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HIV-1/HCV CO-INFECTION: IMMUNITY AND VIRAL DYNAMICS

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ABSTRACT

Human immunodeficiency virus-1 (HIV-1) and hepatitis C virus (HCV) are the agents behind two viral epidemics causing huge morbidity and mortality worldwide. HCV infection is a leading cause of end-stage liver disease. Co-infection with HIV leads to faster progression to cirrhosis and lower clearance rate of HCV infection following current standard treatment with pegylated interferon α (peg-IFN α) and ribavirin. The overall aims of the studies described in this thesis were to gain knowledge of how to best manage HIV/HCV co-infection in clinical practice, to study immunological features in co-infected patients, to search for immunological host factors affecting HCV treatment outcome, and to identify possible predictive markers of treatment response.

In **paper I** we investigated the feasibility of treating HIV/HCV co-infected patients with peg-IFN α and ribavirin in a Swedish HIV outpatient clinic. We found that only a small fraction of the patients were suitable treatment candidates when following international guidelines. However, in those treated the response was good and correlated to HCV RNA kinetics during initial treatment. One of the patients, who was among those screened for participation in the abovementioned study, spontaneously cleared her chronic HCV infection. Since this is a very rare event we further investigated the immune response of this patient, as described in **paper II**. She displayed a low level of T cell activation and a high level of T cell function compared to HIV/HCV co-infected control subjects, thus resembling a healthy individual. In **paper III** we investigated the impact of chronic HIV/HCV co-infection and the effects of treatment with peg-IFN α and ribavirin on NK cells and on NKT cells by flow cytometry. Conventional NK cells were largely unaffected by the co-infection, with only a slight decrease in perforin content in CD56dim cells and an increased CD56bright immunoregulatory population. In contrast, the NKT cells were severely reduced in the co-infected patients and were not restored by HCV therapy. Interestingly, we observed a significant accumulation of unconventional CD56-CD16+ NK cells in these subjects. The expansion of CD56- NK cells was rapidly reverted when HCV replication was suppressed by HCV treatment. In **paper IV**, we observed that the CD56- NK cells in HCV infected patients were functionally skewed, with poor IFN γ production but retained MIP-1 β expression. In addition, we found that pre-treatment levels of CD56- NK cells in peripheral blood correlated with peg-IFN α and ribavirin treatment outcome. Patients with low levels of CD56- NK cells were more likely to clear the HCV infection, and this was not directly linked to other viral and host factors known to influence treatment outcome. In **paper V** we measured the chemokine IP-10 in plasma from HIV/HCV co-infected patients. We found that lower pre-treatment plasma IP-10 levels were associated with faster clearance of the virus. This effect was more pronounced on the first phase viral reduction (day 0-2), than on the second (day 7-28).

In summary, this thesis increases our knowledge of the immune system's interaction with HCV and HIV, and identifies an immunological biomarker that correlates with HCV treatment outcome. In addition, it highlights the need for further implementation of HCV treatment in the HIV/HCV co-infected patient group.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. **Falconer K**, Sandberg JK, Reichard O, Alaeus A. HCV/HIV co-infection at a large HIV outpatient clinic in Sweden: feasibility and results of hepatitis C treatment. *Scandinavian Journal of Infectious Diseases* 2009;41(11-12):881-5.
- II. **Falconer K**, Gonzalez VD, Reichard O, Sandberg JK, Alaeus A. Spontaneous HCV clearance in HCV/HIV-1 coinfection associated with normalized CD4 counts, low level of chronic immune activation and high level of T cell function. *Journal of Clinical Virology* 2008 Feb;41(2):160-3.
- III. Gonzalez VD, **Falconer K**, Michaëlsson J, Moll M, Reichard O, Alaeus A, Sandberg JK. Expansion of CD56- NK cells in chronic HCV/HIV-1 co-infection: reversion by antiviral treatment with pegylated IFNalpha and ribavirin. *Clinical Immunology* 2008 Jul;128(1):46-56.
- IV. Gonzalez VD, **Falconer K**, Björkström NK, Blom KG, Weiland O, Ljunggren HG, Alaeus A, Sandberg JK. Expansion of functionally skewed CD56-negative NK cells in chronic hepatitis C virus infection: correlation with outcome of pegylated IFN-alpha and ribavirin treatment. *Journal of Immunology* 2009 Nov 15;183(10):6612-18.
- V. **Falconer K**, Askarieh G, Weis N, Hellstrand K, Alaeus A, Lagging M. IP-10 predicts the first phase decline of HCV RNA and overall viral response to therapy in patients co-infected with chronic hepatitis C virus infection and HIV. *In manuscript*.

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
Anti-HCV	Antibodies to hepatitis C virus
APC	Antigen presenting cell
ART	Antiretroviral therapy
CD	Cluster of differentiation molecule
cEVR	Complete early viral response
CTL	Cytotoxic T lymphocyte
CXCR3	Chemokine (X-C motif) receptor
DC	Dendritic cell
DNA	Deoxyribonucleic acid
Ds	Double-stranded
EACS	European AIDS Clinical Society
ELISA	Enzyme-linked immunosorbent assay
ESLD	End-stage liver disease
EVR	Early viral response
FACS	Fluorescence-activated cell sorting
FSC	Forward scatter
HAART	Highly active antiretroviral therapy
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
IDU	Injection drug use
IFN	Interferon
IL	Interleukin
IP-10	Interferon γ -inducible protein 10 kDa
IRF	Interferon regulatory factor
ISG	Interferon stimulated gene
IU	International units
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
NK	Natural killer
NKT	Natural killer T
PAMP	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
Peg-IFN	Pegylated interferon
RNA	Ribonucleic acid
RVR	Rapid viral response
SMI	Swedish Institute for Infectious Disease Control
ss	Single-stranded
SSC	Side scatter
STAT-C	Specifically targeted antiviral therapy for HCV

SVR	Sustained viral response
TCR	T cell receptor
Th	T helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T regulatory cell
WHO	World Health Organization

INTRODUCTION

Approximately 33 million people are infected with HIV today (1), and an estimated 25-30% of HIV-positive individuals in the West are co-infected with HCV (2-4). Since the introduction of highly active antiretroviral therapy (HAART) against HIV, AIDS-related mortality has decreased dramatically and end-stage liver disease has become a leading cause of death for HIV-infected individuals (5, 6).

The treatment of HCV in HIV-infected patients has become a priority mainly for two reasons. First, progression to liver cirrhosis and end-stage liver disease occurs more rapidly in this population (7) than in the HCV mono-infected. Second, the tolerance of antiretroviral agents is poorer in the presence of an underlying chronic HCV infection, with a greater risk of hepatotoxicity (8). Furthermore, a pre-existing HIV infection reduces the likelihood of spontaneous HCV clearance, and establishment of chronic HCV infection occurs more often than in mono-infected (9). The response rate to HCV therapy is inferior in HIV/HCV co-infected patients compared to HCV mono-infected, and the reasons for this is incompletely understood. A better understanding of the underlying mechanisms of the reduced response rate is crucial in order to ameliorate the HCV treatment outcome in this patient group. Few studies have focused on the host immune system.

HEPATITIS C VIRUS (HCV)

HCV was initially called non-A non-B hepatitis, as the agent causing post-transfusion hepatitis was unknown, until it was renamed upon its discovery in 1989 (10). HCV belongs to the Flaviviridae family and the genus Hepacivirus. It is a spherical, enveloped RNA virus. The single positive-strand RNA genome contains a large open reading frame flanked by highly conserved untranslated regions at both the 5' and the 3' termini (11).

HCV has a tropism for hepatocytes where it replicates, although the possibility to infect monocytes and lymphocytes has been suggested (12). The replication takes place in the cytoplasm leading to translation of a single polyprotein further cleaved to produce three structural, and seven non-structural proteins; the core protein (C), two envelope proteins (E1, E2), the short membrane peptide (p7), probably promoting assembly and release of virions, and six proteins involved in polyprotein processing and viral replication (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (13, 14). HCV has a very high rate of replication with approximately 10^{12} virions produced per day in the infected host. The virions have an estimated half-life of 3 hours (15). Due to lack of proof reading by the HCV RNA polymerase, the virus has an exceptionally high mutation rate and therefore a high genetic variability (16, 17).

The HCV strains are classified into six major genotypes (1-6), depending on genetic differences with several subtypes within each genotype (represented by letters) (18, 19). Furthermore, within each infected individual the virus exists as a population of closely related viral variants, i.e. viral quasispecies (20). Knowledge of the HCV genotype is important because it helps to predict the outcome of antiviral therapy and influences the choice of the therapeutic regimen (21).

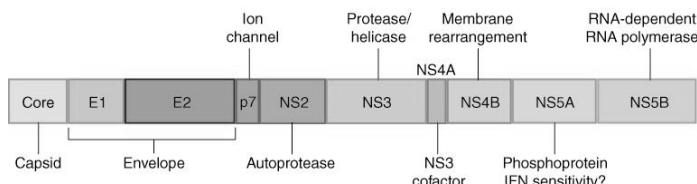


Figure 1. Schematic picture of the HCV genome. Adapted from (16).

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

HIV-1 is a member of the Lentivirus genus of the Retroviridae family, and the causative agent of acquired immunodeficiency syndrome (AIDS). The virus was first identified by the Montagnier/Barré-Sinoussi group in France 1983 (22), and the finding was further confirmed by Robert Gallo in USA (23).

HIV-1 is composed of two copies of positive single-stranded (ss) RNA enclosed in a capsid surrounded by a cell-derived phospholipid envelope. The genome consists of nine genes encoding for 15 viral proteins in total. *Gag* and *env* are the two major structural genes and *pol* encodes for the viral enzymes, reverse transcriptase (RT), protease and integrase. The remaining six (*tat*, *rev*, *nef*, *vif*, *vpr* and *vpu*) are regulatory genes for proteins involved in infection, replication and immune evasion (24). Through genetic characterisation based on phylogenetic analysis, HIV-1 can be classified into three groups: major (M), novel (N), and outliers (O). Just recently, a forth group named P has been identified in a Cameroonian woman (25). Only group M strains are of epidemic importance, and they can be further divided into nine subtypes (A, B, C, D, F, G, H, J and K), and an increasing number of circulating recombinant forms (CRFs) (26). Subtype B is the most prevalent subtype in Europe and North America, while C is the mostly spread subtype globally. For epidemiological research the knowledge of different subtypes is essential, but no important clinical difference has yet been found between the subtypes, although this is an area of conflicting results and somewhat controversial.

HIV infects a variety of immune cells such as T-cells, dendritic cells (DCs) and macrophages that express the CD4 receptor and the chemokine co-receptors CCR5 or CXCR4 (27). Viral replication occurs after the viral capsid has been released into the cytoplasm. The viral ssRNA is transcribed, through reverse transcription into double-stranded (ds) DNA. It is then transported into the cell nucleus and becomes integrated with the host genome where it may lie dormant or be replicated. Due to lack of proof-reading function during reverse transcription, there is a considerable inter- and intra-individual variability of molecular sequences in different HIV-1 strains (28). The HIV infection can produce up to 10^{10} virions per day and the average half-life of free plasma virus has been estimated to be 6 hours (24).

