0.001) and higher albumin (41 vs. 38, p<0.001), but there was no difference in their median ALT or INR.

Tables 6.3 summarize the results of the logistic regression analysis of factors independently associated with being at least 95% adherent to ART. As seen in Table 6.3.1, which presents the results using continuous measures of liver injury, the adjusted odds ratio (AOR) and 95% confidence interval (95%CI) for HCV infection remained significant in the final model (AOR 0.39, 95%CI: 0.22-0.73, p=0.003), as did albumin (AOR 1.07, 95%CI: 1.03-1.11, p=0.002), and male sex (AOR 2.42, 95%CI: 1.18-4.96, p=0.016). Even when only adjusting for biochemical markers of liver injury but not HCV co-infection, IDU became non-significant (AOR 0.67, 95%CI: 0.41-1.14).

As seen in Table 6.3.2, in the final model, both HCV co-infection (AOR: 0.35, 95% CI: 0.24 – 0.51, p<0.001) and liver injury (AOR 0.47, 95%CI: 0.23 – 0.94) were negatively associated with adherence, while male sex remained positively associated (AOR 2.58, 95%CI: 1.59-4.18, p<0.001). The interaction term of HCV*liver injury fell out of the final model (AOR 1.42, 95%CI: 0.62-3.25, p=0.412). Interestingly, although IDU became non-significant in the final model (AOR: 0.80, 95%CI: 0.55-1.16, p=0.243), it did remain significant after adjusting only for liver injury, but not other factors such as HCV.

We then endeavoured to separate the effects of injection drug use and hepatitis C. Of the 544 individuals with no reported history of IDU and negative HCV serology, 400 (74%) were at least 95% adherent, and 144 (26%) were not. Of the 36 individuals who had a reported history of IDU but negative HCV serology, 20 (55%) were adherent, and
16 (45%) were not (p=0.019). Of the 321 individuals with no history of IDU but who had positive HCV antibodies, 134 (42%) were adherent, while 187 (58%) were not (p<0.001). Finally, of the 285 individuals with both a reported history of IDU and HCV infection, 121 (42%) were adherent, while the remaining 164 (58%) were not (p<0.001). Pearson’s Correlation Coefficient between any injection drug use and hepatitis C serostatus was 0.46.

Tables 6.4 summarize the results of the analyses aimed at further separating the effects of HCV and injection drug use. As seen in Table 6.4.1, without exception, the individuals who have a history of injection drug use but no HCV infection have the most favorable results regarding biochemical markers of liver injury, while those with positive HCV antibodies have the worst. In logistic regression analysis only controlling for baseline sociodemographic and clinical factors (Table 6.4.2), while the IDU+/HCV-group are less likely to be at least 95% adherent to medication (AOR 0.47, 95%CI: 0.23-0.93, p=0.030), those with HCV infection with or without IDU history are even less likely to be adherent (IDU-/HCV+ AOR: 0.27, 95%CI: 0.20 - 0.36, p<0.001; IDU+/HCV+ AOR: 0.30, 95%CI: 0.22 - 0.42, p<0.001). When also controlling for biochemical markers of liver injury as continuous measures, those with a history of IDU but no HCV are not independently less likely to adhere to treatment (AOR: 0.19, 95%CI: 0.03 - 1.32, p=0.126), while those with HCV infection (with or without IDU) remain significantly less likely to be adherent (IDU-/HCV+ AOR: 0.34, 95%CI: 0.18 - 0.64, p<0.001; IDU+/HCV+ AOR: 0.44, 95%CI: 0.23 - 0.87, p=0.018).
In summary, our data suggest that HCV co-infection, biochemical markers of liver injury (notably albumin), male sex, and to a lesser extent age, are those factors most strongly associated with adherence to antiretroviral therapy. While our findings do not suggest an interaction between HCV and liver injury, they do support the hypothesis that HCV is more strongly associated with adherence than a history of injection drug use.

Our data support those of others who have found that HCV infection is associated with treatment discontinuations and treatment interruptions. Melvin et al. reported antiretroviral discontinuation rates due to hepatic toxicity of more than two-fold in HCV-co-infected individuals compared to HIV mono-infected persons [22]. Among a population of 465 previously antiretroviral naïve individuals, HCV seropositivity was associated with an adjusted 40% increased risk of discontinuing or changing initial HAART regimens within the first year of treatment [21]. The authors indicate that whether this was due to histological damage, reduced adherence, or increased hepatotoxicity is not clear, because hepatic cirrhosis was also independently associated with HAART discontinuation (AOR 2.1, 95% CI: 1.1-3.8) [21]. Aceti (2002) reported that 83% of those who discontinued antiretrovirals because of hepatotoxicity were HCV co-infected [31]. Of note, D’Arminio Monforte et al. (2000) found no impact of HCV on treatment interruptions due to toxicity [23].

There are a number of important strengths to our analysis. One, the data are drawn from a population-based program, and are therefore more broadly generalizable than other study populations. Secondly, we have endeavoured to untangle the rather
complex relationship between HCV infection, injection drug use, and hepatic injury, enabling a much deeper understanding of adherence issues as they relate particularly to HCV infection and injection drug use. Third, to our knowledge, we are the first to explicitly examine the question of hepatitis C co-infection and adherence to antiretroviral therapy, and to ask the question about the combined effect of HCV co-infection and hepatic injury. Finally, more effective hepatitis C treatment (i.e. pegylated interferon combined with ribavirin) only became available in British Columbia in June, 2003, and as this analysis was restricted to patients first year on HIV treatment, the effect of HCV treatment on our results is negligible.

There may also be potential limitations to our analysis. One, the markers of liver injury are only present for a maximum of 72% of the cohort, and this may limit the results. However, we feel that it is likely the distribution of missing values would remain proportionate across the adherence and liver injury categories, and that having data from more patients would strengthen the results. Two, the measures of liver injury are biochemical, and ALT and AST levels in particular may not be reliable measures of liver inflammation or injury, as others have shown that patients with normal ALT levels may have hepatic scarring [32]. Furthermore, there are many symptoms of HCV disease and ART hepatotoxicity which could impact on adherence, without being captured by these measures, including nausea, malaise, etc. This may be the reason why HCV infection itself remains associated with adherence in adjusted models. A third limitation to our study is that our measure of injection drug use is a composite one, drawing upon both physician and patient self-report data. The fact that only 47% of those with HCV infection have any reported history of IDU suggests that there may be underreporting of this variable. However, as HCV is highly infectious and individuals at risk for HIV infection may also have multiple risk factors for HCV (e.g. certain sexual
practices, tattooing, intranasal cocaine use), this underestimation may be minimal. Further, the IDU measure is based on any history of IDU, which may include those who used injection drugs recreationally many years ago, but who are otherwise not typical of those IDU generally considered at high-risk of non-adherence (i.e. actively street-involved and actively using injection drugs). A final limitation of this analysis is that we were not able to account for the effects of either alcohol use or depression or depressive symptoms on adherence.

Our data have important implications for the conduct of clinical research regarding HIV/hepatitis C co-infection and injection drug users. Accurate estimates of effects due to IDU may be difficult to obtain without adjusting for the effects of HCV infection, and vice versa. More research is urgently needed to elucidate the mechanisms involved in precluding or preventing HIV/HCV co-infected individuals and those with a history of injection drug use from maximally adhering to their antiretroviral therapy.

In conclusion, our data suggest that HCV infection, albumin (as a marker of hepatic injury), and male sex, are independently associated with adherence, and after adjusting for these other factors, injection drug use is not. Further, although HCV co-infected individuals are more likely to have any biochemical evidence of liver injury, the combined effect of HCV and liver injury in this analysis was negligible, although both HCV and liver injury were both independent factors.
6.6 References


Table 6.1. Characteristics in First Year of ART Among HCV-positive and HCV-negative Individuals in the British Columbia HIV/AIDS Drug Treatment Program

<table>
<thead>
<tr>
<th></th>
<th>HCV-positive N=606</th>
<th>HCV-negative n=580</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥95% Adherent</td>
<td>255 (42%)</td>
<td>420 (72%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST ≥ 2.5Xuln (n=846)</td>
<td>59 (15%)</td>
<td>9 (2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT ≥ 2.5Xuln (n=630)</td>
<td>42 (13%)</td>
<td>9 (3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT ≥ 5.0Xuln (n=630)</td>
<td>13 (4%)</td>
<td>4 (1%)</td>
<td>0.049</td>
</tr>
<tr>
<td>Albumin ≤ 30 (n=323)</td>
<td>39 (20%)</td>
<td>13 (11%)</td>
<td>0.034</td>
</tr>
<tr>
<td>INR ≥1.3 (n=260)</td>
<td>24 (15%)</td>
<td>8 (8%)</td>
<td>0.136</td>
</tr>
<tr>
<td>Any liver injury (n=852)</td>
<td>126 (32%)</td>
<td>39 (9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT (median, IQR)</td>
<td>52 (32 – 89)</td>
<td>35 (27 – 51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (median, IQR)</td>
<td>46 (30 – 80)</td>
<td>29 (23 – 36.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALB (median, IQR)</td>
<td>39 (33 – 42)</td>
<td>41 (37 – 43)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR (median, IQR)</td>
<td>1.1 (0.9 – 1.2)</td>
<td>1.0 (0.9 – 1.1)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

*ART: antiretroviral therapy
Table 6.2. Factors associated with Adherence to Antiretroviral Medication

