Complication of managing HIV in relation to HCV in persons seen for routine clinical care in Italy

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Declaration

I, Milensu Shanyinde confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Hepatitis C virus (HCV) is a global public health concern compared to communicable diseases such as HIV and shown to be associated with faster disease progression in PLWH. The introduction of highly effective direct acting antivirals (DAAs) in 2015 revolutionised HCV therapy. In 2015 WHO called for a global strategy in HCV elimination by 2030. Whilst DAA is recommended to all, HIV/HCV coinfected individuals may require special consideration.

My initial research focused on the role of HCV as an effect modifier for the association between alcohol consumption and risk of severe liver disease (SLD) and the association between HCV and risk of specific ARV drug discontinuation in PLWH. This shifted to, real-world estimate of the presence of late HCV presentation and its risk of all-cause mortality. I evaluated regional differences in rate of accessing care with respect to HCV-RNA testing, DAA uptake and achieving sustained virological response (SVR).

The data analysis involved two multicentre observational prospective cohorts enrolling PLWH with/without HCV in routine care across Italy.

There was no evidence that HCV was an effect measure modifier for the relationship between alcohol consumption and risk of SLD. The rate of ARV discontinuation was similar between HIV/HCV coinfected and HIV monoinfected participants, except of darunavir/r for which the risk of discontinuation was higher in the coinfected. There was weak evidence for an association between late HCV presentation and risk of all-cause mortality. Among people enrolled between 2015 and 2018 in Icona, 90% were HCV-RNA tested and among those initiating DAA treatment, 88% achieved SVR. HIV/HCV coinfected individuals receiving care in the South had 50% (95%CI:34%–55%; $p<0.001$) reduced probability of initiating DAA compared to those receiving care in the North and Central regions. Overall, the results indicate that Italy is on course towards meeting the WHO HCV elimination goals in PLWH.

IMPACT STATEMENT

The impact of my thesis is partitioned between my earlier research relating to the role of HCV to predict clinical outcomes and the latter research relating to the impact of DAA as well as remaining barriers in the HCV CoC pathway. One key question was to evaluate whether HCV infection could be an effect measure modifier for alcohol consumption in relation to the risk of developing severe liver disease (SLD). This question is less relevant now although remains important for the management of few PLWH in whom HCV could not be eradicated. Additionally, it remains key for public health authorities to assess whether alcohol consumption acts as a barrier in the HCV CoC pathway.

Alcohol data has been collected in the Icona database but there was need to consolidate the data. My work led to the study team to re-evaluate the modality of data collection of alcohol use by introducing a modified and simplified standardised questionnaire. My work is also likely to benefit researchers leading other HIV cohorts in understanding methodological issues when assessing alcohol consumption.

The data revealed that Italy is on course towards HCV elimination in the population of PLWH. However, my analysis also identified in a sample of newly diagnosed HIV individuals a non-negligible proportion of HIV/HCV coinfected individuals who remained undiagnosed for HCV until they developed advanced liver disease. The data highlighted the need for additional efforts to increase the frequency of HCV testing in PLWH regardless of their modality of HIV acquisition.

The data also revealed regional disparities in terms of access to HCV screening and uptake of HCV therapy and these may represent ongoing barriers towards HCV elimination by 2030.

These findings have important public health implications and they should be key for policy makers evaluating the deployment of more health care resources across the country.

Importantly, I have used well recognised national epidemiological data sets to perform data analysis for these real-world pertinent questions and developed handling a number of methodological challenges. Towards the end of my work, I used direct acyclic graphs (DAGs), a robust methodological tool to depict the underlying assumptions regarding the causal structure of the data. Although it is hard to establish causation using observational data, I tried to be intellectually honest and declared that this was the aim in some of my analyses. Thus, my thesis may benefit researchers within or outside my research area for academic teachings to demonstrate the application of such methods.

I participated in the Icona Think-Tank meetings engaging with clinicians and other statisticians giving input to upcoming projects using my statistical expertise. I disseminated my work through different means by presenting the data to the wider Icona/Hepaicona team in Italy through HIV/HCV coinfection internal meetings, at international conferences, authoring and coauthoring a number of peer reviewed publications relating to HIV/HCV coinfection. All these contributions highlight the importance of my research in the field of HIV/HCV coinfections in real-world setting during my time as a member the statistical team of the Icona Network group.