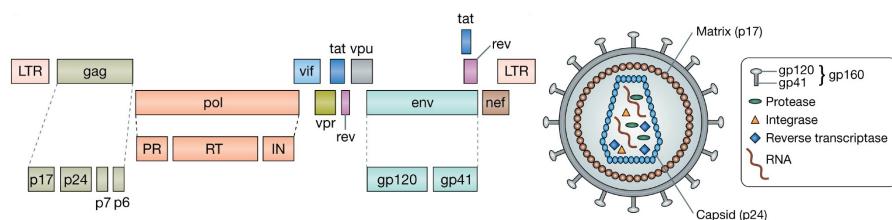


Figure 2. Schematic picture of the HIV-1 genome and the HIV-1 virus. Adapted from (29).

EPIDEMIOLOGY OF HIV-1 AND HCV CO-INFECTION

WHO estimates that approximately 130-170 million people are living with HCV today, and that 33 million are infected with HIV (1). Co-infection with the two viruses is common due to shared routes of transmission. Approximately 10% of those infected with HIV globally is also HCV infected according to one report (30). However, it is difficult to find reliable data on the global prevalence of HIV/HCV co-infection due to limited epidemiological studies in low income countries. In the West, as much as 25-30% of all HIV-positive persons are suffering from concomitant HCV infection, as reported in several studies (2, 3).

Though HIV and HCV share common routes of infection, the viruses are transmitted with different efficacy depending on the mode of transmission. Whereas both viruses are transmitted with high efficacy via direct blood-to-blood contact, HCV is less easily transmitted via the sexual route. Thus, the prevalence of HCV co-infection within different countries and populations is closely related to the prevalence of blood-borne (mainly injection drug use (IDU)) HIV infection. For instance, within the Eastern European countries like Belarus and Ukraine, where IDU is the main route of HIV transmission, HCV co-infection rates as high as 70% can be observed. On the contrary, in central European countries such as Belgium and Germany, where sexual intercourse dominates the mode of transmission HCV co-infection rates are lower, between 10-15% (4). These trends can be observed elsewhere in the world, as for instance in Asia, where co-infection rates among Chinese former plasma donors, are as high as 85% (31), whereas in countries with predominately heterosexual transmission of HIV like Thailand, co-infection rates are around 10% (32, 33). In Sweden, the proportion of HCV co-infection within the HIV-positive population has not been investigated in detail. So far 5170 persons are registered in the national Swedish database for HIV-positive patients (InfCare HIV, January 2010). 771 of these patients lack data concerning concomitant HCV infection, whereas 495 patients are anti-HCV positive (successfully treated patients not included). If patients with unknown hepatitis C status are excluded, the HIV/HCV co-infection rate in Sweden is likely to be approximately 11%. Since this figure is based on the anti-HCV prevalence and not on HCV-RNA in plasma, the true figure is expected to be somewhat lower. Thus, in total, approximately 400-500 HIV/HCV co-infected persons would be expected in Sweden. To conclude, this is in congruence with data from other countries with predominantly heterosexual transmission.

Although the traditional route of HCV infection is blood-borne, epidemic outbreaks among HIV positive men who have sex with men in several high-income countries document that HCV may well be sexually transmitted (34-36). While the exact mode of transmission remains unclear, associations have been seen with HIV-positive status, recent sexually transmitted infections like syphilis and gonorrhoea, multiple sexual partners, traumatic anal intercourse, and non-injecting recreational drug use, particularly snorting of cocaine.

In summary, the prevalence of HCV within the HIV infected population is far higher compared to the general population worldwide, where the global burden of HCV infection is estimated to be 2.2-3% (37).

CLINICAL PICTURE OF HIV-1 AND HCV CO-INFECTION

Natural course of hepatitis C in HIV infected patients

After acute HCV infection, progression to chronicity is increased from 65-85% in those not infected with HIV to over 90% in HIV infected individuals, particularly in those with advanced immunosuppression (9, 38). Individuals with HIV/HCV co-infection have been shown to have higher HCV RNA levels in plasma (39, 40) and, in some studies increased HCV RNA levels have been correlated with more advanced immunosuppression (41).

Although the introduction of highly active antiretroviral therapy (HAART) has markedly reduced HIV-related morbidity and mortality, non HIV-related conditions, particularly liver disease, now constitute a high proportion of causes of death among people with HIV infection (6). In fact, HCV infection is one of the major causes of death in HIV positive individuals (5, 42-44). Chronic HCV infection may result in cirrhosis, end-stage liver disease (ESLD) and hepatocellular carcinoma (HCC), all of which are associated with high morbidity and mortality. Several studies have shown that co-infection with HIV worsens the prognosis of HCV-related liver disease (45-47). In a meta-analysis of eight studies examining the risk of cirrhosis and ESLD in HIV/HCV co-infected compared with HCV mono-infected individuals a two-fold and six-fold higher risk of progression to cirrhosis and liver failure was found (48). Once HIV/HCV co-infected patients have developed cirrhosis, the risk of hepatic decompensation is higher than for HCV mono-infected individuals. Further, survival following decompensation is poor, despite effective HAART. HIV/HCV co-infected patients develop HCC at a younger age, with a mean age of 42 years for co-infected patients compared with a mean age of 69 years for those infected with HCV alone (49).

Studies on the effect of HAART on the natural history of chronic HCV disease have been contradictory (50-52). Qurishi et al reported a lower risk of liver mortality in persons who lived long enough to receive effective HAART (53) and so did Brau et al (54). However, several prospective studies have not detected a beneficial effect of HAART on HCV disease (55, 56). In some studies, HAART has been associated with hepatic injury (57, 58). Additional research is needed to determine the long-term effect of HAART on HCV disease progression.

In summary, HIV infection exacerbates the natural history of HCV infection. HIV infected patients are less likely to clear hepatitis C viremia following acute infection, have higher HCV RNA loads, and experience more rapid progression to HCV-related liver disease than those without HIV infection (9).

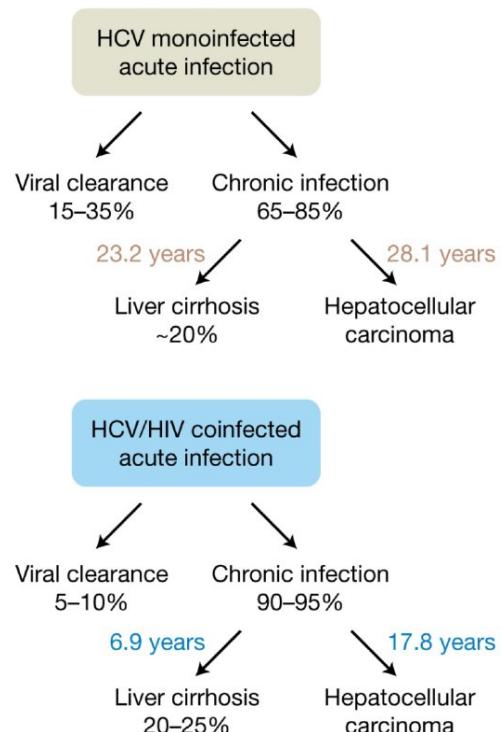


Figure 3. Clinical course of infection in HCV mono-infected and HIV/HCV co-infected patients. Adapted from (29).

The effect of HCV on HIV disease progression

The effect of HCV infection on HIV disease progression is not clear. The majority of studies have found no association between HIV/HCV co-infection and poorer HIV disease outcome. In 2002 a US study of more than 1900 HIV infected individuals demonstrated no differences between HIV mono-infected and HIV/HCV co-infected populations with regard to incidence of AIDS, death, or decline of CD4 count (59). Survival was also examined in a European cohort study, EuroSIDA, together with HIV disease progression, and virological and immunological response in almost 6000 individuals, of whom 33% were HIV/HCV co-infected (4). Here HIV/HCV co-infected persons had much higher rate of liver-related mortality, but there was no increased risk of AIDS, and overall mortality rates were similar to HIV mono-infected individuals. HIV virological suppression and CD4 count responses following HAART were not affected by HCV co-infection. Conversely, some studies have found increased mortality and more rapid HIV-related disease progression in HIV/HCV co-infected populations (60, 61). However, these findings have been associated with limited uptake of HAART, particularly among IDU-acquired co-infection. At present, the overall

literature suggests that the major contribution of HCV to mortality of co-infected individuals is attributable to accelerated liver disease and not increased AIDS-related complications.

Treatment of chronic HCV infection in HIV-positive patients

Because the prevalence of co-infection is high, all HIV infected patients should be screened for HCV and vice versa. Persons found to be positive for HCV antibodies should also be tested for HCV RNA in plasma to confirm an active disease. Patients with advanced HIV disease and patients with acute HCV infection may not have detectable antibodies. Thus, HCV RNA testing should be done if HCV infection is clinically suspected although anti-HCV is negative. Noteworthy, serosilent HCV infections may exist in immune competent persons as well, although those infections are very rare (62).

Acute HCV can be spontaneously cleared by the immune system, but more commonly, a chronic infection is established and consequently treatment might be needed. HCV treatment offers the possibility of eradicating HCV within a defined treatment period. Every patient with chronic HCV, co-infected with HIV or not, should therefore be considered for treatment when the benefits of therapy outweigh the risks. The treatment of hepatitis C in HIV infected individuals has become a priority mainly for two reasons. First, progression to liver cirrhosis and end-stage liver disease occur more rapidly in this population (7, 50, 63). Second, the tolerance of antiretroviral agents is poorer in the presence of underlying chronic hepatitis C, with a greater risk of hepatotoxicity (8).

A variety of viral and host factors influence treatment outcome. Factors associated with poorer response rates are HCV genotypes 1 and 4, high HCV RNA load and slow HCV RNA decline when treatment is started. Host factors like male gender, African American race, old age, obesity, insulin resistance, advanced liver fibrosis, alcohol intake, and coexisting hepatitis are also associated with poorer response to HCV therapy (64, 65). Like in HCV mono-infected patients, the standard treatment today consists of a combination of peg-IFN α and ribavirin. Peg-IFN α is immunomodulatory and ribavirin is an antiviral guanosine analogue, but the exact mode of action for these two drugs is incompletely understood. There are currently two peg-IFN α isoforms used for HCV treatment. The standard dose for peg-IFN α -2a is 180 μ g once weekly, and for peg-IFN α -2b 1.5 μ g/kg body weight once weekly. A weight-based dose of ribavirin (1000 mg if <75 kg, and 1200 if >75 kg) is recommended for all genotypes (66).

