Clinical and Epidemiological Aspects of HIV and Hepatitis C Virus Co-infection in KwaZulu-Natal Province of South Africa

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Declaration

This study represents the original work of the author and has not been submitted in any form to another university. The use of work by others has been duly acknowledged in the text.

The research described in the study was carried out at the Department of Virology, University of KwaZulu-Natal, under the supervision of Prof. Umesh Lalloo.

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Raveen Parboosing

Publications and Presentations arising out of this work

PARBOOSING, R., PARUK, I. & LALLOO, U. G. (2008) Hepatitis C virus seropositivity in a South African Cohort of HIV co-infected, ARV naive patients is associated with renal insufficiency and increased mortality. *J Med Virol,* 80**,** 1530-6.

(Parboosing et al., 2008)

Dr R. Parboosing, Prof AN Smith, Prof UG Lalloo. Epidemiology of HCV in HIV infected and uninfected individuals in KwaZulu-Natal, South Africa.

(Oral Presentation), 9 December 2005

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Dedication

To my parents, Mr. Parboosing Bolasingh and Mrs. Savithri Bolasingh, and my wife Dr Nerisha Tathiah

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List of Abbreviations

ACRiA	AIDS Care Research in Africa
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine aminotransferase
ARVs	Antiretrovirals
AZT	azidothymidine (3'-Azido-2',3'dideoxythymidine)
b-DNA	branch-DNA
CD	cluster of differentiation
CDC	Centers for Disease Control and Prevention (of the USA)
CI	Confidence Interval
CTLs	cytotoxic or cytolytic T lymphocytes
DALYs	disability adjusted life years
DOH(SA)	National Department of Health (of South Africa)
DNA	deoxyribonucleic acid
ECLIA	electrochemiluminescence immunoassay
EIA	Enzyme-Immuno Assay
ELISA	Enzyme-Linked Immunosorbent Assay
GIS	Geographic Information Systems
HAART	Highly Active Antiretroviral Therapy
HAV	Hepatitis A Virus
HBSag	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

HIV	Human Immunodeficiency Virus
HIVAN	HIV associated nephropathy
hSOD	human superoxide dismustase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	interleukin
INR	International Normalised Ratio (of Prothrombin time)
IVDU	intravenous drug use
KZN	KwaZulu-Natal (Province of South Africa)
KZN-DOH	KwaZulu-Natal Department of Health
MHC	Major Histo-compatibility Complex
NASH	non-alcoholic steato-hepatitis
NAT	nucleic acid testing
NEQAS	National External Quality Assessment Service (of the UK)
NS3	Non-structural protein 3
NS4	Non-structural protein 4
NS5A	Non-structural protein 5A
OR	Odds Ratio
PCR	polymerase chain reaction
RT-PCR	reverse transcriptase PCR
RIBA	Recombinant Immunoblot Assay
RNA	ribonucleic acid
SOD	superoxide dismutase
SA	South Africa

STI	sexually transmissible infection
TB	tuberculosis
TMA	transcription mediated amplification
TNF	tumour necrosis factor
USA	United States of America
UTR	untranslated region
VTC	Voluntary Testing and Counseling
WHO	World Health Organisation

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Ethics

The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (E141/02).

Abstract

HIV is known to affect the epidemiology, transmission, pathogenesis and natural history of HCV infection whilst studies on the effects of HCV on HIV have shown conflicting results and are confounded by the influence of intravenous drug use and anti-retroviral therapy. This study was conducted in KwaZulu-Natal Province in South Africa where HIV is predominantly a sexually transmitted infection. Intravenous drug use is rare in this region and the study population was naive to anti-retroviral therapy.

For this study, specimens from selected sentinel sites submitted to a central laboratory for routine HIV testing were screened for anti-HCV IgG antibodies. HIV positive HCV-positive patients were compared to HIV-positive HCV-negative patients in a subgroup of patients within this cohort in order to determine if HCV sero-prevalence was associated with clinical outcomes in a linked anonymous retrospective chart survey.

The prevalence of HCV was 6.4% and that of HIV, 40.2%. There was a significantly higher prevalence of HCV among HIV infected patients as compared to HIV negative patients (13.4% vs. 1.73% respectively). HCV-HIV co-infected patients had significantly increased mortality (8.3 vs. 21%). A significant association was found between HCV serostatus and abnormal urea and creatinine levels. Hepatitis B surface antigen seropositivity was not found to be a confounding factor.

This study has found that hepatitis C co-infection is more common in HIV positive individuals and is associated with an increased mortality and renal morbidity.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 HIV

HIV is a cytopathic retrovirus that targets primarily $CD4^+$ lymphocytes. The destruction of $CD4^+$ lymphocytes results in an inexorable decline in immune function, which leads to AIDS, a syndrome characterized by the development of unusual tumours and infections which are rarely seen in immune-competent individuals. The progression and natural history of HIV is intimately linked to the development of these opportunistic infections. Incidental, concomitant and opportunistic co-infections interact with HIV on an epidemiological, clinical, pathogenic, immunological and therapeutic level. In the absence of a cure for HIV, efforts are being directed at controlling and treating opportunistic infections, particularly where effective therapies or vaccines are available. (Braunwald et al., 2001).

These co-infections play a particularly important role in the African continent where HIV and other infections are highly prevalent (Lawn, 2004). In the province of KwaZulu-Natal in South Africa, for example, the National Antenatal HIV and Syphilis Sero-prevalence Survey found that approximately 39.1 % of antenatal attendees were HIV positive in 2005 (Anonymous, 2006). It is in this context that co-infections such as HCV take on an even greater importance.

1.2 HCV

1.2.1 Virology

HCV, a single stranded RNA virus that belongs to the genus *Hepacivirus* within the family *Flaviviridae*, was discovered in 1989 as a cause of Non-A Non-B hepatitis (Larson and Carithers, 2001). Yellow Fever virus, West Nile virus, Dengue virus and Hepatitis G virus are other examples of viruses belonging to the family *Flaviviridae*. The virus consists of a single strand of positive sense RNA of about 9500 bases, which is surrounded by a nucleo-capsid protein and lipid envelope (Lauer and Walker, 2001).

1.2.2 Pathogenesis

HCV primarily targets the hepatocyte, although monocytes and lymphocytes may also be infected (Thomas, 2002). HCV interacts with Major Histocompatibility Complex (MHC) II molecules on antigen presenting cells which results in the activation of Th1 $CD4^+$ T-cells. This leads to the release of cytokines such as TNF-α, TNF-γ and IL-2 which activate macrophages, Natural Killer cells and Kuppfer cells resulting in the phagocytosis and lysis of infected hepatocytes. Infected and uninfected hepatocytes are also targeted and lysed by cytotoxic $CD8^+$ T-cells. Anti-HCV antibodies play a role by facilitating opsonisation. The lysis of hepatocytes results in increased transaminase levels. The destruction of hepatocytes is therefore immune mediated and not due to the direct effects of HCV, which appears to be non-cytopathic. Destruction of all *infected* hepatocytes by these mechanisms results in viral clearance. The inability to clear infected hepatocytes results in chronic infection (Gonzalez and Talal, 2003, Mohsen et al., 2002). This inability to clear the virus

is largely due to the capacity of the virus to escape from the immune system due to its extraordinary diversity. This diversity is generated by an extremely high viral replication rate and the lack of a proof-reading mechanism (Lauer and Walker, 2001). Patients who clear acute hepatitis C infection are not protected from re-infection and multiple bouts of infection have been reported (Fields et al., 2001).

1.2.3 Epidemiology

Epidemiological studies of HCV are challenging since most cases of HCV infection are asymptomatic and indistinguishable clinically from other causes of hepatitis (see 1.2.5.6). A laboratory diagnosis is therefore essential, but not always available, particularly in resource- limited settings. There are consequently few population-based epidemiological studies of HCV. Most studies are based on select high risk (e.g. intravenous drug users) or low risk (e.g. blood donor) populations which either overestimate or underestimate the true prevalence respectively (Ray Kim, 2002).

HCV is endemic worldwide, with an estimated global prevalence of 3%, which represents 170 million people. HCV is four to five times more prevalent than HIV globally (Winnock et al., 2004) and is the commonest chronic blood-borne infection in the United States (Gonzalez and Talal, 2003). Three to four million people are infected by HCV every year (Madhava et al., 2002).

There is great variation in the geographical distribution of HCV, with the highest prevalence in Africa and Asia, and lower prevalence in industrialized countries (Figure 1). Even within developing nations, there is great variation in prevalence, ranging from 0.9% in India and 3.2% in China, to the highest reported prevalence in Egypt of 22%. Some of this variation may be explained by differences in study and reporting methods. Actual differences in HCV prevalence may be due to variation in risk factors in different parts of the world. Injection drug use is the single most important risk factor in developed regions of the world. Unsafe therapeutic injections and blood transfusions are important risk factors in developing nations where these practices occur. Occupational, peri-natal and sexual exposure, and tattooing, body-piercing, acupuncture and scarification are other modes of HCV transmission. The relative contribution of these risk factors to the prevalence of HCV is poorly defined, particularly in developing regions of the world (Ray Kim, 2002, Shepard et al., 2005).

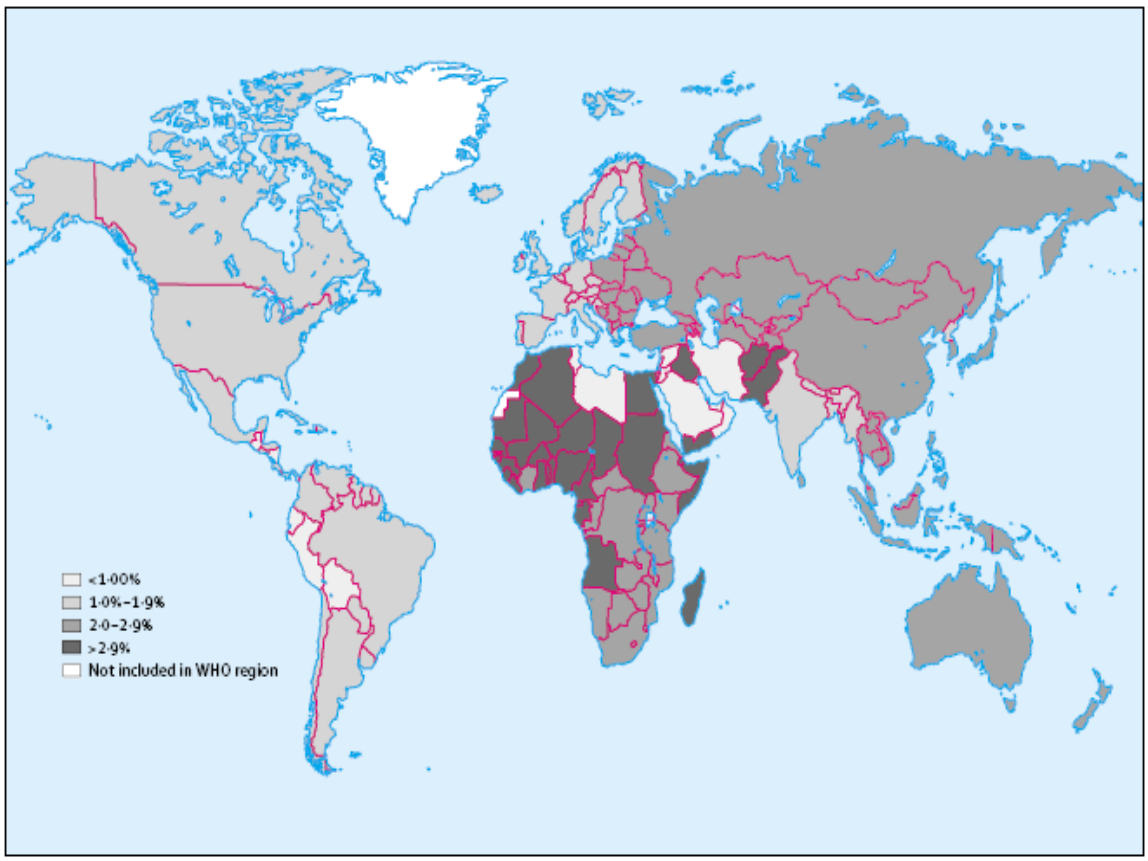


Figure 1 The global prevalence of HCV (Shepard et al., 2005)

Sub-Saharan Africa has the highest prevalence of HCV (5.3%) among the WHO world regions. Within this region there are remarkable differences in prevalence: the central African region has the highest prevalence (6%), and Southern and East Africa, the lowest (1.6%). Cameroon has the highest national prevalence of 12% and South Africa the lowest (0.1%) among blood donors. It is postulated that the HCV epidemic may be at different time points in these regions. Variations in the use of unsterile injections in medical procedures, screening of blood donors and scarification practices may also explain some of these differences. Among countries in Africa, only South Africa and Zimbabwe are known to consistently and routinely screen for HCV in blood donors. The risk of transmission of

HCV through an unsafe injection is estimated to be 6%. Africans are estimated to receive 1.5 unsafe therapeutic injections per year. Studies have demonstrated the association between injections, e.g. hormonal contraceptives, and HCV seropositivity. (Madhava et al., 2002).

The prevalence of HCV may be influenced by the varying prevalence of HIV and other sexually transmissible infections within the continent. HIV is known to increase the mother to child transmission of HCV, and sexually transmitted infections may facilitate the heterosexual transmission of HCV. The magnitude of intravenous drug use and its contribution to the geographical variations in HCV prevalence is unknown (Madhava et al., 2002).

There are several unique factors within the Southern African region which may influence the outcome of prevalence studies: Antibody tests designed for HCV genotypes and subtypes common in Europe and North America may not be as sensitive and specific against viruses circulating in Africa. The immunology of HCV clearance may be different in African patients, who may clear HCV infection more readily and retain antibodies for longer periods. The impact of HCV is exacerbated by the concurrent HIV epidemic within this region. There is a clear need for well designed population based studies of the epidemiology of HCV within this region (Madhava et al., 2002).

There are likewise few representative studies investigating the prevalence of HCV in South Africa. The estimated prevalence in South Africa is 4.3 – 65% (average 23.5%) in high risk cohorts (patients with liver disease, patients receiving multiple blood transfusions or blood products, patients on dialysis and renal transplant patients (Madhava et al., 2002). The estimated prevalence in low-risk cohorts such as blood donors, on the other hand, is as low as 0.1% (Madhava et al., 2002). Seroprevalence in the blood donor population was 0.16, 0.34, 0.75, and 0.22% for whites, Asians, Africans, and Coloureds, respectively, in a study carried out in 1993 (Soni et al., 1993). A survey of blood donors in the Western Cape found a seropositivity of 0.41% (Tucker et al., 1997). HCV antibodies were found in 1.8% of healthcare workers in a large urban referral hospital in South Africa (Vardas et al., 2002). A previous study within KwaZulu-Natal found a low prevalence of HCV of 1.7% and 0.9% among urban and rural blacks, respectively (Abdool Karim and Tait, 1993). The prevalence of HCV among HIV positive patients attending the Chris Hani Baragwanath Hospital in Johannesburg, was also found to be low (1%) (Lodenyo et al., 2000). In contrast, the prevalence of hepatitis C was found to be 11% among patients experiencing early hepato-toxicity in a randomized controlled trial that included nevirapine as part of the antiretroviral regimen (Sanne et al., 2005).

There is a relationship between the prevalence of HCV and the mode of transmission. In regions with low prevalence, such as USA and Europe, most infections are acquired from injection drug use in young adults. In areas with intermediate prevalence, such as Japan and Italy, healthcare related procedures such as transfusions, unsafe injections and acupuncture predominate. In areas with a high prevalence, such as Egypt and Pakistan,

healthcare related procedures are the major source of transmission. Peri-natal transmission, which is enhanced by HIV infection, contributes to a pool of infected individuals who maintain a high prevalence of HCV in these regions (Ray Kim, 2002).

The demographic and socio-economic characteristics of HCV positive individuals are also influenced by the distribution of risk factors. In the United States, for example, high risk drug-taking and sexual behavior and receiving blood donations before 1992 are important risk characteristics. In the USA, HCV prevalence is higher in men than in women and in non-Hispanic blacks than in non-Hispanic whites and Mexican Americans. The peak prevalence is in the 40-49 year age group (Armstrong et al., 2006). The demographic distribution in developing regions is less well studied.

The epidemiology of HCV is affected by HIV and HBV co-infection. HIV, HCV and HBV share common risk factors and routes of transmission. The prevalence of co-infection varies by region and is influenced by the nature of the common risk factor. In HIV-infected individuals with a history of intravenous drug use, for example, the prevalence of co-infection is over 80%. In regions where HIV is predominantly a heterosexually transmitted infection, the prevalence of co-infection is much lower, usually < 7%. There is a paucity of data on the prevalence of HIV-HCV co-infection in developing countries where the administration of unsafe therapeutic injections to HIV positive individuals is postulated to contribute to co-infection. The role of heterosexual transmission remains poorly defined. The prevalence of HBV-HCV co-infection is also uncertain. Most published studies are

based on high risk groups such as intravenous drug users. Co-infection rates in developing countries in particular, are unknown (Shepard et al., 2005).

Assessing mortality and morbidity associated with HCV infection is difficult since most infections remain asymptomatic and complications such as hepatocellular carcinoma, cirrhosis and liver failure occur late and only in a small proportion of those infected. Once they do occur, however, they require intensive utilization of healthcare resources and contribute significantly to the reduction of disability adjusted life years (DALYs). The complications of HCV are similar to those of HBV infection, and available data on the global burden of liver disease do not distinguish between the two infections (Ray Kim, 2002). The potential impact of HCV is more challenging to assess in developing countries where HCV infected individuals die from other causes such as HIV and TB, before the onset of the complications of HCV. More research is clearly needed to assess the impact of HCV on morbidity and mortality in developing countries.

1.2.4 Transmission

There are multiple routes of transmission of HCV. HCV is a blood-borne infection which is transmitted sexually and vertically and by iatrogenic, occupational, cultural and recreational activities. Unsafe transfusions and therapeutic injections and acupuncture are examples of iatrogenic transmission. Intravenous drug use, tattooing, scarification and ear-piercing are examples of recreational and cultural activities that may spread HCV. HCV may also be transmitted by needle-stick injuries (Hughes and Mahy, 1998).

Parenteral person-to-person transmission from a chronically infected individual is postulated to be the commonest mode of spread. Intravenous drug use and blood transfusions have been the most important mechanisms of parenteral transmission in Western countries (Winnock et al., 2004). The predominant route of transmission in developing countries remains to be defined.

Hepatitis C is 10 times more efficiently transmitted than HIV through percutaneous blood exposure such as needlestick injuries, where the risk of transmission is about 30/1000 exposures, and injection drug use, where the incidence of HCV is substantially higher than that of HIV (Sulkowski and Thomas, 2003, Thomas, 2002, Winnock et al., 2004). In contrast, the sexual and vertical transmission of HCV is far less efficient than that of HIV (Thomas, 2002) since mucosal transmission is relatively inefficient. Unlike other flaviviruses, HCV is not thought to be transmitted by insect vectors (Fields et al., 2001).

The risk of transmission of Hepatitis C by blood transfusion and immunoglobulin administration has been drastically reduced by the use of a volunteer donor system, pre-donation risk assessment questionnaire and routine screening of blood donors for antibodies against HCV. This risk has been further reduced by the introduction of NAT testing, which identifies HCV RNA during the antibody window period, and viral inactivation of immunoglobulin products (Fields et al., 2001, Sulkowski and Thomas, 2003, Winnock et al., 2004). In developed countries, transmission by intravenous drug use is more common than transmission by blood transfusions (Winnock et al., 2004). In

countries such as South Africa, where antibody and NAT testing are routinely practiced in the blood transfusion service, transfusion-acquired HCV is unlikely to contribute significantly to the prevalence of HCV (Heintges and Wands, 1997, Madhava et al., 2002).

Perinatal transmission most likely occurs during labor or at the time of delivery. The risk, in the neonate, of chronicity of perinatally transmitted HCV infection is increased by HIV co-infection and is related to maternal HCV viral load. Perinatal infection is unlikely if HCV RNA is undetectable in the maternal circulation. HCV RNA is undetectable in breastmilk; however the risk of HCV transmission by breastfeeding remains undefined (Heintges and Wands, 1997).

The rate of sexual transmission of HCV is low and is much lower than that of Hepatitis B and HIV. The prevalence of HCV in STI clinics is low, and the incidence of HCV in sexual partners of HCV carriers is low. For these reasons, HCV has not usually been considered a sexually transmitted infection (Bouvet, 2005). On the other hand, HCV seropositivity *does* correlate with the number of sexual partners, the presence of sexually transmitted infections and employment in the sex industry (Sulkowski and Thomas, 2003, Fields et al., 2001). These conflicting findings make it difficult to assess the extent of the sexual transmission of HCV infection (Hughes and Mahy, 1998).

The risk of sexual transmission varies geographically and is confounded by factors such as the use of intravenous drugs, shared use of razors and toothbrushes and condom-use. Sexual transmission is also influenced by the number of sexual partners and syphilis and HIV seropositivity. HIV co-infection is thought to enhance the sexual transmission of HCV (Sulkowski and Thomas, 2003, Winnock et al., 2004). The risk in homosexual and heterosexual relationships appears to be similar (Heintges and Wands, 1997, Wejstal, 1999). The relative role of the various routes of transmission is not well defined in developing countries.

1.2.5 Clinical

1.2.5.1 Acute Hepatitis

The average incubation period is 7 weeks, but this may vary widely depending on the inoculum and route of transmission. Prodromal symptoms are rare. Acute hepatitis C infection is icteric in only 20% of patients and other symptoms occur infrequently and are rarely severe. Symptoms are similar to those of other forms of acute hepatitis and include malaise, nausea, right upper quadrant pain, dark urine and jaundice. Symptoms, when they do occur, last for up to 12 weeks. Complications such as fulminant hepatitis are rare. Since most infections are asymptomatic, and the symptoms are non-specific when they do develop, the diagnosis of Hepatitis C is based on laboratory markers rather than clinical symptoms. Biochemical markers such as elevated ALT, occur in 80% of patients with acute hepatitis (Fields et al., 2001, Marcellin, 1999).