<table>
<thead>
<tr>
<th>Factor</th>
<th>Adherent n=675</th>
<th>Non-Adherent n=511</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Gender</td>
<td>614 (91%)</td>
<td>400 (78%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at baseline (median, IQR)</td>
<td>37.6 (32.5 – 44.6)</td>
<td>36.7 (31.1 – 42.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Any IDU</td>
<td>141 (21%)</td>
<td>180 (35%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCV-Antibody positive</td>
<td>255 (38%)</td>
<td>351 (69%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AIDS diagnosis at baseline</td>
<td>109 (16%)</td>
<td>43 (8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any Liver Injury (n=852)</td>
<td>71 (14%)</td>
<td>94 (27%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST ≥ 2.5Xuln (n=846)</td>
<td>27 (5%)</td>
<td>41 (12%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALT ≥ 2Xuln (n=630)</td>
<td>23 (6%)</td>
<td>28 (10%)</td>
<td>0.070</td>
</tr>
<tr>
<td>ALT ≥ 5Xuln (n=630)</td>
<td>5 (1%)</td>
<td>12 (4%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Albumin ≤ 30 (n=323)</td>
<td>14 (8%)</td>
<td>38 (24%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR ≥1.3 (n=260)</td>
<td>13 (10%)</td>
<td>19 (14%)</td>
<td>0.368</td>
</tr>
<tr>
<td>ALT (median, IQR)</td>
<td>39 (29 - 60)</td>
<td>42 (29 - 76)</td>
<td>0.215</td>
</tr>
<tr>
<td>AST (median, IQR)</td>
<td>31 (25 - 44)</td>
<td>37 (27 - 67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALB (median, IQR)</td>
<td>41 (37 - 43)</td>
<td>38 (31 - 41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INR (median, IQR)</td>
<td>1.1 (1.0 – 1.1)</td>
<td>1.1 (1.0 – 1.2)</td>
<td>0.280</td>
</tr>
</tbody>
</table>
Table 6.3.1: Logistic Regression Analysis of Factors Associated with Being At Least 95% Adherent to Antiretroviral Therapy in First Year of Treatment (n=1186)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C positive</td>
<td>0.27 (0.19-0.37)</td>
<td>0.36 (0.21-0.62)</td>
<td>-</td>
<td>0.36 (0.20-0.64)</td>
<td>0.39 (0.22-0.73)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Any History of IDU</td>
<td>0.46 (0.33-0.66)</td>
<td>-</td>
<td>0.67 (0.41-1.14)</td>
<td>1.05 (0.59-1.86)</td>
<td>1.09 (0.60-1.98)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td>0.139</td>
<td></td>
<td>0.772</td>
</tr>
<tr>
<td>ALT (continuous)</td>
<td>0.997 (0.995-0.999)</td>
<td>1.00 (0.99-1.01)</td>
<td>1.00 (0.99-1.01)</td>
<td>1.00 (0.99-1.01)</td>
<td>1.00 (0.995-1.01)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.010</td>
<td>0.972</td>
<td>0.909</td>
<td>0.979</td>
</tr>
<tr>
<td>AST (continuous)</td>
<td>0.994 (0.991-0.996)</td>
<td>0.998 (0.99-1.01)</td>
<td>0.997 (0.990-1.00)</td>
<td>0.998 (0.99-1.01)</td>
<td>1.00 (0.99-1.01)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td>0.592</td>
<td>0.356</td>
<td>0.587</td>
</tr>
<tr>
<td>Albumin (continuous)</td>
<td>1.08 (1.04-1.12)</td>
<td>1.06 (1.02-1.10)</td>
<td>1.07 (1.03-1.11)</td>
<td>1.06 (1.02-1.10)</td>
<td>1.07 (1.03-1.11)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male Sex</td>
<td>3.90 (2.40-6.40)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.42 (1.18-4.96)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td>Age (per 10 yr increase)</td>
<td>1.27 (1.11-1.45)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.32 (0.95-1.85)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td>0.097</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>1.90 (1.20-3.10)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.14 (0.56-2.35)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.012</td>
<td></td>
<td></td>
<td>0.718</td>
</tr>
<tr>
<td>CD4 (per 100 cells)</td>
<td>0.96 (0.90-1.00)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.91 (0.80-1.04)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.302</td>
<td></td>
<td></td>
<td>0.166</td>
</tr>
</tbody>
</table>
Table 6.3.2 Logistic Regression Analysis of Factors Associated with Being At Least 95% Adherent to Antiretroviral Therapy in First Year of Treatment (n=1186)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatitis C positive</strong></td>
<td>0.27 (0.19-0.37)</td>
<td>0.28 (0.20-0.39)</td>
<td>-</td>
<td>0.31 (0.21-0.44)</td>
<td>0.31 (0.21-0.45)</td>
<td>0.35 (0.24-0.51)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>-</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Any IDU</strong></td>
<td>0.46 (0.33-0.66)</td>
<td>-</td>
<td>0.47 (0.33-0.66)</td>
<td>0.82 (0.56-1.22)</td>
<td>0.75 (0.52-1.07)</td>
<td>0.80 (0.55-1.16)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>-</td>
<td>&lt;0.001</td>
<td>0.328</td>
<td>0.115</td>
<td>0.243</td>
</tr>
<tr>
<td><strong>Any Liver Injury</strong></td>
<td>0.40 (0.27-0.60)</td>
<td>0.56 (0.36-0.86)</td>
<td>0.42 (0.28-0.64)</td>
<td>0.55 (0.36-0.85)</td>
<td>0.54 (0.24-1.25)</td>
<td>0.47 (0.23-0.94)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.150</td>
<td>0.033</td>
</tr>
<tr>
<td><strong>HCV*Liver Injury</strong></td>
<td>0.30 (0.19-0.48)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.03 (0.39-2.72)</td>
<td>1.42 (0.62-3.25)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.955</td>
<td>0.412</td>
</tr>
<tr>
<td><strong>Male Sex ~</strong></td>
<td>3.90 (2.40-6.40)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.58 (1.59-4.18)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Age ~ (per 10 yr increase)</strong></td>
<td>1.27 (1.11-1.45)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.27 (1.06-1.52)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>AIDS at baseline ~</strong></td>
<td>1.90 (1.20-3.10)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.47 (0.90-2.42)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>0.012</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.126</td>
</tr>
<tr>
<td><strong>CD4 (per 100 cells) ~</strong></td>
<td>0.96 (0.90-1.00)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.95 (0.88-1.02)</td>
</tr>
<tr>
<td><em>p</em>-value</td>
<td>0.302</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.158</td>
</tr>
</tbody>
</table>
Table 6.4.1. Median results (interquartile range) of biochemical markers of liver injury by the four possible categories of HCV and IDU status

<table>
<thead>
<tr>
<th>Median (interquartile range)</th>
<th>IDU-/HCV- n=544</th>
<th>IDU+/HCV- n=36</th>
<th>IDU-/HCV+ n=321</th>
<th>IDU+/HCV+ n=285</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>35 (27 – 52)</td>
<td>31.5 (27 – 42)</td>
<td>49 (33 – 94)</td>
<td>53 (31 – 88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST</td>
<td>29 (23 – 36)</td>
<td>28 (22 – 39)</td>
<td>46 (30 – 81)</td>
<td>46 (29 – 78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>41 (37 – 43)</td>
<td>41.5 (40 – 43)</td>
<td>39 (32 – 42)</td>
<td>38 (33 – 42)</td>
<td>0.006</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (1.0 – 1.1)</td>
<td>1.0 (1.0 – 1.0)</td>
<td>1.1 (1.0 – 1.2)</td>
<td>1.1 (1.0 – 1.2)</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Table 6.4.2. Logistic Regression Analysis of Factors Associated with Being At Least 95% Adherent to Antiretroviral Medications, Controlling for Gender, Age at Baseline, AIDS Diagnosis at Baseline, and Baseline CD4

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Odds (95% CI)</th>
<th>p-value</th>
<th>Adjusted Odds (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDU-/HCV-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IDU+/HCV-</td>
<td>0.45 (0.23-0.89)</td>
<td>0.022</td>
<td>0.47 (0.23-0.93)</td>
<td>0.030</td>
</tr>
<tr>
<td>IDU-/HCV+</td>
<td>0.26 (0.19-0.35)</td>
<td>&lt;0.001</td>
<td>0.34 (0.18-0.64)</td>
<td>0.001</td>
</tr>
<tr>
<td>IDU+/HCV+</td>
<td>0.27 (0.20-0.36)</td>
<td>&lt;0.001</td>
<td>0.30 (0.22-0.42)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6.4.3. Logistic Regression Analysis of Factors Associated with Being At Least 95% Adherent to Antiretroviral Medications, Controlling for AST, ALT, Albumin, Gender, Age at Baseline, AIDS Diagnosis at Baseline, and Baseline CD4

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Odds (95% CI)</th>
<th>p-value</th>
<th>Adjusted Odds (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDU-/HCV-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IDU+/HCV-</td>
<td>0.45 (0.23-0.89)</td>
<td>0.022</td>
<td>0.19 (0.03-1.32)</td>
<td>0.126</td>
</tr>
<tr>
<td>IDU-/HCV+</td>
<td>0.26 (0.19-0.35)</td>
<td>&lt;0.001</td>
<td>0.34 (0.18-0.64)</td>
<td>0.001</td>
</tr>
<tr>
<td>IDU+/HCV+</td>
<td>0.27 (0.20-0.36)</td>
<td>&lt;0.001</td>
<td>0.44 (0.23-0.87)</td>
<td>0.018</td>
</tr>
</tbody>
</table>
CHAPTER 7: MORTALITY AND HIV/HCV CO-INFECTION

7.1 FORWARD

This chapter is currently has been conditionally accepted by the Canadian Medical Association Journal as:


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7.2 INTRODUCTION

Hepatitis C is widely prevalent among HIV-infected individuals [1]. While several authors have previously found that liver disease has become a leading cause of death of HIV-positive individuals [2-4], debate continues as to the effect of the hepatitis C virus (HCV) on HIV disease progression, as measured either by a new AIDS-defining illness, CD4 decline, or HIV-related mortality [4-9]. Mortality in this population can be strongly confounded by adherence to antiretroviral therapy (ART), injection drug use, and previous ART use.

During the 1990’s, Vancouver, British Columbia, had an explosive epidemic of HIV and HCV infection among the city’s 10,000 injection drug users, and over 30% of this population is co-infected with HIV and HCV [10, 11]. Here we report on the effect of HCV serostatus on the risk of non-accidental mortality in a population-based HIV treatment program of previously ART naïve patients, adjusting for adherence to ART and injection drug use, and describe broad causes of non-accidental death among these individuals.

7.3 METHODS

The British Columbia HIV/AIDS Drug Treatment Program, previously described in detail elsewhere, [12] is the only source of free antiretrovirals in BC, and follows therapeutic guidelines developed consistent with international standards [13]. Data for this analysis is drawn from those previously antiretroviral therapy (ART) naïve individuals who initiated ART with a triple-drug regimen consisting of either two nucleoside reverse transcriptase inhibitors (NRTI) plus either a protease inhibitor (PI),
or a non-nucleoside reverse transcriptase inhibitor (NNRTI). This analysis is restricted to those for whom there was HCV serological data, and who first initiated antiretroviral therapy between August 1996 and July, 2000. The data are censored at June 30, 2003. Mortality data were obtained through a linkage with British Columbia Vital Statistics, and ICD9/10 codes were used to evaluate the underlying cause of death. Accidental deaths were excluded from all survival analyses.

Our definition of adherence is based on the ratio of the time that medication dispensed would last as a proportion of follow-up time, and has previously been validated as a strong predictor of both virologic response [14] and survival [15, 16]. Statistical analyses included parametric and non-parametric methods and standard survival analysis techniques including Kaplan-Meier and Cox Proportional Hazards methods.

7.4 RESULTS

There were 1186 individuals eligible for analysis, including 606 (51%) HCV-antibody positive, and 580 (49%) HCV-antibody negative. Baseline characteristics are summarized in Table 4.1 (page 90). HCV-positive individuals were less likely to be male (78% vs. 93%, \( p < 0.001 \)), to have had an AIDS diagnosis at baseline (11% vs. 15%, \( p = 0.028 \)), and to have a lower median CD4 fraction (0.19 vs. 0.16, \( p < 0.001 \)). There was no statistical difference in their absolute baseline CD4 count, baseline log HIV viral load, or whether they initiated ART with a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor.
There were 163 non-accidental deaths during the study period, including 118 (72%) among the HCV-positive individuals, and 45 (28%) among the HCV-negatives (p<0.001). Figure 7.1.1 displays the results of Kaplan Meier analysis, in which HCV serostatus is strongly associated with time to non-accidental death (p<0.001), even after restricting the analysis to those who were over 95% adherent to their antiretroviral medication (Figure 7.1.2) in the first year of treatment (p<0.001). Table 7.1 summarizes the unadjusted and adjusted hazards of a non-accidental death. After controlling for age at baseline, gender, having any history of injection drug use, baseline CD4 and log HIV viral load, having an AIDS diagnosis at baseline, and adherence to antiretrovirals, HCV serostatus remained strongly predictive of experiencing non-accidental mortality in this population (Adjusted Hazard 2.79, 95% confidence interval: 1.91-4.09, p<0.001).

Table 7.2. describes the causes of death in this population, including accidental deaths. HIV/HCV co-infected individuals were statistically more likely to die an HIV-related cause of death (13% vs. 6%, p<0.001), and appeared more likely to die of accidental causes (24% vs. 13%), liver-related (4% vs. 2%) and unknown causes (6% vs. 0%).

7.5 Discussion

These data strongly support the hypothesis that HCV seropositivity is an independent predictor of non-accidental mortality in this previously ART-naïve HIV-infected population who initiated ART with a triple-combination. Our data suggest that HIV/HCV co-infected patients are more likely to die of HIV-related, liver-related and unknown causes, compared to their HIV mono-infected counterparts.
Several authors have found that HCV co-infection has an adverse impact on HIV disease progression and HIV-related mortality [4, 5, 17]. Indeed, there are a number of ways in which HCV could impact on HIV-related survival and mortality. Liver injury and increased ART toxicity may preclude HIV/HCV co-infected patients from tolerating antiretroviral therapy [18, 19]. Our center has previously shown that 30% of individuals in British Columbia dying of HIV-related causes have never accessed antiretroviral therapy, and these patients' sociodemographic profile is consistent with individuals expected to be co-infected with HCV in this province [20]. In addition, because the progression of HCV disease is exacerbated in the setting of HIV, it is possible that the underlying cause of death may be noted as HIV-related.

There are several key strengths to this analysis. The data are population-based, and are therefore more generalizeable than other studies. Two, by adjusting for adherence to antiretroviral medications and a history of injection drug use, we were able to account for two potentially very important confounding factors. Three, because the analysis is restricted to those individuals who initiated ART since 1996, our analysis is not subject to the kind of survivorship bias inherent in those analysis which analyze the survival of HIV/HCV co-infected individuals before and after 1996. Four, by excluding accidental deaths, we remove those deaths due to overdose, suicide, violence, and other causes of death that may be more strongly associated with HCV co-infection because of the lifestyle associated with injection drug use.

There may also be potential limitations to this analysis. One, the HCV data are based only on serology, and have not been PCR confirmed. Two, our measure of adherence is restricted to the first year of therapy. However, this was done explicitly to avoid the
possible reverse causation that may result from those individuals who become less adherent to their ART because they are too sick to take the medications.