The primary aim of HCV treatment is sustained viral response (SVR), defined as undetectable serum HCV RNA 24 weeks after the end of therapy. If an early viral response (EVR) of at least 2 \log_{10} reduction in HCV RNA compared with baseline is not achieved at week 12, and if HCV RNA is still detectable at week 24, treatment should be discontinued, as an SVR is unlikely. A negative HCV RNA test 4 weeks into therapy is defined as a rapid viral response (RVR), and is associated with a high likelihood of SVR (67, 68).

If chronic hepatitis C is detected early in the course of HIV infection (before the initiation of HAART) treatment for chronic HCV is advised. However, if a co-infected

patient has severe immunodeficiency (CD4 count <200 cells/ μ L) the immune status of the patient should be improved using HAART prior to start of HCV treatment. Recent HIV treatment guidelines suggest that HCV co-infection might present a situation under which HAART should be considered, independent of CD4+ cell criteria, which are otherwise used to determine initiation of treatment (66, 69). During peg-IFN α and ribavirin therapy, didanosine is contraindicated due to the risk of lactic acidosis (70). Stavudine should be avoided for the same reason and zidovudine as well, due to the risk of severe anaemia (66). The role of abacavir is somewhat uncertain. Some studies have shown lower SVR in patients receiving abacavir (71, 72). A possible explanation for this may be the impairment of ribavirin phosphorylation by abacavir.

Several studies assessing the efficacy and safety of peg-IFN α and ribavirin in HIV co-infected patients have been conducted over the last few years. Most of these trials have provided treatment for 12 months, regardless of HCV genotype. Overall, the response rates have been poor. The multicenter French trial, RIBAVIC, and the ACTG 5071 study, performed in the US, both reached an overall SVR of 27 % (73, 74). In the ACTG 5071 the initial ribavirin dose was low, 600 mg/day, which may explain the low response rate. The APRICOT study evaluated HIV/HCV co-infected patients receiving peg-IFN α -2a and a fixed dose of 800 mg ribavirin/day (75). The overall rate of SVR was 40 %, but only 29% in patients with genotype 1. For genotype 2 and 3 patients the SVR was 62%. In the PRESCO trial patients received peg-IFN α -2a + weight-based ribavirin for 48 or 72 weeks (genotype 1) and 24 or 48 weeks (genotype 2 or 3). Here the overall SVR was higher, 50%, underlining the importance of higher ribavirin doses (76). All in all, the response rates have been lower than in studies of HCV mono-infected patients (64, 77, 78).

As a rule of thumb, 48 weeks of HCV combination therapy is recommended for HIV/HCV co-infected patients. However, individualized tailoring of the duration of therapy is advisable, taking into account rapid or delayed viral response, fibrosis stage, and the HCV genotype. A proposed algorithm for peg-IFN α and ribavirin combination therapy in HIV/HCV co-infected individuals is shown in Figure 4. This reflects the recommendation in the last version of the European AIDS Clinical Society (EACS) guidelines (66). The Swedish guidelines are essentially the same, with a difference regarding genotypes 2 and 3. Thus, in Sweden, the shortened therapy of 24 weeks is recommended not only if an RVR is obtained but also if a complete EVR (HCV-RNA negativity week 12) is obtained, provided the dose of ribavirin is not reduced (79).

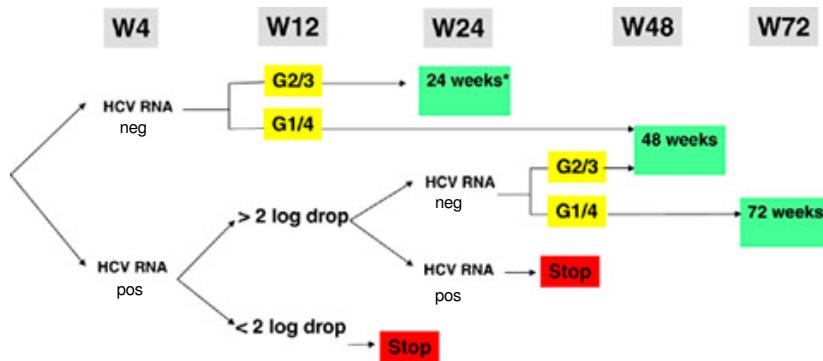


Figure 4. Proposed optimal duration of HCV therapy in HIV/HCV co-infected patients. *Patients with baseline HCV RNA< 400.000 IU/mL and minimal fibrosis. Adapted from (66)

GENERAL IMMUNOLOGY

The immune system

The immune system protects us from invading pathogens. Detailed insight into the interaction of pathogens with this system will lead to better understanding and help to develop efficient protective strategies. The immune system can roughly be divided in the innate and the adaptive immune systems, with the innate system acting as a first line of defence, readily activated to respond within minutes, while an effective adaptive immune response takes days. However, the traditional view of innate and adaptive immunity as two separate entities is increasingly being replaced by a more integrated picture.

The innate system is specialized in the control of invading pathogens during the primary phase of an infection, and it does not require prior exposure to respond rapidly to a given antigen. It acts in a non antigen-specific manner recognizing general pathogen associated molecular patterns (PAMPs) and cellular stress signals indicating infection or cellular dysfunction, which enables invading pathogens and abnormal cells to be phagocytosed or killed via cytotoxicity. The cellular constituents of the innate immune system include granulocytes, dendritic cells (DCs), macrophages and natural killer (NK) cells. The adaptive immune system with its B and T lymphocytes acts in an antigen-specific manner, which leads to the establishment of an immunological memory that persists after the antigen is cleared. The immunological memory established after a successful response allows for a more rapid response upon a secondary infection by the same pathogen. Cells of the innate and adaptive immune systems share features of development, activation as well as function as they synergize and interact in a complex network against immune challenges.

Innate immunity

Natural killer (NK) cells play important roles in the defence against viral infections (80) and in tumor surveillance (81). They are recruited and activated by inflammatory cytokines and chemokines, and when activated, they are able to initiate a response that involves both cellular cytotoxicity and secretion of cytokines. Thus, they may have direct antiviral effects as well as serve to recruit other cell types involved in host defences. NK cells comprise 5-20% of peripheral blood mononuclear cells (PBMCs) and 20-30% of mononuclear cells in the human liver (82). They are classically identified as CD3 negative, CD56 positive and CD16 negative or positive. Two major populations of NK cells are recognized based on the relative level of CD56 expression, CD56 low ($CD56^{\text{dim}}$) or high ($CD56^{\text{bright}}$) cells (83). $CD56^{\text{dim}}$ NK cells express high levels of CD16 and comprise approximately 90% of all NK cells. They are primarily cytotoxic, but can also produce cytokines and chemokines. The $CD56^{\text{bright}}$ NK cells are primarily focused on production of cytokines such as IFN γ upon activation. Recently, an additional CD56 negative CD16 positive NK cell population has been in focus in relation to chronic viral infections. These cells have been regarded to display a largely dysfunctional profile with poor cytokine production, low proliferative and cytotoxic capacity (84-86). NK cells use a complex set of receptor interactions that can differentiate between “self” and “non-self”. The receptors that regulate NK function can be classified as either activating or inhibitory receptors (87). “Non-self” recognition is triggered by activating receptors in the absence of MHC class I. Conversely, “self” recognition is achieved upon binding of NK cell inhibitory receptors with the MHC class I molecule present on the surface of nucleated cells. Since many viral infections lead to down regulation of MHC class I molecules on the surface of infected cells as a means of evading host cytotoxic T lymphocytes (88), “missing-self” recognition represents an important counter measure against such viral pathogens.

Natural killer T (NKT) cells are a diverse group of T cells that share properties of both T cells and NK cells. They constitute a small proportion of peripheral lymphocytes, but are abundant in the liver. They express a T cell receptor (TCR), and a variety of molecular markers that are typically associated with NK cells. They differ from conventional T cells in that their TCRs are far more limited in diversity and in that they recognize lipids and glycolipids presented by CD1d molecules, rather than peptide-MHC complexes, on DCs and monocytes. NKT cells share features with NK cells as well, such as expression of CD161 and the receptor NKG2D, and some expression of CD16, CD56 and perforin. They have the capacity to regulate and activate several other immune cells including DCs, NK cells, T and B cells (89) and may play a role in autoimmunity, allergies, tumor immunity and antimicrobial immune responses (90). Upon activation, NKT cells are able to rapidly produce large quantities of cytokines such as IFN γ , IL-4, IL-2 and tumor necrosis factor α (TNF α). Importantly, depending on location and stimulus, CD1d-reactive NKT cells can modulate their spectrum of cytokine production toward either a pro-inflammatory or a more immunoregulatory profile (91). The importance of NKT cells in viral infections is incompletely understood although evidence suggests that they play a role in the control of hepatitis B virus as well as cytomegalovirus (CMV) infection (92, 93). They are lost

in HIV-1 infection (94, 95), and they might be involved in the immunopathogenesis of HCV infection (96, 97).

Interferon

Interferons (IFNs) are potent antiviral proteins synthesized and released by a number of different cell types in response to the presence of pathogens, such as viruses, bacteria, and parasites. IFNs are named after their ability to “interfere” with viral replication within host cells. Besides inducing the interruption of viral replication, IFNs promote recruitment and activation of NK cells and macrophages (98, 99). In addition, IFNs increase recognition of infected cells by up-regulating antigen presentation to T lymphocytes, and increase the ability of uninfected host cells to resist infection. Thirteen subtypes of IFN α and a single IFN β isoform belong to type I IFNs in humans (100). Type I IFNs bind to a cell surface receptor, the IFN α/β receptor, and induce intracellular signalling through the Jak-STAT pathway (101).

IFN α is one of the cornerstones in the current treatment against HCV infection. However, the exact mode of action in therapy against HCV is not known. The role of IFN α in HIV infection is complex and published data report both protective and pathogenic effects. Both *in vivo* and *in vitro* data support a protective effect in primary infection (102, 103). Studies in chronic HIV infection, however, have suggested that IFN α may promote immunopathogenesis through immune activation (104).

Chemokines

Chemotactic cytokines, or chemokines, are a subfamily of cytokines that primarily coordinate leukocyte recruitment, but may also be involved in activation. They have been recognized in the last few years as important mediators in the pathogenesis of many human diseases. Since the first report in 1977, over 40 such molecules have been identified in humans (105). Chemokines are composed of single polypeptide chains including conserved cysteine residues that have been used for subfamily definition and nomenclature. They are hence divided into four major subfamilies, CXC, CC, C and CX3C, depending on the position of the two N-terminal cysteine residues. Cells that are attracted by chemokines follow a gradient of increasing concentration towards the source of the chemokine, and the migration of cells is regulated through interactions with transmembrane chemokine receptors. Inflammatory chemokines are released from a wide variety of cells in response to stimuli such as bacterial and viral infections in order to recruit monocytes, neutrophils, lymphocytes and other effector cells to the site of infection or tissue damage. In addition to their crucial roles in inflammatory processes and host defence, chemokines also have pivotal roles in processes such as the regulation of embryonic development, angiogenesis and wound healing (106).