1.2.5.2 Chronic Hepatitis

Approximately 85% of patients with acute hepatitis develop chronic hepatitis C infection, where the virus persists for greater than 6 months, and usually, for life. Chronic hepatitis C infection is usually asymptomatic. Two patterns of infection occur, although the presentation varies widely:

Chronic Hepatitis C infection with normal ALT

Approximately 25% patients with chronic hepatitis C infection will have normal ALT levels. These patients are usually asymptomatic. Patients with normal ALT levels have normal liver histology or histology with mild-moderate chronic hepatitis with minimal or absent fibrosis. Cirrhosis is rare in this group (Marcellin, 1999).

Chronic Hepatitis C infection with elevated ALT levels

The remaining 75% of patients with chronic HCV infection have elevated ALT levels. The severity of hepatitis varies widely in these patients; two histological patterns can be distinguished:

Mild Chronic Hepatitis:

These patients have a fibrosis score of ≤ 1 and an activity score of < 6 as described by Knodell (Knodell et al., 1981, Brunt, 2000). Patients with this form of hepatitis complain of mild non-specific symptoms such as fatigue, nausea, anorexia, and weight loss. Overt symptoms of liver disease, such as right upper quadrant pain, dark urine, pale stools, jaundice and pruritis may also occur (Fields et al., 2001). Fatigue is the commonest and

often the only symptom, and may affect the patient's quality of life (Marcellin, 1999, Fields et al., 2001).

Moderate and Severe Chronic Hepatitis

These patients have marked necro-inflammatory lesions and extensive fibrosis corresponding to a fibrosis score of ≥ 3 and an activity score of ≥ 6. This group includes patients with bridging fibrosis and cirrhosis. Neither the severity of symptoms nor the level of ALT is consistent in predicting the severity of hepatitis, and liver biopsy remains the most reliable method of predicting or identifying cirrhosis. Patients, who are older, are immunocompromised or who consume alcohol are more likely to have severe chronic hepatitis (Marcellin, 1999).

1.2.5.3 Cirrhosis

Cirrhosis develops after 2 to 3 decades of Hepatitis C infection. The development of cirrhosis is influenced by several factors including the consumption of alcohol, co-infection with Hepatitis B virus or HIV, and age. Cirrhosis remains silent for many years, until the symptoms of end-stage liver disease appear. These include marked fatigue, muscle wasting, fluid retention, pruritis, jaundice and bleeding tendencies. Symptoms related to portal hypertension (oesophageal varices, splenomegaly, ascites) and liver failure/hepatic encephalopathy (confusion, coma, asterixis, fetor hepaticus, and construction apraxia) represent the late stage of the disease (Braunwald et al., 2001). Patients with chronic liver disease die from portal hypertension, liver failure or

hepatocellular carcinoma, although a proportion die of other causes unrelated to liver disease (Marcellin, 1999).

1.2.5.4 Hepatocellular Carcinoma

Hepatocellular carcinoma is the most serious outcome of cirrhosis in patients with Hepatitis C infection. Chronic infection with Hepatitis C virus results in chronic inflammation, necrosis, regeneration and cirrhosis, which contribute to oncogenesis. Hepatitis C virus is an unusual oncogenic virus in that it does not possess DNA in its genome or its replication cycle, and therefore cannot integrate into the human genome. Its oncogenic potential is thought to arise from the continuous insult on hepatocytes and the resulting regeneration and inflammation. The HCV core protein, NS5A and NS3 proteins are also thought to play a role in oncogenesis (Fields et al., 2001). Hepatocellular carcinoma may remain asymptomatic for many years.

1.2.5.5 Extrahepatic Manifestations

The liver is not the only organ affected by hepatitis C infection. A range of extra-hepatic manifestations are described in chronic hepatitis C infection. There is a strong association between HCV infection and mixed essential cryoglobulinemia, memranoproliferative glomerulonephritis, sicca syndrome and polyarteritis nodosa. Other manifestations such as porphyria cutanea tarda, low grade lymphoma, automimmune thyroiditis, lichen planus, aplastic anaemia, thrombocytopenia, erythema nodosum, diabetes mellitus and neuropathy

are less well documented and further research is needed (Marcellin, 1999). Many of these conditions are thought to arise from an autoimmune mechanism (Fields et al., 2001, Manns and Rambusch, 1999, Marcellin, 1999).

Mixed cryoglobulinemia is the most well described extrahepatic manifestation of Hepatitis C infection. Cryoglobulins consist of immune complexes of HCV and its antibody, rheumatoid factor, immunoglobulins and complement, which usually cause no symptoms, although rarely, arthralgia, Raynaud's disease, vasculitis, glomerulonephritis and purpura result (Fields et al., 2001).

1.2.5.6 Asymptomatic Infection

Most HCV infections are asymptomatic. The prodrome, acute infection and chronic infection are usually clinically silent. The symptoms of acute and chronic HCV infection, when they do occur, are nonspecific and indistinguishable from other causes of hepatitis such as HAV, HBV, alcohol and toxins. Consequently, most HCV infections are diagnosed not by symptoms, but fortuitously by laboratory tests. Many patients therefore remain undiagnosed, untreated and infectious, particularly in resource poor settings where screening for HCV is not readily available. The clinically silent nature of HCV infections necessitates the use of laboratory tests, rather than clinical case definitions, in epidemiological investigations (Marcellin, 1999, Fields et al., 2001).

1.2.6 Natural History

The natural history and disease spectrum of Hepatitis C infection are variable and incompletely defined. The asymptomatic course, slow progression and effects of treatment and multiple cofactors contribute to the complexity of studying the natural history of HCV. The onset of acute infection and transition from acute to chronic infection are most often covert; overt symptoms may occur only in the final stages of infection, and many patients die from other causes before this. The critical events in the natural history of HCV infection are therefore inconspicuous and the natural history difficult to study (Alberti et al., 1999, Seeff, 2002). The natural history of HCV is summarized in Figure 2.

A few patients who are repeatedly exposed to HCV develop a self-limiting sub-clinical HCV infection with no detectable anti-HCV. Most patients who are exposed to HCV develop acute hepatitis C and *detectable* anti-HCV antibodies. Most patients with acute hepatitis C progress to chronicity. Only 10-15% of patients resolve the infection completely, although more recent studies in non-transfusion related HCV infection indicate that the clearance rate may be as high as 50%, particularly with low dose inoculums. It is not known why some patients clear the infection while others fail to do so, but a vigorous T-cell immune response is likely to play a role. Clearance may occur early i.e. less than 6 months post-infection, or late (1-4 years later). Determining the rate of progression to chronicity is difficult because most acute infections are asymptomatic and remain undiagnosed. The progression to chronicity is influenced by the route of infection, dose of the inoculum, immunosuppression, HIV co-infection, and host immunogenetic factors.

Younger, female, non-black immunocompetent patients are less likely to develop chronic infection (Alberti et al., 1999, Hoofnagle, 2002, Thomas et al., 2000).

The chronicity rate of HCV infection is lower in younger patients, which contrasts with HBV infection where infection early in life is likely to become chronic. The chronicity rate of HCV in patients who are less than 20 years of age is estimated to be 30%; the rate in those older than 20 is 76%. The chronicity rate is lower in women, particularly younger women, although this is controversial. Similarly, black patients are less likely to clear HCV infection than Caucasians and Hispanics. Black men in particular have the highest rates of chronicity, approaching 98% (Hoofnagle, 2002, Thomas et al., 2000).

Chronic infection may develop with or without biochemical abnormalities, inflammation, fibrosis or cirrhosis. Viremia may be persistent or intermittent. A patient with chronic hepatitis will rarely clear HCV spontaneously. Spontaneous clearance occurs mainly with end-stage liver disease and hepatocellular carcinoma. Up to 15% of patients develop a carrier state with normal ALTs. Histological progression is rare in those with normal ALTs. A large proportion of patients with chronic HCV infection have a mild form of hepatitis, which remains stable for many years and rarely progresses. Approximately 20% of patients with abnormal ALT levels develop cirrhosis after 20 years of infection. This includes 5-10% of patients who develop end-stage liver disease, 1% who develop hepatocellular carcinoma and 4-8% who die of liver related causes. The risk of decompensation and cancer increases if the patient has cirrhosis: Up to 20% of patients

with cirrhosis decompensate within 5 years, 10 % develop hepatocellular carcinoma and 9% die from liver related causes (Alberti et al., 1999).

Several factors influence the natural history of hepatitis C infection, including the age of the patient when infected, gender, race, alcohol consumption (>50g per day), HBV or HIV co-infection , $CD4^+$ cell count (<200cells/ml), the presence of metabolic liver disease, use of antiviral treatment and host genetics (MHC class II alleles). The influence of viral factors such as HCV viral load, quasi-species diversity and HCV genotype on disease progression is contentious (Alberti et al., 1999, Seeff, 2002, Massard et al., 2006, Thomas et al., 2000).

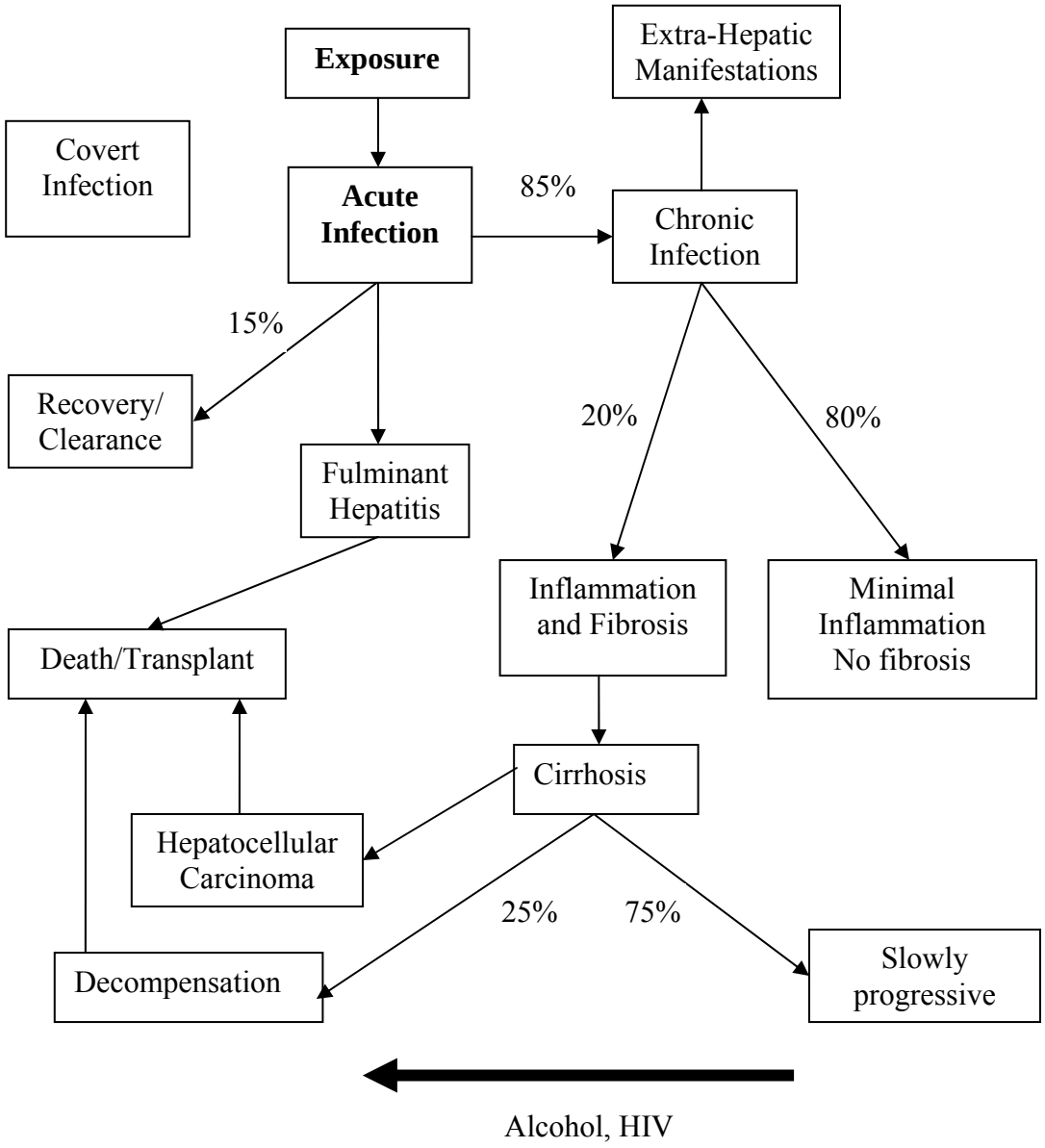
The age at which an individual is initially infected is an important determinant of disease progression. Individuals who are younger (< 40 years) at the time of infection have slower disease progression. It is not known, however, if the rate of disease progression increases with increasing age. Males progress 10 times more rapidly to cirrhosis than females, an effect probably related to the "protective" effects of estrogen. The rate of progression to cirrhosis is lower in African Americans than Caucasians (Seeff, 2002, Massard et al., 2006, Thomas et al., 2000).

Disease progression may also be influenced by the presence of increased hepatic iron stores, non-alcoholic steato-hepatitis (NASH), obesity, type II diabetes and co-infections.

HIV and HBV infection have a profound influence on HCV disease progression due to their immunosuppressive and synergistic pathogenic effects, respectively. Concomitant infection with schistosomiasis is thought to accelerate liver fibrosis by impairing the immune response (Seeff, 2002, Massard et al., 2006). The effect on disease progression of alcohol intake of > 50 g per day is beyond dispute. The effect of lower quantities of alcohol is less clear. The effects of cigarette smoking may influence the development of hepatocellular carcinoma, but this remains controversial. The effect of environmental factors such as diet and toxins remain speculative (Seeff, 2002, Massard et al., 2006, Thomas et al., 2000).

Patients may be classified as slow or non- progressors (cirrhosis > 50 years), intermediate progressors (cirrhosis within 20 – 50 years) and rapid progressors (cirrhosis within 20 years). The subgroup classification correlates with mean ALT and histological activity grade on the first liver biopsy. Patients with normal ALTs have less fibrosis and are less likely to progress to cirrhosis (Alberti et al., 1999, Seeff, 2002).

Figure 2 Spectrum of Natural History of Hepatitis C infection. (Adapted from (Alberti et al., 1999)



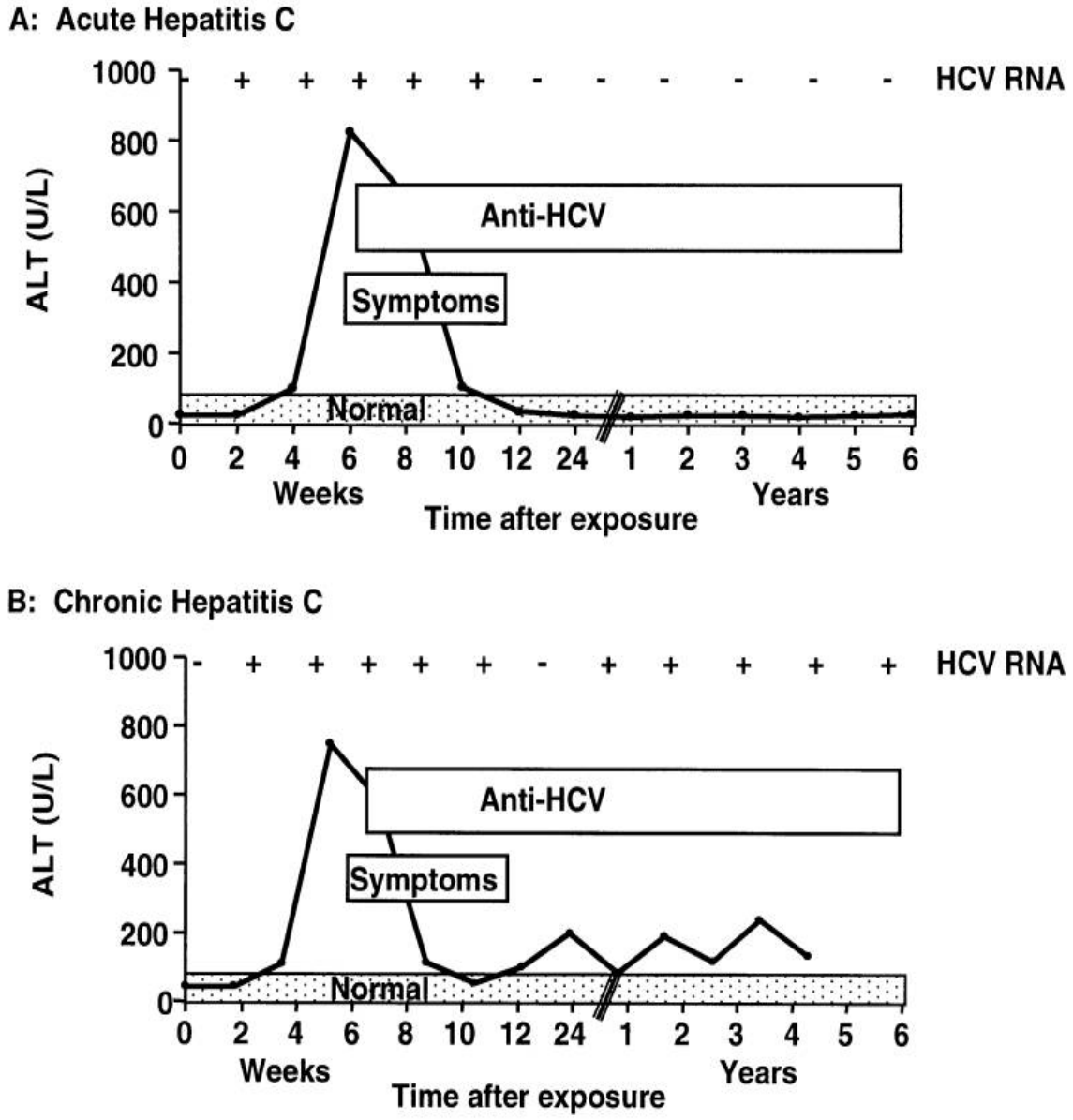
1.2.7 Diagnosis

Serological and molecular assays are available for the diagnosis of hepatitis C infection. A reliable culture system is not available for diagnostic purposes. Handling, transport and storing of specimens is of critical importance to the quality of laboratory results (Erensoy, 2001).

1.2.7.1 Serological Course of Acute Hepatitis

The course of acute resolving hepatitis C infection is illustrated in Figure 3. HCV RNA, detectable in serum 1-2 weeks after infection, rises rapidly and peaks just before the onset of symptoms. Increased ALT levels, which may be up to 10 times the normal, represent hepatic injury and also peak at the time of onset of symptoms. Symptoms, which include jaundice, occur 3-12 weeks after infection in about one third of patients. In acute resolving HCV infection, symptoms disappear, ALT levels return to normal and HCV RNA disappears from the bloodstream. Anti-HCV antibodies appear at the time of onset of symptoms and usually persist for life, except in a proportion of patients who lose their antibodies and therefore have no serological evidence of previous HCV infection (Hoofnagle, 2002).

Figure 3 The course of acute (A) and chronic (B) HCV infection (Fields et al., 2001). Most patients are asymptomatic.



1.2.7.2 Serological Course of Chronic Hepatitis

The early phase of chronic HCV infection is similar to that of the acute infection (Figure 3). HCV RNA persists for at least 6 months in the case of chronic infection. Patients who develop chronic infection are less likely to have symptoms during the acute phase. Anti-HCV antibody levels are higher and more likely to persist with chronic infection. Patients who evolve from acute to chronic infection have a "stuttering" pattern of infection in which HCV RNA levels fluctuate and may temporarily be undetectable, and ALT levels are transiently normal. A normal ALT and negative HCV RNA PCR therefore does not necessarily equate to resolution of infection. HCV RNA levels stabilize once chronic infection has been established and spontaneous resolution is unlikely after 6 months of infection (Hoofnagle, 2002).

1.2.7.3 Serology

Testing for antibodies to HCV is the initial laboratory screening method for diagnosing past and current HCV infection. Enzyme immunoassays (EIAs) are commercially available in high-through-put formats, and play a key role in diagnostic laboratories and blood-banks. Three generations of enzyme immunoassays have been developed to detect antibodies against epitopes of the polyprotein encoded by the hepatitis C genome. *First generation EIAs* were developed to detect antibodies against the c100-3 nonstructural protein (NS4). This assay had low specificity and sensitivity (~80%) and yielded a high number of false positives, especially when used in low risk populations e.g. blood donors. *Second generation EIAs* incorporated the c33c non-structural (NS3) and c22-3 core

structural proteins which significantly enhanced the sensitivity and specificity of the assay. The second generation assays are significantly more sensitive than the first, and detect 10-20% more infections and reduces the window period by up to 90 days. *Third generation EIAs* added the NS5 antigen, and reconfigured the core and NS3 antigens, which further improved the sensitivity and specificity of the assay (Richter, 2002, Erensoy, 2001).

1.2.7.4 RIBA

A supplemental or confirmatory strip immuno-assay, the *R*ecombinant *I*mmuno*b*lot *A*ssay or RIBA was developed to distinguish true and false positive EIA results by identifying antibodies to *individual* antigens in the EIA. The RIBA is an analytical serological assay and is analogous to the Western Blot for HIV. The RIBA contains the EIA antigens, human superoxide dismustase (hSOD) and an IgG control band. hSOD, which is used to fuse the recombinant proteins in third generation EIAs, is included in the RIBA strip to detect non-specific antibodies. A RIBA is considered positive if there are reactions against at least two antigens on the strip, with intensity greater than the IgG control, and no reactivity against hSOD. Reactions against only one antigen, or reactions against more than one antigen *and* the hSOD are considered indeterminate. The use of synthetic antigens in third generation RIBA has greatly reduced the number of indeterminate RIBAs (Richter, 2002, Erensoy, 2001).