In summary, these data suggest that hepatitis C positive antibody status is independently predictive of non-accidental mortality in this population-based program of HIV-infected individuals receiving antiretroviral therapy, even after controlling for adherence to ART and injection drug use, and these deaths are largely HIV-related. ICD codes are notoriously problematic indicators of causes of death, particularly for diseases like hepatitis C or syndromes like AIDS. However, further work is urgently needed to fully characterize the mechanisms responsible for the increased mortality observed in HIV and HCV co-infected patients.
7.6 References


Table 7.1. Unadjusted and Adjusted Cox Models of Predictors of Non-Accidental Mortality

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Unadjusted Hazard (95% C.I.)</th>
<th>p-value</th>
<th>Adjusted Hazard (95% C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV serostatus</td>
<td>2.81 (1.99 – 3.96)</td>
<td>&lt;0.001</td>
<td>2.79 (1.91 – 4.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at baseline</td>
<td>1.37 (1.17 – 1.59)</td>
<td>&lt;0.001</td>
<td>1.33 (1.13 – 1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adherence</td>
<td>0.39 (0.29 – 0.54)</td>
<td>&lt;0.001</td>
<td>0.40 (0.29 – 0.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any IDU</td>
<td>0.96 (0.68 – 1.36)</td>
<td>0.831</td>
<td>0.59 (0.41 – 0.85)</td>
<td>0.005</td>
</tr>
<tr>
<td>Baseline CD4</td>
<td>0.73 (0.67 – 0.80)</td>
<td>&lt;0.001</td>
<td>0.74 (0.67 – 0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline log viral load</td>
<td>2.25 (1.37 – 3.71)</td>
<td>0.002</td>
<td>1.70 (1.06 – 2.73)</td>
<td>0.028</td>
</tr>
<tr>
<td>AIDS at baseline</td>
<td>1.66 (1.12 – 2.45)</td>
<td>0.011</td>
<td>1.10 (0.72 – 1.68)</td>
<td>0.670</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.77 (0.51 – 1.16)</td>
<td>0.208</td>
<td>1.02 (0.66 – 1.56)</td>
<td>0.940</td>
</tr>
</tbody>
</table>
Table 7.2. Causes of Death Among Previously Treatment Naïve HIV Mono- and HIV/HCV Co-infected Individuals in a Population-Based HIV Treatment Program (July 1, 2000 – June 30, 2003)

<table>
<thead>
<tr>
<th>Cause</th>
<th>HIV</th>
<th>HIV/HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52/580 (9%)</td>
<td>156/606 (26%)</td>
</tr>
<tr>
<td>Accidental</td>
<td>7 (1.2%)</td>
<td>38 (6%)</td>
</tr>
<tr>
<td>HIV-related*</td>
<td>35 (6%)</td>
<td>79 (13%)</td>
</tr>
<tr>
<td>Liver-related</td>
<td>1 (0.2%)</td>
<td>7 (1%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>2 (0.3%)</td>
<td>3 (0.5%)</td>
</tr>
<tr>
<td>~Other</td>
<td>7 (1.2%)</td>
<td>20 (3%)</td>
</tr>
<tr>
<td>Unknown Cause</td>
<td>0 (0%)</td>
<td>9 (1%)</td>
</tr>
</tbody>
</table>

*p for HIV-related cause between HIV/HCV co-infected and HIV monoinfected is <0.001
Figure 7.1. Probability of Survival by HCV Serostatus Among HIV-infected Individuals Initiating Antiretroviral Therapy (accidental deaths excluded)

**Figure 1a. Probability of Survival by HCV Serostatus**

![Graph showing probability of survival by HCV serostatus among HIV-infected individuals initiating ART.](image)

- HCV-negative
- HCV-positive

<table>
<thead>
<tr>
<th>Months from Starting ART</th>
<th>HCV-negative</th>
<th>HCV-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>580</td>
<td>606</td>
</tr>
<tr>
<td>12</td>
<td>547</td>
<td>553</td>
</tr>
<tr>
<td>24</td>
<td>526</td>
<td>504</td>
</tr>
<tr>
<td>36</td>
<td>502</td>
<td>464</td>
</tr>
<tr>
<td>48</td>
<td>365</td>
<td>327</td>
</tr>
<tr>
<td>60</td>
<td>245</td>
<td>196</td>
</tr>
<tr>
<td>72</td>
<td>109</td>
<td>62</td>
</tr>
</tbody>
</table>

**Figure 1b. Probability of Survival by HCV Serostatus Among Those More than 95% Adherent to their ART**

![Graph showing probability of survival by HCV serostatus among those more than 95% adherent to their ART.](image)

- HCV-negative
- HCV-positive

<table>
<thead>
<tr>
<th>Months from Start of ART</th>
<th>HCV-negative</th>
<th>HCV-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>420</td>
<td>255</td>
</tr>
<tr>
<td>12</td>
<td>407</td>
<td>231</td>
</tr>
<tr>
<td>24</td>
<td>397</td>
<td>216</td>
</tr>
<tr>
<td>36</td>
<td>384</td>
<td>203</td>
</tr>
<tr>
<td>48</td>
<td>283</td>
<td>138</td>
</tr>
<tr>
<td>60</td>
<td>189</td>
<td>85</td>
</tr>
<tr>
<td>72</td>
<td>86</td>
<td>29</td>
</tr>
</tbody>
</table>
CHAPTER 8: SUMMARY, RECOMMENDATIONS, FURTHER RESEARCH, AND CONCLUSIONS

8.1 SUMMARY OF STUDY FINDINGS

At the beginning of this doctoral project, a review of available research on the issue of antiretroviral therapy in HIV/HCV co-infected patients was undertaken [1]. It became apparent that while work in this area has not been extensive, what research is available suggests there are numerous outstanding questions related to HIV/HCV co-infection, including issues regarding the antiretroviral management of HIV/HCV co-infected patients. Indeed, this literature review identified several possibilities of how antiretroviral interventions could be tailored in order to maximize HIV treatment success, and mitigate HCV disease progression. These possibilities may include initiating antiretroviral therapy earlier in HIV disease progression, choosing drugs which are less hepatotoxic and avoiding certain combinations of these agents, and close monitoring of metabolic and mitochondrial abnormalities because of the potential overlap between ART toxicity and HCV morbidity.

This review also identified several areas requiring further research that subsequently formed the core of this doctoral project. These questions include the population-level prevalence of HCV in the HIV-infected population of British Columbia receiving antiretroviral treatment, and the potential impact of immune reconstitution on the accurate diagnosis of HIV/HCV co-infected individuals, the effect of HCV on the immunologic response to ART, issues regarding the safety and tolerability of antiretroviral agents in HIV/HCV co-infected patients, and ultimately, the effect of HCV on mortality in HIV-positive individuals receiving antiretroviral therapy.
In Chapter 4, I describe the findings of a CIHR funded study investigating hepatitis C diagnostic issues in HIV infected individuals initiating antiretroviral therapy. This research provides among the first population-based estimates of HIV/HCV co-infection in the Developed World. The findings of this research indicate that the prevalence of HCV seropositivity in HIV-infected individuals in British Columbia is much higher than previously believed, at 51%. Although sample quality may have been a limitation, a very high proportion (30%) of the HCV antibody positive patients had no detectable RNA at baseline. These findings are much higher than other reports of possible HCV RNA spontaneous clearance in HIV-infected populations [2, 3]. Further, our findings suggest that 20% of previously HCV RNA negative individuals will develop detectable HCV RNA following ART initiation, and confirm a previous pilot report [4]. Even after taking into consideration these additional 24 patients who became HCV RNA detectable post-antiretroviral treatment, 25% of this population remained HCV RNA undetectable.

These data have important implications regarding the accurate diagnosis and management of HCV in HIV-infected patients. They underscore the importance of testing all HIV-infected individuals for HCV, using the HCV-antibody test, prior to starting antiretroviral therapy. In spite of the cost of PCR testing, patients should be tested for the presence of HCV RNA as well in order to make informed therapeutic decisions (e.g. when and whether to treat HCV, alcohol reduction, diet and lifestyle changes, etc.). If PCR negative, then patients can have peace of mind and yet know the absence of HCV RNA may not be permanent and that they should be re-tested following ART initiation or at regular intervals. These data suggest that an undetectable HCV RNA result does not necessarily mean an HIV co-infected individual has spontaneously cleared the virus. However, if PCR positive, this knowledge provides crucial health information. A treatment plan can be developed, including the possible effects of HCV genotype. HCV genotype has important implications for the
outcome of antiviral treatment [5], on the development of hepatic steatosis [6, 7], and on the development of acute transaminitis in HIV/HCV co-infected patients [8, 9]. Testing prior to ART can enable HCV treatment prior to taking ART [10], as well as tailored ART if HCV treatment is not an option or has not been effective [1]. Our data and others [2] suggest that the diagnosis of chronic HCV infection can be altered by a significant change in immune function [11, 12]. Therefore, HCV testing should be redone following antiretroviral treatment initiation.

An important reason why knowing a patient’s HCV status prior to initiation of ART is described in Chapter 5, whereby some of the possible effects of HCV on the immunologic response to ART are demonstrated. The issue of immunologic response to treatment is a complex domain, and one requiring further study, particularly at the basic science level to understand the immunologic interactions between HIV and HCV. In this study, I showed that while HCV co-infected and HIV mono-infected adults have statistically the same baseline absolute CD4 count, the HCV co-infected have a profoundly altered absolute CD4 response to treatment. Further, the HCV co-infected have a significantly higher CD4 fraction at baseline, and perhaps because of this or because of other factors, do not have a significantly altered CD4 fraction response. This may be due to physiologic factors related to HCV infection, including the possible sequestration of CD4 cells in the lymph nodes or other organs and cells as a result of HCV infection [13]. These data suggest that baseline absolute CD4 count does not significantly alter the impact of either absolute or fraction CD4 response.

Fundamental to the success of antiretroviral therapy in any population is adherence [14]. In Chapter 6, I question whether the effect of injection drug use on adherence is real, given that the vast majority of these individuals are co-infected with HCV. Indeed, these data suggest that HCV infection is one of the strongest predictors of adherence to antiretroviral therapy. In multivariate logistic regression, HCV serostatus at baseline is associated with a 60% reduced probability of being at least 95%
adherent, while in the same model, injection drug use has no independent effect. After creating a 4-level variable intended to disentangle some of the confounding effects of IDU and HCV, these findings suggest that HCV has a stronger effect on the probability of adherence than does injection drug use. These data should be interpreted cautiously, as the measure of IDU in this cohort is a composite one and has not been validated.

I also hypothesized that hepatitis C would be associated with poor adherence through the mechanism of increased hepatic injury, and tested this hypothesis by creating an interaction term between HCV and having any biochemical marker of liver injury. Although liver injury remains independently associated with poor adherence after adjustment for HCV infection (particularly a low albumin level), the interaction term is not significant, suggesting that the decreased adherence among HCV co-infected patients is not due to more biochemical liver injury among them. The interpretation of these findings should be cautioned by noting that many individuals may experience increased toxicity through symptoms such as nausea, malaise, etc., that may reduce adherence, but not be captured through these dichotomized biochemical markers. Further research in these areas is urgently needed.

The combined effects of under-diagnosed HCV infection, impaired immune response to ART, increased liver injury and poorer adherence to ART are culminated in Chapter 7. Chapter 7 shows the effect of HCV serostatus at baseline on non-accidental mortality in this population of HIV-infected patients receiving antiretrovirals. In short, being HCV-positive is associated with a 3 times greater risk of non-accidental death, after adjusting for adherence to antiretroviral therapy and injection drug use (and other relevant factors such as age and gender). People with HCV are more likely to die an HIV-related death, and appear more likely to die of liver-related, accidental, and unknown causes. There is debate regarding the effect of HCV on the progression of HIV disease and HIV-related mortality in the era of highly active antiretroviral therapy [15, 16]. Although this study was not designed to answer this particular question, it
does suggest that HCV has a strong and independent effect on the non-accidental mortality of individuals receiving antiretroviral therapy, highlighting the need for better characterization of the causes of death in this population.

Together, the data presented here demonstrate that hepatitis C co-infection is an important clinical and public health problem in British Columbia. HCV co-infection poses substantial challenges to patients, physicians, and policy-makers. HIV/HCV co-infection is a very complex therapeutic domain, and the people in whom both infections are more likely to co-exist are more likely to be marginalized socioeconomically [17, 18]. These individuals may be at higher risk of concomitant mental illness and addictions [19-21], both complicating factors in the clinical setting.