Adaptive immunity

CD4 T cells recognize antigens when presented by MHC class II molecules expressed by antigen presenting cells (APCs). They are generally called helper T cells and produce a variety of cytokines in response to antigen recognition, directing and

regulating immune responses. The nature of the antigen and the type of co-stimulatory signal provided by the APC drives differentiation and activation of distinct CD4 T cell subsets with different cytokine profiles. This generates immune responses to be mediated against intracellular or extracellular pathogens. After antigen contact the originally undetermined T helper (Th) cell can differentiate into Th1 or Th2 cells. Th1 cells secrete cytokines such as IFN γ and IL-2 effective against intracellular pathogens, and whose principal functions are to activate CD8 T cells and macrophages. Th2 cells mainly produce IL-4, IL-5, and IL-10 supporting B-cell activation and differentiation (107, 108). It is, however, important to note that CD4 T cell responses in humans do generally not display strong Th1 or Th2 bias, as both these phenotypes are represented in a normal healthy immune response.

About a decade ago a CD4 T cell subtype able to suppress effector T cell proliferation and cytokine production was reported, and these cells were named T regulatory cells (Tregs) (109). Increasing evidence suggests that Tregs can be generated during chronic viral infections such as HIV, HCV and herpes virus infection, and may contribute to the suppression of virus specific immune responses. This immune suppression may favour viral persistence, but it may also protect the host from immunopathogenic tissue damage at the site of infection. Tregs are believed to be reduced in numbers or functionally impaired in certain disease settings where chronic immune activation is the key disease mediator (110). Recently, two additional CD4 T cell subsets, called Th17 (111) and follicular helper T cells (T_{FH}) (112) have been described. Th17 cells have been associated with autoimmune diseases and chronic inflammatory diseases (113). They are also believed to play a role in late HIV infection as preferential loss of Th17 cells in the gut leads to increased permeability and bacterial translocation resulting in chronic immune activation and thereby exhausted T cells unable to control viral replication. T_{FH} cells have a crucial role as a helper cell for antibody responses and B-cell memory.

CD8 T cells are able to recognize and kill host cells infected by viruses or other intracellular microbes. They recognize microbial peptides presented by MHC class I molecules that are expressed on virtually all nucleated cells. The release of cytokines and chemokines, such as IL-2, IFN γ , TNF α and MIP-1 α/β by CD8 T cells facilitates and strengthens the immune defence by attracting other T cells, macrophages, NK cells and B cells to the site of infection (114). Upon expansion some CD8 T cells gain effector function to become cytotoxic T lymphocytes (CTLs). These are able to release granules containing membrane pore-forming proteins and enzymes, such as perforin and granzyme, inducing apoptosis of target cells (115, 116). Alternatively CD8 T cells can kill target cells via death receptors such as TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand. During the final stages of an immune response a majority of the CD8 T cells responding to an antigen undergo apoptosis while a portion become long-lived memory cells that remain for years in the host (117). Certain pathogens have evolved mechanisms to suppress the MHC class I presentation to avoid recognition by T cells. However, such virus infected cells may instead become targets for NK cells which detect the pathogen's effect on MHC class I expression.

IMMUNITY IN HIV-1 AND HCV INFECTION

HCV can be spontaneously cleared by the host immune system. However, in a majority of cases a chronic infection is established. Acute HIV infection, on the other hand, always leads to the establishment of a chronic infection. Both viruses have evolved abilities to evade innate and adaptive immune responses by mechanisms that will be discussed in the following section.

Innate immunity

Cells sense the presence of extracellular pathogens using cell surface toll-like receptors (TLRs) which are responsible for sensing microbial infection by recognition of PAMPs, such as dsRNA and ssRNA. After binding pathogens, TLR signalling ultimately leads to activation of transcription factors such as NF- κ B and interferon regulatory factor 3 (IRF-3), which in turn leads to production of type I IFNs (118). Whether the hepatocytes, DCs or the liver resident macrophages, the Kupffer cells, are the first interferon producer upon HCV infection remains unknown (119). However, endogenous type I IFN induces a cascade of IFN regulatory factors (IRFs) leading to up-regulation of interferon stimulated genes (ISGs), that generates an antiviral state in cells to prevent spread of infection and activates innate immune cells able to kill infected cells (120).

HCV proteins perturb innate antiviral signal transduction pathways at many levels suggesting direct evasion mechanisms to circumvent the immune system. For example, the HCV protein E2 was suggested to inhibit NK cell signalling and cytotoxicity through cross-linking of CD81, which is expressed on most nucleated cells (121). However, more recent data did not support this finding (122). Furthermore, HCV infection has been reported to induce changes in NK cell natural cytotoxicity receptor and cytolytic function (123-125). NK cell dysfunction may lead to improper DC maturation and insufficient T cell priming, thus facilitating establishment of chronic HCV infection. Both HCV infection and HIV infection are associated with dysfunctional DC maturation, and these effects may synergize in HIV/HCV co-infection (126). In HIV infection, several studies have shown functional impairment in NK cell cytokine secretion and cytotoxicity (127-129). Both acute and chronic untreated HIV infection is associated with alterations in NK cell subset distribution, with partial loss of CD56+CD16+ cells and expansion of CD56-CD16+ NK cells (84-86, 127-129), that have been reported to have an impaired cytolytic function and cytokine production (86).

NKT cells are susceptible to HIV, and infected subjects display a loss of these cells (94, 95, 130), whereas the impact of HCV on NKT cells remains controversial (97, 131, 132). In some subjects with chronic HIV infection, NKT cells are retained despite ongoing viral replication. However, in these circumstances NKT cells display a functional impairment. The physical loss or functional impairment of these cells is likely to result in secondary impairments in cellular immunity given the role these cells play in activation of NK cells and DCs.

In addition, HCV efficiently interferes with type I interferon production, and with ISGs, as well as with signalling through interferon receptors (120, 133, 134). For example, the two HCV proteins NS5A and E2 interact with the important ISG protein kinase C (PKC) (135, 136). This capacity of HCV to interfere with the IFN pathway at many levels is a likely mechanism underlying the virus success to establish a chronic infection (120). Still, induction of ISGs was found in pre-treatment liver biopsies of patients with chronic HCV, demonstrating that HCV infection can lead to activation of the endogenous IFN system (137). Notably, in the same study, patients with pre-elevated expression of ISGs tended to respond poorly to HCV therapy when compared with patients with low initial expression. In a recent study patients without a pre-activated IFN system experienced a robust up-regulation of many ISGs in the liver 4 hours after injection of peg-IFN α , unlike the patients with high expression of ISGs before treatment (138). Why the induction of the endogenous IFN system before treatment start seems to compromise the success of exogenous IFN remains to be elucidated.

Adaptive immunity

The next line of host defence is the adaptive immune system which is compromised by HIV and in most cases fails to contain HCV. HIV causes significant depletion of CD4 T cells already early in the acute phase of infection. This early loss of CD4 T cells is most severe in the gut, and later becomes significant also in the periphery (139). T helper cell depletion is only partially explained by death through HIV cytopathicity, or by CTL-mediated killing (140). Enhanced turnover due to chronic immune activation seems to be the main mechanism behind the gradual CD4 T cell depletion (141-143). HIV has evolved a variety of mechanisms to evade CTL responses, for example the ability to down-regulate MHC class I molecules (144).

There is growing consensus on the importance of T-cell responses in determining HCV clearance, and there is evidence of protective immunity, since those with a history of spontaneous viral clearance are more likely to clear the virus upon re-exposure (38, 145). Patients exhibiting spontaneous viral clearance mount vigorous multispecific CD4 and CD8 T cell responses. By contrast, patients who progress to chronic disease tend to have weak narrowly focused responses (146-148). CD4 T cells secrete cytokines capable of helping the specific CD8 T cell responses; without CD4 T cells, the induction of immune responses is impaired and CTL memory is poorly maintained (149). It is clear that HCV, just like HIV, is capable of evading the cell-mediated immune response, and that the high levels of viral production and the variable viral genome leads to emergence of immune escape mutations. Amino acid substitutions resulting in impaired recognition by HCV-specific T cells have been observed in chronically infected patients (150, 151), and it is well established that mutations within CTL epitopes promote HCV and HIV persistence (152, 153). The strength and breadth of HCV-specific CD4 and CD8 T-cell responses are reduced in HIV/HCV co-infected patients compared to those infected with HCV alone (154, 155). This suggests that the immunosuppression induced by HIV compromises immune responses to HCV.

Although the immune system is likely to be involved in the fibrosis related to HCV, immunosuppression does not ameliorate fibrosis but instead accelerates it. Accelerated

fibrosis is most pronounced when peripheral CD4 T cell counts are decreased (156-158). The increased rate of fibrosis in HIV/HCV co-infection may partly be explained by the HIV-induced massive depletion of CD4 T cells in the gut, which may in turn lead to increased microbial translocation and immune activation (159). Microbial products, such as lipopolysaccharide (LPS) enter the bloodstream and reach the portal system, where it may cause infection and inflammation in periportal spaces, resulting in hepatic fibrosis. This theory is supported by a recent study by Balagopal et al. where increased levels of LPS were associated with progression of HCV infection to cirrhosis (160).

Interestingly, we found that individuals with HIV/HCV co-infection display sharply elevated immune activation as determined by CD38 expression in T cells. This occurred, despite effective HAART, in both CD8 and CD4 T cells and was more pronounced than in the mono-infected control groups (161).

Interferon γ -inducible protein 10 kDa (IP-10)

IP-10 or CXCL10 targets T cells, NK cells, and monocytes through binding to the CXCR3 receptor, but unlike other CXC chemokines, IP-10 lacks chemotactic activity for neutrophils (162-164). Of the CXC chemokines, IP-10 has been studied most extensively in the context of chronic HCV infection. IP-10 was originally discovered as a 10kDa molecule released from cells stimulated *in vitro* by IFN γ , hence the name IP-10 (165). Besides IFN γ , other stimuli typically elevated during infection or inflammation, such as type I IFNs, TNF α , dsRNA, LPS, and certain viruses can also induce IP-10 expression (162, 166, 167). IP-10 has been implicated in the pathophysiological progression of multiple sclerosis, diabetes mellitus, and HIV (168).