1.2.7.5 Limitations of Serological Methods

Serological assays are not reliable in diagnosing acute infection since antibodies are detectable in only 50% of cases at this time. Antibodies are more consistently measured 3 months after the infection i.e. beyond the serological "window period". Serological assays cannot distinguish between acute and chronic infection. IgM, for example, cannot be used as a marker of acute infection since it is detectable in up to 50% of chronic infections as well. Patients who are immunocompromised, undergoing dialysis, or who have cryoglobulinemia, may yield false negative results on EIA. In these cases, sensitive molecular detection techniques are essential for diagnosis (Richter, 2002). False positives may also occur due to non-specific binding of serum immunoglobulins to contaminants in the antigen preparations or to coating or blocking reagents (Erensoy, 2001). Confirmatory tests such as RIBA are essential in such cases.

The RIBA is more specific, but less sensitive than EIAs and may therefore yield indeterminate or even negative results. This may occur, for example, during early sero-conversion, where antibody levels are yet to rise to detectable levels, or in organ transplant patients, where antibody production is blunted. RIBA is particularly ineffective in resolving weakly positive EIAs. In this case, a more sensitive molecular method, such as PCR, is required. Alternatively, the serological assay may be repeated at a later date, when antibody titres have increased (Erensoy, 2001, Richter, 2002).

Confirmation of the presence of anti-HCV antibodies does not imply active disease or recovery (Erensoy, 2001). Patients may remain HCV seropositive for life even after the infection has cleared. On the other hand, patients with active disease may be anti-HCV antibody positive.

1.2.7.6 Antigen detection

A commercially available ELISA kit is available which detects HCV core antigen. The assay employs an immune-complex dissociation step which vastly improves its sensitivity, but the lower level of detection is still higher than that of molecular methods (Richter, 2002). The assay has not been validated for routine clinical use (Erensoy, 2001).

1.2.7.7 Molecular Diagnosis

HCV RNA can be detected as soon as one week post-infection and is regarded as the gold standard in defining active HCV infection. A single negative RNA test, however, does not exclude the possibility of active infection, since RNA levels fluctuate and may transiently drop to below-detectable limits. HCV RNA may be detected using commercially available kits or in-house "home-brew" methods. A target amplification method such as reverse transcriptase PCR (RT-PCR) or transcription mediated amplification (TMA) or a signal amplification method such as branch-DNA (b-DNA) may be used (Erensoy, 2001, Richter, 2002).

RT-PCR uses primers corresponding to the highly conserved 5' untranslated region (5' UTR) and a HCV-specific oligonucleotide probe. Primer design is of particular significance because of the genetic heterogeneity of the HCV genome. A commercially available semi-automated RT-PCR kit is available (Roche AMPLICOR®, Roche Diagnostics, Branchburg, NJ, USA). TMA employs the enzymatic activities of a T7 RNA polymerase promoter, reverse transcriptase and RNase H in an isothermal reaction that results in detectable copies of template. The bDNA method uses solid phase oligonucleotide capture probes, secondary branch probes and an enzyme linked tertiary probes, which results in an amplified chemiluminescent signal, which is proportional to the amount of input RNA (Richter, 2002, Erensoy, 2001).

Carry-over amplicon contamination and PCR inhibitors in the sample may cause false positives and negatives, respectively, and are a major drawback of PCR. Sample collection, transport and storage affect the yield of RNA and hence the sensitivity of PCR (Damen et al., 1998, Halfon et al., 1996, Jose et al., 2003, Wang et al., 1992). Full automation, availability of quality control panels and standardized commercial kits, and advances such as real time PCR and microchip technology have nevertheless placed molecular techniques in the forefront of HCV diagnosis (Erensoy, 2001).

1.2.8 Treatment

Pegylated interferon α and ribavirin have antiviral and immunomodulatory effects and are currently the treatment of choice for HCV infection. HCV, unlike HIV, does not integrate

into the host genome, and eradication by antiviral therapy *is* possible. However, a virological response i.e. undetectable HCV RNA 6 months following therapy, is achieved in only 56% of patients. Liver transplantation is a consideration in decompensated liver disease due to chronic HCV infection.

1.3 Similarities and Differences between HIV and HCV

Like HIV, HCV is a blood-borne enveloped RNA virus characterised by high viral replication, remarkable viral diversity, an acute infection that is frequently undiagnosed, the ability to escape the immune system, persistent viraemia, long periods of clinical latency, multiple routes of transmission and lack of an effective cure or vaccine.

HIV maintains its persistence by integrating its genome into host DNA. HCV on the other hand is sustained by ongoing replication. HCV can be cleared and become undetectable in the bloodstream. In contrast, HIV never resolves and remains in the bloodstream for the life of the individual (Sulkowski and Thomas, 2003). HIV and HCV share common risk factors and routes of transmission (Gonzalez and Talal, 2003).

1.4 Co-infection (Figure 4)

HCV is a common infection in HIV infected patients and represents a complex public health and clinical challenge, complicated by immune suppression, drug interactions and

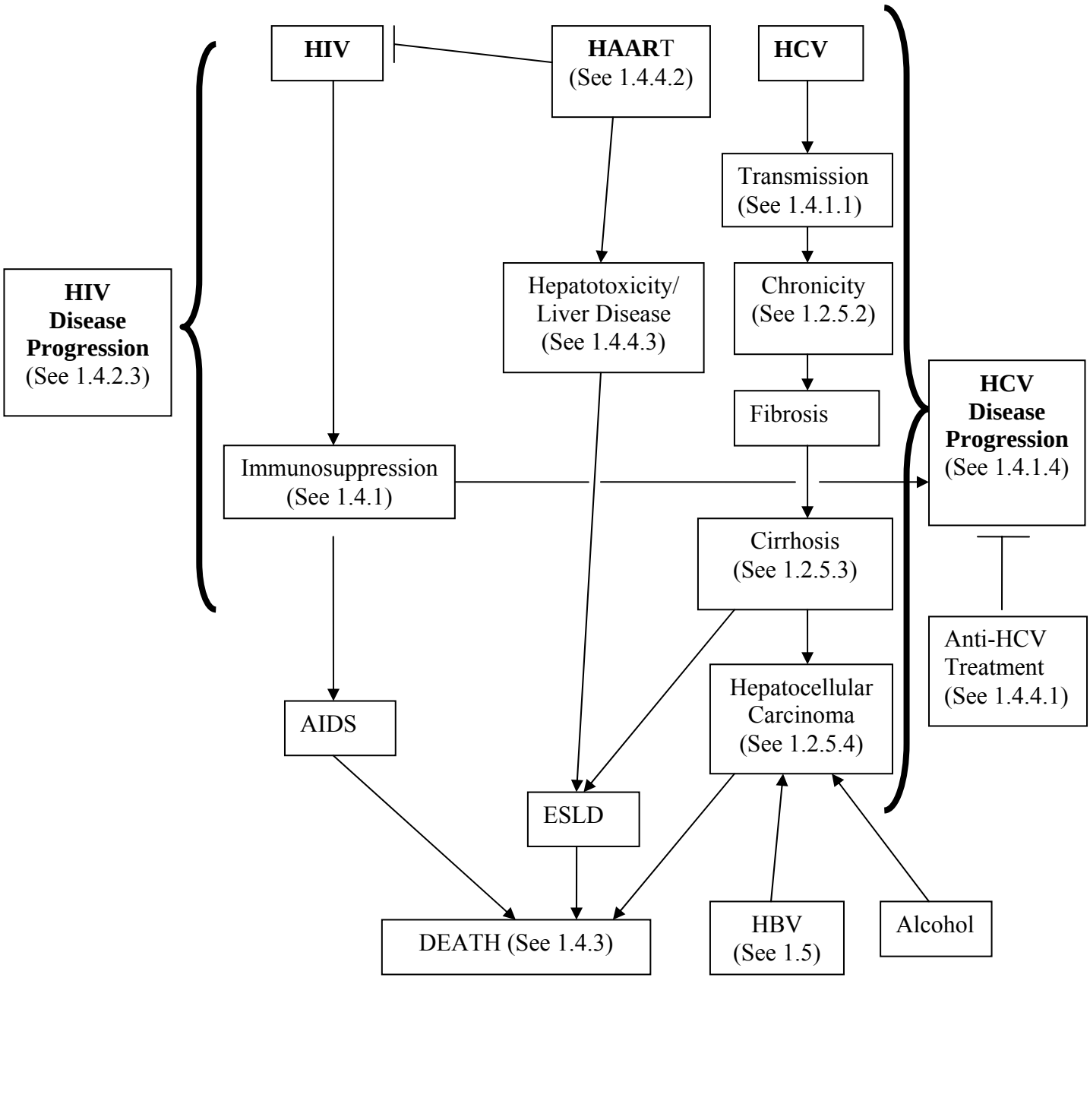
toxicities and paucity of data (Sulkowski and Thomas, 2003, Thomas, 2002). The long term effects of HCV-HIV co-infection are only now becoming more apparent due to the improved survival of HIV positive patients with the use of Highly Active Antiretroviral Therapy (HAART) (Winnock et al., 2004). Co-infection may have an impact on the epidemiology, transmission, pathogenesis, clinical features, natural history and management of both HCV and HIV infection. Liver disease due to HCV infection is now one of the leading causes of morbidity and mortality in HIV-infected patients in the United States (Falusi et al., 2003, Fuster and Clotet, 2003, Khalili and Behm, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Thomas, 2002). In developed countries, approximately 50% of mortality in HIV infected patients is due to HBV-, HCV- or HAART-related liver disease (Sherman, 2004).

HCV and HIV have common routes of transmission. The prevalence of co-infection depends on the common risk factor and route of transmission (Sherman, 2004, Sulkowski and Thomas, 2003, Thomas, 2002, Winnock et al., 2004). Data from several studies in developed countries indicate that in HIV patients who have a history of intravenous drug use, 50% - 95% are co-infected by HCV. Similarly, 4% - 15% of HIV positive homosexuals, 14.3% of heterosexuals and 60 – 90 % of hemophiliacs are co-infected (Bonacini and Puoti, 2000, Dieterich, 2004, Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Thomas, 2002). The overall prevalence of HCV infection in HIV infected individuals in the United States is approximately 25-30% (Khalili and Behm,

2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Thomas, 2002). Data on the epidemiology of co-infection in developing countries is lacking.

The complex interaction between HIV and HCV is shown in Figure 4 and discussed in 1.4.1, 1.4.2, 1.4.3 and 1.4.4.

Figure 4 Complex interaction between HCV and HIV



1.4.1 Effect of HIV on HCV

1.4.1.1 Transmission

It is postulated that horizontal and vertical transmission of HCV is enhanced in co-infected patients due to higher HCV viral loads (Sulkowski and Thomas, 2003). HIV infection increases the peri-natal transmission of HCV two to three fold (Mohsen et al., 2002, Sherman, 2004, Thomas, 2002, Winnock et al., 2004). Peri-natal transmission is enhanced with low $CD4^+$ cell count, high maternal HCV viral load, vaginal delivery and infant HIV infection, and decreased by the use of HAART and delivery by caesarean section (Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003). The effect of HIV on sexual transmission of HCV remains controversial. Studies on sexual transmission are potentially confounded by other risk factors such as intravenous drug use. The prevalence of anti-HCV antibodies in partners of HCV carriers ranges from 2.6 to 7.1%, which is increased in co-infected individuals to 9.1%. HIV is therefore thought to enhance the sexual transmission of HCV (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Dieterich, 2004, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Winnock et al., 2004), although this has been disputed (Thomas, 2002). Further studies are needed to clarify the role of HIV on HCV transmission, particularly in Sub-Saharan Africa, where the prevalence of both infections is among the highest in the world (Anonymous, 2006, Madhava et al., 2002).

1.4.1.2 Pathogenesis

HIV may have a direct cytopathic effect on hepatocytes or induce apoptosis by an "innocent bystander" effect. HIV may facilitate HCV infection of extra-hepatic cells. These mechanisms may exacerbate liver damage in HCV infection and lead to higher HCV viral loads (Rockstroh and Spengler, 2004).

Co-infected patients have higher serum and liver HCV RNA levels than patients who are infected with HCV alone (Sherman, 2004, Thomas, 2002). HCV RNA levels increase by approximately 0.5 to 1 log following HIV sero-conversion (Bonacini and Puoti, 2000, Dieterich, 2004, Khalili and Behm, 2002, Rockstroh and Spengler, 2004) and coinfected patients have on average a ten-fold higher HCV viral load than mono-infected patients (Winnock et al., 2004). This may reflect increased viral replication due to immuno-suppression. This is suggested by the observation that HCV viral load is inversely related to $CD4^+$ cell counts, although this effect has not been consistently demonstrated (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Maier and Wu, 2002, Sherman, 2004, Winnock et al., 2004, Khalili and Behm, 2002). It has alternatively been postulated that HIV facilitates HCV infection of extrahepatic cells, leading to increased viral production and higher HCV viral loads (Rockstroh and Spengler, 2004). Increased HCV levels may have an impact on response to anti-HCV therapy (Sherman, 2004).

Spontaneous clearance of acute HCV infection occurs only in 5 to 10% of co-infected patients compared to 15 to 30% of HCV mono-infected patients, possibly due to impaired

cellular immune response to HCV due to HIV infection (Sulkowski and Thomas, 2003, Thomas, 2002). Co-infected patients, especially those with low $CD4^+$ cell counts, are therefore less able to spontaneously clear HCV infection and more likely to develop chronic infection, (Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Sherman, 2004, Thomas, 2002, Winnock et al., 2004), although this has been disputed(Dieterich, 2004).

Clearance and containment of HCV infection is mediated by cytotoxic $CD8^+$ T lymphocytes (CTLs). CTLs are supported by cytokines released by $CD4^+$ T cells. The decrease in $CD4^+$ T cells in HIV infection leads to diminished support for CTLs and hence failure to clear acute HCV infection, or to control chronic HCV infection (Rockstroh and Spengler, 2004). The use of HAART restores $CD4^+$ and hence $CD8^+$ T-cell functions. The subsequent CTL-mediated destruction of hepatocytes leads to a flare in hepatitis. Immune restoration, however, rarely leads to clearance of HCV or reduction of HCV replication (Rockstroh and Spengler, 2004).

1.4.1.3 Clinical and Histological features

Patients with $CD4^+$ cell counts of less than 0.4 $x10^9$ cells/l have less piecemeal necrosis and portal inflammation, which is in keeping with the immune-mediated basis of HCV induced liver disease. Some studies have found no difference in the rate of fibrosis and cirrhosis between HCV mono-infected and co-infected patients, whereas others have found a difference. The conflicting findings may relate to the different $CD4^+$ cell counts in the cohorts (Bonacini and Puoti, 2000).

Some of the extrahepatic manifestations of HCV infection such as aphthous ulceration, immune thrombocytopenic purpura and sialoadenitis occur in HIV infection as well (Gonzalez and Talal, 2003).

1.4.1.4 Disease Progression

Studying the natural history in co-infected patients is difficult because of the indolent course of HCV infection and the potential confounding effects of alcohol consumption, antiretroviral therapy and Hepatitis B infection (Bonacini and Puoti, 2000, Graham et al., 2001). Disease progression is potentially influenced by race, gender, genotype of HCV and risk factors in the population being studied (Graham et al., 2001). HIV, nevertheless, *does* appear to affect every stage of the natural history of HCV infection (Winnock et al., 2004, Thomas, 2002, Graham et al., 2001), resulting in higher viral loads, increased activity on liver histology, more rapid fibrosis and cirrhosis and an increased risk of liver failure and hepatocellular carcinoma (Sulkowski and Thomas, 2003, Thomas, 2002, Graham et al., 2001). Patients with co-infection are therefore more likely to die from advanced liver disease than mono-infected patients (Rockstroh and Spengler, 2004).

This acceleration in the course of HCV infection, due to HIV-induced immunosuppression, occurs particularly when there is an AIDS defining condition or if the $CD4^+$ cell count drops to below 100 cells/μl (Khalili and Behm, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Graham et al., 2001). Lower $CD4^+$ cell counts are associated with increased fibrosis, cirrhosis and hepatic decompensation. It is postulated that a decline in

$CD4^+$ cell counts leads to a shift in cytokine response from a Th1 to a Th2 pattern. Th2 cytokine production has been shown to be linked to increased fibrogenesis in mice (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003). The increased mortality and morbidity associated with liver disease in HIV-infected patients has nevertheless been noted irrespective of $CD4^+$ cell counts (Monga et al., 2001).

The synergistic effect of HIV-HCV co-infection results in more rapid progression of fibrosis and cirrhosis and an increased likelihood of hepatic failure and death (Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Sherman, 2004, Graham et al., 2001). The rate of fibrosis is reported to be increased at least two-fold in co-infected patients particularly when the $CD4^+$ cell count is below 200 cells/mm^3 (Thomas, 2002). Co-infected patients develop cirrhosis on average 7 years after infection, as compared to 23 - 30 years in HCV mono-infected patients (Maier and Wu, 2002, Winnock et al., 2004). The relative risk of developing cirrhosis or decompensated liver disease in co-infected patients is approximately 2.9 (Mohsen et al., 2002, Graham et al., 2001) which is comparable to the effect of alcohol on the progression of chronic liver disease (Graham et al., 2001).

HIV infection is a risk factor for the development of hepatocellular carcinoma in HCV-infected patients (Gonzalez and Talal, 2003). Co-infected patients develop hepatocellular carcinoma earlier than HCV mono-infected patients (Mohsen et al., 2002, Rockstroh and Spengler, 2004).

HCV-related liver disease is a leading cause of death in HIV/AIDS clinics in North America (Dieterich, 2004) and an important cause of hospitalization in HIV infected patients (Dieterich, 2004, Gonzalez and Talal, 2003, Maier and Wu, 2002, Mohsen et al., 2002, Sulkowski and Thomas, 2003). Between 12 to 45 % of deaths in HIV infected patients are due to liver disease (Rockstroh and Spengler, 2004).The effect of co-infection on mortality, however, remains controversial and reports are inconsistent with regard to the effect of HCV infection on survival in the HIV positive patient (Khalili and Behm, 2002).

Further studies are therefore essential to determine the effect of co-infection on survival, particularly in Sub-Saharan Africa, where the prevalence of both viruses has reached alarming proportions (Anonymous, 2006, Madhava et al., 2002).

1.4.1.5 Diagnosis and Monitoring

HIV positive patients, particularly those with low $CD4^+$ cell counts, may have falsely negative HCV ELISAs (i.e. detectable HCV RNA and negative ELISA) (Sherman, 2004, Sulkowski and Thomas, 2003). HCV ELISAs can result in 4 -6 % false negatives in HIV co-infected patients (Sherman, 2004, Thomas, 2002), due to impaired antibody production (Maier and Wu, 2002, Mohsen et al., 2002, Thomas, 2002) or loss of antibodies due to immunosuppression in advanced HIV infection (Rockstroh and Spengler, 2004). Antibodies may become detectable following HAART, presumably due to immune restoration (Bonacini and Puoti, 2000, Thomas, 2002). HIV infection can also lead to false positive ELISA results due to hypergammaglobulinaemia associated with

immunosuppression (Khalili and Behm, 2002, Sherman, 2004). There may also be an increased number of indeterminate RIBA in the co-infected patient (Khalili and Behm, 2002, Thomas, 2002), due to the differential loss of anti-HCV antibodies following immunosuppression (Sherman, 2004). Other studies, however, have shown no difference in the sensitivity of HCV ELISAs in HIV positive and negative patients (Gonzalez and Talal, 2003).

1.4.2 Effect of HCV on HIV

1.4.2.1 Transmission

HIV vertical transmission may be increased if the mother is HCV co-infected (Bonacini and Puoti, 2000, Khalili and Behm, 2002, Mohsen et al., 2002, Winnock et al., 2004). The effect of HCV on the sexual transmission of HIV remains controversial (Sherman, 2004).

1.4.2.2 Pathogenesis

The mechanisms of direct viral interactions between HIV and HCV remain an area of unexplored research. Although the primary site of HCV infection is the liver, HCV is also known to infect lymphocytes and monocytes. HCV may lead to immune mediated activation of $CD4^+$ T cells and thereby enhance HIV replication (Sulkowski and Thomas, 2003).

1.4.2.3 Disease Progression

The effect of HCV co-infection on the prognosis and progression of HIV infection remains controversial (Rockstroh and Spengler, 2004, Sherman, 2004, Sulkowski and Thomas, 2003, Monga et al., 2001). Some studies have indicated no difference (Staples et al., 1999) while others have shown accelerated progression to an AIDS defining condition and death in co-infected patients (Bonacini and Puoti, 2000, Dieterich, 2004, Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002). The Swiss Cohort study, for example, showed increased risk of progression to AIDS and death and diminished recovery of $CD4^+$ cells following HAART in co-infected patients (Greub et al., 2000). HCV therefore seems to blunt immune recovery (Sulkowski and Thomas, 2003, Thomas, 2002). However, this effect is true only in short term follow-up. On extended follow-up, there seems to be no difference in mortality between HIV mono-infected and HIV-HCV co-infected patients, particularly if the patients are on HAART (Rockstroh, 2006, Rockstroh et al., 2005).

Initiation of HAART is often deferred or delayed in co-infection due to the complexities of managing co-infected patients (Sulkowski and Thomas, 2003, Thomas, 2002, Rockstroh and Spengler, 2004). Differences in the level of compliance to HAART (Sherman, 2004) and other confounding factors, such as intravenous drug use (Thomas, 2002) may also explain the difference in studies comparing outcomes in mono-infected and co-infected patients. Studies carried out on ARV-naïve patients in areas where intravenous drug use is uncommon, are therefore essential.