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To my daughters Funmilayo (my joy) and Ntanda (my premmie star), your unwavering patience as mummy worked on her thesis all the time. May you be inspired to never give up and achieve big goals, anything is possible my cuddle bugs.

LIST OF ABBREVIATIONS

Abbreviation	Description
3TC	Lamivudine
AASLD	American Association for study of liver diseases
Abbvie 3D	Paritaprevir/ombitasvir/ritonavir plus dasabuvir
ABC	Abacavir
AIDS	Aquired Immuno Deficiency Syndrome
AIFA	Agenzia Italiana del Farmaco (Italian Medicines Agency)
ALD	Advanced Liver Disease
ALT	Alanine Aminotransferase
AMACS	Athens Multicentre AIDS Cohort Study
ANRS	National Agency for AIDS Research
APRI	AST to Platelet Ratio Index
ARFI	Acoustic Radiation Force Impulse
ARH	Adjusted relative hazard
ARV	Antiretroviral
AST	Aspartate transaminase
ATV	Atazanavir
AUDIT	Alcohol disorders identification test
BCLC	The Barcelona-Clinic Liver Cancer
BMI	Body Mass Index
CAGE	Cut - Annoyed -Guilty-Eye
cART	Combination Antiretroviral therapy
CCC	Canadian Coinfection Cohort study
CCR5	Cross-Cohort Collaboration Consortium
CD4	cluster of differentiation 4
CDT	Carbohydrate deficient transferrin
CEASE	Control and Elimination within Australia of Hepatitis C From People Living With HIV
95% CI	95% Confidence Interval
COVID-19	Coronavirus disease 19
CRF	Case report form
CXCR4	C-X-C Motif Chemokine Receptor 4
CYP3A	Cytochrome P450, family 3, subfamily A
CYP450	Cytochrome P450
D4T	Stavudine
DAA	Direct acting antiviral
DAG	Direct acyclic graphs
DDI	Drug-Drug interactions
DLG	Dolutegravir
DNA	Deoxyribonucleic acid
DRV	Darunavir

Abbreviation	Description
DTG	Dolutegravir
EACS	European AIDS Clinical Society
EASL	European Association for the study of the liver
EFV	Efavirenz
ELV	Elvitegravir
ETR	Etravirine
EVG	Elvitegravir
FIB	Fibrosis
FTC	Emtricitabine
GEHEP	Group for the Study of Viral Hepatitis
HAART	Highly active antiretroviral therapy
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	human immunodeficiency virus
HR	hazard ratio
IASL	International Association for the Study of the Liver
IDU	Injecting drug use
IDV	Intravenous drug use
IFN	Interferon
INI	Integrase inhibitors
INSTI	Integrase strand transfer inhibitors
IQR	Interquartile range
I-ROBINS	Risk Of Bias In Non-randomised Studies - of Interventions
IRR	Incidence risk ratio
IU	International Unit
IWHOD	The International Workshop on HIV and Hepatitis Observational Databases
LPV	Lopinavir
LRD	Liver related death
MAR	Missing At Random
MELD	Model for End Stage Liver Disease
MI	Multiple Imputation
MSM	Men having sex with men
NFV	Nelfinavir
NHS	National Health Service
NIAID	National Institute of Allergy and Infectious Diseases
NIFN	National Institute of Food and Nutrition
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NOAH	The New Orleans Alcohol Use in HIV
NRTI	Nucleoside reverse transcriptase inhibitors
NS3	Non-structural -3

Abbreviation	Description
NS3A	Non-structural -3A
NS4A	Non-structural -4A
NS5A	Non-structural -5A
NVP	Nevipirine
OR	Odds Ratio
PEG	Pegylated
PI	Protease Inhibitor
PLWH	People Living with HIV
PrEP	pre-exposure prophylaxis)
PWID	People who inject drugs
PY	Person years
PYFU	Person year follow-up
RAL	Raltegravir
RBV	Ribavirin
RCT	Randomised Controlled Trial
RH	Relative Hazard
RPV	Rilpivirine
RR	Relative Risk
RRR	Relative Risk Ratios
RTV	Ritonavir
RVR	Rapid Virological response
SD	Standard Deviation
SLD	Severe Liver disease
SQV	Saquinavir
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SVR	Sustained Virological response
SVR12	Sustained Virological response 12 weeks
SVR24	Sustained Virological response 24 weeks
TAF	Tenofovir alafenamide
TDF	Tenofovir disoproxil fumarate
TOXPC	Toxicity Patient Choice
UNAIDS	United Nations AIDS
WHO	World Health Organisation
ZDV	Zidovudine