Although HCV infection can and should be treated, the majority of HIV co-infected persons will not fully benefit from HCV treatment, and this places extraordinary burdens on the HIV management of these patients. This dissertation is a broad look at some of the implications of HCV co-infection on antiretroviral management, and as importantly, provides the basis and a framework for more extensive HIV/HCV co-infection research in the future.

8.2 UNIQUE CONTRIBUTION, IMPACT, AND IMPLICATIONS

This thesis makes several unique contributions to the expansion of knowledge regarding HIV/HCV co-infection. First, I initiated and prepared the review that is presented in Chapter 2, and will be published in the international medical journal *AIDS*. This work provides a comprehensive summary of the research in the area of HIV/HCV co-infection and antiretroviral therapy to date, and identifies several opportunities for improving the antiretroviral management of co-infected patients. This review paper contains recommendations for further areas of research and intervention. To ensure that these recommendations were relevant and comprehensive, I invited international
experts to provide feedback and suggestions on these recommendations and to be co-authors on this paper. When published, the review will be available on Medline and will be a comprehensive and thought-provoking resource for all those interested in this area of HIV/HCV co-infection treatment and research.

Second, my work now enables the characterization of hepatitis C within the HOMER cohort, and allows for the characterization of the natural history of HIV/HCV co-infection in the context of antiretroviral therapy. As a direct result of this thesis, there now exists a population-based cohort of HCV co-infected and HCV un-infected previously treatment naïve individuals who initiated ART with a triple-combination. There is now data on HCV antibody status at baseline for the entire population, qualitative HCV RNA data on those individuals who tested antibody positive at baseline, plus 10% of those individuals who tested antibody negative at baseline. The formation of this cohort will have major implications on the capacity to lead HIV/HCV co-infection research in British Columbia for years to come.

Third, this study provides among the first population-based measures of HIV/HCV co-infection prevalence in the world. It suggests that HCV co-infection is prevalent in over half of HIV-infected individuals who first initiated HIV treatment between August 1996 and July 2000 in British Columbia, and is probably a conservative estimate of HCV co-infection in British Columbia because of the increasing number of injection drug users and others accessing ART since 2000. The high prevalence of HCV, particularly among those with no reported history of injection drug use, suggests that there may be significant under-reporting of HCV infection secondary to under-testing of individuals not generally perceived to be at risk for HCV. In addition, this study has shown that initiation of antiretroviral treatment may be related to the development of detectable HCV RNA in those patients in whom it was undetectable prior to starting treatment.
Fourth, I have added substantially to the literature regarding immunologic response to ART in HIV/HCV co-infected adults. A question of debate among researchers and clinicians, I have highlighted some of the complexity of this question by providing novel information regarding the difference in absolute and CD4 fraction measures of immunologic function and response, showing that HCV has an important impact on both baseline immune function and immunologic response to ART. As a result of this work, I provide a basis for multiple other research questions and a possible framework for understanding some of the conflicting results currently existing in the literature on this question. Further, through this work I have identified that HIV/HCV co-infected individuals immunologic function and response should be measured through both outcomes. Although not found to be an important contributing factor, I tested the novel hypothesis that baseline CD4 would be associated with the magnitude of immune response, and found that baseline CD4 has little or no effect.

Fifth, I have significantly elucidated our understanding of adherence issues in this population. I tested the hypothesis that hepatitis C would be a stronger factor associated with adherence compared to injection drug use, and I examined whether the effect of HCV was related to increased liver injury among these patients. I have shown that the question of adherence in HIV/HCV co-infected patients is complex, and is the result of the interplay between HCV infection, more liver injury (and therefore less tolerability), and a history of injection drug use. This research is the first to specifically examine the effect of HCV on adherence, and is the first to examine both HCV and injection drug use together, with an attempt to separate the effects. This research opens several new lines of inquiry regarding adherence, hepatitis C, and toxicity, and challenges assumptions and previous data regarding adherence among injection drug users [22]. Due in part to the success of this effort, the BC Center for Excellence in HIV/AIDS has initiated discussions with Providence Health Care to obtain real time access to safety laboratory profiles for all HIV/AIDS Drug Treatment Program patients.
Finally, the study I present in Chapter 7 is among the first to describe the profound effect of HCV serostatus on non-accidental mortality in a population-based program of individuals receiving antiretroviral therapy. It provides substantial justification for further research in the domain of HIV/HCV co-infection, and provides support for initiatives aimed at increasing access to HIV/HCV treatment, care, and support.

These data have important implications for health care planners and providers. There are an estimated 15,000 HIV-positive individuals in the province of British Columbia [23]. Only 4500 are or have ever received antiretroviral therapy in the province. If over 50% of these individuals are co-infected with HCV, then a total of over 7500 people may be expected to come forward for treatment and care related to their HIV and/or HCV infection, including the 606 individuals in the HOMER cohort. Many of these patients may not be aware of their HCV infection. These individuals will have multiple needs, and will pose among the most challenging patient population for health care providers. Adequate HIV/HCV care must address the social determinants of health, addictions, and mental health issues. Optimally, patients would be treated in holistic models, whereby both infectious diseases are treated in consideration of each other, and in the context of improving patient’s health and quality of life more broadly. It should be anticipated that these patients may require more than average health care resources [24-31].

Indeed, as the epidemic of HIV among Vancouver’s injection drug users began in the mid-1990’s [18], assuming an average progression rate of 7 to 15 years for both HIV and HCV, it can be expected that the effect of injection drug use and HIV/HCV co-infection on the health of these patients has begun to take its toll, and currently published data support this [22, 25-34].
8.3 Recommendations

The strengths and limitations of the studies presented here have been outlined in Chapter 3, and in detail as part of each study. For instance, although antiretrovirals are centrally distributed enabling population level analyses to be conducted, a significant proportion of individuals dying of HIV infection in British Columbia die without accessing antiretrovirals, and are probably more likely to be co-infected with HCV [31]. These patients are therefore not captured by this population-based study, and suggest our prevalence finding may be a very conservative estimate.

An important first recommendation therefore, is to expand and enhance efforts to bring people who are at risk for HIV and/or HCV infection into contact with health care. This includes addiction management, primary care, and mental health care. Further, I recommend that criteria for accessing HCV treatment and HCV treatment guidelines in British Columbia and elsewhere be expanded, and that HIV co-infected specific guidelines in Canada be significantly elaborated, in particular to include issues related to antiretroviral therapy. Hepatitis C is considered to be a potentially curable infection, because a sustained virologic response (no evidence of HCV RNA 6 months after completing treatment) is believed to indicate that the infection has been eradicated [5]. Appropriate side effect management and adherence supports, and treating HCV while immune function is still strong regardless of markers of hepatic injury, will maximize the probability of having a successful HCV treatment outcome in HIV-infected patients [10, 35]. HIV has become a chronic manageable condition for many, but because of viral integration into the host’s DNA, it is doubtful that a cure can ever be found. Therefore, antiretroviral therapy is currently anticipated to be a life-long treatment requiring a long-term strategy [36]. Effectively treating HCV will maximize HIV therapeutic options and prevent downstream morbidity. More research into the clinical and economic implications of treating HCV preemptively in HIV-positive populations is needed.
There are a number of clinical implications arising from this body of work for which I propose recommendations. Many of these are contained within the initial literature review I conducted and have co-authored with leading clinician scientists in the field [1]. Summarized in more detail in Table 2.3, they include considering initiating ART at higher absolute CD4 counts than might otherwise be done (e.g. >350 cells/mm3) and anticipating a blunted CD4 response; avoid using drug or combinations of drugs known to cause mitochondrial toxicity (such as ddI, d4T, and/or ribavirin); avoid using nevirapine and full dose ritonavir because of their propensity for severe hepatotoxicity [37]; rely on a variety of markers of liver disease, including biopsy and ultrasound, because biochemical markers of liver injury are not very reliable; and close monitoring of HIV/HCV co-infected patients for insulin resistance and diabetes because of their heightened risk [38, 39].

Current guidelines do recommend HCV antibody testing among HIV positive individuals. Based on the literature as reviewed, and as a result of findings related to this study, it is recommended that physicians and patients consider re-testing following antiretroviral initiation. When RNA is found to be positive, it should be immediately genotyped because genotype 3 in particular is prone to severely elevated liver enzymes and development of fatty liver [6, 7, 37, 40]. In addition, genotype also has a favourable HCV treatment profile [5, 35].

A number of important research recommendations arise from this work. One, I have shown that absolute and fraction CD4 measures at baseline may be significantly different in HCV co- and HIV mono-infected adults, and that this can have important implications for measuring immune response. Thus, future research regarding clinical issues in HIV/HCV co-infection should examine both measures of immunologic function. The question of immune response to ART could be tested in a randomized clinical trial, which could obviate most major potential biases. A second research recommendation regards clinical research among people with a history of injection
drug use. This research and others indicate the substantial clinical morbidity that can arise from HCV infection, ultimately resulting, in this study population, in a 3 times higher rate of non-accidental mortality. In British Columbia and elsewhere, over 90% of IDU are infected with HCV [18]. Therefore the question of injection drug use in clinical research cannot be properly examined when not also examining the effect of HCV disease. The injection drug use variable used in this dissertation does need validation.

Finally, through research conducted related to this dissertation and related activities, I authored a report entitled 'Roadmap for Improving Access to Care and Treatment for People Co-Infected with HIV and Hepatitis C in Canada'. This report was the result of a national symposium sponsored by the Canadian Treatment Action Council and co-chaired by myself. It brought together clinicians, scientists, industry, government, and consumers and their advocates to identify barriers to effective care and treatment among persons HIV/HCV co-infected, and strategies for overcoming them. The issues and recommendations arising from this meeting are summarized in the Final Report, and cover issues regarding clinical care, research, community supports, federal and provincial policies, and industry initiatives. The Executive Summary of this report is included as Appendix 4 of this dissertation. Key recommendations arising from this initiative include the establishment of a national database of disease and treatment outcomes among HIV/HCV co-infected patients, establishment of national and provincial guidelines regarding HIV and organ transplantation (currently BC has the only policy in Canada), elaborate on treatment and management guidelines for HIV/HCV co-infected, and broaden criteria for accessing HCV treatment in HIV co-infected patients.
8.4 Future Research

While specific future research needs and opportunities are contained within each chapter, this thesis points towards several broad areas in need of future research.

One is related to the issue of immune reconstitution and its effects on fibrosis progression and related issues. As noted in chapter 2, it is a difficult question to study because of the need for at least two liver biopsies pre- and post-ART initiation; liver biopsies are associated with severe adverse events [5]. Biopsies themselves have limited reliability, and for these reasons, other measures of hepatic disease and hepatic injury are urgently needed [5]. The effect of immune reconstitution has not been well established and represents an important concern given the prevalence of HCV infection and current treatment guidelines which recommend delaying ART [36]. This issue would be ideally tested in a controlled clinical trial, with HIV mono- and HIV/HCV co-infected patients initiating HIV treatment at different CD4 thresholds, comparing immune response to treatment, and in a sub-study, effects of immune reconstitution on the progression of liver disease.

As chapters 4 and 5 illustrate, there is a great deal about the pathophysiology of HIV/HCV co-infection that is not well understood [41]. In fact, although it is increasingly understood that HCV is an immune-mediated disease, there remains more questions than answers in the field of hepatitis C chronic infection [5]. Indeed, although some factors are known to be associated with spontaneous clearance and viral eradication upon treatment (including younger age, higher CD4 counts, etc), the pathophysiology of HIV/HCV co-infection may hold clues to understanding more about chronic HCV infection specifically and human immunology generally. More bench science regarding HIV/HCV co-infection is needed, and immunologists, virologists and microbiologists working in both HIV and HCV should seek opportunities for cross-collaboration.
Qualitative and quantitative research is needed to elucidate issues related to toxicity of and adherence to ART in HIV/HCV co-infected patients. Although the study presented in chapter 6 found that there was not an interaction between any biochemical markers of liver injury and HCV-positivity, this does not mean that HCV co-infected patients are less able to fully adhere to treatment because of having more toxicity. Indeed, this study supports other literature that HCV co-infected have significantly elevated levels of transaminases and other markers of hepatic injury [37], but indicates that there is something else about having HCV leading to significantly poorer adherence levels (including symptoms related to liver disease not captured by the measures used). Our data suggest that the effect of HCV is independent of injection drug use. In addition to the need for more research regarding adherence issues, more research is also needed to examine the additional burden of morbidity and toxicity that HCV confers to HIV infection. This includes the entire spectrum of potential toxicity, from mitochondrial and metabolic changes, to nausea, fatigue, and other non-life threatening symptoms that nonetheless have important implications for patients' quality of life.