In patients with chronic HCV infection, IP-10 expression is elevated both in the liver and in the peripheral blood (169, 170). Intrahepatic IP-10 reportedly is produced by hepatocytes (167, 170) and sinusoidal endothelial cells (169), and prior studies suggest that both increased peripheral and intrahepatic IP-10 levels are associated with severity of liver fibrosis and inflammation (168, 171) (172). Elevated IP-10 levels have also been reported in HIV mono-infected patients (171, 173). However, Reiberger et al found no differences in IP-10 levels between HCV mono-infected and HIV/HCV co-infected patients with similar HCV viral loads, suggesting that HIV does not have a measurable additive effect on IP-10 production (174). Roe et al, reported that increased IP-levels in HCV/HIV co-infected patients may be linked to the accelerated fibrosis in these patients. Additionally, they observed a negative correlation between IP-10 levels and CD4 cell counts; HCV/HIV co-infected with CD4 counts below 400 cells/ μ L had significantly higher IP-10 levels than those with higher CD4 counts (171).

Aside from its role in inflammation, IP-10 is a marker of successful response to HCV combination therapy with peg-IFN α and ribavirin. Pre-treatment peripheral IP-10 levels are significantly lower in patients achieving SVR as compared with those who do not. This was originally reported in HCV mono-infected (169, 175-177), and later confirmed in HIV/HCV co-infected patients (174, 178).

AIMS

The overall aims of this thesis were to investigate the impact of HIV-1/HCV co-infection on the innate immune system and to gain knowledge of how to best manage this difficult-to-treat patient population in clinical practice. The specific aims were:

- To investigate the feasibility of treating chronic HCV infection in HIV-1/HCV co-infected patients at an HIV-outpatient clinic in Sweden, and to correlate early HCV RNA kinetics to treatment outcome (Paper I).
- To investigate the T-cell immune responses in a HIV-1/HCV co-infected patient who spontaneously cleared a chronic HCV infection (Paper II).
- To study the impact on NK cells and NKT cells by chronic HCV infection in subjects with and without HIV-1 co-infection, and to understand their role in HCV infection as well as in viral clearance upon treatment with peg-IFN α and ribavirin (Paper III and IV).
- To determine host immune correlates predictive of HCV treatment outcome (Paper IV and V).
- To measure plasma levels of the chemokine IP-10 before and during treatment with peg-IFN α and ribavirin, and to correlate the levels to HCV-RNA kinetics (Paper V).

MATERIALS

The patients in paper I-V are from the Viral Dynamics and Immunology in HIV-1/HCV Co-infection (DICO) study. Treated patients were recruited from the HIV outpatient clinic at Karolinska University Hospital, Solna, Sweden (DICO-S) and from 4 Danish centres (Hvidovre University Hospital, Rigshospitalet, Aalborg and Aarhus University Hospitals) (DICO-D). All patients were Caucasian adults (>18 years), HBsAg negative, and treatment naïve for HCV. Furthermore, they all had CD4 counts ≥ 300 cells/mm³ or a percentage $\geq 12\%$, and had a compensated liver disease. Patients on HAART had undetectable HIV-RNA (<50 copies/mL) and patients without HAART had a stable status of the HIV infection in the opinion of the investigator. All patients were anticipated to be treated with peg-IFN α 180 µg/week and ribavirin (800 mg/day if genotype 2 or 3 and 1000-1200 mg/day if genotype 1) for 48 weeks.

Paper I

This study is based on the 11 Swedish HIV/HCV co-infected patients in the DICO-S cohort. Ten of them were on HAART, and one was untreated for the HIV-infection.

Paper II

This case report describes one of the patients screened for participation in the abovementioned study, who spontaneously cleared the chronic HCV infection between screening and study start. HIV/HCV co-infected control subjects were from the DICO-S cohort (n=10), and uninfected control subjects (n=10) were healthy volunteers donating blood at Stockholm Blood bank.

Paper III

The HCV/HIV co-infected patients were from the DICO-S cohort. 13 patients were sampled (9 patients that started HCV treatment + 4 patients that were screened, but did not start treatment) before initiation of treatment. All co-infected patients were on HAART. HIV mono-infected (n=10) and HCV mono-infected (n=14) control groups were from the outpatient clinic at Karolinska University Hospital/Solna. The uninfected control group (n=21) were healthy volunteers donating blood at Stockholm Blood bank. The three groups were matched for age and sex. The HCV mono-infected group was matched for HCV genotype, and the HIV mono-infected group was matched for CD4 count relative to the co-infected study group (Table 1, paper III).

Paper IV

HIV/HCV co-infected patients on HAART from the DICO-S cohort were studied (n=10). In addition, 32 HCV mono-infected patients followed at the outpatient clinic at Karolinska University Hospital/Huddinge were included and analysed retrospectively. None of the patients had received prior HCV therapy. The healthy controls (n=34) were healthy volunteers donating blood at Stockholm Blood bank.

Paper V

All treated HIV/HCV co-infected patients included in the DICO-S and DICO-D cohorts (n=21) were studied.

METHODS

PCR

Polymerase chain reaction (PCR) is a method for detecting specific sequences in the genome or genome products by means of amplification of the sought DNA sequence. The process requires a heat-stable DNA polymerase and primers containing sequences complimentary to the target region. The method relies on thermal cycling, consisting of repeated cycles of heating and cooling allowing for the melting and enzymatic replication of the DNA.

In real-time PCR, the method used for HCV RNA quantification in this thesis, the quantity of amplified DNA formed is continuously monitored during the course of the reaction by registration of the amount of fluorescent light emitted. Real-time PCR assays thus have a broad dynamic range of quantification, are more sensitive, and allow faster analysis than end-point PCR assays. We used PCR for **quantification of HIV RNA** in plasma by the Amplicor HIV-1 Monitor Test with a lower limit of quantification of 50 copies of RNA/mL (version 1.5, Roche Diagnostics Systems, Hoffman-La Roche, Basel, Switzerland). In addition, PCR was used for **HCV RNA quantification** by the COBAS TaqMan HCV test with a lower limit of detection of 15 IU/mL (Roche Diagnostics, Branchburg, NJ, USA).

Genotyping

Genotyping of HCV was performed using the line-probe assay INNO-LiPA HCV II (Innogenetics N.V., Ghent, Belgium). In brief, probes from the 5'region of the different genotypes are attached on strips of nitrocellulose, and upon adding denatured HCV-RNA it will bind to the probe with complimentary sequence.

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a technique used to detect the presence of an antibody or an antigen in a sample. For **IP-10 quantification** in plasma the Quantikine (R&D SYSTEMS Minneapolis, MN), a solid-phase ELISA, was utilized. In brief, a monoclonal antibody specific for IP-10 has been pre-coated onto 96-well plates. Standards and samples are added into the wells and any IP-10 present is bound by the antibody. After washing, an enzyme-linked polyclonal antibody specific for IP-10 is added. Subsequently, substrate solution is added and measurable color develops in proportion to the amount of IP-10 bound in the initial step. An enzyme immunoassay was also used for the **detection of antibodies to HCV** (HCV version 3.0 AxSYM, ABBOTT). This test is based on 4 recombinant HCV antigens, representing the core, NS3, NS4 and NS5.

Flow cytometry

Flow cytometry is a widely used technique for counting, examining, and sorting cells and micro organisms while suspended in a stream of fluid. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second. A common application is to physically sort particles based on their properties, so as to purify populations of interest.

Fluorescence-activated cell sorting (FACS) has been used for the analysis of lymphocytes in paper II, III and IV. FACS is used to study the properties of cell subsets identified using monoclonal antibodies to cellular proteins. Individual cells within a mixed population are first tagged by treatment with specific monoclonal antibodies labelled with fluorescent dyes, called fluorochromes. The mixture of labelled cells is then forced in a fluid of saline through a nozzle, creating a fine stream of liquid containing cells spaced singly at intervals. As each cell passes through a laser beam it scatters the laser light, and any dye molecules bound to the cell will be excited into emitting light at longer wavelength than the light source. A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam detecting particle size (Forward Scatter or FSC) and several perpendicular to it, one measuring granularity and inner complexity (Side Scatter or SSC) and one or more fluorescence detectors. By analysing fluctuations in brightness at each detector it is then possible to derive various types of information about each individual particle. Sensitive photomultiplier tubes detect both the scattered light, and the fluorescence emissions, which give information on the binding of the labelled monoclonal antibodies and hence on the expression of cell-surface proteins. An Analogue to Digital Conversion system generates FSC, SSC and fluorescence signals from light into electrical signals that can be processed by a computer (179).

There are dozens of fluorescent molecules with a potential application in flow cytometry. With the advancement of technology, proper execution and analysis has become more complicated requiring expertise knowledge behind fluorochrome selection and combination. One consideration to be aware of when performing multicolour fluorescence studies is the possibility of spectral overlap. When two or more fluorochromes are used during a single experiment there is a chance that their emission profiles will coincide making measurement of the true fluorescence emitted by each difficult. Therefore the contribution in a specific detector that derives from fluorochromes not assigned to that channel must be subtracted in a process called compensation. Modern flow cytometry analytical software applies fluorescence compensation mathematics automatically, which simplifies the procedure considerably. However, software compensation is limited by the fact that conversion of the electronic signal from linear to logarithmic is approximate and thereby sometimes inaccurate.

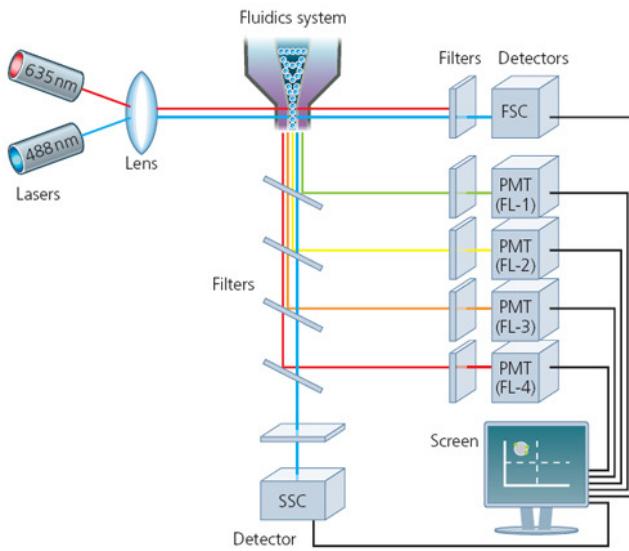


Figure 5. Schematic overview of a flow cytometer. Adapted from (180)

Histology

In paper I and V the liver biopsies were scored according to the Ishak protocol (181). In brief, fibrosis stage is ranked from 0-6, where 0 represents no fibrosis, 1-2 portal fibrosis, 3-4 bridging fibrosis, and 6 cirrhosis.

Statistics

For detailed information about statistical analysis used in this thesis, I refer to the respective study.