1.4.3 Survival and Mortality in Co-infected Patients

Co-infected patients have been shown to have reduced survival and/or increased HIV disease progression in some studies (Anderson et al., 2004, Braitstein et al., 2005, Greub et al., 2000, Rosenthal et al., 2003, Monga et al., 2001) but not in others (Sulkowski et al., 2002, Cacoub et al., 2001, Rancinan et al., 2002, Staples et al., 1999, Tedaldi et al., 2003, Bonacini et al., 2004). Several factors may explain the differences in study outcomes. Differences in study design, cohort selection and length of follow-up may be important factors (Anderson et al., 2004, Greub et al., 2000, Rancinan et al., 2002, Staples et al., 1999). Differing levels of adherence to HAART, the use of intravenous drugs, hepatotoxic medication and alcohol and co-infections such as hepatitis B may also be important confounders (Braitstein et al., 2005, Greub et al., 2000, Rosenthal et al., 2003, Salmon-Ceron et al., 2005, Staples et al., 1999, Tedaldi et al., 2003, Bica et al., 2001, Monga et al., 2001).

Increased mortality may be due to AIDS-related causes (Braitstein et al., 2005), HAART-associated hepatotoxicity and immune-reconstitution (Anderson et al., 2004, Rosenthal et al., 2003, Graham et al., 2001), limited initiation or tolerance of HAART (Braitstein et al., 2005) or liver-related causes such as cirrhosis, liver failure, end-stage liver disease and hepatocellular carcinoma (Cacoub et al., 2001, Greub et al., 2000, Rosenthal et al., 2003, Monga et al., 2001). The increased mortality may also be due to the association of HCV infection with intravenous drug use (Greub et al., 2000).

It is postulated that hepatotoxicity associated with HAART has contributed to the increase in liver-related mortality. HCV infection is a risk factor for HAART-related hepatotoxicity (Bica et al., 2001) and conversely, HAART exacerbates HCV-related liver disease (Rosenthal et al., 2003). Many studies in the pre-HAART era have therefore not demonstrated increased mortality. On the other hand, immune restoration due to effective HAART has been shown to reduce liver-related mortality in HIV-HCV co-infection (Sherman, 2004) and to lessen the difference in survival between mono and co-infected patients (Tedaldi et al., 2003, Bonacini et al., 2004). It has therefore been suggested that the control of HIV using HAART, and maintenance of $CD4^+$cell counts above 200 x 10^6 cells/l is the first priority in co-infected patients (Bonacini et al., 2004). Co-infected patients nevertheless do not always benefit fully from the improved survival associated with HAART since they are less likely to initiate and/or tolerate antiretroviral medication (Anderson et al., 2004, Braitstein et al., 2005, Rancinan et al., 2002). Furthermore, HCV infection in itself blunts HAART-associated $CD4^+$ cell recovery, possibly due to the direct pathogenic effect of HCV on lymphocytes (Greub et al., 2000, Rossi et al., 2002) although not all studies have shown this effect (Anderson et al., 2004, Sulkowski et al., 2002).

End-stage liver disease, mainly due to HCV infection, is an important cause of (non-AIDS) deaths in HIV-infected patients in developed countries (Rosenthal et al., 2003, Salmon-Ceron et al., 2005, Bonacini et al., 2004, Bica et al., 2001, Dieterich, 2004, Graham et al., 2001). Up to 45% of deaths in HIV-infected patients are due to liver disease (Rockstroh and Spengler, 2004), which may manifest as cirrhosis, ascites, variceal bleeding or encephalopathy (Monga et al., 2001). HIV infection accelerates the course of HCV

infection resulting in an increase in the risk of end-stage liver disease and death (Sulkowski and Thomas, 2003, Thomas, 2002, Rockstroh and Spengler, 2004, Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Sherman, 2004, Mohsen et al., 2002, Monga et al., 2001). This partly explains the high number of liver-related deaths in co-infected patients.

Results from studies on the effect of co-infection on survival have been inconsistent (Khalili and Behm, 2002), due to differences in study design (Anderson et al., 2004, Greub et al., 2000, Rancinan et al., 2002, Staples et al., 1999) and the effect of multiple confounding factors such as antiretroviral therapy (see 1.4.4.5) and intravenous drug use (Braitstein et al., 2005, Greub et al., 2000, Rosenthal et al., 2003, Salmon-Ceron et al., 2005, Staples et al., 1999, Tedaldi et al., 2003, Bica et al., 2001). Further research is therefore critical.

1.4.4 Management of co-infected patients

1.4.4.1 Anti-HCV Treatment

The response rate to anti-HCV therapy is lower in HIV co-infected patients (Rockstroh and Spengler, 2004, Sherman, 2004). It is hypothesized that the higher HCV viral loads and lower response rates may necessitate longer duration and/or higher doses of interferon α and ribavirin in co-infected patients (Fuster and Clotet, 2003, Gonzalez and Talal, 2003). The incidence of adverse events necessitating withdrawal of anti-HCV therapy is also higher in co-infected patients (Rockstroh and Spengler, 2004, Sherman, 2004).

There are also concerns about the safety and tolerability of interferon/ribavirin therapy in HIV co-infected patients, particularly those on HAART. Ribavirin at high doses may have a lymphotoxic effect and may lead to a decrease in absolute $CD4^+$cell counts (Rockstroh and Spengler, 2004).

Interferon α may also lead to a drop in white cell and absolute $CD4^+$ cell counts due to its myelosuppressive effects (Thomas, 2002). Up to 80% of patients treated with interferon experience a drop in $CD4^+$ cell counts (Maier and Wu, 2002). A drop in the $CD4^+$ cell count may in turn increase the risk of developing opportunistic infections (Mohsen et al., 2002), although other authors have disputed this on the basis that the $CD4^+$cell *percentage* remains unaltered despite the drop in the absolute $CD4^+$ cell count (Sulkowski and Thomas, 2003, Thomas, 2002).

Cytopenias are an important concern in the treatment of co-infected patients. Neutropenia and anaemia due to interferon therapy are of particular concern in the immunocompromised patient (Sherman, 2004). Co-infected patients treated with ribavirin and AZT are also more prone to anemia (Maier and Wu, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Thomas, 2002).

The incidence of side effects of anti-HCV therapy such as influenza-like symptoms, haematological abnormalities and neuropsychiatric disorders is higher in co-infected

patients. This necessitates dose reductions or treatment discontinuation (Rockstroh and Spengler, 2004).

1.4.4.2 Anti-HIV Treatment

$CD4^+$ cell recovery following HAART may be impaired in co-infected patients (Dieterich, 2004, Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Greub et al., 2000). Conversely, others have not demonstrated this effect, and have shown that response to HAART and risk of disease progression and death is not influenced by HCV co-infection (Sulkowski et al., 2002, Rancinan et al., 2002).

Initiation of HAART may lead to transient increases in HCV viral load and liver enzymes (Dieterich, 2004, Mohsen et al., 2002, Winnock et al., 2004). In patients with low $CD4^+$ cell counts, the increased HCV RNA levels may persist for up to 48 months following initiation of HAART. This paradoxical increase in HCV viral loads may reflect increased replication in an extra-hepatic reservoir or decreased immune-mediated clearance of HCV (Sherman, 2004). On the other hand, immune reconstitution following 12 months of HAART is associated with decreases in HCV viral load (Rockstroh and Spengler, 2004), and even spontaneous clearance (Winnock et al., 2004).

HAART seems to modify the natural history of HCV infection. Patients on protease inhibitors, for example, have decreased intrahepatic viral loads (Winnock et al., 2004),

lower rates of fibrosis (Fuster and Clotet, 2003, Khalili and Behm, 2002, Mohsen et al., 2002, Thomas, 2002), improved outcome of HCV disease and decreased liver related mortality (Rockstroh and Spengler, 2004). These benefits of HAART may outweigh the associated risk of hepatotoxicity.

1.4.4.3 Hepatotoxicity

HCV infection is a risk factor for HAART induced hepatotoxicity (Maier and Wu, 2002, Mohsen et al., 2002, Sherman, 2004, Sulkowski and Thomas, 2003, Thomas, 2002). HAART-related hepatotoxicity is 3 to 4 times more common in HCV infected patients (Fuster and Clotet, 2003). This may lead to discontinuation of ARVs (Dieterich, 2004). Nevirapine and ritonavir, in particular, are associated with an increased risk of hepatotoxicity, and didanosine and stavudine with hepatic steatosis, in co-infected patients. Hepatic steatosis is associated with an increased risk for fibrosis and decreased response to interferon therapy (Rockstroh and Spengler, 2004). Co-infected patients who are on HAART therefore require closer monitoring of liver function particularly in the first few months after initiation of therapy (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Sulkowski and Thomas, 2003, Mohsen et al., 2002).

The mechanisms of hepatotoxicity in co-infected patients include altered drug metabolism, HCV-specific immune reconstitution and mitochondrial dysfunction (Sulkowski and Thomas, 2003). HAART decreases HIV viral load and restores $CD4^+$cell counts. This is

postulated to enhance the intra-hepatic immune response against HCV-infected hepatocytes, resulting in necroinflammation, hepatic decompensation and liver failure.

Co-infected patients are less likely to be prescribed HAART due to hepatic complications, poorer compliance or substance abuse (Falusi et al., 2003, Gonzalez and Talal, 2003).

1.4.4.4 Drug Interactions

Therapy of the co-infected patients is complex and challenging due to drug-drug interactions and additive toxicities. Ribavirin may inhibit mitochondrial DNA polymerase. There are therefore concerns about adverse effects of ribavirin, including the risk of lactic acidosis, in the context of mitochondrial toxicity associated with antiretroviral therapy (Bonacini and Puoti, 2000, Khalili and Behm, 2002, Mohsen et al., 2002). The concurrent use of didanosine and ribavirin, for example, is contraindicated due to the increased risk of mitochondrial toxicity and associated pancreatitis, liver failure and lactic acidosis (Rockstroh and Spengler, 2004, Sherman, 2004). Furthermore, ribavirin, a nucleoside analogue, increases the intracellular availability of phosphate, thereby facilitating the conversion of didanosine to its active triphosphorylated form. This leads to increased drug levels and exacerbation of toxicities (Sherman, 2004, Thomas, 2002). Conversely, ribavirin decreases the efficacy of zidovudine and stavudine by interfering with their intracellular phosphorylation (Bonacini and Puoti, 2000, Khalili and Behm, 2002, Maier and Wu, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Thomas, 2002, Gonzalez

and Talal, 2003). The adverse effects of anti-HCV therapy may impact on the adherence to HAART (Bonacini and Puoti, 2000).

1.4.4.5 Antiretroviral therapy as a confounding factor in co-infection studies

HAART significantly reduces mortality and morbidity in patients with HIV (Thomas, 2002, Mohsen et al., 2002, Khalili and Behm, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Bica et al., 2001, Martinez-Sierra et al., 2003, Klein et al., 2003, Law et al., 2004). HCV-HIV co-infected patients, however, may not derive benefits from HAART (Klein et al., 2003) because they are less likely to receive or initiate HAART (due to concerns regarding hepato-toxicity (Fuster and Clotet, 2003, Anderson et al., 2004, Dorrucci et al., 2004, Rockstroh et al., 2005, Rossi et al., 2002, Sulkowski et al., 2002) or the use of intravenous drugs (Sulkowski et al., 2002)). Furthermore, tolerance to ARVs may be reduced because HAART-associated liver injury and toxicity is worsened by HCV infection (Braitstein et al., 2005, Khalili and Behm, 2002). This leads to decreased adherence (Falusi et al., 2003) or earlier discontinuation of HAART in HCV positive patients (Rancinan et al., 2002, Bica et al., 2001, Klein et al., 2003). Co-infected patients may also have poorer compliance to HAART, specifically if they abuse intravenous drugs (Dorrucci et al., 2004, Klein et al., 2003). Antiretroviral therapy therefore confounds outcomes analysis because HAART affects survival and outcome (Dorrucci et al., 2004), but its use is different in HCV negative and positive patients.

Furthermore, HAART affects HCV-HIV co-infected and HIV mono-infected patients differently. HAART causes a transient increase in HCV viral load (Bonacini and Puoti, 2000, Dieterich, 2004, Sherman, 2004, Staples et al., 1999), increases serum transaminase levels (Sherman, 2004, Staples et al., 1999, Graham et al., 2001), exacerbates hepatic necro-inflammation (Bonacini and Puoti, 2000, Mohsen et al., 2002, Rosenthal et al., 2003) accelerates the progression of liver disease (Rosenthal et al., 2003) and precipitates liver failure (Bonacini and Puoti, 2000) and hepatic decompensation (Graham et al., 2001) in co-infected patients. HCV infection is a risk factor for HAART-related hepatotoxicity (Fuster and Clotet, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Sherman, 2004, Sulkowski and Thomas, 2003, Winnock et al., 2004, Bica et al., 2001, Rosenthal et al., 2003, Law et al., 2004) and lipo-atrophy (Mohsen et al., 2002). Indinavir can cause severe hyperbilirubinaemia (Maier and Wu, 2002, Mohsen et al., 2002), while the risk of hepatic steatosis in patients using the "d-nucleosides" (didanosine and stavudine) (Rockstroh and Spengler, 2004) is increased in co-infected patients. On the other hand, protease inhibitors may have a protective effect in HCV-related liver disease by decreasing fibrosis rates (Rosenthal et al., 2003, Fuster and Clotet, 2003, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Graham et al., 2001, Marine-Barjoan et al., 2004, Martinez-Sierra et al., 2003).

Anti-retroviral therapy is therefore a confounding factor in HIV-HCV co-infection studies (Braitstein et al., 2005, Rosenthal et al., 2003, Salmon-Ceron et al., 2005, Staples et al., 1999, Tedaldi et al., 2003, Monga et al., 2001, Rockstroh, 2006, Rockstroh et al., 2005,

Lincoln et al., 2003) because its use and effects are different in HIV mono-infected and HIV-HCV co-infected patients.

1.4.4.6 Recommendations

HIV infected individuals should be screened for anti-HCV antibodies and Hepatitis B surface antigen, using EIAs, when clinically indicated (Gonzalez and Talal, 2003, Mohsen et al., 2002, Sulkowski and Thomas, 2003). HCV RNA PCR should be used to confirm HCV infection (Thomas, 2002). Co-infected patients should have their liver function (aminotransferases, albumin and bilirubin) and prothrombin time/INR and platelet counts assessed. A liver biopsy and HCV RNA levels should also be performed in patients being considered for treatment (Thomas, 2002).

Co-infected patients should be immunized against hepatitis A and B and be strongly advised to completely abstain from alcohol. (Bonacini and Puoti, 2000, Mohsen et al., 2002, Sulkowski and Thomas, 2003, Thomas, 2002)

HAART is highly recommended in co-infected patients because it lowers fibrosis rates (Fuster and Clotet, 2003). Concerns over hepatotoxicity should not be a reason to withhold HAART in co-infected patients (Thomas, 2002).

Pegylated Interferon-α and ribavirin in combination lowers the risk of hepatocellular carcinoma and liver failure and may lead to viral clearance. This combination is considered the optimal form of therapy and should therefore be considered in co-infected patients (Sulkowski and Thomas, 2003).

1.5 HCV and Hepatitis B Virus (HBV)

The clinical features of acute and chronic hepatitis B infection may be indistinguishable from those of Hepatitis C infection (Fields et al., 2001). HCV and HBV may also have similar extra-hepatic manifestations (Fields et al., 2001, Han, 2004). Both HBV and HCV are associated with increased liver mortality in HIV-infected patients (Bonacini et al., 2004, Law et al., 2004). Patients who have infection with both HBV and HCV have a poorer prognosis than those infected with either one of the viruses alone (Brook, 2006, Gaeta et al., 2006). HBV infection may therefore be a powerful confounding factor in studies investigating HCV clinical outcomes and interactions, particularly in regions where HIV, HBV and HCV are endemic.

Chronic Hepatitis B virus infection is endemic in Sub-Saharan Africa. The hepatitis B surface antigen carrier rate in South Africa averages 9%, but varies widely between 1 – 17% in various cohorts (Firnhaber et al., 2008, Sanne et al., 2005, Hoffmann et al., 2007, Kotzee et al., 2006, Kew, 2008, Burnett et al., 2005). The prevalence of hepatitis B infection in HIV infected individuals may furthermore be increased by up to 2 fold in Sub-Saharan Africa and by up to 10-fold in certain risk groups (Alter, 2006, Burnett et al.,

2005). There may also be an epidemiological association between HBV and HCV infection (Lincoln et al., 2003).

AIMS AND OBJECTIVES

AIM

To elucidate the clinical and epidemiological interactions between HCV and HIV

OBJECTIVES

1. To determine the prevalence of HCV in this study population
2. To determine the difference, if any, in the prevalence of HCV in HIV positive and negative subjects
3. To determine the clinical and laboratory correlates of HCV–HIV co-infection

CHAPTER TWO: MATERIALS AND METHODS

2.1 Study Design and Rationale

A representative sampling frame was required in order to achieve the first objective of the study, which was to determine the prevalence of HCV in this region. The study was therefore conducted in a centralised virology laboratory which received the majority of specimens for viral assays in the region. The majority of the 198 hospitals and 428 clinics in KwaZulu-Natal in 2003 were public health facilities. Only 10 - 11% of the population had medical aid coverage and the rest relied primarily on state health-care services (Lehohla, 2006). Approximately 90% of the population was therefore served by the Provincial Laboratory Services, which included the Virology laboratory in which this study was based. The population served by the laboratory was therefore a representative sampling frame of the population of KwaZulu-Natal.

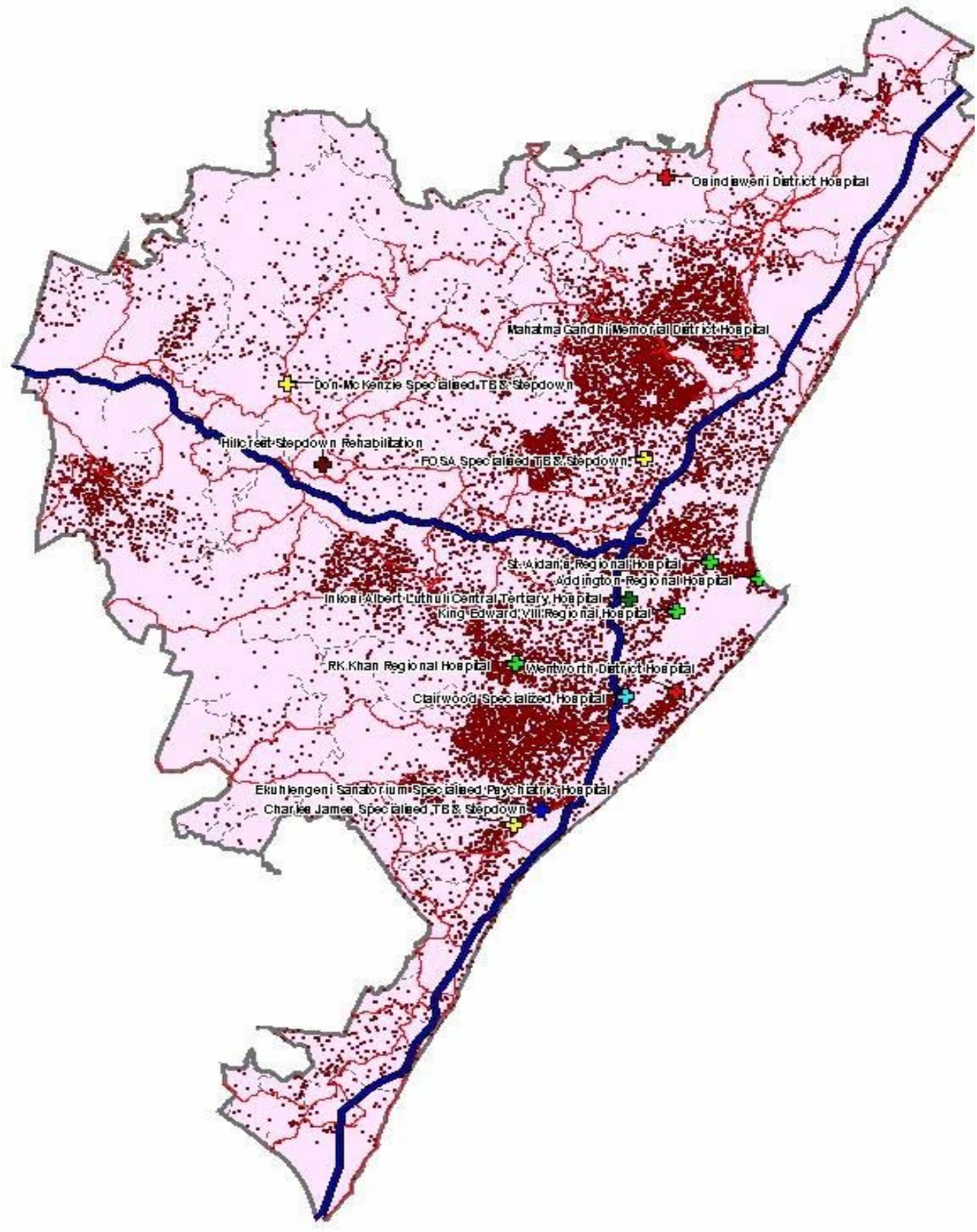
It was also essential to ensure that the sentinel sites selected were representative of this sampling frame. Of the 9.4 million people in the Province of KwaZulu-Natal, aproximately 46% live in urban areas, of which the majority (over 3 000 000) reside in the Ethekwini District, in which our sentinel sites are located (Lehohla, 2006). The selected sentinel sites are referral hospitals within the region and receive a representative spectrum of patients from all regions of the province. The samples were from inpatients and outpatients and included medical, surgical, obstetric and gynaecological patients. The sentinel sites include

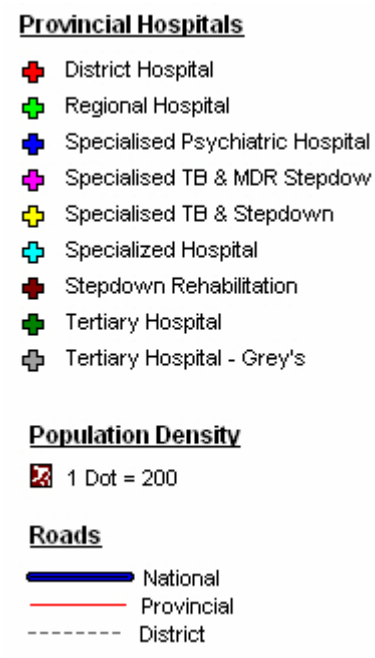
a flagship tertiary and quaternary hospital and several regional and district hospitals (Table 1 and Figure 5).