The above are areas for future research that have emerged from this specific doctoral project. There are, however, many other critical areas for future research. For example, natural history studies of HIV/HCV co-infection, treated and untreated, are urgently needed. Secondly, the area of HCV treatment in HIV co-infected patients also demands more attention. More research is needed into maximizing HCV treatment, including the use of red blood cell growth factors, increasing adherence, and the economic costs of delaying treatment. Understanding to what extent treatment can confer histologic improvement in spite of the continued presence of HCV RNA is crucial in this population, because their liver disease will progress more rapidly. Knowing the dynamics of why HIV co-infected patients respond less well to existing HCV treatments is critical.
The World Health Organization estimates that Africa has the highest prevalence of HCV globally [42]; it also has the highest prevalence of HIV [43]. However the prevalence of HCV/HIV co-infection is not established, and a subject of debate because the primary HIV risk factor in most places in Africa is heterosexual sex. However, HCV is highly infectious, and more likely to be transmitted through health care settings, circumcision, or the use of certain traditional medicines. As antiretrovirals are becoming more widely available throughout much of Africa and elsewhere in low-income settings, surveillance systems are needed to monitor emerging trends and conditions that may include HCV co-infection.

The area of primary prevention has not yet been addressed in this dissertation, but not because of its insignificance. Preventing HIV and HCV disease transmission requires a variety of approaches, including harm reduction in both drug use and sex, and providing the means necessary so that people have options in terms of their lifestyle, and health management. The prevention of these infectious agents cannot be accomplished without addressing underlying social determinants of health, including poverty, housing, and gender equality and the social and economic empowerment of women.

8.5 CONCLUSIONS

HIV co-infection significantly exacerbates HCV infection, leading to more fibrosis, cirrhosis, end-stage liver disease, and liver-related mortality [10, 41]. This study has shown that HCV significantly complicates antiretroviral management because of the effects of immune suppression and restoration on HCV disease, an impaired immune response to treatment, increased toxicity of, and poorer adherence to, antiretroviral therapy, together resulting in elevated rates of non-accidental mortality in
spite of accessing ART. It is the interactions between the two viruses and their associated effects and complications that results in a disease process that goes beyond the sum its respective parts.

In many places in the world, including Thailand [44, 45], Russia [45], and China [46], burgeoning HIV epidemics are the result of widespread injection drug use. It is of paramount importance to know the burden of HIV/HCV co-infection globally, and to not assume infection rates simply from primary HIV transmission categories. Both HIV and HCV can be managed to some degree, but to do so effectively requires addressing them together, because they significantly affect each other. Therefore, HIV and HCV clinicians and scientists must collaborate together along with bench scientists, policy makers, and people living with HIV/HCV co-infection to move forward on those issues which can significantly enhance access to treatment and care, and maximize their effectiveness.
8.6 REFERENCES


42. World, H.O., *Hepatitis C (Factsheet #164)*. 2000, WHO.


Appendix 1

Statement of Authorship

(Appendix Withdrawn)
A. Specific Aims
The overarching aim of this project is to develop an appropriate testing algorithm for hepatitis C virus (HCV) among an HIV-infected population seeking HIV treatment in a Developed World setting with universal access to health care (British Columbia, Canada). To this end, this study has the following objectives and hypotheses:

Primary Objective:
To measure the point prevalence of Hepatitis C infection among HIV-positive individuals initiating Highly Active Antiretroviral Therapy (HAART) in a population-based HIV treatment cohort.

Primary Hypothesis:
That there will be an overall Hepatitis C prevalence of 30% among this population (defined as having positive HCV antibodies and a positive HCV RNA test; or being antibody-negative but having evidence of HCV RNA; or being antibody-positive without evidence of HCV RNA) at the time of initiation of antiretroviral therapy, but a true prevalence of hepatitis C (defined only as either having positive antibodies and a positive RNA test; or being antibody-negative but having evidence of HCV RNA) of 20%. (Please see Appendix One for a Glossary of terms and acronyms.)

Secondary Objectives:
- To describe the sociodemographic and clinical factors associated with HCV prevalence. (Hypothesis: That male gender, older age, and a history of injection drug use will be associated with prevalent HCV cases, in both definitions of prevalent.)
- To measure the positive and negative predictive values of the currently used HCV-antibody test among HIV-positive individuals who have initiated HAART since 1996.
- To quantify the prevalence of discordant HCV-antibody and PCR test results among HIV-positive individuals. (Hypothesis: That there will be a prevalence of 5% of antibody-negative but RNA-positive discordant responses; and a prevalence of 15% of antibody-positive, but RNA-negative discordant responses.)
- To describe the sociodemographic and clinical factors associated with discordant test results. (Hypothesis: That compared to people who are True HCV Positives, people with antibody-positive, but RNA-negative discordant responses will be more likely to be female and to be of median younger age; and that antibody-negative but RNA-positive discordant responses...
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will be more likely to have a baseline CD4 count below 200 cells/mm$^3$.) (Please see Methods section and/or Figure 1 for definitions of True HCV Positives, etc.)

B. Background and Rationale

It is estimated that as much as 3% of the world's population is infected with the Hepatitis C virus (HCV) [1], including an estimated 2% of people from the United States [2], and 1% of Canadians [3]. As of December, 2002, the United Nations had estimated that there were 42 million people living with HIV/AIDS in the world [4], including approximately 40,000 in Canada [5]. Due to shared routes of transmission such as receiving contaminated blood products and injection drug use, the prevalence of co-infection by both HIV and Hepatitis C is considerable, particularly in some populations. In Canada and elsewhere in the Developed World including the United States, it is estimated that as many as 30% of individuals who have HIV also have Hepatitis C [6-8].

There is an important negative synergy between HIV and Hepatitis C whereby people who are co-infected with both viruses have faster rates of liver fibrosis and development of cirrhosis compared to HCV mono-infected individuals [9-17], poorer responses to Hepatitis C treatment [8, 18-23], and overall worse survival [8, 12, 24-28].

These negative outcomes appear to be at least partially mediated through factors that are sometimes modifiable and sometimes not. These factors include CD4 count and other immunological factors [29-32], type and length of Hepatitis C treatment [18-20, 33-37], use of antiretroviral therapy for HIV infection [22, 31, 38-45], age and gender [10, 16, 46, 47].

Due to these co-factors, a consensus is developing that among HIV-infected individuals, Hepatitis C needs to be detected and managed as early as possible [17, 22, 45, 48-51]. However, several issues inhibit the timely and accurate diagnosis of Hepatitis C among HIV-infected individuals. One of these may include a misperception among some primary health care providers that only high-risk individuals (i.e. injection drug users, hemophiliacs) are at risk for Hepatitis C, and therefore do not proactively test all HIV-positive patients. Indeed, only 552 of the 1422 individuals who have initiated antiretroviral therapy in British Columbia since 1996 have documentation of HCV serology. Factors associated with not getting tested include being female, and having a physician with less HIV experience (unpublished data).

A second factor preventing the timely and accurate diagnosis of hepatitis C in the HIV-infected population is the predisposition, due to an altered immunologic capacity, of HIV-infected individuals to test negative for HCV antibodies, but to have detectable HCV RNA when tested using PCR methods. Although Thio et al. (2000) found that inconsistent antibody and PCR results were primarily related to seroconversion [52], our center has confirmed other reports [23, 53] which suggest that approximately 5% of individuals who test antibody-negative may have detectable HCV RNA with no evidence of seroconversion [54]. If accurate, this would mean that at least 50 of the 1422 individuals who have initiated antiretroviral therapy since 1996 may have been misdiagnosed as not having Hepatitis C. PCR testing for the detection of HCV RNA is not routinely done in British Columbia and elsewhere because of the cost of the test, reinforcing the need for the development of an appropriate testing algorithm in this population.
A third factor in the potential for misdiagnosis of Hepatitis C is the unknown rate of HCV clearance in the HIV infected population. In the HCV mono-infected population, it is estimated that approximately 15% of antibody-positive individuals will not develop chronic infection due to spontaneous clearance of HCV viremia [55, 56]. It is not currently known whether the same proportion of individuals with HIV infection will test antibody-positive but not have any detectable virus. People who are infected with HIV at the time of HCV acquisition may have higher HCV viral loads during seroconversion compared to people without HIV [57], and this would suggest that HIV-infected individuals may be less likely to spontaneously clear HCV. Factors associated with spontaneous clearance in the non-HIV-infected population include younger age, and female gender [2].

Distinguishing between Overall Prevalence and True Prevalence has a number of important clinical and policy related implications. Although it is important to know who has positive antibodies for Hepatitis C, these individuals do not represent the burden of hepatitis C disease. In contrast, individuals who have evidence of HCV RNA are those who are at risk for disease progression, who may require treatment, and who are at greater risk of transmitting the virus.

Although the use of Highly Active Antiretroviral Therapy (HAART) has largely transformed HIV disease into a chronic, manageable illness [40], most if not all antiretroviral agents are primarily metabolized through the liver, and can cause varying degrees of hepatotoxicity [43, 44]. Hepatotoxic drugs are known to exacerbate HCV infection [2]. Converesely, viral co-infection can further exacerbate drug-related hepato-toxicities [58-60]. Despite the gains made by the use of antiretroviral therapy, viral co-infection with Hepatitis C has already become a leading cause of morbidity and mortality among people living with HIV [14, 59, 61-63]. These issues all accentuate the importance of early and accurate diagnosis of Hepatitis C in the HIV-infected population.

In summary, although the timely and accurate diagnosis of HCV in HIV-positive individuals is of the utmost clinical importance, there are several issues which prevent a valid and reliable estimate of active HCV infection in this population. While it is generally considered that 30% of persons with HIV are co-infected with Hepatitis C, there are several reasons why these estimates may be either under or over-estimates. The burden of Hepatitis C among HIV-positive individuals is clinically significant and complex, and the accurate and timely diagnosis of these individuals is essential.

C. Roles and Contributions of Investigators
Robert Hogg, PhD. Principal Investigator (5% effort). Dr. Hogg is responsible for overseeing and advising on study design, interpretation and application of results, and development of publications.

Paula Braitstein, PhD (Cand). Co-investigator, and graduate student whose dissertation topic is HIV/Hepatitis C co-infection (30% effort). Responsible for developing project design and implementation, in consultation with co-investigators, including all statistical analysis and development of publications.

P. Richard Harrigan, PhD. Co-investigator (1% effort), virologist and head of the HIV laboratory, responsible for advising on technical components of the assays and virologic aspects of the study.

Mel Krajden, MD. Co-Investigator (5% effort), responsible for advising on technical components of assays, and interpretation and implications of findings on a policy level.
Val Montessori, MD. Co-investigator (5% effort), a physician specializing in HIV/Hepatitis C co-infection, responsible for advising on clinical aspects and implications of research questions and results.

Julio S.G. Montaner, MD. Co-investigator (2% effort), physician specializing in HIV and antiretroviral therapy, responsible for advising on clinical aspects and implications of research questions and results.

Michael V. O'Shaughnessy, PhD. Co-investigator (2% effort), a virologist and senior policy director, responsible for advising on both scientific and policy implications of study in design, analysis, interpretation.

Chris Sherlock, MD. Co-investigator (10% effort), Director of lab where testing will be performed, Dr. Sherlock is responsible for leading technical aspects of assay utilization and testing of samples.

Martin T. Schechter, MD, PhD. Co-investigator (2% effort), a clinical epidemiologist responsible for advising on study design and analysis.

D. Research Design and Methods

Data Source: The HIV/AIDS Drug Treatment Program (DTP)
Antiretroviral medications have been centrally distributed at no cost to eligible HIV-infected individuals since 1986. In October 1992, the distribution of antiretroviral agents became the responsibility of the HIV/AIDS Drug Treatment Program of the British Columbia Center for Excellence in HIV/AIDS. This antiretroviral drug distribution program remains the only free source of antiretroviral medication in this Canadian province (and is a unique program in Canada). The Centre’s HIV/AIDS Drug Treatment program has received ethical approval from the University of British Columbia Ethics Review Committee at its St. Paul’s Hospital site, and the program conforms with the province’s Freedom of Information and Protection of Privacy Act.