RESULTS AND DISCUSSION

HIV/HCV co-infected patients can achieve good response rates to HCV treatment, but we treat too few

In paper I we investigated the feasibility of treating HIV/HCV co-infected patients with peg-IFN α and ribavirin at the HIV outpatient clinic at Karolinska University Hospital/Solna, which, at the time of study start, was one of the largest HIV outpatient clinics in Sweden. In November 2004, 658 HIV positive patients were followed at this clinic. Of these, 644 had had an anti-HCV test performed and 97 (15%) were found to be positive. Of these, 87 were tested for HCV-RNA whereof 61 (70%) were found to be positive. Remarkably, only 17 of these 61 patients (28%) were considered suitable for participation in the treatment study (Figure 1, paper I/Figure 6, below) when following international guidelines. The reasons for not being included were mainly ongoing drug abuse (n=19), but also poor patient motivation (n=5), psychiatric disease (n=5), previous HCV treatment (n=5), co-existing hepatitis B (n=3), decompensated liver cirrhosis (n=3), other co-morbidities (n=1), frequent travelling (n=2), and pregnancy (n=1). Furthermore, 6 out of the 17 patients who were screened did not start treatment due to relapse of severe psychiatric problems (n=2), withdrawal of consent (n=2), relapse in IDU (n=1), and in 1 case, a late spontaneous clearance of chronic HCV, described in paper II. Hence 11 patients (18% of the whole group of co-infected) were included in the treatment study, which is indeed a low number. Similar observations have been made in a large retrospective study by Mehta et al (182), and in a prospective study by Fleming et al (183), although the percentage of treated patients was even smaller in these studies. This clearly illustrates the dilemma when treating HIV/HCV co-infected patients. Many have co-morbidities such as psychiatric disease and ongoing drug abuse, and consequently there are concerns about adherence. These co-morbidities are naturally present also in HCV mono-infected subjects. Another reason for withholding therapy to the co-infected group may be a historically strong focus on the HIV infection. Lack of experience of treating HCV infection in HIV-positive patients, and fear of drug-drug interactions and adverse events may also hamper treatment in this group. Moreover, as shown by Mehta et al, in many cases, there is reluctance to treatment from the patient's side.

The overall treatment response in our study was high with a SVR rate of 73%. This is considerably higher than what has been reported in large previous trials (73, 75, 76). There may be several reasons for this; we had a relatively high percentage of genotype 2 and 3 patients in the cohort (45 %), and the patients had a relatively well preserved immunological profile. Furthermore, none of the patients were cirrhotic, although in the 6 patients with retrievable biopsies, 4 had bridging fibrosis (Table 1, paper I). The use of weight-based ribavirin for genotype 1 and very few dose reductions of ribavirin (only one case) have probably also contributed to the high SVR rate. Furthermore, frequent contact with nurses, doctors and counsellors may be an important reason for the good adherence and thereby high SVR in this study.

Obviously, our study material is small. Thus, we can not draw any firm conclusions from the HCV RNA kinetics closely monitored during the study period. However, in congruence with other studies we observed that RVR is a good predictor of SVR, and that genotype 2 and 3 patients had a more rapid viral decline than genotype 1 patients (Figure 2, paper I). We measured ribavirin concentrations at treatment week 2, 4 and 8. Notably, at week 2 and 4 all 3 patients that did not achieve SVR had ribavirin concentrations below the median for respective genotype, whereas all 3 patients requiring erythropoietin or blood transfusion due to anaemia had ribavirin concentrations above the median for respective genotype (data not shown, paper I). Interestingly, occurrence of anaemia during the early phase of peg-IFN α and ribavirin treatment has been linked to better treatment outcome in patients with genotype 1 (184). As mentioned above, 3 patients in our study developed anaemia, and they all obtained a SVR.

Since co-infected patients experience faster progression to cirrhosis and an increased risk of ESLD compared to HCV mono-infected patients this patient group should be prioritised regarding HCV treatment. Clinicians should be encouraged to be more liberal in their treatment decisions, and to put effort into making patients more suitable candidates for treatment, i.e. consult psychiatrists and specialists in addiction medicine to optimise the status of the patient. Importantly, these patients can obtain good treatment response under optimised circumstances, as shown in our study.

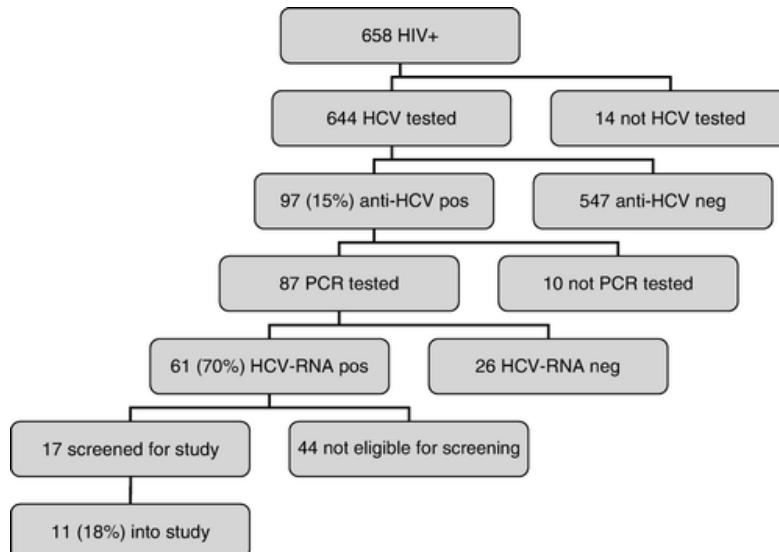


Figure 6. Anti-HCV seroprevalence and HCV RNA positivity in the HIV-positive patients followed at the HIV-outpatient clinic at Karolinska University Hospital/Solna, at the time for study start.

A plausible reason for spontaneous HCV clearance

One of the patients screened for participation in the DICO-S HCV treatment study became spontaneously HCV-RNA negative somewhere between October 2004 and March 2005 after being positive for several years, as described in paper II. The patient was a 48-year-old female on HAART since 2001 (Figure 1, paper II/Figure 7, below) that probably contracted both viral infections during a period of IDU in the 1980s.

Spontaneous clearance of chronic HCV in patients co-infected with HIV is likely to be very rare given the damage to the immune system caused by HIV and few cases are described in the literature (185, 186). This spurred our interest to study the adaptive immune response of this particular patient more closely.

Analysis of T cell activation and function revealed several interesting findings when comparing the response of this patient with healthy controls and other HIV/HCV co-infected patients on HAART. PBMC was analysed by flow cytometry from the time point where HCV RNA negativity was first observed. We found that her CD4 T cell counts were approaching those observed in healthy controls. As immune status and disease progression in HIV-infected subjects is known to correlate with the down regulation of the early differentiation marker CD7 in CD8 T cells (187), and with the level of immune activation as determined by CD38 expression in these cells (188-190) we next assessed these parameters. Interestingly, she displayed a low level of CD38 expression in T cells, compared to the co-infected controls. When looking at CD7 expression in T cells her levels were in line with the levels of healthy individuals (Figure 2A, paper II). We also assessed the functional profile of T cells in this patient in terms of the ability to produce IFN γ , TNF α and IL-2 in response to polyclonal stimulation. We observed a more polyfunctional response in T cells from this patient as compared to the co-infected controls (Figure 2B, paper II). Moreover, when assessing the presence of CD8 T cell responses to an immunodominant HCV epitope (NS3 1073-1081), we found that CD8 T cells specific for this epitope were more numerous in this patient compared to the other co-infected patients (Figure 2C, paper II). In addition, CD8 T cells responding with IFN γ and TNF α to this epitope were observed after *in vitro* stimulation with the corresponding synthetic peptide.

The precise mechanism of HCV clearance remains unknown. However, our data support that this patient displayed a normalized CD4 count, low level of T cell activation and a high level of T cell function compared to other HIV/HCV co-infected subjects. This suggests that a beneficial immune status with regard to these parameters may have contributed to clearance of HCV. Low level of immune activation, which is strongly correlated with slow HIV disease progression, may also be a factor promoting HCV control and clearance. We believe that long-term HAART is likely to contribute strongly to strengthen the immune system to a level where spontaneous HCV resolution may occur. Moreover, we suggest that clinicians treating HIV/HCV co-infected patients with HAART should continuously monitor for spontaneous resolution of the HCV infection.

Finally, one could speculate that the shift in HAART before the planned HCV treatment start, where didanosine was replaced by tenofovir, may have had something to do with the HCV clearance. However, tenofovir has no documented effect on HCV. Still, the shift in treatment regimen may well have lead to a more effective HIV therapy, contributing to an improved immunological status, and indirectly to the viral clearance.

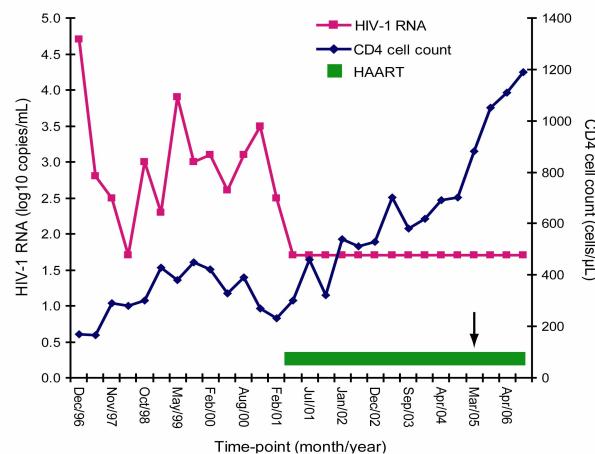


Figure 7. Changes in plasma HIV-1 RNA, CD4 cell counts and ART regimen during the clinical course of the patient. First time point with negative HCV RNA is marked by an arrow.

Innate cellular immunity – changes induced by HIV/HCV co-infection, and by HCV treatment

The role of innate cellular immunity in HIV/HCV co-infection is incompletely understood. In paper III we have investigated NK cells and NKT cells, both key players in the innate immune response, in HIV/HCV co-infected patients on HAART.

We initially analyzed the percentage and absolute numbers of CD56+ NK cells in PBMC from patients with combined chronic untreated HCV infection and chronic HIV infection controlled by HAART. For comparison, we also assessed these cells in control groups of HCV mono-infected and HIV mono-infected subjects, and in healthy controls (Table 1, paper III). CD56+ NK cell percentages and absolute counts in peripheral blood were not significantly different between the co-infected group as compared to the two mono-infected or healthy control groups (Figure 1B and 1C, paper III). Interestingly, the percentage of CD56+ CD16+ NK cells with high perforin expression was significantly different between the groups ($p=0.044$), and was lower in the co-infected group compared to the other three groups (Figure 1E, paper III).

After HCV treatment start with peg-IFN α and ribavirin, HCV viral load was efficiently suppressed in the co-infected subjects, and decreased from a median viral load of 4.33×10^6 IU/mL to 62 IU/mL at 4 weeks of therapy. Notably, the percentage of CD56+ NK cells doubled in PBMC in response to 12 weeks of treatment (Figure 1F, paper III). When treatment was ended the percentages returned to pre-treatment levels. Interestingly, this treatment was associated with an internal redistribution among NK cell subsets, with a doubled proportion of CD56^{bright} NK cells ($p=0.007$) (Figure 1H, paper III). Absolute CD56+ counts were largely unchanged, indicating that peg-IFN α and ribavirin treatment supports a relative NK cell expansion in blood with significant redistribution within the NK cell compartment towards a CD56^{bright} immunoregulatory profile.