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CHAPTER 1

1 INTRODUCTION

People living with human immunodeficiency virus (PLWH) may present or become infected with other chronic diseases that may be detrimental to the progression of human immunodeficiency virus (HIV) disease or challenging to manage. The simultaneous presence of more than one infectious disease in one person is termed as coinfection. Coinfection with hepatitis (a virus causing inflammation of the liver) is particularly important as this leads to higher burden of morbidity and mortality. They are five types of hepatitis viruses (A, B, C, D and E). Briefly, hepatitis (A and E) are obtained from eating and drinking contaminated food and water [13]. Hepatitis (B, C and D) are obtained from infected bodily fluids[13]. This thesis will focus on chronic hepatitis C virus infection in PLWH termed as HIV/HCV coinfection. In particular, the thesis focuses on HIV/HCV coinfection in Italy as it has one of the highest HCV prevalence in Europe: six percent overall. This high prevalence is mostly due to an increase in HCV transmission from transfusion with infected blood and unsafe medical procedures pre 1980s.

The overall aim of the thesis is to investigate a series of related research questions concerning the impact of HIV/HCV coinfection on specific adverse outcomes in PLWH in Italy, the impact of late HCV diagnosis on prognosis among HIV/HCV coinfected individuals, and the continuum (or cascade) of care (CoC) for HCV among PLWH.

First, the thesis investigates the role of HCV infection on the relationship between alcohol consumption and risk of severe liver disease (SLD) (potentially caused by HCV infection) among PLWH. Lifestyle factors can potentially play a crucial role in affecting treatment outcomes that can lead to poor prognosis of HIV/HCV coinfection. PLWH are now living longer due to improved HIV therapy and this has resulted in the emergence of liver disease, which is now a major public health concern. Alcohol and HCV are both common risk factors for liver disease with

greatest risk in HIV/HCV coinfected people. However, debate in the literature continues about the interaction between HCV and alcohol consumption in terms of risk of liver disease, and therefore this remains an important question to investigate.

Second, the role of HCV coinfection on discontinuation of specific HIV antiretroviral (ARV) drugs was studied. Hepatitis C virus affects the liver, which is where most drugs are metabolised. Therefore, presence of liver damage may compromise this process and thus lead to difficulties or toxicities with ARV treatment which could potentially compromise the effectiveness of the HIV therapy.

Thirdly, the timing of HCV diagnosis is another challenge as being diagnosed late may impact the prognosis of HIV/HCV coinfected PLWH. It is well established that HIV treatment has advanced, and people are now living normal life expectancies if on antiretroviral treatment (ART). Since I started this thesis, treatment for HCV has also advanced with availability of very effective direct acting antivirals (DAAs) with cure rates of more than 95%, meaning prompt diagnosis and treatment of HCV are particularly relevant. Therefore, the thesis investigates the association of late diagnosis of HCV with subsequent risk of all-cause mortality and probability of starting HCV treatment in HIV/HCV coinfected individuals.

Finally, this thesis evaluates the HCV CoC pathway among HIV/HCV coinfected individuals in the DAA era of effective HCV therapy. This era of DAAs relates to the revolutionised treatment of HCV with newer, safer, and more tolerable effective drugs to treat HCV. The data analysis for this thesis objective was post introduction of DAAs and involved investigation of specific outcomes along the HIV/HCV CoC pathway including; HCV testing, initiation of DAAs HCV cure and the impact of geographic region within Italy on these outcomes. This addressed the challenges of potential regional disparities across Italy in terms of management of HIV/HCV coinfection. This is important in ensuring optimal use of new effective treatment and tackling the eradication of HCV infections.

My research questions were investigated using HIV cohorts established from the Foundation Icona Network of individuals seen as part of routine clinical care in a number of infectious disease units in Italy. All clinical questions investigated in this thesis use two data sources of cohorts of HIV-positive individuals. The first is the Italian Cohort of Naïve to Antiretroviral Foundation study (Icona), which started in 1997 in which approximately 30% of participants are coinfected with HIV/HCV. The second is a cohort of HIV/HCV coinfected individuals (Hepatitis Icona - Hepaicona), which started in 2013; the cohort was started with the specific aim of studying access and response to DAAs in the HIV/HCV coinfected population.