The Center distributes antiretroviral drugs based on specific guidelines generated by the Therapeutic Guidelines Committee [64]. In 1992, the HIV/AIDS Drug Treatment Program made available double combination therapy for individuals with CD4 cell counts of 350/mm$^3$ or less. In December 1995, double combination therapy was made available to everyone with CD4 cell counts of 500/mm$^3$ or less. In June 1996 the Centre adopted plasma viral load driven antiretroviral therapy guidelines, consistent with those put forward by the International AIDS Society — USA[65]. In brief, antiretroviral therapy na ve individuals with plasma viral load > 100,000 copies/mL were offered triple drug regimens (i.e. two nucleosides plus a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI)), while those with plasma viral loads from 5,000 to 100,000 copies/mL were offered dual nucleoside therapy. The Centre guidelines were revised in July 1997 to recommend triple combination therapy for all antiretroviral na ve individuals with plasma HIV-1 RNA levels greater than 5,000 copies/mL or CD4 cell counts below 500 cells/mm$^3$. The Centre’s guidelines recommend further that plasma HIV-1 RNA levels be monitored at baseline, at 4 weeks after starting antiretroviral therapy and every three months thereafter. Plasma viral loads are measured using the Amplicor HIV-1 Monitor“ (Roche Diagnostics Branchburg, NJ).

All classes of federally licensed antiretroviral drugs are currently available through the program, including all nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse
transcriptase inhibitors (NNRTI), and protease inhibitors (PI). Tenofovir, Atazanavir, and Enfuvirtide are also available. Eligibility for antiretroviral medication has remained consistent with current international recommendations [45].

Individuals are automatically entered into the DTP when they are first prescribed any antiretroviral agent. At DTP entry and with each subsequent physician visit, the participant’s complete history (if any) of antiretroviral use, CD4 cell count, plasma viral load, and disease stage are recorded. Typically, patients are followed-up at 3 month intervals, at which time prescriptions are renewed or altered based on treatment success and other clinical factors. Blood drawn for the purposes of CD4 and viral load testing is stored for each individual at each follow-up visit for future research activities related to HIV disease.

Study Population:
The population of interest for this study are the 1422 individuals whose first ever antiretroviral combination consisted of a triple-drug regimen of either two NRTI s plus a PI, or two NRTI s plus a NNRTI, and who initiated therapy between July 1996 and August, 2000.

Study Design and Outcome Measures:
Frozen, archived samples of blood plasma are available to the Drug Treatment Program. The first sample taken, that from just prior to HAART initiation, will be tested for HCV antibodies (Ab) and HCV RNA, the latter using polymerase chain reaction (PCR) technology. This will establish a baseline point prevalence of Hepatitis C for this cohort, and help to inform the degree to which immune suppression contributes to the test’s predictive values, and discordant test results. For individuals with negative-antibody but positive PCR results, a sample obtained from them at least six months later will be tested to determine whether the discordant result was due to seroconversion. For individuals with positive antibodies but negative PCR results, a second test will be performed to confirm the absence of HCV replication, as recommended by the National Institutes of Health [2].

To determine sociodemographic and clinical factors associated with prevalent cases (defined in two ways to evaluate differences in associated factors depending on the definition), and having discordant test results (each of false-antibody-negative and false-antibody-positive, the following outcomes will be assessed in a cross-sectional analysis using multivariate logistic regression (see Table 1):

- True Prevalence (true Ab-positives plus false Ab-negatives versus true Ab-negatives)
- Overall prevalence (true Ab-positives plus false Ab-negatives plus false Ab-positives versus true Ab-negatives)
- Discordant results:
  a) Antibody-positive, RNA negative versus true Ab-positives
  b) Antibody-negative, RNA positive versus true Ab-positives

<table>
<thead>
<tr>
<th>Table 1. Dependent Variables Defined</th>
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<tbody>
<tr>
<td><strong>Outcome</strong></td>
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<tr>
<td>True HCV</td>
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</table>
**Prevalence**

<table>
<thead>
<tr>
<th>Overall HCV Prevalence</th>
<th>true positives plus false negatives plus false positives</th>
<th>true negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discordant (Antibody positive, RNA negative)</td>
<td>Antibody-positive, RNA negative</td>
<td>true positives</td>
</tr>
<tr>
<td>Discordant (Antibody negative, RNA positive)</td>
<td>Antibody-negative, RNA positive</td>
<td>true positives</td>
</tr>
</tbody>
</table>

**Definitions (see Figure 1):**

- **True-negative**: those individuals who test HCV negative on both antibody and PCR;
- **True-positive**: those individuals who test HCV positive on both antibody and PCR;
- **Seroconverters**: those whose first HCV test showed negative antibodies but was positive on PCR, and whose follow-up test at least six months later shows positive antibodies; these individuals will subsequently be classed as true positives.
- **False antibody negative**: those individuals whose initial and confirmatory tests showed negative antibodies and positive PCR.
- **False antibody positive**: those whose initial and confirmatory tests showed positive antibodies and negative PCR, and for whom there is no record of hepatitis C treatment prior to testing. (Please note that the term "false antibody positive" is used for convenience to refer to people who it is presumed have spontaneously cleared the hepatitis C virus.) If an individual is found, upon confirmatory testing, to have both positive antibodies and a positive PCR test, they will subsequently be classified as a true-positive.

**Figure 1.**

```
+ +  true positives
| - |
+ true negatives
| false antibody negative |
- false antibody positive
```
Principal Applicant: Hogg et al.  
Research Proposal

**HCV Screening:**
Blood samples are collected and stored frozen at -20C until time of processing. Plasma is separated within 6 hours of collection by centrifuging at 800-1600xg for 15 minutes at room temperature. Each consenting individual’s sample will be tested for both HCV antibodies and for the presence of HCV RNA.

Blood samples will be tested for HCV antibody using the dual enzyme immunoassay (EIA) algorithm (Abbott primary screen followed by Ortho HCV test if primary screen is positive). These third generation EIA assays have a sensitivity greater than 99% and a specificity of 99%, in immune-competent patients [2].

HCV RNA will be detected using the Roche Cobas HCV AmpliPrep/COBAS Amplicor test. The AmpliPrep/COBAS AmplicorHepatitis C Virus test (v.2.0) is a qualitative nucleic acid amplification test for the detection of HCV in clinical specimens of human plasma. Sample preparation is automated using the COBAS AmpliPrep Instrument, and amplification and detection are automated using the COBAS Amplicor Analyzer. This is a qualitative assay with a lower level of detection of HCV RNA of 100 copies per ml. The specificity for this assay exceeds 98% [2]. All testing will be completed at the University of British Columbia Virology Laboratory at St Paul’s Hospital. The Standard Operating Procedures for these assays have been included as an appendix to this proposal (Appendix Two).

**Statistical Methods:**
Hepatitis C prevalence will be calculated at baseline for the entire cohort of 1422 individuals. Individuals will be considered true prevalent cases if their sample is antibody-positive and PCR-positive or if they have been found to be false negative cases. A second overall prevalence measure will be calculated to include both of these groups, in addition to individuals with positive antibodies but negative PCR results.

Standard calculations of sensitivity and specificity, and proportions of false-positives and false-negatives for the antibody tests will be performed. We will calculate the positive and negative predictive values of the HCV antibody test, stratifying by baseline CD4 count (>500 cells/mm$^3$, 200-500 cells/mm$^3$, 100-199 cells/mm$^3$, and <100 cells/mm$^3$), enabling the development of an appropriate testing algorithm for an HIV infected population (based on the hypothesis that immune suppression is related to discordant results).

To describe factors associated with prevalent cases, and discordant test results, cross-sectional logistic regression multivariate analysis will be used. To assess bivariate associations, Student’s t-test will be used for normally distributed continuous variables, and the Wilcoxon Rank Sum test for non-normal continuous variables. Pearson’s Chi-Square test will be used for categorical data in which all the cells have a minimum of five observations, and if any of the cells contain five or fewer observations then Fisher’s Exact Test will be used. Factors independently associated with the outcomes will be assessed using multivariate logistic regression. The multivariate model will be built by entering those variables which are bivariately statistically significant (p<0.05) (all variables assessed in bivariate analyses will have previously been hypothesized to be of potential clinical significance), assessing potential statistical interactions and confounders as appropriate, beginning with the most significant variables first.

Independent variables to be considered will be:
**Baseline sociodemographics:** gender (male/female), age (continuous measure), primary HIV risk group (sexual, drug use, other) (note that ethnicity will not be examined as data on ethnicity is not routinely collected)

**Baseline clinical characteristics:** CD4 count (continuous measure), HIV viral load (continuous measure), AIDS diagnosis (yes/no)

**Power for Primary Hypothesis:** *(see Figure 2)*
At an alpha of 0.05, a sample size of 1422 individuals, using a null probability of 15%, and an alternative probability of 30% (the primary hypothesis being that there will be a 30% overall prevalence of hepatitis C at baseline in this cohort) this study has over 95% power.

**Power for Secondary Objectives:**
If, as the literature suggests, it is assumed that 5% of those who test antibody negative in fact have detectable HCV RNA (this would be the secondary outcome with the least power), at an alpha of 0.05, sample 1 consisting of 50 false negatives, sample 2 consisting of 363 true positives, assuming that 25% of false negatives have a baseline CD4 below 200 cells/mm³ compared to 15% of true positives (please refer to secondary study hypotheses), this analysis would have over 90% power.

**Figure 2.**

<table>
<thead>
<tr>
<th>1422 individuals in cohort</th>
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<tr>
<td>(Assume 30% HCV prevalence based on antibody test)</td>
</tr>
<tr>
<td>427 people antibody-positive and 995 people antibody-negative</td>
</tr>
<tr>
<td>15% of these Ab+ may be PCR- (15% of 427) = 64</td>
</tr>
<tr>
<td>True positives (427-64) = 363</td>
</tr>
<tr>
<td>5% of Ab- may be PCR+ (5% of 995) = 50</td>
</tr>
</tbody>
</table>

**E. Study Limitations**
Due to cost constraints, we are not able to test the entire group of nearly 4000 individuals currently receiving antiretroviral therapy in the province of British Columbia. We hope that selecting antiretroviral naive individuals who have initiated HAART will enable us to control for the potential confounding effects of pre-treatment, while providing extensive information regarding HCV testing in individuals initiating and receiving antiretroviral therapy in the HAART-era.
A second limitation of this study is that we have chosen not to quantify the HCV RNA or to genotype the samples. We feel that to do so would be beyond the scope of this particular project, and is considered a next step.

Third, obtaining consent for each individual may present logistical difficulties, perhaps resulting in a selection bias. However, because of the important nature of the information to patients and their physicians, we anticipate that there will be a high degree of compliance. A comparison of those individuals who provide consent to those who do not will be performed to elucidate any bias occurring. There may be a response bias introduced into the study by obtaining consent from patients via their physicians, in that patients who have been lost-to-follow up in their routine clinical care may be more likely to have hepatitis C (by virtue of being more likely to be an injection drug user and therefore not consistently followed). We will have these patients baseline information, including risk group, and will endeavor to quantify the bias related to this issue, and control for it as much as possible in the analysis.

Fourth, although we are endeavoring to accurately classify individuals as true cases or not, there is potential for misclassification if a seroconversion takes longer than the period over which individuals are to be tested (6 months) (i.e. they would be counted as a discordant response rather than as a true positive). Misclassification may also occur if individuals who had initially tested antibody-negative but PCR-positive, but who upon re-testing developed positive-antibodies but due to an activated immune response (since by this point they will have started antiretroviral therapy), rather than due to seroconversion (i.e. they would be classified as a true positive rather than discordant).

A fifth and final limitation of this analysis is that ideally we would calculate the incidence of hepatitis C in this population from 1996 until the end of 2002, thereby enabling both an understanding of the incidence of HCV in this population, as well as an accurate and current point prevalence, and the ability to conduct prospective analyses. Unfortunately, the costs of PCR testing are prohibitively expensive, resulting in a modified proposal that will at least enable us to target our PCR testing in the clinical setting for all individuals.

F. Study Strengths
This investigation has several important strengths. The study population is a population-based cohort, and therefore has the advantage of being broadly representative of the population of people living with HIV and Hepatitis C who have initiated antiretroviral therapy in the HAART era, in the Developed World. Thus, the study will be more generalizable than other patient populations in whom such an investigation could occur (eg. hospital cohorts, clinical trial, etc.).