Untreated viremic HIV infection has been associated with the expansion of a CD16+ NK cell subset lacking CD56 expression, and these cells have been suggested to be functionally impaired (84-86). We identified this cell subset in peripheral blood of HIV/HCV co-infected, and in our three control groups. The CD56-CD16+ NK cells were sharply elevated in co-infected subjects, both as percentage and absolute numbers, over those found in healthy as well as mono-infected subjects ($p<0.05$) (Figure 3A and 3B, paper III). Moreover, the co-infected subjects displayed a further decreased perforin expression in CD56-CD16+ NK cells as compared to healthy controls ($p=0.003$) (Figure 3C, paper III). This aberrant change in the NK cell compartment was much less pronounced in HCV mono-infection, which suggests that the accumulation of CD56- NK cells may be driven by high HCV loads. When HCV treatment was initiated this unconventional NK cell subset rapidly contracted and reached relative and absolute counts similar to those observed in healthy control subjects ($p=0.029$ and $p=0.011$, respectively) (Figure 3D and 3E).

NKT cells were significantly reduced in co-infected patients in comparison to healthy controls. This reduction occurred to a similar extent in the mono-infected control groups (Figure 5B and 5C, paper III). However, the difference between mono-infection and co-infection was in the overall presence of NKT cells. A detectable population of NKT cells was more commonly observed in mono-infected control groups than in HIV/HCV co-infection (Figure 5D, paper III). Interestingly, peg-IFN α and ribavirin treatment had no detectable impact on the relative or absolute NKT cell counts in circulation (Figure 5E and 5F, paper III).

In conclusion, conventional CD56+ NK cell subsets suffer only minor alterations in HIV/HCV co-infected with regard to the parameters we have assessed, although reduced perforin content was detected. CD56- NK cells, on the other hand are largely expanded. These alterations are more severe in HIV/HCV co-infected patients than in HCV mono-infected patients, suggesting that the higher HCV load in co-infected subjects may drive this phenomenon. Furthermore, we observe several potential beneficial effects on NK cells by peg-IFN α and ribavirin treatment. First, there is a relative increase in CD56+ NK cells in peripheral blood which may be important given their potent antiviral activity. Second, there is an increase specifically in the CD56^{bright} NK cell subset which is primarily focused on cytokine production and has therefore been described as immunoregulatory (191). It is possible that such an NK cell subset more focused on cytokine-mediated effector mechanisms may be both more effective

and cause less immunopathogenesis in the liver as compared to perforin-mediated lysis of the infected cells. Third, there is a sharp drop in the CD56- NK cell subset previously described as functionally impaired. Moreover, we conclude that HIV/HCV co-infection is associated with a severe loss of NKT cells that cannot be reversed by treatment with peg-IFN α . Permanent loss of NKT cells may hamper NK cell activation, and may in the setting of HIV/HCV co-infection contribute to increased susceptibility to opportunistic infections, higher HCV viral load and a more rapid disease progression. However, because HCV infection is a hepatic disease and a large fraction of NKT cells reside in the liver, it is important to keep in mind the possibility that reduction of peripheral blood NKT might partially be due to redistribution to organs other than blood.

The results reported in paper III have limited clinical impact, but contribute to an improved understanding of the innate immunity in HIV/HCV co-infection. Clarifying the molecular and immunological interactions between these two chronic viral infections could form the basis for development of new therapies aimed at restoring a functional immune system.

HCV infection drives expansion of CD56- NK cells – a possible marker for HCV treatment outcome?

In paper IV we further studied the subset of CD56- NK cells by including a group of HCV mono-infected patients analyzed retrospectively. As previously mentioned, accumulation of CD56-CD16+ NK cells with an altered functional profile has been observed in viremic HIV-1 infection (84-86). Furthermore, reversion of the expansion of CD56- NK cells upon effective ART has been observed suggesting that high levels of viral replication may be responsible for driving and maintaining increased numbers of those cells (192). However, these cells had not previously been studied in relation to HCV infection.

In both groups of HCV-infected patients, the percentages of CD56-NK cells out of the total lymphocytes were elevated compared with levels in healthy blood donors (Figure 2A, paper IV). Because the viral genotype is clinically important, we investigated possible differences in the CD56 NK cell subset between genotype 1 and genotype 2 and 3 patients. Interestingly, we found no difference in CD56- NK cell percentages between these two patient groups (Figure 2C, paper IV). We further evaluated the functional capacity of NK cells in blood samples from the chronically HCV-infected patients. Responses in CD56-, CD56^{dim}, and CD56^{bright} subsets were assessed by intracellular cytokine flow cytometry (Figure 3A, paper IV). Levels of the inflammatory chemokine MIP-1 β was comparable between the three subsets (Figure 3B, paper IV). However, expression of IFN γ and TNF α was impaired in the CD56- NK cells, and perforin expression was significantly lower in these cells as compared with the CD56^{dim} cells (Figure 3C and 3D, paper IV). The CD56- NK cells also displayed sharply reduced polyfunctionality in their response compared with both CD56^{dim} and CD56^{bright} subsets (Figure 4A). This was most pronounced in the limited ability to express all four functions (MIP-1 β , IFN γ , TNF α , and CD107a) (Figure 4B, paper IV).

MIP-1 β monofunctionality was the only functional profile which was significantly more common in the CD56- NK cells.

We next investigated whether the marked intragroup variability in CD56- NK cell levels among infected patients before initiation of peg-IFN α and ribavirin treatment was associated with the variable treatment outcome observed in HCV infection. When subdividing the patients in two groups, one that obtained SVR, and one that did not, we found that levels of CD56- NK cells were significantly higher in the non-SVR group (Figure 5A, paper IV/Figure 8A, below). Only 29% of HCV mono-infected subjects with high levels of CD56- NK cells achieved SVR, whereas 76% of subjects with low levels achieved SVR. For the co-infected group the numbers were 25% and 100%, respectively (Figure 5B, paper IV/Figure 8B, below). High levels of CD56- NK cells were not directly associated with previously described factors influencing treatment outcome, like HCV viral load, genotype, age or gender (Figure 6, paper IV). However, one should keep in mind that a larger prospective study would be required to ascertain whether CD56- NK cell numbers can be considered an independent predictor of treatment outcome. It would have been of interest to investigate a plausible association between high CD56- NK cell counts and liver fibrosis. Unfortunately, we did not have access to such data but for a minor part of the population studied.

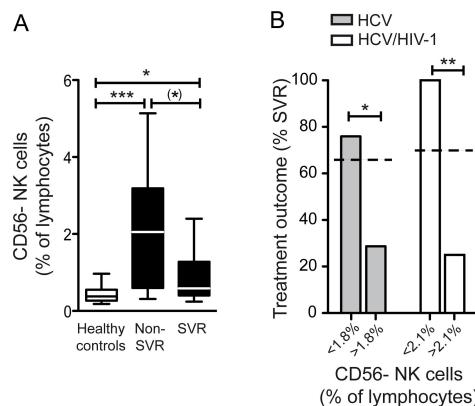


Figure 8. Baseline levels of CD56- NK cells in responders (SVR) and non-responders (non-SVR) to HCV therapy, and in healthy controls (A). Treatment outcome in HCV mono- and HIV/HCV co-infected patients grouped by high and low levels of CD56- NK cells (B). * indicates $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

From an immunological point of view, it is interesting that pre-treatment levels of plasma chemokines can correlate with HCV treatment outcome (168, 175, 176). However, cellular immunological correlates of treatment outcome are less well described. Our data suggests that elevated levels of this aberrant CD56- NK cell subset is an indication of a disturbance in the NK cell compartment, which correlates with the patient's ability to respond to peg-IFN α and ribavirin treatment. Furthermore, the data suggests that CD56- NK cells could have a role as an immunological biomarker for

prediction of the likelihood of response to HCV treatment. We showed that the function of CD56- NK cells in HCV-infected patients was impaired compared with that of the conventional CD56+ NK cells, with a profoundly reduced polyfunctionality in response to K562 target cells. However, they retain the capacity to respond with MIP-1 β production. Interestingly, heterozygosity for the $\Delta 32$ deletion mutant of the MIP-1 β receptor CCR5 was found to be associated with significantly lower hepatic inflammation and milder fibrosis (193, 194). It is thus possible that an abundance of NK cells skewed to produce large amounts of MIP-1 β may exacerbate liver inflammation and fibrosis. We also found that the CD56- NK cells had a severely impaired capacity to produce IFN- γ and had decreased perforin expression, which may inhibit their ability to control the virus.

We speculate that CD56- NK cells are aberrantly differentiated NK cells failing to reach full effector function. Our finding that they lack CD57 expression might argue against the interpretation that they are late-stage differentiated cells that have down-regulated CD56 in response to activation, because at least in CD8 T cells CD57 expression seems to indicate a highly differentiated effector stage (195). On the other hand, the function of CD57 and the role of CD57 as a differentiation marker in NK cells has not yet been fully elucidated.

Independently of the immunological interpretations, the data indicates that pre-treatment measurement of CD56- NK cells could potentially be used as a clinical predictor of peg-IFN α and ribavirin treatment outcome provided that the results can be verified in a larger prospective study. Due to the limited response to therapy and the frequent side effects, prediction of response prior to HCV treatment initiation is important. Though factors like genotype, baseline viral load, and histological staging are well-characterised predictors of response, foreseeing treatment responses in individual patients remain difficult. Use of new predictive markers can allow us to identify patients that are less likely to respond to peg-IFN α and ribavirin treatment. These patients may be in need of higher doses and longer treatments and they are likely to benefit the most from novel combination regimens with specifically targeted antiviral therapy for HCV (STAT-C).

IP-10 correlates with hepatitis C viral response to therapy

In HCV mono-infection, baseline levels of IP-10 are elevated in patients not achieving SVR (168), and in difficult-to-treat HCV genotype 1 patients cut-off levels of 150 and 600 pg/mL have yielded a positive predictive value (PPV) of 71% and a negative predictive value (NPV) of 100%, respectively (177). A few studies have been published that confirms the predictive value of IP-10 in HIV/HCV co-infected patients, and it was reported that pre-treatment IP-10 levels ≥ 400 pg/mL predict non-responsiveness to therapy with peg-IFN α and ribavirin (174, 178). In these studies the number of patients was limited, and the majority of patients were on HAART.

We aimed to further characterize the putative association between IP-10 levels in HIV/HCV co-infected patients and the HCV viral kinetic response to peg-IFN α and ribavirin, as described in paper V. We thus measured plasma IP-10 levels and HCV-

RNA on days 0, 1, 2, 7, 14, 28 and weeks 8, 12, 24, 36, 48 on therapy, and 24 weeks after completion of therapy. The patients were divided into three groups according to baseline IP-10 levels, based on the cut-off levels previously suggested (177). No significant differences in baseline characteristics were detected between the three groups (Table 1, below).