Prior to my thesis, some of the previous research in Icona focused on questions addressing impact of HCV disease progression on HIV outcomes following combination antiretroviral treatment (cART) initiation, such as a virological response, or the risk of non-AIDS events. Additionally, the research team previously looked at the prognostic value of predicting liver related outcomes and mortality using a measure of fibrosis – that is the extent of scarring of the liver. Most of the research questions before 2015 were addressed prior to the introduction of DAAs

Therefore, my research questions were developed considering findings from previous Icona work and remaining gaps in knowledge. At the start of my PhD, my research questions were focused on assessing the impact of HIV/HCV coinfection on clinical outcomes among PLWH. Therefore, earlier chapters 4 and 5 addressed questions relating to role of HCV on clinical outcomes such as risk of SLD and the association of alcohol with SLD, and risk of discontinuation of specific ARV drugs.

With evidence of the effectiveness of DAAs, the diagnosis, management and treatment of HCV in PLWH became particularly relevant. As the World Health Organisation (WHO) has deemed HCV as a global health concern, investigation of these issues using real world data is essential, to highlight challenges that impact on the prognosis of HIV/HCV coinfection. In chapters 6 and 7 I addressed

questions related to the diagnosis and management of HIV/HCV co-infection, and specific potential barriers in the HCV CoC pathway.

The outline of the thesis is as follows. The first three chapters present a general introduction to HIV, HCV and HIV/HCV coinfection, and consideration of the rationale for my thesis objectives (chapter 1) data and study methodology (chapter 2) and the prevalence of HCV and participant characteristics (chapter 3). These are followed by results chapters (chapters 4 to 7). The first two results chapters (4 and 5) are analyses including both HIV mono-infected and HIV/HCV coinfected individuals. Chapter 4 assesses the role of alcohol consumption on risk of SLD and whether this association is exacerbated by infection with HCV. Chapter 5 assesses the role of HCV in discontinuation of specific ARV drugs for any reason in the era of modern ARV drugs.

The second two results chapters (6 and 7) focus on late HCV diagnosis and part of the CoC pathway of HIV/HCV coinfection and so include only HIV/HCV coinfected individuals. Chapter 6 investigates the potential impact of late HCV diagnosis on all-cause mortality and starting HCV therapy. Chapter 7 develops and evaluates the HCV CoC in HIV/HCV coinfected individuals in the DAA era and assesses regional differences. Finally, chapter 8 brings the results together and discusses overall conclusions and clinical and public health implications of my findings. The appendix includes publications or posters arising from the thesis and snapshots of the electronic case report forms (eCRF).

With exception of chapters 1, 2 and 8, all results chapters have broadly the following structure; introduction (incudes aims and specific objectives), literature review (specific to the research question addressed in the chapter), methods (specific statistical methodology), results, discussion, strengths and limitations and conclusions.

1.1 Summary outline of each chapter

Chapter 1: Introduction/background to HIV, HCV and HIV/HCV coinfection
In this chapter, I present my thesis aims and structure, and give a background on HIV, HCV and HIV/HCV coinfection diseases. The chapter includes the history of HIV and HCV diseases and global epidemics, but specifically focuses on the Italian epidemics. I give an overview of the natural history and current treatment available for both HIV and HCV. I also give an overview of the impact of HIV infection on HCV disease and vice versa, as well as current challenges of managing HIV/HCV coinfection, and present the rationale for my thesis objectives. Although there is a general literature search included in chapter 1, I have included a detailed literature review giving a background to the specific thesis research questions in the introduction section of each of the results chapters (4 to 7).

Chapter 2: Data and Methodology
In this chapter, I give detailed information on the methodology of the two cohort studies used in this thesis, especially those methods related to data collection. Most of the subsequent analyses carried out in this thesis are prospective in nature, involving a sample of PLWH with or without HCV infection. The analyses use data extracted from the cohorts of PLWH enrolled in Icona and Hepaicona cohorts, with inclusion criteria specific to the research question being addressed, which are detailed in the relevant results chapters. This chapter also describes the statistical methods used in the thesis; however more specific statistical methodology used in each chapter is described as relevant in those chapters.