Second, it is an investigation that, although relatively simple and straightforward in its concept and approach, will have significant clinical implications. While the cohort will be small enough to make the project feasible, the cohort is large enough to enable powerful results.

A third important strength is that while this particular investigation is cross-sectional, because the testing will be conducted on samples drawn and stored at baseline, and because the testing will not be anonymous and will eventually be linked with patients other records existing through the HIV/AIDS Drug Treatment Program, the potential exists for extensive subsequent prospective analyses to be performed regarding the impact of Hepatitis C on HIV-positive individuals. Conversely, without doing this kind of study, any further analyses conducted on co-infected individuals in our cohort will be potentially biased both through selection bias of those
who have received a hepatitis C test, and through misclassification bias related to the HCV antibody vs. PCR status issue.

Fourth, all the testing will be done using the same assays, in the same laboratory, eliminating any bias due to differences in assays or laboratories.

G. Projected Outcomes
It is anticipated that this research will yield important results of direct clinical interest to patients, their physicians, and policy makers. Specifically, this project will:
✓ quantify the prevalence of hepatitis C infection in a population-based cohort of HIV infected individuals initiating antiretroviral treatment;
✓ contrast HCV prevalence depending on how it is defined;
✓ provide important information to clinicians and policy-makers regarding the burden of Hepatitis C among HIV-positive individuals initiating antiretroviral therapy;
✓ calculate the sensitivity, specificity, positive and negative predictive values of the HCV antibody test relative to a qualitative PCR test for HCV RNA in an HIV-infected population;
✓ describe characteristics associated with prevalent cases, and discordant test results;
✓ enable the development of an appropriate HCV testing algorithm for the HIV clinical setting.

H. Tentative Timetable
August 2003: Obtain Ethics Approval
January 2004: Receive notification of funding
January 2004 — October 2004: Obtain informed consent from participants
February 2004 — October 2004: Test stored samples
November 2004 — December 2004: Analyze results and prepare abstracts/ manuscripts; Communicate results to patients through their physicians.

I. Ethical and Informed Consent Issues

Human Subjects Involvement:
As this will be a retrospective analysis, the involvement and enrolment of participants will be restricted to requesting their consent to test their previously stored blood samples. Human subjects will only be involved via requests, through their physicians, to sign informed consents. No new data, blood samples, or interventions of any kind will be required for this study.

Sources of Material:
No new data or blood will be required of participants. Just prior to initiation of antiretroviral therapy (at baseline), participants provided blood samples for storage for future clinical research purposes. Participants will be asked, by their family physicians, to sign an informed consent allowing us to test their stored blood samples for the purposes of the study. The samples are stored by identification numbers for which only two people in the BC Center for Excellence have links to personal identifying data (Data Managers). All analyses, including both the testing of the blood and the statistical analyses, will be done using only the identification number, and there will not at any point be an opportunity for anyone involved in the study to know the names of the individuals being tested.

Potential Risks:
As there is no intervention intended for this study, the risks to study participants are minimized, especially physical risks. There is a psychological risk to finding out that one has Hepatitis C when one previously did not know (because of not being tested) or because one had falsely tested antibody-negative. However, the benefit of knowing whether one has hepatitis C or not would outweigh the risk associated with the knowledge, given the seriousness of having hepatitis C. The fact that the results will be communicated by patients' physicians will alleviate psychological risks to the patient. Patients may request a Hepatitis C antibody test from their physicians in the context of routine care. However, qualitative PCR tests are not routinely done.

Recruitment and Informed Consent:
Recruitment for this study will be limited to requesting individuals' consent, through their physicians, to test their previously stored blood samples. An explanatory letter to all antiretroviral prescribing physicians will be sent, with copies of the informed consent for them to present to patients. Patients will not be directly contacted by the BC Center for Excellence in HIV/AIDS. Patients will be requested by their physicians to sign the informed consent, in the context of their routine care. The informed consent is a 2-page form which explains in lay language the purpose, reason, and methods of the study, clearly articulating that only stored blood will be tested (see Appendix 3). The consent forms will then be sent back to the Data Managers of the BC Center for Excellence in HIV/AIDS, by the physician, in the same manner that antiretroviral prescriptions, requests for HIV resistance testing, and a number of other confidential requests are communicated to the BC Center for Excellence in HIV/AIDS. The Data Managers will document whether consent was given or denied, and whether the patient is lost-to-follow-up, in an Oracle Database that contains all other patient information. From this point, all data that is accessed utilizes only identification numbers, and is stripped of all personal identifying information. To date, there has not yet been a known breach of confidentiality within the BC Center for Excellence in HIV/AIDS. We are therefore reasonably confident that the system that exists for protecting patient confidentiality will be reliable.

Potential Benefits of Proposed Research
Although screening for hepatitis C among HIV-infected persons is considered standard of care in British Columbia, many physicians do not routinely test their patients. Routine screening involves only antibody testing, with qualitative PCR testing only in exceptional circumstances. Therefore, a direct benefit for patients will be the dual testing of their blood, enabling a much more accurate diagnosis of their hepatitis C status. If positive, patients will be then be enabled to pursue appropriate treatment and management options (e.g. HCV treatment, adapting lifestyle, etc.), and if negative, patients will have the opportunity to be appropriately counseled regarding avoiding hepatitis C transmission. This study will help raise the profile of the burden of hepatitis C in British Columbia and the rest of Canada, and will encourage patients and physicians to test all HIV-infected patients.

Benefits from this study will also be conferred on a population-level. This study will provide comprehensive diagnostic data that will enable a wide variety of epidemiological and clinically relevant investigations in the future (with appropriate ethical approval for each). This research can then be applied to improved patient management.

Importance of the Knowledge to be Gained
As previously discussed, the knowledge to be gained from this project will be substantial on both an individual patient-level, and on a population-level. To our knowledge, such a study has not yet been performed, particularly in an HIV-infected population, in whom there is evidence to
suggest the results may be different. This study will contribute substantially to the field of Hepatitis C diagnostics, and may also provide insight on an epidemiological level into the interaction between HIV and Hepatitis C.

Also to our knowledge, there is no other population-based HIV treatment cohort for whom extensive hepatitis C data also exists. This cohort has contributed significantly to the field of HIV clinical management [40, 66-68], and has the potential to address many of the important, and largely understudied, questions outstanding in the area of the clinical management of people who are co-infected with HIV and Hepatitis C [2].
References Cites


46. Hayashi, J., et al., Age-related response to interferon alfa treatment in women vs. men with chronic hepatitis C virus infection: women 39 years or less of age respond better to HCV treatment than men and women older than 40 years. Archives of Internal Medicine, 1998. 158(2): p. 177-81.
Appendix 3

Certificates of Ethics Approval

Appendix 3.1 HCV Testing (original and amendment)
Appendix 3.2 Linkage with Providence Laboratory
Appendix 4

Executive Summary:

Roadmap for Addressing the Epidemic of HIV and Hepatitis C Co-infection in Canada:

Issues, Recommendations, Priorities, and Next Steps

Roadmap for Addressing the Epidemic of HIV and Hepatitis C Co-Infection in Canada:

Issues, Recommendations, Priorities and Next Steps

June, 2004

Report from the National Stakeholders Meeting on Improving Access to Care, Treatment, and Support for People Living with HIV and Hepatitis C Co-infection, Montréal, Québec, January 2004.
This report is dedicated in loving memory to

Glen Edward Hillson

for whom knowledge about and action on these issues did not happen fast enough.
Preamble

The Canadian Treatment Action Council (CTAC) is a national, non-profit, consumer-driven organization dedicated to improving the lives of people living with HIV/AIDS by promoting informed public policy and public education, and promoting awareness of issues that impact access to treatment and health care for people living with HIV/AIDS.

Over the course of 2003, CTAC, with the financial support of Schering-Plough Canada and Agouron/Pfizer, sponsored a series of regional fora in Vancouver, Montreal, Toronto, and Halifax, regarding treatment and care issues in HIV and Hepatitis C co-infection. In January, 2004, these fora culminated in a multidisciplinary gathering of 50 people living with HIV and/or HCV co-infection, physicians (including general practitioners, hepatologists, and gastroenterologists), epidemiologists, and people working in community organizations, correctional settings, government, and the pharmaceutical industry (see Appendix One). The national meeting was supported by Schering-Plough, Hoffmann-LaRoche, the Anemia Institute, Agouron/Pfizer, Shire BioChem/GlaxoSmithKline, Bristol Myers Squibb, Abbott Laboratories, and Boehringer Ingleheim.

The purpose of CTAC’s regional fora and national meeting was to identify barriers to the appropriate treatment, care and support of people who are co-infected, and to identify mechanisms and the key players involved in moving past those barriers. The national meeting was intended to produce a report that would serve as a ‘roadmap’ of where we are in Canada with the epidemic of HIV/hepatitis C co-infection in terms of treatment and care issues, where we need to go, and how we can get there.

This document is a summary and synthesis of that national meeting, bolstered by other relevant information and recommendations where appropriate, and was prepared by Paula Braitstein (Board Member, Canadian Treatment Action Council (CTAC), Senior Policy Advisor on Health Promotion, BC Persons with AIDS Society (BCPWA)).

CTAC would like to thank the organizers of the meeting, including Louise Binder, James Kreppner, Philip Lundrigan, Paula Braitstein, Sheena Sargent, Patrick McIntyre, Claire Checkland, Kim Thomas, Lorne Fox, Marie Prevost, Susan Redgrave, Daryle Roberts, Daryn Bond, Françoise Grothe, and Marlene Allan. CTAC would like to gratefully acknowledge the work of the notetakers, Chantale Perron and Terry Pigeon. Last but not least, the organizing committee would like to acknowledge and thank Mardie Serenity for her logistical wizardry, excellent minutes, attention to detail, and generally keeping it all together!
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Appendix One: List of meeting participants  
Appendix Two: Availability of Treatments by Province and Territory for Hepatitis C  
Appendix Three: Letter to BC Pharmacare by coalition of BC activists regarding lack of evidence used in setting criteria in British Columbia for accessing HCV treatment.
List of Acronyms

AIDS Acquired Immune Deficiency Syndrome
ALT Alanine aminotransferase (a liver enzyme)
APRICOT AIDS Pegasys Ribavirin International Co-Infection Trial
ASO AIDS Service Organization
BCPWA British Columbia Persons with AIDS Society
CAHR Canadian Association for HIV Research
CanFAR Canadian Foundation for AIDS Research
CASL Canadian Association for the Study of the Liver
CIHR Canadian Institutes of Health Research
CSHA Canadian Strategy on HIV/AIDS
CTAC Canadian Treatment Action Council
CTN Canadian HIV Trials Network
EPO erythropoietin
FRSQ Fédération de Recherche de la Société Québécoise
HAART Highly Active Antiretroviral Therapy
HCV Hepatitis C Virus
HIV Human Immunodeficiency Virus
LCDC Laboratory Centre for Disease Control
MAT/DOT Maximally Assisted Therapy/Daily Observed Therapy
MSFHR Michael Smith Foundation for Health Research
OHTN Ontario HIV Treatment Network
PASAN Prisoners' HIV/AIDS Support Action Network
VANDU Vancouver Area Network of Drug Users
Executive Summary

Hepatitis C and HIV co-infection is an important Canadian health issue that is not receiving the attention it demands. Hepatitis C affects approximately 30% of people living with HIV (and approximately 10% of those infected with hepatitis C are also infected with HIV). Co-infected individuals are more likely to be Aboriginal, young, current or former injection drug users, current or former inmates, and people who received contaminated blood or blood products in the course of their healthcare. The majority of people who are co-infected live in Montreal or Vancouver, with emerging epidemics in Ottawa, Toronto, Calgary, and Edmonton.

In the presence of HIV, hepatitis C disease progression takes place 2 to 3 times faster, compared to people who only have hepatitis C. Thus, in seven to fifteen years after becoming infected, approximately 50-70% of co-infected people will begin to develop liver inflammation, and at least 20-30% will progress to liver fibrosis and cirrhosis, including end-stage liver disease. Because of the rapidity of disease progression in co-infected individuals, and their often unique constellation of social and health needs (e.g. in dealing with addiction, mental health, and HIV treatment issues), co-infected people represent a distinct population, falling between the cracks of both HIV and Hepatitis C treatment systems. HIV care providers and community services are ill-equipped, under-funded, and often lack sufficient or appropriate information on hepatitis C. Hepatitis C care providers are sometimes uninterested in HIV issues or HIV-positive people and often also require education and awareness training on HIV. Hepatitis C community services are almost non-existent, in part because of the lack of leadership by the Federal government (approximately 1% of Canadians are believed to be infected with hepatitis C, yet Canada has no national Hepatitis C Strategy or dedicated funding).