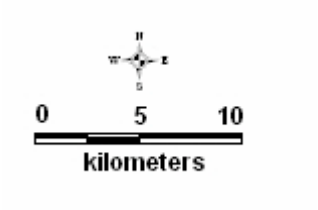
Table 1 Description of the Sentinel Sites

Hospital	Description
Inkosi Albert Luthuli Central	A flagship tertiary and quaternary hospital providing patient care to all persons in KwaZulu-Natal and part of the Eastern Cape. **(http://www.ialch.co.za)**
King Edward	King Edward VIII Hospital is the second largest hospital in South Africa providing a tertiary service for the entire province of KZN and parts of Mpumalanga and Eastern Cape provinces. **(http://www.kznhealth.gov.za/kingedwardhospital.htm)**
Mahatma Gandhi Memorial	MGMH District Hospital serves the population of Phoenix, Inanda, Amaoti, Mt Edgecombe and Duffs Road and also provides Regional services in Internal Medicine, Obstetrics and Gynaecology, Neonatology and Paediatrics to the population of Kwa-Mashu, Tongaat, Verulam and Ndwedwe. **(http://www.kznhealth.gov.za/mahatma/history.htm)**
Addington	Addington is a district and regional hospital, situated on South Beach, Durban. There are 16 clinics in Addington's catchment area. **http://www.kznhealth.gov.za/addingtonhospital.htm**
RK Khan	RK Khan is a regional and district hospital located in Chatsworth, a suburb in the eThekwini health district. RK Khan hospital serves the population of Chatsworth and surrounding areas. It is also a referral hospital for St Mary's hospital and KwaDabeka clinic and functions as a Regional Hospital. (**http://www.kznhealth.gov.za/rkkhanhospital.htm**)

Figure 5 Sentinel Sites and Catchment Population of KwaZulu-Natal







(Provided by Geographic Information Systems (GIS), KwaZulu-Natal Department of Health, Pietermaritzburg: http://healthmap.kznhealth.gov.za)

In order to achieve the second and third objectives of this study, which entailed studying the effects of HIV-HCV co-infection, it was necessary to select a population with a high prevalence of HIV, but one in which confounding factors such as the use of anti-retroviral drugs and intravenous drug abuse were absent. There were several reasons which made this a unique population in which to achieve the study objectives.

1. The prevalence of HIV in KwaZulu-Natal is among the highest in the world. In 2005, 39.1% of women attending antenatal clinics in the public sector were HIV positive (Anonymous, 2006) .
2. HIV is predominantly a sexually transmitted infection in this region (Buve et al., 2002, Simon et al., 2006) and the use of intravenous drugs is rare (Parry et al., 2005).
3. The study population was naïve to antiretroviral therapy. During the study period, <1% of the number of HIV infected patients in the province were on antiretroviral treatment in the public sector (ASSA, 2005). Moreover, the patients in this study could not have been on antiretroviral therapy since patients already on antiretroviral therapy would not have required an HIV test and would therefore not have fulfilled the inclusion and exclusion criteria for this study (see 2.7).

In order to achieve the aims of this study it was necessary to conduct a HCV sero-prevalence survey to obtain prevalence and demographic data. It was also necessary to carry out a retrospective cross-sectional chart review to compare the effect of HCV on

outcomes in HIV positive patients. It was deemed necessary to combine these strategies (by using a subset of patients from the sero-prevalence survey in the cross-sectional chart review) because this was a unique time point in the evolving HIV epidemic:

1. With time large numbers of patients would be recruited into the Antiretroviral Rollout programme and commence antiretroviral therapy (DOH(SA), 2000, DOH(SA), 2003). As a result, the study of HIV-HCV co-infection in *ARV-naïve* patients would become more difficult and less representative
2. With time, the Voluntary Testing and Counselling (VTC) programme would result in the widespread availability of "rapid" point-of care HIV tests (DOH(SA), 2000, Ramkissoon et al., 2004). Subsequently, the number of samples referred to the central virology service for formal HIV ELISAs would diminish and the sampling frame would no longer be representative.

2.2 Overview of the Study Design

This study was a HCV sero-prevalence survey conducted on all samples submitted for routine HIV testing from selected sentinel sites to a central laboratory. A subset of patients in the sero-prevalence survey was entered into a retrospective cross-sectional chart review in order to compare the effect of HCV on outcomes in HIV positive patients.

2.2.1 Seroprevalence Survey

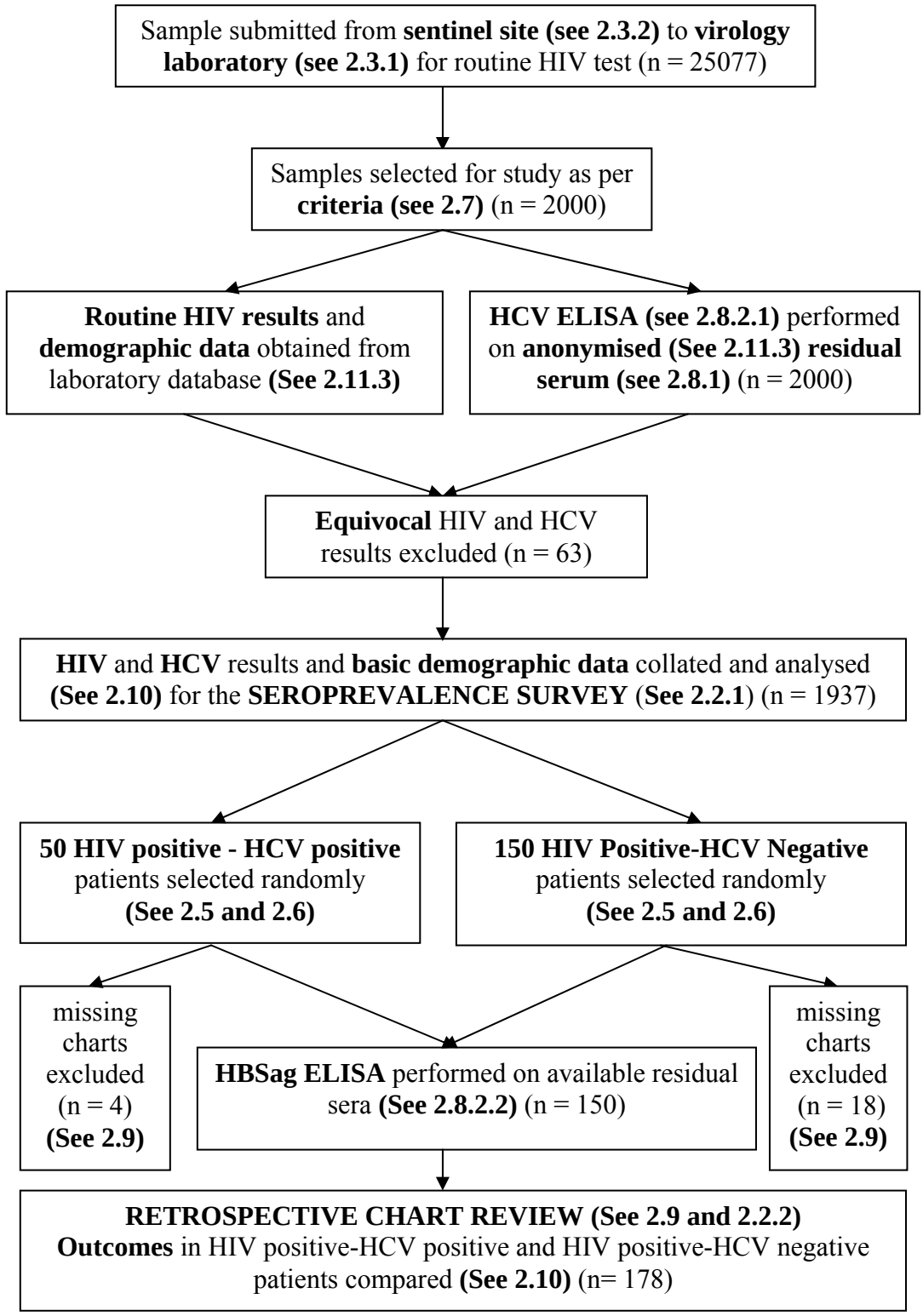
All specimens from subjects aged 18 years and older, submitted for routine HIV testing, from the selected sentinel sites to the Department of Virology Laboratory at Inkosi Albert Luthuli Central Hospital, during the 18 month period ended June 2005 were tested, additionally, for anti-HCV antibodies. Samples were selected sequentially. Repeat, unsuitable or inadequate samples were excluded. HIV and HCV sero-status and basic demographic data (age and gender) were collated on 1937 sequential samples.

2.2.2 Retrospective Chart Review

Morbidity and mortality data was collected on a randomly selected subset of patients within the sero-prevalence survey. 50 HIV-HCV co-infected patients and 150 HIV-positive, HCV-negative patients were selected for this purpose. Hepatitis B Virus (HBV) surface antigen ELISA was performed in this subset of patients.

An overview of the study methodology is shown in Figure 6

Figure 6 Flow Chart Summarising Study Methodology



2.3 Study Location

2.3.1 The Laboratory

This laboratory-based study was conducted in the Department of Virology at Inkosi Albert Luthuli Central Hospital, in Durban. This laboratory is the reference virology laboratory in the province of KwaZulu- Natal, South Africa. Specimens for HIV ELISA testing from patients presenting to public health facilities in the province are submitted primarily to this laboratory.

2.3.2 The Sentinel Sites

The sentinel sites are referral hospitals within the region which receive a representative spectrum of patients from all regions of the province (Table 1 and Figure 5). The samples were from inpatients and outpatients and included medical, surgical, obstetric and gynaecological patients. *All* patients from the sentinel sites who required an HIV ELISA would have had a sample submitted to the Department of Virology Laboratory at Inkosi Albert Luthuli Central Hospital.

2.4 Study Period

The study was conducted during the 18 month period from January 2004 to June 2005. The study period was determined by the time taken to achieve the required sample number (see 2.6).

2.5 Sampling Strategy

Five sentinel sites were selected based on the human resources available to obtain and record good clinical notes. *All* specimens from the selected sentinel sites, submitted to the Virology Laboratory for routine HIV testing, were entered into the sero-prevalence study. Samples were selected sequentially. All samples fulfilling the inclusion criteria were selected. Only samples with exclusion criteria were excluded (See 2.7 for exclusion and inclusion criteria).

After completion of sample collection, a subset of the patients from the sero-prevalence survey was entered into a retrospective chart review. This subset included 50 HIV-HCV co-infected patients and 150 HIV positive-HCV negative patients randomly selected from the 1937 patients used in the sero-prevalence survey. Randomisation was performed on all 1937 study numbers, using random numbers generated without replacement by STATA™10 (StataCorp, College Station, Texas, USA).

2.6 Statistical Plan and Sample Size Estimation

The sample size required to estimate the prevalence of HCV was calculated according to a standard formula (Kirkwood et al., 2003):

$n > [\pi(1- \pi)]/e^2$

where n = required sample number,

π = proportion,

and e = required size of standard error

Estimates of the prevalence of HCV in this region vary from 1 to 6 % (Abdool Karim and Tait, 1993, Lodenyo et al., 2000, Madhava et al., 2002, Soni et al., 1993). A sample size of ≥ 1521 is required if the prevalence is 1% with a 95% confidence interval of 0.5 to 1.5 %, assuming a power of 90%.

If the prevalence is 6%, a sample size of 2166 yields a 95% confidence interval of 5 to 7 % (power = 90%). Therefore, a sample size of 2166 was adequate for the most likely estimates.

The sample size required to detect a difference in HCV prevalence between HIV positive and negative groups was calculated according to a standard formula (Kirkwood et al., 2003):

Sample size per group =

$$[u[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]^{1/2} + v[\pi'(1-\pi')]^{1/2}]^2[\pi_2 - \pi_1]^{-2}$$

where

π_1 and π_2 are the assumed proportions (0.01 and 0.02 respectively) and π' their mean.

u = 1.28 and v = 1.96, corresponding to a power of 90% and significance level 5%, respectively.

Assuming a minimum HCV prevalence of 1.5% and assuming a difference of 1% between groups to be meaningful at the 5% significance level, the minimum sample size per group is 2095. A difference between the groups, of 1%, was selected, based on the observation that HCV prevalence varies by at least 1% between HIV positive and negative groups in numerous studies (Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Sherman, 2004, Sulkowski and Thomas, 2003, Thomas, 2002, Winnock et al., 2004, Soriano et al., 1999, Armstrong et al., 2006, Shepard et al., 2005). Assuming that 45% of HIV results are positive would require a minimum total sample size of 4656. A sample size of approximately 5000 seemed to be the most appropriate based on the lowest anticipated estimates of the HCV and HIV prevalence. Interim, 6-monthly analyses were planned to assess the accuracy of the initial assumed estimates.

An accurate sample size could not be determined for the morbidity and mortality analysis because the data required for the assumptions of the sample size calculation were lacking and the number of variables that were to be analysed was large. This component of the study was regarded as *hypothesis-generating* rather than *hypothesis-testing*. The sample size was limited by practical considerations which included the following:

- The personnel at the medical registries of the respective hospitals had limited time and resources to find and retrieve patients' charts
- It was time consuming and laborious to extract the required information from patients' charts.
- The charts were made available to the investigators for a limited time period, after which they had to be returned to the medical registry

Limiting the sample size to 200 charts allowed the study to be completed within the required time-frame with the available resources. A randomly selected subset of 50 HIV-HCV positive patients and 150 HIV positive-HCV negatives patients were entered into the retrospective chart survey. A 3:1 ratio of HCV positive to negative patients was opted for in order to increase the statistical confidence of the study (Barker et al., 1997).

At the 18 month interim analysis, 2000 samples had been collected and the prevalence of HCV and HIV was found to be 6.4% and 40% respectively. The number of samples collected at this point was therefore adequate for the purposes of the prevalence estimates (Objective 1). Furthermore, the actual prevalence of HCV exceeded the minimum prevalence required to detect a difference between HIV positive and negative subjects, according to our initial sample size calculations (Objective 2). Since the primary objectives of our study were already achieved with the number of samples collected at this point, we could not justify further sample collection. Sample collection was therefore stopped and the chart review and analysis was completed (Objective 3).

2.7 Exclusion and Inclusion Criteria

Inclusion Criteria

1. All specimens from the selected sentinel sites (Inkosi Albert Luthuli Central Hospital, Mahatma Gandhi Memorial Hospital, RK Khan Hospital, King Edward VIII Hospital and Addington Hospital) submitted to the Virology Laboratory for routine HIV testing
2. Subjects aged 18 years and older

Exclusion Criteria:

1. samples submitted for a repeat (confirmatory) HIV test on the same patient within the defined study period, or samples repeated from the same patient for any other reason
2. samples of inadequate volume
3. Samples unsuitable for ELISA testing as determined by the manufacturers guidelines e.g. haemolysed samples
4. those samples that required residual serum to be stored for medico-legal or other reasons as per routine laboratory standard operating procedures e.g. samples taken from patients following sexual assault and rape or needle-stick injuries
5. Patients who have been, or are, on antiretroviral therapy

2.8 Laboratory Methods

2.8.1 Specimen Collection and Processing

Residual serum from specimens that had been subjected to an HIV test, and which would have otherwise been discarded, was utilised.

2.8.2 ELISA

The residual serum was tested for anti-HCV IgG antibodies and Hepatitis B Surface antigen.

2.8.2.1 HCV ELISA

A commercially available 3rd generation ELISA (ABBOTT AxSYM® HCV v 3.0, Abbott Laboratories, Abbott Park, IL, USA) was utilised according to the manufacturer's protocol. This is a microparticle enzyme immunoassay for the qualitative detection of anti-HCV antibodies in human serum or plasma. The assay uses proteins coated on a microparticle solid phase to capture antibodies against structural and nonstructural proteins of HCV. The bound analyte is detected by an antibody coupled to an enzyme. This enzyme acts on a substrate to produce a fluorescent signal. The fluorescent signal is proportional to the amount of analyte present in the initial sample.

Several recombinant proteins are included in the assay. Collectively they contain sequences of putative core structural proteins and nonstructural proteins NS3, NS4 and NS5 chimerically bound to superoxide dismutase (SOD)

The kit contains positive and negative controls and a calibrator for quality control purposes. The specificity and sensitivity of the assay are stated to be 99.84% and 100%, respectively (Package insert - ABBOTT AxSYM® HCV v 3.0, Abbott Laboratories, Abbott Park, IL, USA).

2.8.2.2 HBV Surface Antigen ELISA

The Cobas® HBsAg II and Roche Modular Analytics E170 were used for the qualitative determination of hepatitis B Surface Antigen in serum (Roche Diagnostics GmbH, Mannheim). This is an electrochemiluminescence immunoassay (ECLIA) based on the sandwich principle. Biotinylated anti-HBsAg antibodies and anti-HBsAg antibodies labeled with a ruthenium complex are mixed with the sample to form a sandwich complex. This complex binds to a streptavidin-coated microparticle via the interaction of streptavidin with biotin. This complex is magnetically captured onto the surface of an electrode. Unbound substances are removed. A voltage applied across the electrode induces a chemiluminescent signal which is measured by a photomultiplier. The software automatically determines the result by comparing this signal to calibrated signal-cutoff values.

The kit contains controls and a calibrator for quality control purposes. The specificity and sensitivity of the assay are stated to be 100% (Package insert: Cobas® HBsAg II, Roche Diagnostics GmbH, Mannheim).

2.8.2.3 Quality Control Procedures

The kits contain negative and positive controls. Routine maintenance and calibration is performed on the equipment as per manufacturers' recommendations. The laboratory uses the United Kingdom National External Quality Assessment Service (NEQAS) to ensure quality of serology results (NEQAS).

2.9 Data Collection Method: Clinical records

A morbidity and mortality study was performed on a subset of patients within the seroprevalence study. 50 HIV-HCV co-infected and 150 HIV-positive, HCV-negative patients were randomly selected for this purpose. 178 charts were reviewed systematically. The remaining 22 patients (4 co-infected and 18 from the HIV positive-HCV negative subset) were excluded from the analysis because their charts were missing or contained inadequate data. The difference in the number of missing charts between the two groups was not statistically significant ($p = 0.4$).

The visit at which the patient had the HIV test (that was sent to the Virology laboratory) was the time point of analysis of the retrospective chart review. The patient's morbidity and mortality status at *this visit* was assessed. The patients were not followed up, and events beyond this visit were not included in the study. A standardized form was used to record the clinical and laboratory data available at this visit (see 2.9). Data was extracted from the patients' hospital charts by the investigators who were blinded to the HCV status

of the patient. Where there was more than one measurement for a particular variable, the earliest (i.e. baseline) reading was taken. The WHO (WHO, 2006) (See Appendix B), CDC (Castro et al., 1992) (See Appendix C), and Karnofsky (O'Dell et al., 1995) (See Appendix D) scores were inferred by the investigators using the available clinical information. The diagnosis of liver failure, cirrhosis and chronic liver disease, were similarly inferred by the investigators using the criteria listed in Appendix E (Braunwald et al., 2001). Particular note was made of recreational drug use, and risk factors for transmission such as blood transfusion, tattoos and scarification. If the patient died during the visit, the cause of death was ascertained from the death certificate.

The Clinical Record Form was used to enable standardized collection of data (see Appendix A)

2.10 Statistical Techniques and Analysis

The sero-prevalence of HCV, together with 95% confidence intervals, was determined in the HIV positive and negative groups. The HCV sero-positivity in the HIV infected and uninfected groups were compared using the χ-squared test. A morbidity and mortality chart review was performed on randomly selected HIV-HCV co-infected patients and randomly selected controls from the HIV-positive, HCV-negative group. The information was entered into a Clinical Record Form (see 2.9 and Appendix A) and thereafter collated and entered into Access® (MicroSoft Corporation). The data fields included clinical information, laboratory data and other investigations as recorded in the patients' charts.

The data was exported to Excel® (MicroSoft Corporation) and then STATA™ 9 (StataCorp, College Station, Texas, USA), where statistical analysis was performed. For categorical data, Chi-squared test was used when Cochran's criterion was fulfilled and Fisher's Exact tests, when it was not. Student's t test was used for continuous variables. A normal distribution was assumed (Bland, 2000).

2.11 Ethical Considerations

2.11.1 Ethics Approval

Ethics approval for the study protocol was obtained from the University of KwaZulu-Natal Bioethics Committee (Ref. E141/02).

This was a laboratory-based study utilising residual sera for hepatitis C and B testing and clinical data obtained from the patients' hospital records. There was no direct patient interaction or contact. No additional blood samples were taken. No tests other than those for Hepatitis C and Hepatitis B were performed for the purposes of this study. Laboratory test results, where available, were obtained from the patients' clinical records. These tests were performed routinely by the patients' care provider independently of the study.

The study was designed on the basis that only samples submitted to the laboratory for routine HIV testing were to be selected. Patients would therefore already have had HIV

results prior to entry into the study. HIV testing was therefore not performed solely for purposes of this study.