Table 1. Baseline demographics of study subjects grouped by baseline IP-10

	<150 pg/mL (n=4)	150-600 pg/mL (n=9)	>600 pg/mL (n=8)
Age (years)	40 (20-48)	43 (35-48)	46 (38-55)
Gender (Male/Female)	3/1	7/2	6/2
BMI (kg/m ²)	21.1 (17.9-29)	23 (21.4-27.2)	23.6 (21.8-34.6)
CD4 baseline	675 (600-950)	500 (300-1370)	565 (380-1000)
HCV RNA level (log IU/mL)	5.87 (5.77-7.04)	6.26 (4.83-7.29)	6.56 (5.83-7.3)
HCV Genotype (1/2/3)	3/1/0	7/1/1	6/0/2
Ongoing HIV therapy	4	7	5
Normalized ALT (ALT/ULN)	0.69 (0.51-3.21)	1.61 (0.78-4.83)	1.39 (0.64-3.66)
Platelet Count (10 ⁹ /L)	199 (173-225)	212 (113-409)	178 (94-318)
Fibrosis Stage (Ishak 0/1/2/3/4/5/6)	0/1/0/0/0/0/0	0/0/1/2/1/1/1	0/1/1/1/1/1/0
Steatosis Grade (0/1/2/3)	1/0/0/0	5/1/0/0	3/1/1/0

Data presented as n (%) or median (range); ^an=7

The clearance of HCV viremia during treatment was most rapid in patients with baseline IP-10 levels <150 pg/mL, the slowest in patients with baseline IP-10 levels >600, and intermediate for those with baseline IP-10 levels between 150 and 600 pg/mL (Figure 1A, paper V/Figure 9, below). When limiting the analysis to genotype 1, the association between baseline IP-10 and HCV clearance was statistically significant when comparing the patients with IP-10 levels below 150 with each of the other two groups (<150 vs >600, p=0.003; <150 vs 150-600, p=0.03). Interestingly, the association between baseline IP-10 levels in plasma and HCV kinetic response was most pronounced for the first phase decline (day 0-2) in HCV RNA. Thus, low levels of IP-10 were associated with a pronounced viral elimination during the first 48 hours of combination therapy ($r_s=-0.68$, $p=0.004$) (Figure 1B, paper V). A similar association was observed when the analysis was limited to patients with genotype 1 ($r_s=-0.66$, $p=0.01$), and an analogous non-significant trend was observed for patients with genotype 2 and 3. A significant albeit weaker correlation was also observed between baseline IP-10 levels and the second phase decline in HCV RNA between treatment

days 7 to 28 ($r_s=-0.51$, $p=0.03$). Furthermore, HCV-genotype 1-infected patients achieving HCV-RNA <50 IU/mL at week 4 had significantly lower baseline IP-10 levels than those having HCV RNA ≥ 50 IU/mL.

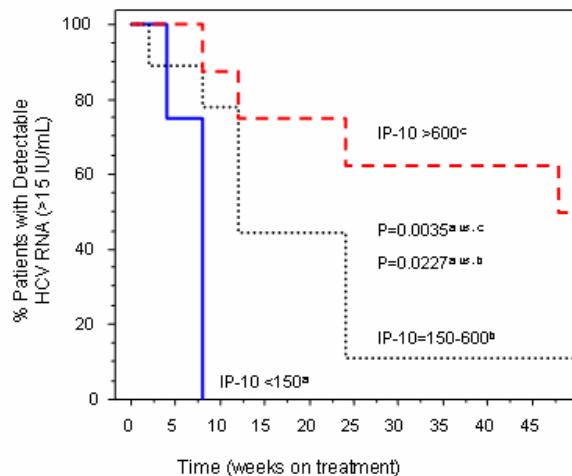


Figure 9. Kaplan-Meier curves of cumulative HCV RNA undetectability (HCV RNA ≤ 15 IU/mL) during HCV treatment, with patients grouped as having baseline IP-10 concentration <150 , 150-600, or >600 .

Plasma IP-10 levels peaked 24 hours after initiation of therapy in all but 3 patients (Figure C, paper V), and then gradually declined. In contrast to the finding of response prediction by baseline IP-10 levels, no association was observed between HCV viral kinetics and the IP-10 kinetics during therapy. Twenty-four weeks after discontinuation of therapy, IP-10 levels were significantly lower in patients achieving SVR as compared with those who did not (Figure 1D, paper V). However, 3 of the 8 patients achieving SVR continued to have IP-10 > 150 pg/mL, suggesting IP-10 is here driven by another mechanism than HCV.

The results of our study confirm the utility of pre-treatment levels of plasma IP-10 for predicting the viral decline during HCV therapy in patients co-infected with HIV and HCV. Unfortunately, our study was limited by the relatively small sample size, due to the factors highlighted in paper I. Thus, though we could demonstrate a significant association between baseline IP-10 and the overall viral response, we note a non-significant trend towards lower baseline IP-10 levels in patients achieving SVR than in those who did not ($p=0.14$).

In our study 4 of 10 patients (2 out of 6 HCV genotype 1 infected) achieved SVR in spite of having baseline IP-10 above the previously suggested predictor of non-response in co-infected, 400 pg/mL. Thus, it may be more appropriate to restrict the clinical utility of IP-10 to identifying and encouraging treatment in patients for whom current HCV therapy is likely to be beneficial and not primarily for excluding patients.

The mechanisms underlying the association between IP-10 and impaired response to HCV combination therapy remain to be elucidated. Interestingly, increased intrahepatic expression of other interferon stimulated genes have been described in non-responders to antiviral treatment (137, 196), which could be explained by increased activation of the endogenous IFN pathway in these individuals. A recent study by Sarasin-Filipowicz affirmed the assumption of a blunted response to IFN in the setting of an endogenously pre-activated IFN pathway (138). The patients with high expression of ISGs thus seem to respond weekly to both endogenous and exogenous IFN.

We thus conclude that IP-10 is an interesting potential predictor of response to peg-IFN α and ribavirin treatment in HIV/HCV infected patients, and that the prognostic value of IP-10, alone or together with other prognostic factors, as well as its potential role for the antiviral actions of interferon warrants further investigation.

MAIN CONCLUSIONS

- HIV/HCV co-infected patients can achieve good response rates to treatment with peg-IFN α and ribavirin.
- Probably, too few HIV/HCV co-infected patients are treated for their hepatitis C virus infection.
- Chronic HCV may rarely be spontaneously cleared in HIV co-infected patients.
- HIV/HCV co-infection induces considerable disturbance in the innate cellular immunity, and this disturbance is partially restored by therapy with peg-IFN α and ribavirin.
- A functionally skewed subset of NK cells that lack CD56 expression is expanded in chronic HCV infection.
- The level of CD56- NK cells at treatment baseline correlates with HCV treatment outcome. Whether this can be a useful immunological predictive marker remains to be verified in future studies.
- Baseline levels of plasma IP-10 correlate with HCV RNA decline during treatment with peg-IFN α and ribavirin in HIV/HCV co-infected patients.

FUTURE PERSPECTIVES

The area of HIV and HCV co-infection is intriguing and presents many challenges for the future. With approximately 10% of HIV-infected persons globally (25-30% in the West) also being HCV infected this co-infection indeed has an important impact on global health. With access to powerful HIV treatment, the co-infected patients are expected to live longer which may lead to a higher incidence of liver failure and an augmented need for liver transplants. Thus, it is of great importance to evaluate all HIV/HCV co-infected patients for HCV treatment and to treat them provided the benefits outweigh the risks.

The epidemiological situation of HIV/HCV co-infection in Sweden is not clear. Having access to unique data bases of HIV-infected (InfCare HIV), and just recently, also of HCV and HBV infected patients (InfCare Hepatit) makes it possible to perform a nationwide epidemiological study of HIV/HCV co-infection. Questions to be answered are the true prevalence of the co-infection, verified by HCV-RNA, and the number of patients that have received HCV treatment so far. Moreover, the prevalence of cirrhosis and HCC, and mortality will be addressed. It would certainly be of interest to look more closely into why the untreated patients have not started HCV therapy. Such a study could help encourage clinicians to treat co-infected patients and thereby avoiding severe complications like ESLD and HCC, and diminishing the need for liver transplants.

In my thesis, I have partially focused on the important, and relatively poorly explored, immune response in HIV/HCV co-infection. It is obvious that the abilities of HIV and HCV to establish persistent infections present unique challenges to the immune system. Gaining insight into the mechanisms that underlie the immunopathogenesis of these viral infections could lead to new therapeutic strategies for infected patients.

In paper IV we found a correlation between the level of CD56- NK cells in peripheral blood and HCV treatment response. This finding needs to be verified in a larger prospective study. Are CD56- NK cells a possible marker for treatment outcome to be used together with already known markers like genotype? The treatment regime for HCV infection is about to change fundamentally, and many new drugs with a direct antiviral effect will be on the market in a near future. When drugs acting directly on the virus are used, we would expect the immune response of the patient to have a smaller impact on the treatment outcome. However, expert panels agree on that there will still be a need for peg-IFN α and ribavirin in the new treatment regimes. Therefore, it would be of interest to study the impact of immune correlates like CD56- NK cells and plasma IP-10 on treatment outcome, also when using specifically targeted antiviral therapy against HCV (STAT-C). In our future studies of the correlation between CD56- NK cells and SVR we plan to use the non-invasive method Fibroscan (197) for assessing the liver fibrosis stage of the patients. It is possible that we will find an association between this cell subset and liver fibrosis.

Another topic of interest is the possible correlation between CD56- NK cells and IP-10. IP-10 targets CXCR3 expressing cells like NK-cells. Do the functionally skewed CD56- NK cells that we have studied express CXCR3, and do these cells in that case respond to IP-10 signalling? Such a question could be addressed by leukocyte migration assays.

Recently, genetic studies of HCV mono-infected patients have reported a strong association to SVR within the gene region encoding interleukin 28B (198). To study this gene polymorphism in co-infected patients would be interesting, and to correlate the result to levels of CD56- NK cells and IP-10. Is the reason for elevated levels of these immune correlates to be found on a genetic level?

Finally, IFN-induced psychiatric problems are common and cumbersome side-effects that sometimes leads to disruption of therapy. Therefore, we prospectively collected data on mental health by validated questionnaires given to the patients in the DICO-S and DICO-D cohorts throughout the treatment period. This data will be evaluated together with a specialist in psychiatry, who will help us understand if the clinical observations, as stated in the patient records, are in congruence with the outcome of the questionnaires. We will investigate the incidence of depression in the cohort and evaluate if these standardized questionnaires might be a useful complement to the clinical observations.

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