The average onset of HIV symptoms in the absence of treatment is seven to ten years after infection. The onset of fibrosis or scarring of the liver due to hepatitis C in the presence of HIV is seven to fifteen years. Many individuals who are currently co-infected acquired their two viruses together, and many of them became dually infected in the 1990's. Because of this, there is urgency to address the unique issues arising from the convergence of these epidemics.

The purpose of CTAC’s regional fora and national meeting was therefore to identify barriers to the appropriate treatment, care and support of people who are co-infected, and to identify mechanisms and the key players involved in moving past these barriers. The meeting was intended to produce a report that would serve as a ‘roadmap’ of where we are in Canada with the epidemic of HIV/hepatitis C co-infection in terms of treatment and care issues, where we need to go, and how we can get there.

Areas identified as needing particular attention were:

- Clinical issues including access to treatment, management of side effects, balancing HIV management, psychiatric and mental health supports, transplantation, and health care delivery
- Defining research priorities
- Policy issues ranging from federal and provincial strategies and funding, to formulary drug coverage and transplantation
• Prevention education, including primary and secondary prevention
• Community support services
• Correctional settings

Advocacy Priorities

Short-term:
• Identify best practices and standards of care for HIV/HCV co-infection.
• Advocate with provincial and private payers to broaden criteria for accessing treatment, and to cover concomitant Growth Factors, if necessary.
• Update treatment and management guidelines for co-infection, including HCV treatment issues, HIV treatment issues, side effect management, transplantation, nutritional issues, and psychiatric issues (expand upon what is contained in the CASL 2004 viral hepatitis guidelines).
• Disseminate treatment and management guidelines widely.
• Work with CTN to expand their HCV co-infection ‘Core’ as a basis for a network of investigators in co-infection.
• Have provincial transplant centers and the Canadian Society of Transplantation develop appropriate guidelines for the assessment and transplantation of HIV co-infected individuals.
• Identify pharmacoeconomists who can conduct research into the cost-effectiveness of properly addressing HIV/HCV co-infection, and the cost-effectiveness of treatment, and those who are willing to work with community activists to decipher existing materials.
• Use advocacy issues, such as lack of access to treatment, to raise public awareness and apply pressure on government through media campaigns.

Long-term:
• Develop a national observational cohort of co-infected individuals, on and off treatment.
• Expand CTAC’s Post-Approval Surveillance System project to incorporate HCV treatments.

Next Steps
1. CTAC to disseminate meeting report to all meeting participants, and other relevant stakeholders who did not attend.
2. CTAC to organize a national meeting of key clinicians, scientists, and consumers, to develop a research agenda for distribution to all research funding bodies (including the pharmaceutical industry, CIHR, OHTN, and CTN).
3. National state-of-the-art co-infection treatment and management guidelines should be developed and published.
4. CTAC's Co-Infection sub-committee to identify individuals interested in working together to develop workplan priorities to continue this work, including following-up on priorities highlighted in this report.


6. PASAN to circulate updated information regarding co-infection in correctional settings.

7. HepCure BC to conduct a survey on the availability of HCV treatment across the country, and disseminate findings (see Appendix Two).
1. Summary of Recommendations by Stakeholder

**Federal Government**

- Fund and implement an ongoing national hepatitis C strategy that significantly incorporates HIV co-infection.
- Incorporate priorities regarding co-infected individuals into the CSHA, with extra dollars attached.
- Devote more money to researching clinical aspects of co-infection, including natural history and pathogenesis issues.
- Create a network of databases for sharing data regarding treatment outcomes among co-infected individuals, and a network of physicians and researchers focussing on co-infection.
- Establish Centers for Excellence in Hepatitis C with expertise in HIV co-infection.
- Provide sufficient financial resources to allow integrated and specialized clinics to operate.
- Immediately implement and fund more harm reduction and addiction treatment services.
- Fund AIDS Service Organizations and other community-based organizations to provide resources and materials to co-infected individuals.
- Pressure the Canadian Society for Transplantation to develop appropriate guidelines for transplantation in HIV co-infected individuals.
- The Ministerial Council on HIV/AIDS should advise the federal HIV/AIDS Division to incorporate HCV co-infection into the revised CSHA, with the recommendation of attaching not currently allocated dollars.
- Include significant participation from both HIV and hepatitis C groups in the membership of all federal government committees addressing either HIV or hepatitis C issues.
- Classify hepatitis C as an AIDS defining illness, and classify addiction as a disability (where it is not already).
- Support, fund, and implement general and targeted education campaigns aimed at increasing the number of people getting tested for both viruses, and at decreasing the stigma associated with having them. Health Canada should lead and fund these initiatives, in collaboration with grassroots organizations and the provincial government.

**Provincial Governments**

- Expand eligibility criteria for accessing and remaining on hepatitis C treatment for as long as patient and physician believe it necessary and appropriate.
- Recognize and accept that the cost of growth factors is part of the cost of HCV treatment; then negotiate with the companies that make hepatitis C treatments, and those that make the growth factors, to enable the combined usage at a reduced cost.
- Develop provincial Hepatitis C Strategies, with devoted money, paying particular attention to addressing clinical management and community support issues.
• Identify what specialized services, in terms of HCV treatment and HIV co-infection, are available in Canada, and where they are available.

• Regularly revise treatment and management guidelines based on current evidence, and disseminate widely to physicians and patients.

• Ensure that each province has appropriate guidelines for liver transplantation in HIV-infected individuals.

• Develop Centers for Excellence in Hepatitis C with expertise in HIV co-infection.

• Provide sufficient financial resources to allow integrated and specialized clinics for coinfection to operate.

• Immediately fund and implement more harm reduction and addiction treatment services.

• Support, fund, and implement general and targeted education campaigns aimed at increasing the number of people getting tested for both viruses, and at decreasing the stigma associated with having them. Health Canada should lead and fund these initiatives, in collaboration with grassroots organizations and the provincial government.

• Include significant participation from both HIV and hepatitis C groups in the membership of all provincial government committees addressing either HIV or hepatitis C issues.

• Classify addiction as a disability (where it is not already).

• Fund AIDS Service Organizations and community-based organizations to provide resources and materials to co-infected individuals.

**Correctional Services**

• Immediately implement recommendations from existing reports regarding safe drug use and tattooing, methadone treatment, addiction treatment, harm reduction, and unhindered access to knowledgeable care providers and specialists.

• Enhance collaboration between existing clinics and hepatitis, HIV, and infectious disease specialists.

• Integrate other health care modalities into all clinics, and move toward a holistic and patient-centered model of care.

• Provide opportunities to see patients in health care settings daily or weekly to assist them with receiving and tolerating their treatments (e.g. daily observed therapy, maximally assisted therapy), and provide adequate nutritional and mental health supports.

• Develop new, or adapt existing, drop-in day clinics to help patients receive and tolerate their treatments.

• Immediately implement more harm reduction and addiction treatment services.

• Launch general and targeted education campaigns aimed at increasing the number of people getting tested for both viruses, and at decreasing the stigma associated with being infected.
Industry

- Make products, including both HCV treatments such as pegylated interferon and also supportive therapies such as erythropoietin (EPO), more accessible through price reduction and other means.
- Work with public payers to expand eligibility criteria for accessing and remaining on hepatitis C treatment for as long as patient and physician believe it necessary and appropriate.
- Conduct pharmacoeconomic studies to show the cost-effectiveness of supportive treatments such as EPO, and early treatment of HCV infection.
- Participate in the development of a HIV/HCV co-infection research agenda, and integrate these priorities into their drug development plans.
- Devote more money to researching clinical aspects of co-infection, including natural history, pathogenesis issues, HCV treatment, and HIV treatment.
- Always provide an expanded access program for new drugs for hepatitis C, with designated spaces reserved for HIV co-infected individuals.
- Include, and separately analyze, HCV co-infected individuals in research for HIV and HIV-related products.
- Support community initiatives in the areas of harm reduction, addiction treatment, and poverty reduction.
- Support the development of drop-in centers for co-infected persons, including explicit supports for co-infected people on treatment.

Clinicians, Health Care Services, and Health Authorities

- Use clinical authority to advocate that third party payers expand their eligibility criteria for accessing and remaining on hepatitis C treatment for as long as patient and physician believe it necessary and appropriate.
- Encourage public payers and pharmaceutical manufacturers to negotiate the costs of HCV treatment, including growth factors.
- Identify best practices and standards of care elsewhere in the world for the treatment and management of HIV/HCV co-infection.
- Identify what specialized services, in terms of HCV treatment and HIV co-infection, are available in Canada, and where they are available.
- Regularly revise treatment and management guidelines based on current evidence, in consultation with consumers, and disseminate widely to other physicians and patients.
- Refer HIV-positive patients to transplant centers for assessment, even if the transplant center does not have an HIV infection policy, and not wait until the patient has decompensated cirrhosis to refer them.
• Pressure organ transplant centers to develop appropriate policies and guidelines for assessing and performing liver transplants on people living with HIV. Clinicians and surgeons working in transplant centers should be proactive in developing these policies and guidelines.

• Encourage and support existing HIV clinics in working more collaboratively with hepatitis experts.

• Hepatitis experts should become more proactive in learning about HIV and collaborating with HIV experts.

• Move all clinics toward integrating other health care modalities, and toward a holistic and patient-centered model of care.

• Develop Centers for Excellence in Hepatitis C with expertise in HIV co-infection.

• Provide opportunities to see patients in health care settings daily or weekly to assist them with receiving and tolerating their treatments (e.g. daily observed therapy, maximally assisted therapy).

• Develop or adapt drop-in day clinics to help patients receive and tolerate their treatments.

• Implement more harm reduction and addiction treatment services.

• Develop Continuing Medical Education programs specifically to train physicians on co-infection, and develop and offer more training to front-line workers.

• Develop mentorship and training programs in co-infection for physicians and researchers.

• Support efforts to classify hepatitis C as an AIDS defining illness, and addiction as a disability.

• Develop more drop-in centers for persons co-infected with HIV and HCV, including explicit supports for people on treatment.

**Scientists**

• Conduct pharmaeconomic studies to show the cost-effectiveness of early HCV treatment and the use of supportive therapies such as EPO.

• Regularly revise treatment and management guidelines based on current evidence, in collaboration with consumers, and disseminate widely to physicians and patients.

• Develop an HIV/HCV research agenda in order to identify research priorities across the Four Pillars Drug Strategy (harm reduction, prevention, treatment, enforcement).

• The Canadian Association for HIV Research (CAHR) should write a letter to CIHR, OHTN, CTN, and other research institutions such as CanFAR, to advocate for more money to be devoted to researching various aspects of co-infection, including natural history and pathogenesis issues.

• Develop Centers for Excellence in Hepatitis C with expertise in HIV co-infection.

• The CIHR Advisory Committee on HIV/AIDS and the Federal Ministerial Council on HIV/AIDS should recommend the same thing to these research organizations.
• The Canadian HIV Trials Network should create a network of databases for sharing (anonymized) data regarding treatment outcomes among co-infected, and a network of physicians and researchers focusing on co-infection.

• Research funding bodies such as CIHR, CanFAR, the CTN, and the OHTN should solicit research proposals specific to the issue of HCV/HIV co-infection.

**Community Organizations**

• Develop and implement more harm reduction services.

• Develop targeted education campaigns aimed at increasing the number of people getting tested for both viruses, and at decreasing the stigma associated with having them.

• Lobby for funding dedicated to HCV/HIV co-infection to be built into the Canadian Strategy on HIV/AIDS, with devoted materials and resources developed as a result.

• Make hepatitis C co-infection a priority in organizational workplans.

• Encourage participation of both HIV-positive and hepatitis C-positive individuals on organizational committees.

• Provide resources and materials to HCV/HIV co-infected individuals about co-infection.

• Develop peer-driven networks and groups to foster mutual support and collective action.

• Develop more drop-in centers for persons co-infected, including explicit supports for people on treatment.

**Activists and Consumers**

• Have courage, be tenacious, and know your stuff.

• Familiarize yourself with the recommendations for all stakeholders, select the issue(s) that is/are of highest personal importance and/or interest, and actively work towards the achievement of the recommended actions, either as an individual or through an affiliate organization.