2.11.2 Consent

Informed consent was not obtained for the non-routine tests since these were performed anonymously on sera that would have otherwise been discarded. Informed consent for the routine HIV test would have been obtained by the patient's care-giver, in accordance with Department of Health policies and guidelines (Tshabalala-Msimang, 2003). Use of anonymised discarded samples is acceptable in minimal-risk research, where the risk of breaching confidentiality by attempting to obtain informed consent is greater than the risk of conducting the research in an anonymous unlinked manner. The ethical basis and design of this protocol are in keeping with Section 9 of the South African National Department of Health Guidelines for good practice in the conduct of clinical trials in human participants in South Africa (Rees et al., 2000).

In respect of the retrospective chart review, Guideline Four of the International Ethical Guidelines for Biomedical Research Involving Human Subjects, prepared by the Council for International Organizations of Medical Sciences in collaboration with the World Health Organization, Geneva (2002) states the following:

"…when the research design involves no more than minimal risk and a requirement of individual informed consent would make the conduct of the research impracticable (for

example, where the research involves only excerpting data from subjects' records), the ethical review committee may waive some or all of the elements of informed consent."

(CIOMS and WHO, 2002)

2.11.3 Patient Confidentiality

The protocol was designed in a linked, anonymous manner to protect the confidentiality of patients with respect to clinical data and HIV, HBV and HCV status. This was achieved by adhering strictly to the following procedures:

1. The Principal Investigator identified samples fulfilling the study criteria. Original sample tubes are routinely labeled with a unique HOSPITAL NUMBER. The hospital number facilitates retrieval of the patients' medical records. A "master list" was created linking the hospital number to a unique, sequentially-generated, anonymous STUDY NUMBER. It is impossible to identify patients using the study number alone. This "master list" was available only to the Principal Investigator. The HIV result, generated for routine clinical purposes, was recorded by the Principal Investigator, per study number (the "HIV result list"). The "HIV result list" was anonymous since it did not contain the hospital number or any other patient identifiers. The original specimen was aliqouted into an anonymous vial bearing only the study number.

2. The anonymous vials, bearing only the unique study numbers, were received by the technologist. The “HIV result list”, with the HIV result per study number, but without any personal patient identifiers, was provided to the technologist. The technologist was not given the “master list” or the patients’ hospital number or any other personal identifiers. The technologist performing the test was therefore blinded to the identity and personal and clinical details of the patients. The technologist performed the HCV and HBV serology and recorded this result, together with the HIV result (from the “HIV result list”), against the anonymous study number. The technologist randomly selected 50 HCV positive- HIV positive study numbers and 150 HCV negative-HIV positive study numbers as per randomization method (See 2.5). The technologist listed these study numbers, in ascending order, but WITHOUT the HCV/HBV/HIV results (the “randomized study number list”). It was therefore impossible to deduce the HCV/HBV results from this list, since the list consisted of study numbers only and these could belong to either HCV positive or HCV negative specimens. This list was provided to the Principal Investigator.

3. The Principal Investigator used the Master List to link the anonymous study number in the “randomized study number list”, to the hospital number, and provided a list of corresponding hospital numbers, but NOT study numbers, to the medical practitioner, for collection of clinical data. The medical practitioner was therefore blinded to the HCV/HBV status of the patients. The medical practitioner extracted the clinical data using the hospital numbers as patient identifiers. The data

was recorded in the Clinical Record Form (see Appendix A). This data was provided to the Principal Investigator.

4. The Principal Investigator collated the data into a spreadsheet, in which the clinical data was arranged per hospital number. Patient names and other personal details or patient identifiers were not recorded on the spreadsheet. The Principal Investigator replaced the hospital number with the unique study number, using the Master List. The Master list was then destroyed. It was thereafter impossible to link the data in the spreadsheet to individual patients i.e. the spreadsheet was anonymous.

5. The spreadsheet containing the anonymised clinical data, with no personal patient identifiers, was provided to the technologist who entered the HCV/HBV results per study number.

6. The list was returned to the Principal Investigator, who collated and analysed the anonymised data.

2.11.4 Patient Impact

A consequence of the design of this study was that the HCV and HBV results could not be made available to the patients even if this may have been desirable or even lifesaving (consider unlinked anonymous HIV surveillance as an analogy)

"Unlinked anonymous testing without informed consent is only conducted in clinical settings in which a specimen of blood originally collected for other purposes, such as syphilis testing at antenatal clinics, is tested for HIV after all information that could identify the source of the blood is removed from the specimen. Thus, the test result may not be traced back to the patient nor may he or she be informed of the test results."

(WHO, 2001)

The patient and care-provider still had access to his/her HIV results and routine care, which is not compromised by this study. The study did not interfere with or diminish the standard of care available to the patient. At the time that this study was conducted, treatment for hepatitis C infection was not routinely available in the study population. The research or surveillance purpose was separate from and did not intrude on the clinical function.

CHAPTER THREE: RESULTS

3.1 Prevalence

Table 2 shows the HIV and HCV results of 1937 samples entered into the sero-prevalence survey (of the 2000 samples screened, 63 had equivocal HIV and/or HCV ELISA results and were excluded from the analysis). The prevalence of HCV in this study population was 6.4 % and that of HIV, 40.2%. There was a significantly higher prevalence of HCV among HIV infected patients as compared to HIV negative patients (13.4% vs. 1.73%) ($p < 0.001$, Odds Ratio (OR) 8.8, 95% confidence interval (CI) of the OR 5.4 - 14.3). 83.9 % of individuals who were HCV positive were HIV positive as well.

Table 2 Distribution of HCV among HIV Positive and Negative Individuals

	HCV Negative	**HCV Positive**	**Totals**	**HCV Prevalence**
HIV Negative	1139	20	1159	1.7% (0.98 – 2.48%)
HIV Positive	674	104	778	13.4% (11.0 – 15.8%)
Total	1813	124	**1937**	**6.4%** **(5.3 – 7.5%)**
HIV Prevalence **(% and 95%CI)**	37.2% (35.0 – 39.4%)	83.9% (77.4 – 90.4%)	**40.2%** **(38.8 – 42.4%)**	

$\chi^2 = 105$, $p < 0.001$, $n = 1937$

3.2 Demographic Profile of HIV Positive and Negative Individuals

Females were significantly more likely to be HIV positive than males (42.2 vs. 37.5 %, respectively, p = 0.043) (Table 3). The ratio of HIV seropositivity in females vs. males was 1.12. There was a significant difference between the mean age of HIV positive and negative patients (p < 0.0001)

Table 3 Demographic Profile of HIV Positive and Negative Individuals

Variable	HIV Negative	HIV Positive	% positive	Total	Significance
Total	1159	778	40.2%	1937	
Male	478	287	37.5%	765	p = 0.043[a]
Female	656	478	42.2%	1134	
Unknown	25	13		38	
Female to Male ratio			1.12		
Age (years, 95%CI)	42.6 (41.7 – 43.5)	35.0 (34.3 – 35.8)			p < 0.0001[b]

[a] Chi-squared test [b]t-test with equal variances

3.3 Demographics Profile of HCV Positive and Negative individuals

In comparison, the HCV seropositivity was 6.0 and 6.7 % in males and females, respectively (Table 4). The difference in HCV seropositivity between males and females did not reach statistical significance. The ratio of HCV seropositivity in females vs. males was 1.12. There was no statistically significant difference in the mean age between the HCV positive and HCV negative groups.

Table 4 Demographic Profile of HCV Positive and Negative Individuals

Variable	HCV Negative	HCV Positive	% Positive	Total	Significance
Total	1813	124	6.4%	1937	
Male	719	46	6.0%	765	p = 0.548[a]
Female	1058	76	6.7%	1134	
Unknown	36	2		38	
Female to Male ratio			1.12		
Age (years, 95%CI)	39.6 (39.0 - 40.3)	38.7 (36.4 - 41.0)		39.6 (39.0 – 40.2)	p = 0.476[b]

CI Confidence Interval

[a]Chi-squared test [b]t-test with equal variances

3.4 Demographic Profile of patients selected for chart review

Table 5 shows the baseline demographics in the subset of patients selected for the detailed chart review. There was no statistically significant difference in the female-to-male ratio or mean age between the HCV positive and HCV negative groups.

Table 5 Demographic Profile of HCV Positive and Negative Individuals in the Subset of Patients Selected for the Chart Review

Variable	HCV Negative	HCV Positive	% positive	Total	Significance
Total	132	46	25.8%	178	
Male	46	14	23.3%	60	p = 0.586[a]
Female	86	32	27.1%	118	
Female to Male ratio			1.16		
Age (years, 95%CI)	34.8 (33.2 - 36.5)	36.3 (33.7 - 38.8)		35.2 (33.8 – 36.6)	p = 0.366[b]

CI Confidence Interval

[a] Chi-squared test [b] t-test with equal variances

3.5 Age-Gender Distribution

Figure 7 shows the prevalence of HIV in men and women in different age groups. Figure 8 shows the prevalence of HCV in men and women in different age groups. Both HIV and HCV prevalence peak in the 30 – 34 and 25 – 29 year age groups, in males and females, respectively.

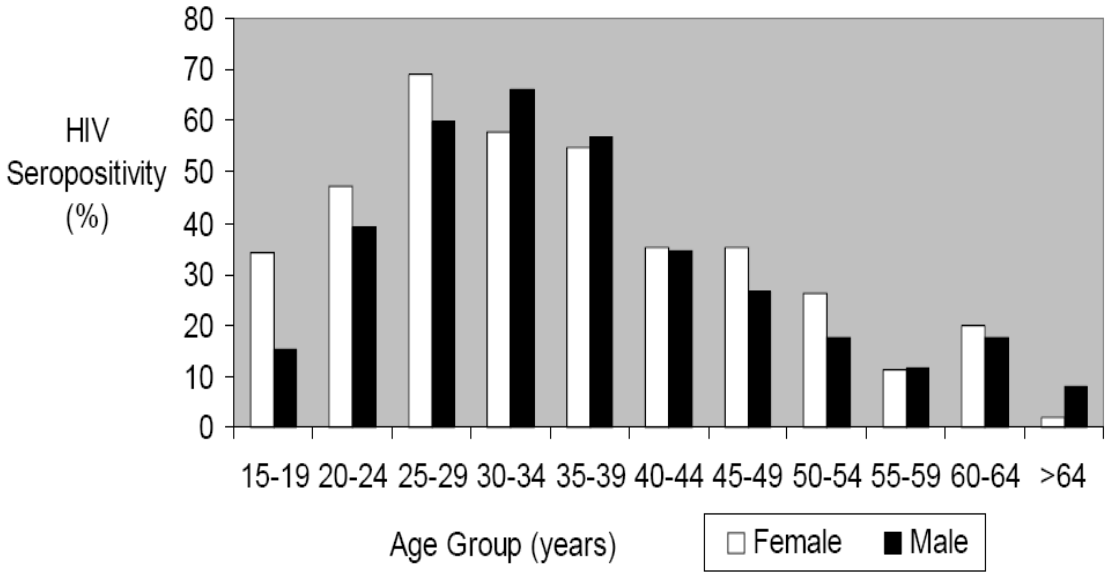


Figure 7 Age-Gender Distribution of HIV

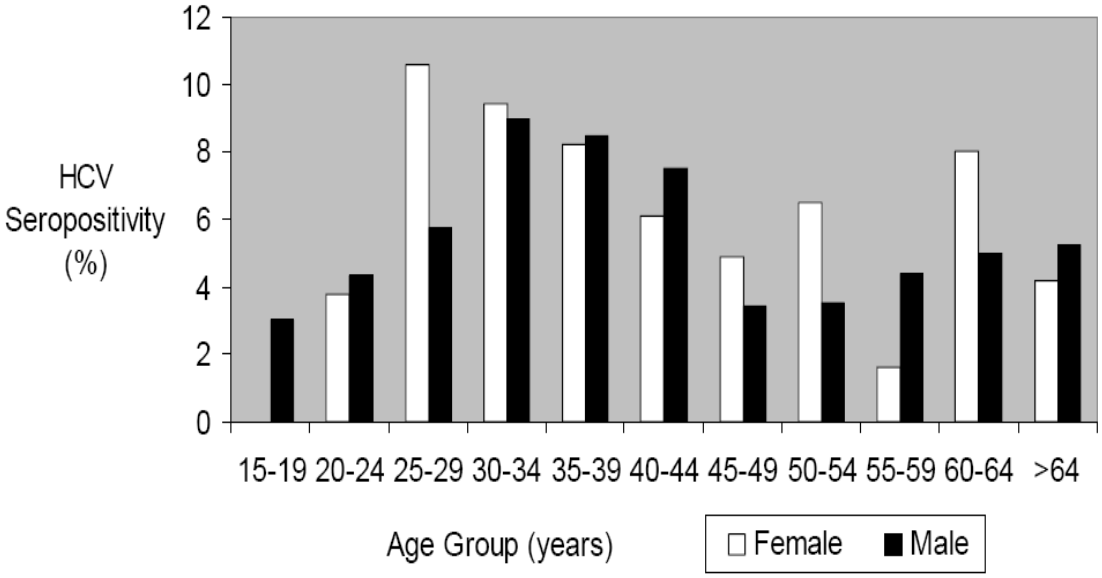


Figure 8 Age Gender Distribution of HCV

3.6 Clinical Outcomes

There was no difference in the proportion of patients admitted or length of hospital stay between HCV positive and negative patients (**Table 6**). Similarly, there was no difference in HIV disease progression, as measured by CDC classification and WHO staging, between HCV positive and negative patients. There was also no difference between these groups in terms of ambulation and Karnofsky score. HIV infected patients who were seropositive for HCV had significantly increased mortality (8 vs. 21 %) ($p < 0.02$, OR = 3.0, 95% CI 1.12 – 8.00).

Table 6 Clinical Outcomes in HCV Positive and Negative HIV-infected Individuals

Variable	HCV Negative	HCV Positive	Significance
Ambulant	90/117 (76.9%)	26/35 (74.3%)	P = 0.747[a]
Number Admitted to Hospital	94/125 (75.2%)	34/42 (81.0%)	P = 0.446[a]
Average number of days in Hospital (95%CI)	8.14 (6.33-9.94)	8.42 (6.10-10.73)	P = 0.87[b]
Number with AIDS-defining condition	66/123 (54%)	26/42 (62%)	P = 0.353[a]
Median WHO score	3	3	
Median Karnofsky score	70	70	
Number of Deaths (Mortality rate)	10/120 (8.3%) (3.4% - 13.3%)	9/42 (21.4%) (9.0% - 33.8%)	**p < 0.05[c]**
	Odds Ratio = 3.0		95% CI of OR: 1.12 – 8.00

CI Confidence Interval, p-values in bold are significant at $\alpha = 0.05$.

[a]Chi-squared test [b]t-test, [c]Fisher's Exact test

3.7 Causes of Death

The causes of death are shown in Table 7. The average age of death was 36 years in HIV mono-infected patients and 38 in co-infected patients. It is noted that the cause of death was an AIDS-defining condition or an AIDS-related condition in almost all patients. There were no patients whose death was attributed to an HCV-related condition such as end-stage liver disease or hepatocellular carcinoma. Renal failure or renal impairment appears in the death certificates of 5 patients (in bold italics). This finding, the statistical significance of which is uncertain because of the small numbers, was explored further in Table 8.

Table 7 Causes of Death in HCV Positive and Negative Patients

Patient Number	HCV STATUS	DIAGNOSES AT DEATH	AGE
1	N	PTB	45
2	N	SAH, TCP, Kaposi's, Histoplasmosis	32
3	N	Bronchopneumonia	50
4	N	PCP	29
5	N	Bronchopneumonia	48
6	N	Lymphoma(spine)	27
7	N	TB Meningitis/ miliary TB	16
8	N	Meningitis	24
9	N	Anemia CCF ***Renal Failure***	36
10	N	GE, PTB	54
11	P	***Renal Failure***	29
12	P	***HIVAN - renal failure***	40
13	P	Toxoplasmosis meningitis	51
14	P	PTB, PCP, ***renal impairment***	34
15	P	Pancytopenia, meningitis, pneumonia	33
16	P	TB Meningitis	29
17	P	Pneumococcol Meningitis	36
18	P	***Renal failure***, disseminated TB	57
19	P	Disseminated TB	35

3.8 Renal and electrolyte abnormalities

Table 8 explores renal and electrolyte abnormalities in HCV negative and positive patients by analyzing the association between HCV sero-status and sodium, bicarbonate, urea and creatinine levels. There was a statistically significant association between HCV serostatus and abnormal urea and creatinine levels. A statistically significant association was also found between HCV seropositivity and hyponatremia, hyperkalemia and low bicarbonate levels.

Table 8 Renal and Electrolyte Abnormalities in HCV Positive and Negative HIV-infected Individuals

Variable	HCV Negative	HCV Positive	Significance
Renal Failure or Impairment Noted in Death Certificate	1/10 (10%)	4/9 (44%)	p = 0.141[a]
Mean Sodium (mmol/l) Reference Range: 132-146	135 (134 - 136)	131 (130 -133)	**p < 0.005[b]**
Hyponatremia	30/100 (30%)	20/35 (57%)	**p < 0.005[b]**
Hypernatremia	1/100 (1%)	0/35 (0%)	p = 0.553[c]
Mean Potassium mmol/l Reference Range: 3.3 – 5.1	4.01 (3.85 - 4.18)	4.37 (3.97 - 4.78)	p = 0.0538[b]
Hyperkalemia	5/99 (5%)	7/34 (21%)	**p < 0.05[a]**
Mean Bicarbonate (mmol/l) Reference Range: 22 – 29	22.03 (20.94 - 23.12)	19.05 (17.35 - 20.75)	**p < 0.01[b]**
Low Bicarbonate	42/95 (44%)	23/33 (69.7%)	**p < 0.05[c]**
Mean Urea (mmol/l) Reference Range: 1.7 - 8.3	7.33 (5.65 - 9.01)	15.39 (8.28 - 22.50)	**p < 0.005[b]**
High urea	24/99 (24%)	16/35 (46%)	**p < 0.05[c]**
Mean Creatinine (μmol/l) Reference Range: 0 – 80	145 (103 - 186)	252 (126 - 378)	**p < 0.05[b]**
High creatinine	51/99 (52%)	25/35 (71%)	**p < 0.05[c]**

NOTE. CI Confidence Interval, Reference Ranges defined by the laboratory performing the test; p-values in bold are significant at $\alpha = 0.05$;

[a]Fisher's Exact test, [b]t-test, [c]Chi-squared test

3.9 Liver Function

Table 9 shows the relationship between HCV seropositivity and liver dysfunction. HIV positive patients who were HCV seropositive were twice as likely to have hepatomegaly as HIV mono-infected patients ($p < 0.05$). However, we found no statistically significant association between HCV seropositivity and jaundice, signs of chronic liver disease or liver failure. Patients with HCV infection did have slightly higher bilirubin levels, but this was not statistically significant. HCV seropositivity was associated with low albumin and high ALT levels.

Table 9 Clinical and Laboratory Indicators of Liver Function in HCV Positive and Negative HIV-infected Individuals

Variable	HCV Negative	HCV Positive	Significance
Hepatomegaly (%) **(95% CI)**	19/100 (19%) (11.3 – 26.7%)	15/40 (37.5%) (22.5 - 52.5%)	**p< 0.05**[a]
Liver Failure	0/100 (0%)	2/39 (5%)	p = 0.077[b]
Chronic Liver Disease	0/100 (0%)	1/40 (2.5%)	p = 0.286[b]
Jaundice	1/100 (1%)	2/40 (5%)	p = 0.196[b]
Mean Albumin (g/l) **Reference Range: 34-48**	24.3 (22.1 – 26.4)	18.0 (15.4 - 20.6)	**p < 0.005**[c]
Hypoalbulinaemia	55/69 (80%)	22/22 (100)	**p < 0.05**[a]
Mean Bilirubin (μmol/l) **Reference Range: 0-17**	14.2 (6.4 - 21.9)	19.1 (8.9 - 29.3)	p = 0.51[c]
Hyperbilirubinaemia	9/69 (13%)	7/22 (32%)	p = 0.057[b]
Mean (ALT) (U/l) **Reference Range: 0-31**	29.2 (23.4 -34.9)	52.0 (24.9 - 79.0)	**p < 0.05**[c]
High ALT	20/69 (29%)	12/22 (55%)	**p < 0.05**[a]

NOTE. CI Confidence interval, Reference Ranges defined by the laboratory performing the test; p-values in bold are significant at $\alpha = 0.05$.

[a]Chi-squared test, [b]Fisher's Exact test, [c]t-test

3.10 Hepatitis B Virus

Table 10 shows the differences between HBV positive and negative HIV infected patients in terms of several clinical and laboratory markers which were found to be significantly different in *HCV* negative and positive patients. None of the variables that were found to be significantly different in HCV negative and positive patients reached statistical significance in HBV negative and positive individuals.