Chapter 3: Prevalence of HCV in Icona at enrolment and characteristics of Icona and Hepaicona study participants according to HCV related factors
The overall aim of this chapter was to describe the prevalence of HCV at study enrolment and describe participant characteristics of the study populations of Icona and Hepaicona up to 30th of June 2016. This date corresponds to the data lock date for the versions of the databases that were used for the first two of the results chapters, while the final two results chapters used data locked on 31st January

2018. I present the number of participants enrolled in the cohorts over time and the prevalence of HCV and describe participants' characteristics at the time of enrolment in terms of demographics, HIV related, lifestyle and socio-economic factors stratified by HCV infection status. In HIV/HCV coinfected individuals, participant characteristics are presented stratified by stage of liver disease. This helps to illustrate the differences between PLWH with and without HCV infection. I describe rates of HCV seroconversion over follow-up in the Icona cohort. In addition, I also identified individuals under active follow-up and naïve to DAAs and described the main characteristics.

The purpose was to set the scene for the analyses presented in the subsequent chapters, which are based on subsets of these populations purposely extracted to address the specific research questions. In these subsequent chapters, the sample size varied due to both specific inclusion/exclusion criteria date of data lock for the analysis.

Chapter 4: What is the role of HCV coinfection on the association between alcohol and liver disease in people living with HIV?

The overall aim of this chapter was to investigate whether HCV was an effect measure modifier for the relationship between alcohol consumption and risk of SLD. I included PLWH from the Icona and Hepaicona cohorts with or without HCV infection enrolled between 1st January 2002 and 30th of June 2016. Individuals had to be free from SLD at enrolment to be included in this analysis.

The research questions relate to a routinely collected, physician-documented measure of alcohol consumption in this population. Firstly, I mapped physicians' assessments of patients' alcohol consumption reported on the electronic case report form to those used in national drinking guidelines from the National Institute for Food and Nutrition (NIFN) in Italy. This has never been done before and this was helpful in quantifying the usefulness of data collected relating to alcohol consumption, data that is not routinely collected in most of the European clinical HIV cohorts. I then investigated the association of this physician assessment of alcohol consumption with the risk of SLD in PLWH with or without HCV. I then

investigated the interaction between HCV and alcohol consumption in terms of the SLD outcome. Finally, focussing on a key methodological question, I assessed the impact of missing data on alcohol consumption on the above mentioned association. This further highlights challenges in assessing this particular risk factor in PLWH with or without HCV.

I used the measure of alcohol consumption derived in this chapter in the subsequent analyses in this thesis. I developed the study hypothesis, devised the analysis plan and carried out the statistical analysis for this study. This work was initially accepted as a poster presentation at the 20th International Workshop on HIV observation databases (IWHOD) in Hungary (April 2016). Following this, I drafted a manuscript with the input of co-authors, which has been published in the BMC journal in 2019 [14].

Chapter 5: What is the role of HCV coinfection on discontinuation of specific antiretroviral drugs in people living with HIV?

The aim of this chapter was to assess the impact of HIV/HCV coinfection on stopping specific ARV drugs among HIV-positive people seen for care in Italy. Specifically, I assessed the association between HIV/HCV coinfection and risk of stopping cART for any reason, aiming to identify drugs which were more likely to be discontinued among HIV/HCV coinfected people. I then repeated the analyses among individuals who were HCVAb positive by assessing the association between HCV-RNA status and risk of stopping specific ARV drugs. This analysis is restricted to the Icona cohort data only and included people enrolled up to 30th of June 2016. Individuals who started cART defined as at least three ARV drugs of any drug class were included in the analysis.

The project was led by Prof Antonella D'Monforte and Dr Sabastiano Leone of the Icona Network, and myself. I had input into the study hypothesis, devised the analysis plan and carried out the statistical analysis for this study and contributed to the drafting of the manuscript which has been published in the European Clinical Microbiology of Infectious disease in 2018 [14]. As mentioned previously, at the time of the analyses, this was an important clinical question, however treatment for HCV has greatly advanced since then and this is no longer such a relevant an issue.

Chapter 6: What is the role of late HCV presentation on all-cause mortality and HCV treatment initiation among newly diagnosed coinfected HIV individuals seen for routine clinical care in Italy?

The aim of this chapter was to investigate the prevalence of late HCV presentation among PLWH and assess its association with risk of all-cause mortality and probability of starting HCV therapy.