Table 10 Selected Clinical and Laboratory Variables in Hepatitis B surface antigen Positive and Negative HIV-infected Individuals

Variable	HBV Negative (n=124)	HBV Positive (n=26)	Significance
Age (years)	35.7	33.4	p = 0.273[a]
Deaths; (mortality, %) (95% CI of mortality)	16/124 (12.9) (7.0 -18.8 %)	2/26 (7.69) (-2.5 – 17.9%)	p = 0.457[b]
Hepatomegaly (%), (95% CI)	23/106 (21.7%) (13.9 – 29.5%)	8/24 (33.3%) (14.5 – 52.2%)	p = 0.227[c]
Serum sodium (mmol/l); (95% CI) Reference Range: 132-146	134 (133 – 135)	133 (130 – 136)	p = 0.454[a]
Serum bicarbonate (mmol/l); (95% CI) Reference Range: 22-29	22.0 (20.8 – 23.1)	20.2 (18.8 – 21.7)	p = 0.152[a]
Blood urea (mmol/l); (95% CI) Reference Range:1.7-8.3	9.4 (6.5 -12.3)	9.7 (5.2 – 14.1)	p = 0.925[a]
Serum creatinine (μmol/l); (95% CI) Reference Range: 0-80	161 (111 – 211)	170 (86 – 255)	p = 0.859[a]
Serum albumin (g/l); (95% CI) Reference Range: 34-48	23.7 (21.5 – 25.8)	19.6 (15.4 – 23.8)	p = 0.107[a]
Serum alanine transaminase (U/l);(95% CI) Reference Range: 0-31	36.5 (26.7 – 46.4)	29.9 (13.5 – 46.5)	p = 0.553[a]

NOTE. CI Confidence interval, Reference Ranges defined by the laboratory performing the test; [a]t-test, [b]Fisher's Exact test, [c]Chi-squared test

3.11 Risk Factors

There was no record of intravenous drug use in the clinical notes of any of the patients in this study. One patient, who was HIV positive but HCV antibody and HBV surface antigen negative, had evidence of traditional scarification. Nine patients had received a blood transfusion, 6 of whom had the study sample taken within 5 days of the transfusion, which would have been too early to detect hepatitis C antibodies or hepatitis B surface antigen (Fields et al., 2001). The remaining 3 patients (none of whom were HCV or HBV surface antigen sero-positive) had their study blood sample taken 12, 15 and 20 days post-transfusion.

CHAPTER FOUR: DISCUSSION

Hepatitis C Virus (HCV) is a common infection in Human Immunodeficiency Virus (HIV) positive patients because of shared routes of transmission and common risk factors. HIV is known to affect the epidemiology, transmission, pathogenesis and natural history of HCV infection, primarily due to the immunosuppressive effects of HIV. Patients with co-infection, for example, have higher HCV viral loads and are more likely to progress to chronic liver disease, cirrhosis, end-stage liver disease and hepatic failure, than HCV mono-infected patients. Treatment of both HIV and HCV is more complex in co-infected patients because of liver toxicity and drug interactions (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Sterling et al., 2003, Sulkowski and Thomas, 2003, Thomas, 2002, Winnock et al., 2004).

On the other hand, the effect of HCV on HIV infection is controversial and less well understood. Some studies have shown that patients who have HCV have increased progression to an AIDS-defining condition and death and diminished $CD4^+$cell responses while on potent anti-retrovirals (ARVs) (Greub et al., 2000). Conversely, other studies have not demonstrated these effects (Sulkowski et al., 2002, Staples et al., 1999). These studies are confounded by several factors including the use of intravenous drugs and variable anti-retroviral regimens. The effect of HCV on HIV disease progression in ARV-naïve subjects, in particular, has not been well studied.

There are no representative population-based studies investigating the prevalence of HCV in the province of KwaZulu-Natal in South Africa, where HIV is highly prevalent. The magnitude of the above issues is therefore unknown in this population. This laboratory-based study was conducted in the Department of Virology Laboratory at Inkosi Albert Luthuli Central Hospital, in Durban in order to determine the prevalence of HCV and to elucidate the clinical and epidemiological interactions between HCV and HIV in this study population.

There are several factors which make this a unique population and a unique time point in which to study HIV-HCV co-infection. Firstly, the prevalence of HIV in KwaZulu-Natal is among the highest in the world. In 2005, 39.1% of women attending antenatal clinics in the public sector were HIV positive (Anonymous, 2006) . The prevalence of HCV in this population, and hence the potential magnitude of the problems associated with HIV-HCV co-infection, has not been described. The high prevalence of HIV may also reveal effects of co-infection which may not be apparent in areas of lower HIV prevalence. The high prevalence of HIV has largely contributed to the low life expectancy of 43.3 years in this region (Lehohla, 2006). To our knowledge, there have been no population-based studies on HIV-HCV co-infection in regions with such high prevalence of HIV. Furthermore, the role of HCV in contributing to the low life expectancy has not been assessed.

Secondly HIV is predominantly a sexually transmitted infection in this region (Buve et al., 2002, Simon et al., 2006) and the use of intravenous drugs is rare (Parry et al., 2005). Most

co-infection studies have been conducted in populations where intravenous drug use is the predominant risk factor for transmission of both viruses. Intravenous drugs are a confounding factor in morbidity and mortality studies and furthermore seroprevalence data in high risk cohorts cannot readily be extrapolated to the general population (Anderson et al., 2004, Braitstein et al., 2005, Greub et al., 2000, Dorrucci et al., 2004, Rosenthal et al., 2003, Tedaldi et al., 2003, Sterling et al., 2003, Carter, 2004, Jenny-Avital, 2000, Klein et al., 2003, Rockstroh et al., 2005, Sulkowski et al., 2002). This is one of the first HIV-HCV co-infection studies where the effect of intravenous drug use is not a confounding factor.

Thirdly, the study population was naïve to anti-retroviral therapy. Anti-retroviral therapy is a potential confounding factor in outcomes analysis of HIV-HCV co-infection (Braitstein et al., 2005, Rosenthal et al., 2003, Salmon-Ceron et al., 2005, Staples et al., 1999, Tedaldi et al., 2003, Monga et al., 2001, Rockstroh, 2006, Rockstroh et al., 2005, Lincoln et al., 2003) because while its use significantly reduces mortality and morbidity in patients with HIV (Thomas, 2002, Mohsen et al., 2002, Khalili and Behm, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Bica et al., 2001, Martinez-Sierra et al., 2003, Klein et al., 2003, Law et al., 2004), HCV-HIV co-infected patients may not derive such benefits (Klein et al., 2003) because they are less likely to initiate or remain on HAART (Fuster and Clotet, 2003, Anderson et al., 2004, Dorrucci et al., 2004, Rockstroh et al., 2005, Rossi et al., 2002, Sulkowski et al., 2002, Braitstein et al., 2005, Khalili and Behm, 2002, Falusi et al., 2003, Rancinan et al., 2002, Bica et al., 2001, Klein et al., 2003).

Furthermore, the risk of ARV-associated hepato-toxicity is greater in co-infected patients (Fuster and Clotet, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Rockstroh and Spengler, 2004, Sherman, 2004, Sulkowski and Thomas, 2003, Winnock et al., 2004, Bica et al., 2001, Rosenthal et al., 2003, Law et al., 2004) and HAART exacerbates the severity and progression of liver disease in HIV-HCV co-infection (Sherman, 2004, Staples et al., 1999, Graham et al., 2001, Bonacini and Puoti, 2000, Mohsen et al., 2002, Rosenthal et al., 2003). Therefore, the utilisation, risks and benefits of HAART are different in HIV mono-infected and co-infected patients.

The National Department of Health of South Africa initiated the rollout of anti-retroviral therapy in 2004 (DOH(SA), 2003)**.** Between March 2004 and May 2005, only 12 007 patients commenced anti-retroviral therapy in public health facilities in KwaZulu-Natal (KZN-DOH). Based on actuarial estimates, this represents <1% of the number of HIV infected patients in the province (ASSA, 2005). Moreover, the patients in this study could not have been on anti-retroviral therapy since patients already on anti-retroviral therapy would not have required an HIV test and would therefore not have fulfilled the inclusion criteria for this study. It was essential to conduct the study at this unique time point, since with time large numbers of patients would be recruited into the Anti-retroviral Rollout programme and commence anti-retroviral therapy. As a result, the study of HIV-HCV co-infection in ARV-naïve patients would become more difficult and less representative.

Finally, the existence of a centralised Virology service which received the majority of the specimens for viral assays in this region was a source of a representative sampling frame for this population. This laboratory serves approximately 90% of the population of the province, which consists of 9.4 million people (Lehohla, 2006). This was in a unique time-point, prior to the widespread availability of "rapid" point-of care HIV tests. The Department of Health in South Africa was at this juncture implementing its Voluntary Testing and Counselling (VTC) programme in which easily accessible point-of-care rapid tests for the diagnosis of HIV were being prioritised (DOH(SA), 2000, Ramkissoon et al., 2004). Subsequently, the number of samples referred to the central laboratory for formal HIV ELISAs would diminish and be less representative as they were being replaced by the widespread use of rapid tests.

At the time this study was conducted, HCV serology for clinical or surveillance purposes was not routinely available in the public sector. There was a paucity of data on the sero-prevalence and clinical impact of HCV in this study population. By conducting a seroprevalence survey, large numbers of HCV-HIV co-infected samples could for the first time be identified. For these reasons, it was essential and informative not only to conduct the seroprevalence survey at this unique time point but also to combine this with a retrospective cross-sectional chart review based on clinical information anonymously linked to the HIV and HCV results.

This study has found the overall prevalence of hepatitis C sero-positivity to be 6.4% (95% CI 5.3 – 7.5%). This is more than double the estimated global prevalence of 3% (Winnock et al., 2004), and higher than previous estimates of HCV seropositivity in this province, of 1.7% and 0.9% among urban and rural blacks, respectively (Abdool Karim and Tait, 1993). The prevalence of HCV in this study is, as expected, higher than that of most developed countries (Ray Kim, 2002, Shepard et al., 2005), and is slightly higher than that of previous estimates within Sub-Saharan Africa (5.3%) (Madhava et al., 2002). The geographical variation in HCV prevalence is well known and reflects the relative contribution of the risk factors to the epidemiology of HCV in different parts of the world. These risk factors are poorly defined in developing regions of the world (Ray Kim, 2002, Shepard et al., 2005).

There are few representative population-based studies investigating the prevalence of HCV in South Africa. The average prevalence was found to be 23.5% in high risk cohorts (patients with liver disease and patients receiving multiple blood transfusions, blood products, dialysis and renal transplants) and < 1% in low-risk blood donors (Madhava et al., 2002, Soni et al., 1993, Tucker et al., 1997). HCV antibodies were found in 1% of AIDS patients admitted to the Chris Hani Baragwanath Hospital in Johannesburg (Lodenyo et al., 2000), in 1.8% of healthcare workers (Vardas et al., 2002) in a large, urban referral hospital and in 11% of HIV-positive patients on antiretroviral drugs who experienced hepatoxicity (Sanne et al., 2005). The prevalence in KwaZulu-Natal was found to be 1.7% and 0.9% among urban and rural blacks, respectively (Abdool Karim and Tait, 1993). .

Studies in high or low risk cohorts are non-representative and may either over-estimate or under-estimate the true prevalence of HCV. The findings in low risk blood bank cohorts (Madhava et al., 2002, Tucker et al., 1997, Soni et al., 1993), in healthcare workers (Vardas et al., 2002) and in HIV-positive patients experiencing hepato-toxicity (Sanne et al., 2005) cannot be extrapolated to the general population. The sample size in Lodenyo's study was only 100 (Lodenyo et al., 2000). Studies performed in the early 1990's before the development of new more sensitive tests for HCV may have under-estimated the true sero-prevalence. Furthermore, these estimates were made prior to the effects of the HIV pandemic and therefore do not reflect the effects of HIV on HCV epidemiology. Indeed, the prevalence of HCV in the *HIV negative* group of our study (1.7%) is remarkably similar to the estimates of the prevalence of HCV in the studies conducted in the early 1990s (Abdool Karim and Tait, 1993, Soni et al., 1993), perhaps reflecting the role of the subsequent HIV pandemic in the dramatic increase of HCV seropositivity. This finding is evidence of the validity and representativeness of our sampling frame. Slight differences in the estimates may be explained by the increased sensitivity of ELISA techniques with advances in technology. Our study is therefore representative of the population that is affected by the HIV pandemic within this province and is furthermore the first to estimate hepatitis C prevalence outside a risk-defined population.

This study has found a significantly higher prevalence of HCV among HIV infected patients as compared to HIV negative patients (13.4% vs. 1.73%). This is one of the first studies to investigate the epidemiology of HCV in HIV-infected individuals in Africa. The paucity of information on co-infection rates in developing countries has been noted by

several authors (Shepard et al., 2005, Madhava et al., 2002). The only other study investigating co-infection in South Africa found the prevalence of HCV among HIV positive patients to be low (1%) (Lodenyo et al., 2000). The sample size in Lodenyo's study, however, was only 100, and the sample was non-representative.

The prevalence of co-infection depends on the common risk factor and route of transmission (Sherman, 2004, Sulkowski and Thomas, 2003, Thomas, 2002, Winnock et al., 2004). The prevalence of co-infection in our study (13.4%) is comparable to the prevalence of co-infection in heterosexual cohorts in other parts of the world (14.3%) and much lower than the prevalence in hemophiliacs (60 – 90 %) or in individuals who have a history of intravenous drug use (50% - 95%) (Bonacini and Puoti, 2000, Dieterich, 2004, Fuster and Clotet, 2003, Gonzalez and Talal, 2003, Khalili and Behm, 2002, Maier and Wu, 2002, Rockstroh and Spengler, 2004, Sulkowski and Thomas, 2003, Thomas, 2002). This suggests that HCV is predominantly a sexually transmitted infection in this region. Furthermore, there were no other risk factors such as intravenous drug use, traditional scarification or blood transfusion that adequately explained the high prevalence of HCV in this population. There was no record of intravenous drug use in the clinical notes of any of the patients in this study. One patient had evidence of traditional scarification and nine patients had received a blood transfusion. The outcomes of the study were not influenced when these patients were analyzed separately. The Blood Transfusion Service routinely screens donor samples serologically for HIV, HCV and Hepatitis B (Madhava et al., 2002). It is therefore unlikely that any of these risk factors could explain the high seroprevalence of HCV in this study population.

In our study, females were more likely to be HIV seropositive than males (42.2 vs. 37.5%, $p < 0.05$, Odds Ratio: 1.12). Females were similarly more likely to be HCV seropositive than males (6.7 vs. 6.0%, Odds Ratio: 1.12), although this did not reach statistical significance. The ratio of female-to-male seropositivity for HIV was remarkably similar to that of HCV (both approximately 1.12). This is in contrast to the USA, where HCV prevalence is higher in men than women and probably reflects high risk drug-taking, which is a risk factor more common in men than women in that country (Armstrong et al., 2006). The distribution of risk factors in developing regions has not been well described, and the demographic profile of HCV seropositive patients has not been previously documented. HIV is more common in females due to biological reasons related to sexual transmissibility (Buve et al., 2002). Similar biological factors could explain the female to male ratio of HCV and HIV in our study.

The prevalence of HIV in females and males peaks in the 25 - 29 and 30 - 34 age groups, respectively (Figure 7). A similar trend has been shown in other studies and reflects, among other factors, the tendency for young women to have older men as partners (Glynn et al., 2001, Buve et al., 2002). The peak for HCV positive females and males are virtually identical to those of HIV (Figure 8). A possible explanation of this phenomenon is that HIV and HCV are associated because of common risk factors which define their age and gender distribution. Since HIV is a heterosexually transmitted disease in this population (Buve et al., 2002), it seems likely that HCV shares the same route of transmission.

The rate of sexual transmission of HCV is generally regarded to be minimal (Wyld et al., 1997) and the prevalence of HCV in sexual partners of HCV carriers is low (Bouvet, 2005, Fields et al., 2001, Heintges and Wands, 1997) but significantly higher than that of the general population (Wejstal, 1999). On the other hand, HCV seropositivity *does* correlate with the number of sexual partners, the presence of sexually transmitted infections, total number of sexual intercourse (Tahan et al., 2005), length of sexual exposure (Tong et al., 1995) and employment in the sex industry (Sulkowski and Thomas, 2003, Fields et al., 2001, Wejstal, 1999). Furthermore, the risk of HCV in female partners of HCV-infected men is reported to be increased 3.7 fold (Hisada et al., 2000). These conflicting findings make it difficult to assess the extent of the sexual transmission of HCV. Furthermore, studies on sexual transmission are confounded by factors such as the use of intravenous drugs, number of sexual partners and syphilis and HIV seropositivity (Sulkowski and Thomas, 2003, Winnock et al., 2004, Wejstal, 1999, Hughes and Mahy, 1998).

This study provides evidence that HCV is sexually transmitted. Firstly, the high rates of HCV-HIV co-infection suggest that the risk factors responsible for HIV transmission favour HCV transmission as well. Secondly, both HIV and HCV have a female preponderance in this region. It is likely that the factor(s) responsible for the female preponderance of HIV apply to HCV as well. Thirdly, the age-gender distribution of HCV and HIV seropositivity are remarkably similar suggesting that HIV and HCV are associated because of common risk factors which define their age and gender distribution. Since HIV is predominantly a heterosexually transmitted infection in this region, a similar route of transmission for HCV would explain these phenomena. This contrasts with the

findings of other studies which regard sexual transmission of HCV to be relatively inefficient and not as important in the epidemiology of HCV as IVDU, blood transfusion and other parenteral routes (Wyld et al., 1997, Tor et al., 1990, Conry-Cantilena et al., 1996, Gordon et al., 1992).

The discrepancy between our study and those which regard the sexual transmission of HCV as inefficient, may be explained by the controversial postulate that the sexual transmission of HCV is enhanced in co-infected patients (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Dieterich, 2004, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Winnock et al., 2004, Sulkowski and Thomas, 2003, Thomas, 2002, Eyster et al., 1991). It has been suggested that the sexual transmission of HCV is enhanced in co-infected patients due to higher HCV viral loads (Sulkowski and Thomas, 2003), in analogy to the documented increase in peri-natal transmission of HCV in co-infected mothers (Mohsen et al., 2002, Sherman, 2004, Thomas, 2002, Winnock et al., 2004). This is supported by the increased prevalence of anti-HCV antibodies in partners of co-infected patients (9.1%) compared to the partners of HCV mono-infected patients (as low as 2.6%) (Bonacini and Puoti, 2000, Gonzalez and Talal, 2003, Dieterich, 2004, Khalili and Behm, 2002, Maier and Wu, 2002, Mohsen et al., 2002, Winnock et al., 2004).

In contrast to the hypothesis of sexual transmission of HCV, the increased prevalence of HCV in HIV positive individuals in previous studies has been attributed to the use of intravenous drugs, which is regarded as the common risk factor in these individuals

(Sherman et al., 2002, Armstrong et al., 2006). In our study population, HIV is transmitted predominantly by the sexual route (Simon et al., 2006). The use of intravenous drugs in this population is rare as evidenced by epidemiological studies (Parry et al., 2005). Furthermore, there was no record of intravenous drug abuse in the clinical notes of any of the patients in this study. We therefore postulate that the common risk factor in this population is sexual transmission rather than intravenous drug use and that HCV, like HIV, is predominantly a sexually transmitted infection in this population. The increased sexual transmissibility of HCV in HIV positive individuals (Eyster et al., 1991, Filippini et al., 2001) is the most likely explanation for the high prevalence of HCV among HIV positive patients in our study. This has important public health implications, because it suggests that HIV is fuelling the HCV epidemic and that HCV prevalence may rise to unprecedented levels.

In this study, patients with HIV-HCV co-infection had a higher mortality than HIV mono-infected patients (8.3 vs. 21 %). This difference was statistically significant. Increased mortality in co-infected patients has been demonstrated previously (Monga et al., 2001, Greub et al., 2000, Anderson et al., 2004, Braitstein et al., 2005, Klein et al., 2003). The increased risk of liver disease and hepato-toxicity, decreased use of anti-retroviral therapy, diminished response to HAART and the deleterious effects of intravenous drugs and alcohol have been suggested as possible reasons for the increased mortality in co-infected patients (Anderson et al., 2004, Bica et al., 2001, Braitstein et al., 2005, Greub et al., 2000, Monga et al., 2001, Rancinan et al., 2002, Rosenthal et al., 2003, Graham et al., 2001, Mohsen et al., 2002, Klein et al., 2003, Rockstroh et al., 2005, Rockstroh, 2006, Rossi et

al., 2002). An analysis of the causes of death (as documented in the death certificates) showed that the increase in mortality in this study was unrelated to HAART, intravenous drug use or alcohol and was due to an AIDS-defining condition rather than to liver disease. These findings are in contrast to other studies which suggest that the increased mortality in co-infected patients is due to liver disease and *not* advanced HIV disease (Monga et al., 2001, Bica et al., 2001, Rosenthal et al., 2003). Furthermore, the study population was naïve to anti-retroviral therapy, and there was no record of intravenous drug use in the clinical notes of any of the patients in this study. This eliminates anti-retroviral therapy and intravenous drugs as contributory factors to the increased mortality, unlike most other co-infection studies which fail to exclude these confounding factors.

It may be argued that HCV sero-positivity is merely a marker for more advanced HIV disease and immuno-suppression and that there is no cause-effect relationship between HCV sero-positivity and mortality. However, we found no association between HCV serostatus and markers of HIV disease progression (World Health Organization(WHO) staging (WHO, 2006), Centers for Disease Control (CDC) classification (Castro et al., 1992)) or between HCV serostatus and admission rates, length of hospital stay, Karnofsky Score (O'Dell et al., 1995) and whether the patient was ambulant or not. This suggests that the association between HCV and morbidity and mortality, as described in this study, is unlikely to be confounded by these factors. Also, there was no significant difference in mean age between HCV positive and negative individuals (36.3, 95% CI 33.7-38.8 and 34.8, 95% CI 33.2-36.5 years respectively, $p = 0.366$). This implies that HCV infection and outcomes are not merely associated by the accumulation of risk with age.

There was a non-significant trend towards mortality due to renal failure and a tendency for renal related problems in co-infected patients. There was a statistically significant association between HCV serostatus and abnormal urea and creatinine levels and between HCV seropositivity and hyponatremia, hyperkalemia and low bicarbonate levels. It is possible that the increased mortality associated with HCV sero-positivity may in part be explained by renal insufficiency and renal failure.

The association between HCV and renal insufficiency was recently noted and the authors suggested chronic liver disease, intravenous drug use or other high risk behaviours as an explanation for the renal insufficiency in HCV positive patients (Franceschini et al., 2006). Renal disease has also been noted in HCV-HIV co-infected patients in another study, in which differences in the patients' ages was offered as an explanation (Tedaldi et al., 2003).

However, the patients in our study were naïve to anti-retroviral therapy and did not have evidence of intravenous drug use or stigmata of chronic liver disease. There was also no statistically significant difference in the mean age between HCV positive and HCV negative patients in this study. We therefore postulate that this unexpected association between co-infection and renal abnormalities is more likely to be due to other mechanisms.

Several mechanisms may explain renal involvement in HCV-HIV co-infection (di Belgiojoso et al., 2002, Cheng et al., 1999). Hepatorenal syndrome, mixed

cryoglobulinemia, fibrillary and immunoactoid glomerulonephritis and other glomerulopathies may be the mechanism of renal involvement in HCV infection. HIV associated nephropathy (HIVAN), immune complex glomerulonephritis, and various other forms of glomerular diseases are the underlying mechanism in HIV infection. These mechanisms may work in concert or even synergistically in co-infection.

A previous paper from our center has reported the lack of association between HCV and membranoproliferative glomerulonephritis (Madala et al., 2003). However, their study specifically excluded HIV positive patients and therefore the findings cannot be extrapolated to HIV co-infected patients. Patients with HCV-HIV co-infection have more rapid progression of liver disease than HCV mono-infected patients (Graham et al., 2001, Mohsen et al., 2003). It is therefore not unlikely that the immunosuppressive effects of HIV may also influence the progression of HCV related renal disease. An alternate hypothesis is that a novel pathophysiological mechanism may exist. It is not known which of these mechanisms accounts for the renal involvement in HCV-HIV co-infected patients in our study.

We noted that patients with HCV co-infection, when compared to HIV mono-infected patients, were twice as likely to have hepatomegaly but not other signs of chronic liver disease. None of the variables that were found to be significantly different in HCV negative and positive patients were significantly different between HBV negative and positive individuals, suggesting that HBV is not a confounding factor in this study.

Several limitations to the study design and methods are acknowledged and have been addressed. It may be argued that the difference in outcome between HCV positive and negative patients could have been better assessed within a longitudinal design rather than a cross-sectional study. The retrospective design of this study also makes the timing of infection difficult since it was not possible to tell which came first: HIV or HCV infection. The outcomes in HCV infection, however, would have required long follow-up periods in a longitudinal study design. A longitudinal study would also depend on the drop-out and mortality rate and HCV-negative patients remaining negative during follow-up (Tedaldi et al., 2003). Patients may also commence HAART at varying time points within the follow-up period, making comparisons of outcomes difficult. This study was conducted at an opportune time, prior to the rollout of anti-retrovirals within this region. This allowed evaluation of outcomes without the confounding effects of anti-retroviral therapy. This study has also revealed certain outcomes, such as renal dysfunction, which may be the focus of future cohort studies.

There may be sample bias, in that specimens selected from patients in whom an indication exists for an HIV test may have risk factors for HCV as well. This may have led to an overestimate of the prevalence of HCV by over-representing patients with these risk factors. On the other hand, not all samples that are received for HIV testing have a risk factor for HIV. HIV testing, for example, may be performed routinely preoperatively and in renal transplant and antenatal clinics, whether a risk factor exists for HIV or not. Also, samples for this study were from a central laboratory in the region that received *all* specimens for HIV testing from the sentinel study sites at the time the survey was

conducted. This laboratory is the reference virology laboratory in the province and provides diagnostic virology services to 90% of the population in this region. The results can therefore be more readily extrapolated to the general population served by the laboratory. This study provides an estimate that is far more accurate than previous studies which used high risk cohorts or low-risk blood donors and therefore either over-estimated or under-estimated the prevalence, respectively (Madhava et al., 2002, Soni et al., 1993).

It may be argued that limiting the study to five sentinel sites may have also introduced sample bias. Limiting the study to the sentinel sites was based on the human resources available to obtain and record clinical notes. Limiting the data collection to two medically qualified practitioners reduced potential inter-observer bias. Limiting the number of sites to academic centres where good clinical records are maintained also reduces the bias that might be introduced due to variations in the quality of note-keeping. The selected sites are, nevertheless, referral hospitals within the region and receive a representative spectrum of patients from all regions of the province. The samples were from a wide range of inpatients and outpatients and included medical, surgical, obstetric and gynaecological patients.

The HCV ELISA results were not confirmed by Polymerase Chain Reaction (PCR) or Recombinant Immunoblot Assay (RIBA). False negative HCV ELISAs may occur in the window period, in patients who are severely immuno-compromised or with mixed cryoglobulinemia (Richter, 2002, Colin et al., 2001, Pawlotsky, 1999, Pawlotsky, 2002, Patel et al., 2006). False positives also occur, even with third generation HCV ELISAs,

particularly in patients with low risk of HCV infection (Alter et al., 2003, Erensoy, 2001). False positives occur due to cross –reactivity (Pawlotsky, 1999) or nonspecific binding of serum immunoglobulins to contaminants in the ELISA kit (Erensoy, 2001). Hypergammaglobulinemia, liver disease, auto-immune disease and HIV infection may also cause false positive results (Colin et al., 2001).

Positive HCV ELISAs must be confirmed by a supplemental test such as RIBA or nucleic acid detection methods such as PCR (Erensoy, 2001, Alter et al., 2003, Patel et al., 2006). We feel, however, that although confirmatory testing is obligatory for the clinical diagnosis in individual patients, that the relatively small number of false positives do not significantly affect the interpretation of epidemiological studies. The Abbott AxSym HCV version 3.0 has a specificity of 99.6% and sensitivity of 100% respectively (Package insert - ABBOTT AxSYM® HCV v 3.0, Abbott Laboratories, Abbott Park, IL, USA). If all false positives were excluded by a confirmatory test, the true prevalence would be 6.04%. This calculation is based on the stated sensitivity and specificity (see calculations in Appendix F). The 95% confidence interval of the estimated prevalence of HCV is 5.3 – 7.5% (Table 2). The true prevalence, measured by a test with no false positives, therefore falls within the confidence interval of the actual prevalence estimated without confirmatory testing. Confirming the false positives would therefore not have improved our prevalence estimates significantly. Approximately 2.8 - 5% of patients who are sero-negative will have detectable HCV RNA, and 5-10% of HCV seropositive patients will have undetectable HCV RNA. Therefore misclassification of HCV positive vs. HCV negative groups is minimal (Braitstein et al., 2005, Tedaldi et al., 2003). Numerous studies in co-

infected patients have used seropositivity on a single ELISA (without PCR or RIBA) to define HCV infection (Anderson et al., 2004, Braitstein et al., 2005, Law et al., 2004, Gebo et al., 2003, Cacoub et al., 2001, Rosenthal et al., 2003, Tedaldi et al., 2003, Rockstroh et al., 2005, Lichterfeld et al., 2005, Staples et al., 1999, Bica et al., 2001).

It was also considered that RIBA is not routinely performed in this laboratory and is costly and time-consuming. Furthermore, RIBA is known to yield indeterminate or even negative results and is particularly ineffective in resolving weakly positive EIAs (Erensoy, 2001, Richter, 2002). PCR could not be performed optimally in the context of this study design. The sensitivity of PCR is affected by sample collection, transport and storage (Damen et al., 1998, Halfon et al., 1996, Jose et al., 2003, Wang et al., 1992). It has been recommended that serum be separated immediately upon clot formation, transported on ice, exposed to minimal freeze-thaw cycles (Halfon et al., 1996) and frozen immediately (Wang et al., 1992). These conditions could not be consistently maintained in this study since the samples were collected specifically for routine serology and not molecular analysis. The sample would usually be kept on the bench-top for prolonged periods, for example, prior to selection into the study. This is acceptable for serology, but not for PCR. We therefore did not consider PCR to be reliable in the context of this study design. PCR would have been useful in differentiating active from past infection (Erensoy, 2001, Richter, 2002). Approximately 15% of patients have acute HCV infection that is cleared without proceeding to chronicity (Marcellin, 1999, Fields et al., 2001). Although PCR would have identified such patients in our study, a prospective study using specimens collected specifically for that purpose would be a more appropriate means to achieve this.

Information on $CD4^+$ cell counts and HIV viral loads were lacking, since these tests were not standard of care at the time of the study. Differences in $CD4^+$ cell counts and HIV viral loads might explain differences in survival (Fields et al., 2001), and may be a confounding factor in this study . In the context of this study, however, an endpoint indicator of disease progression such as death or an AIDS defining condition is more useful than a measurement such as viral load and $CD4^+$cell counts taken at single time point. Viral load and $CD4^+$cell counts are inherently variable measurements, affected by intercurrent illnesses and other biological and technical factors (Bartlett and Gallant, 2005). While serial measurements are useful in monitoring of patients, measurement at a single time point in a cross-sectional study design is open to question. For these and practical reasons, we relied on CDC (Castro et al., 1992), WHO (WHO, 2006) and Karnofsky (O'Dell et al., 1995) scores to assess disease progression. These scores were inferred by the investigators using the available clinical information and were not recorded *per se* by the attending physician. Scoring depends on crude clinical information, which was readily available in the patients' charts. Relying on the investigators to calculate the scores standardized the scoring and limited inter-individual variability in assigning these scores.

The retrospective chart review is dependent on the quality and consistency of the clinical records. Records kept for clinical purposes may not necessarily fulfill the requirements of a clinical protocol. This study maintained a consistent quality of data by using a standardized clinical record form. This ensured that the necessary details were extracted from the clinical records as accurately and thoroughly as possible. The findings of hepatomegaly and increased ALT in HCV seropositive patients are well-known and expected features of

viral hepatitis (Monga et al., 2001) and therefore indicate that the study design was sufficiently sensitive to detect them. Caregivers may not have routinely enquired about or recorded scarification practices and the use of intravenous drugs in the clinical notes. Furthermore, since the latter may be (or may be perceived to be) a stigmatizing and illegal activity, responses may not reflect the true prevalence of intravenous drug use in this population. This limitation is inherent to the retrospective nature of this study and can only be addressed by conducting further research designed specifically to gather information about these risk factors.

CONCLUSIONS

This study has found a significantly higher prevalence of HCV among HIV infected patients as compared to HIV negative patients (13.4% vs. 1.73%) ($p < 0.001$, OR 8.8, 95% CI 5.4 -14.3). We postulate that HCV is sexually transmitted based on the observation that the age and gender distribution of HCV and HIV are similar. We also observed that HCV co-infection was associated with renal insufficiency and higher mortality (8.3 vs. 21 %, $p < 0.02$, OR = 3, 95% CI: 1.12 to 8.00). There was a non-significant trend towards mortality due to renal failure. We noted that patients with HCV co-infection, when compared to HIV mono-infected patients, were twice as likely to have hepatomegaly but not other signs of chronic liver disease. These findings are not confounded by differences in age, stage of HIV disease or HBV surface antigen carrier status.

The findings of this study have important implications for the diagnosis and management of co-infected patients. In view of the high prevalence of HCV in this population, screening of patients for HCV prior to commencing HAART or other potentially hepatotoxic agents should be considered in HIV positive patients. HCV should similarly be excluded in HIV infected patient who have elevated liver enzymes and abnormal renal function. Preventative measures against the sexual HIV transmission of HIV, such as barrier contraceptives, are necessary to prevent the transmission of HCV as well and should be reinforced, particularly in sero-discordant couples. There is a need for periodic surveillance to monitor trends in HCV seroprevalence (Hughes and Mahy, 1998). The

National HIV and Syphilis Antenatal seroprevalence surveys (Anonymous, 2006) provides a useful opportunity to conduct such studies.

Finally, the surprising observation of renal abnormalities in co-infected patients is of great interest and has direct management implications. Our study demonstrates that renal abnormalities may exist in co-infected patients even in the absence of chronic liver disease or risk factors such as the use of intravenous drugs or antiretroviral therapy. Screening for HCV should be part of the routine workup of patients who are HIV positive who demonstrate renal insufficiency. Certain anti-retrovirals such as indinavir and tenofovir are nephrotoxic (Roling et al., 2006). This needs to be taken into consideration in the HCV-HIV co-infected patient who may require closer monitoring of renal function when treated with these or other nephrotoxic drugs.

Although HCV infection does occur in the absence of HIV, the natural history and prognosis of HCV infection is markedly different in HIV infected patients. The effect of co-infection on renal function, as demonstrated in this study, is clear evidence of this. Based on the results of this study we propose that HCV infection be regarded as a sexually transmitted, opportunistic infection (Graham et al., 2001) in HIV and that co-infection is a distinct entity from HIV and HCV mono-infection (Gonzalez and Talal, 2003, Winnock et al., 2004). This viewpoint necessitates a different approach to screening, diagnosis, treatment and prevention.

APPENDICES

APPENDIX A: Clinical Record Form

CONFIDENTIAL

HEPATITIS C AND HIV STUDY DEPARTMENT OF VIROLOGY, UKZN

PATIENT INFORMATION

HOSPITAL NUMBER:_____**HOSPITAL**_____

AGE(YEARS)_____**SEX**____

CLINICAL INFORMATION

ADMITTED(Y/N)_____DATES: ADMISSION_____DISCHARGE_____

DIAGNOSIS ON ADMISSION_____

DIAGNOSIS ON DISCHARGE_____

HAS PATIENT DIED?_____DATE OF DEATH_____

CAUSE OF DEATH ON AUTOPSY(IF KNOWN) _____

IS PATIENT AMBULANT(Y/N)_____

PRESENTING COMPLAINTS AND DURATION OF ILLNESS

_____

_____

_____

_____

PAST MEDICAL AND SURGICAL HISTORY

_____

PREVIOUS ADMISSION HISTORY

_____

_____

HISTORY OF LIVER DISEASE

_____

DRUG AND MEDICATION HISTORY

_____

_____

ANTIRETROVIRALS(Y or N, name and duration)

_____

VACCINATION AGAINST HEPATITIS(Y or N, HBV or HAV)_____

HISTORY OF TRANSFUSION/TATTOOS/SCARIFICATION/TRANSPLANT

_____

ALCOHOL/RECREATIONAL DRUGS(Y or N, TYPE, DURATION)

_____

SPECIFIC SYMPTOMS(Y OR N, DETAILS IF Y)

JAUNDICE_____PRURITIS_____

HAEMATEMESIS/MALENA_____

ABDOMINAL SYMPTOMS_____ANOREXIA_____

DARK URINE/PALE STOOLS_____

OTHER:_____

_____

_____

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HEPATITIS C AND HIV STUDY DEPARTMENT OF VIROLOGY, UKZN

HOSPITAL NUMBER _____

EXAMINATION

JAUNDICE_____PALLOR_____FEVER(T°)_____EDEMA_____
WASTING(Y OR N)_____WEIGHT(KG)_____
HYDRATION_____RASH_____
SYSTEMIC FINDINGS
_____
_____
NEUROLOGICAL SIGNS_____GCS E____M____V____
HEPATOSPLENOMEGALY_____
LIVER FAILURE[1]_____
CHRONIC LIVER DISEASE[2]_____
CHILD-PUGH CATEGORY [3]_____
WHO CATEGORY[4]_____CDC CATEGORY[5]_____
CDC DIAGNOSIS[5]_____
KARNOFSKY SCORE[6]_____

LABORATORY DATA(INDICATE NA IF NOT AVAILABLE OR APPLICABLE)

U&E_____
ALBUMIN_____BILIRUBIN_____ AFP_____
ALK.PHOS_____ALT_____AST_____GGT_____
PI/INR_____PTT
HEPATITIS SEROLOGY_____
DATE OF FIRST POSITIVE HIV TEST_____
DATE OF LAST NEGATIVE HIV TEST_____
VIRAL LOAD_____
CD4_____CD8_____
TOTAL WCC_____HB_____PLATELETS_____
NEUTROPHILS_____LYMPHOCYTES_____

OTHER INVESTIGATIONS(IF AVAILABLE OR APPLICABLE)

ULTRASOUND_____
_____
XRAYS_____
_____
CT_____
OTHER INVESTIGATIONS
_____
_____

SIGN

DATE

APPENDIX B: WHO Staging (WHO, 2006)

Clinical stage I

1. Asymptomatic
2. Persistent generalized lymphadenopathy

Performance scale 1: asymptomatic, normal activity

Clinical stage II

3. Weight loss, <10% of body weight
4. Minor mucocutaneous manifestations (seborrheic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)
5. Herpes zoster within the last five years
6. Recurrent upper respiratory tract infections (i.e. bacterial sinusitis)

And/or performance scale 2: symptomatic, normal activity

Clinical stage III

7. Weight loss, >10% of body weight
8. Unexplained chronic diarrhoea, >1 month
9. Unexplained prolonged fever (intermittent or constant), >1 month
10. Oral candidiasis (thrush)
11. Oral hairy leukoplakia
12. Pulmonary tuberculosis within the past year
13. Severe bacterial infections (i.e. pneumonia, pyomyositis)

And/or performance scale 3: bedridden <50% of the day during the last month

Clinical stage IV

14. HIV wasting syndrome, as defined by the Centers for Disease Control and Prevention[a]
15. *Pneumocystis carinii* pneumonia
16. Toxoplasmosis of the brain
17. Cryptosporidiosis with diarrhoea >1 month
18. Cryptococcosis, extrapulmonary
19. Cytomegalovirus disease of an organ other than liver, spleen or lymph nodes
20. Herpes simplex virus infection, mucocutaneous >1 month, or visceral any duration
21. Progressive multifocal leukoencephalopathy
22. Any disseminated endemic mycosis (i.e. histoplasmosis, coccidioidomycosis)
23. Candidiasis of the oesophagus, trachea, bronchi or lungs
24. Atypical mycobacteriosis, disseminated
25. Non-typhoid *Salmonella* septicaemia
26. Extrapulmonary tuberculosis
27. Lymphoma
28. Kaposi's sarcoma
29. HIV encephalopathy, as defined by the Centers for Disease Control and Prevention[b].

And/or performance scale 4: bedridden >50% of the day during the last month

Note: both definitive and presumptive diagnoses are acceptable.

a. HIV wasting syndrome: weight loss of >10% of body weight, plus either unexplained chronic diarrhoea
   (>1 month) or chronic weakness and unexplained prolonged fever (>1 month).
b. HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings.

APPENDIX C: CDC Classification(Castro et al., 1992)

The clinical categories of HIV infection are defined as follows:

Category A

Category A consists of one or more of the conditions listed below in an adolescent or adult (greater than or equal to 13 years) with documented HIV infection. Conditions listed in Categories B and C must not have occurred.

- Asymptomatic HIV infection
- Persistent generalized lymphadenopathy
- Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Category B consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria: a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples of conditions in clinical Category B include, but are not limited to:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting greater than 1 month
- Hairy leukoplakia, oral
- Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess
- Peripheral neuropathy

For classification purposes, Category B conditions take precedence over those in Category A. For example, someone previously treated for oral or persistent vaginal candidiasis (and who has not developed a Category C disease) but who is now asymptomatic should be classified in clinical Category B.

Category C

Category C includes the clinical conditions listed in the AIDS surveillance case definition. For classification purposes, once a Category C condition has occurred, the person will remain in Category C.

Conditions included in the 1993 AIDS surveillance case definition

- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain

- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV

Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

APPENDIX D: Karnofsky Score (O'Dell et al., 1995)

100: Normal, no complaints or evidence of disease

90: Able to perform normal activity; minor signs and symptoms of disease

80: Able to perform normal activity with effort; some signs and symptoms of disease

70: Cares for self, unable to perform normal activity or to do active work

60: Requires occasional assistance but is able to care for most of own needs

50: Requires considerable assistance and frequent medical care

40: Requires special care and assistance; disabled

30: Hospitalization indicated, although death not imminent; severely disabled

20: Hospitalization necessary; active supportive treatment required, very sick

10: Fatal processes progressing rapidly; moribund

0: Dead

APPENDIX E: Signs and Symptoms of Liver Disease

SIGNS AND SYMPTOMS OF LIVER FAILURE

- Change in level of consciousness(confusion, coma or subtle perceptual abnormalities)
- Other neurological manifestations(seizures, asterixis, hyper-reflexia)
- Foetor hepaticus
- bleeding tendencies
- hypoglycemia

SIGNS AND SYMPTOMS OF CIRRHOSIS

- weight loss
- chronic malaise
- impotence, loss of libido, menstrual irregularities
- oedema and/or ascites
- distended abdominal veins
- spider naevi and palmar erythema
- gynaecomastia
- testicular atrophy
- hepatomegaly
- splenomegaly
- bleeding tendencies

- leuconychia
- Dupuytren’s contracture
- clubbing
- abnormal pigmentation
- jaundice
- complications of portal hypertension(oesophageal varices, hematemesis, melena)

APPENDIX F: The influence of test validity on prevalence estimates

		GOLD STANDARD		
TEST		+	-	TOTAL
	+	TP	FP	*124*
	-	0	1813	*1813*
	TOTAL			1937

T = total, TP = true positives, TN = true negatives, FP = false positives

FN = False Negatives, P = study positives

By definition:

True Prevalence = TP/T, Study Prevalence = P/T, P = TP + FP

Sensitivity = Se = [TP/(TP + FN)] Specificity = Sp = [TN/(TN + FP)]

(Bland, 2000)

In this study :

Total = 1937, Study Positives = 124 ∴ Study Negatives = 1937 – 124 = 1813

The kit package insert states the sensitivity and specificity of the Abbott AxSym HCV Version 3.0 to be 100% and 99.6%, respectively (Abbott Laboratories, Abbott Park, IL, USA)

∴ Sensitivity = Se = [TP/(TP + FN)] ~ 1 ∴ FN = 0 and TN = 1813

and specificity = Sp = [TN/(TN + FP)] ~ 0.996

→ TN/(TN + FP) = 1813/(1813 + FP) = 0.996

→ 1813 + FP = 1813/0.996 ~ 1820

∴ FP = 1820 – 1813 = 7

P = TP + FP ∴ TP = 124 – 7 = 117

∴ True prevalence = TP / T = 117/1937 = 6.04 %.

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