This chapter is restricted to Icona cohort data only and included individuals enrolled up to 31st of January 2018. Individuals who were newly diagnosed with HIV within six months of the date of enrolment and subsequently had at least one month of follow-up in the cohort were included in the analysis.

In this chapter I used the consensus definition of late HCV presentation developed by a group of experts in viral hepatitis within the European Association for the study of the Liver (EASL) and HIV in the Europe Initiative in 2015 in people with newly diagnosed with HIV. Firstly, I estimated the prevalence of individuals tested for HCV among newly diagnosed HIV-positive people, this started to set the scene for chapter 7 when I looked at HCV care pathway. Secondly, I estimated the prevalence of late HCV presentation at entry into the Icona cohort and assessed whether sociodemographic, lifestyle and HIV related factors were associated with late HCV presentation. Thirdly, I evaluated prevalence of late HCV presentation over time among HIV/HCV coinfected individuals and finally assessed the impact of late HCV presentation on the risk of all-cause mortality and probability of starting HCV therapy.

The project was led by Prof Enrico Girardi of the Icona Network and myself. I had input into the study hypothesis, devised the analysis plan and carried out the statistical analysis for this study. A manuscript is in draft form for this analysis.

Chapter 7: Are there regional differences in terms of continuum of care for HCV among HIV/HCV coinfected individuals seen for routine clinical care in Italy since January 2015?

The aim of this chapter was to investigate the different stages of the HCV CoC in relation to specific outcomes (testing, treatment uptake, achieving sustained

virological response) and assess the role of geographic region on these outcomes In this chapter, both the Icona and Hepaicona cohorts' data were used. The analysis included individuals who were alive and in active follow-up from 1st January 2014 to 31st January 2018. Active follow-up defined as having their last clinical visit registered after 01st January 2014 (maximum 1 year prior to the baseline date of 01st January 2015).

In this chapter, I developed and evaluated the HCV CoC for Italy in HIV/HCV coinfected individuals seen for routine clinical care since January 2015. The stages considered as outcomes in the HCV CoC pathway were; testing for HCV, treatment uptake of DAAs among those testing positive and achieving SVR amongst those initiating treatment. I specifically focused on describing regional differences of health care (between northern, southern and central enrolling centres) and whether access to health care in terms of each of these outcomes varied by geographical region. I devised the project hypothesis and analysis plan and carried out the statistical analysis for this study. A paper is planned for this analysis.

Chapter 8: Implications and final concluding remarks

Each results chapter contains an introduction as well as literature review and extensive discussion of the findings, in the context of previous literature and limitations specific to the research question addressed. This final chapter brings together the findings from the results chapters. Both clinical and public health implications and implications on future research of the findings of the thesis are discussed and final conclusions are drawn.

Chapter 9: Appendix

Attachments of all submitted abstracts, posters, manuscripts and snapshots of eCRFs are included.

1.2 Human Immunodeficiency Virus

1.2.1 A brief overview

The Human immunodeficiency virus is a retrovirus that attacks and depletes specific cells in an individual's immune system. These specific cells are T-helper lymphocyte cells, known as CD4. Once the immune system is weakened this makes the person more susceptible to other illnesses [15]. There is no cure for HIV and once infected, the virus is present for life. When left untreated HIV results in progressively increased risk of opportunistic infections and certain cancers known as AIDS-defining conditions, and ultimately death [16]. The period from infection (if left untreated) to death varies but generally has a median time of 10.2 years (95% CI: 9.7–10.5 years) [17].

However, since the 1990s there has been effective treatment for HIV known as combination antiretroviral (cART) treatment which enables people to live long healthy lives and prevent HIV transmission. [15]

A person infected with the HIV virus can transmit the virus via bodily fluids, in particular through sexual contact [15]. After HIV infection not everyone will have symptoms and the only way of knowing the infection status is to get tested [15]. Transmission of HIV can occur through contact with bodily fluids of the person infected with HIV, in particular through having unprotected sexual intercourse, sharing injection equipment [16] and through use of infected blood products. Vertical transmission of HIV can occur from mother to child during pregnancy, birth and breastfeeding. Prevention of HIV transmission is possible through a number of methods including: condom use, the use of pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) [16, 18] ,and when the HIV-positive partner has an undetectable HIV viral load on treatment [16, 18].

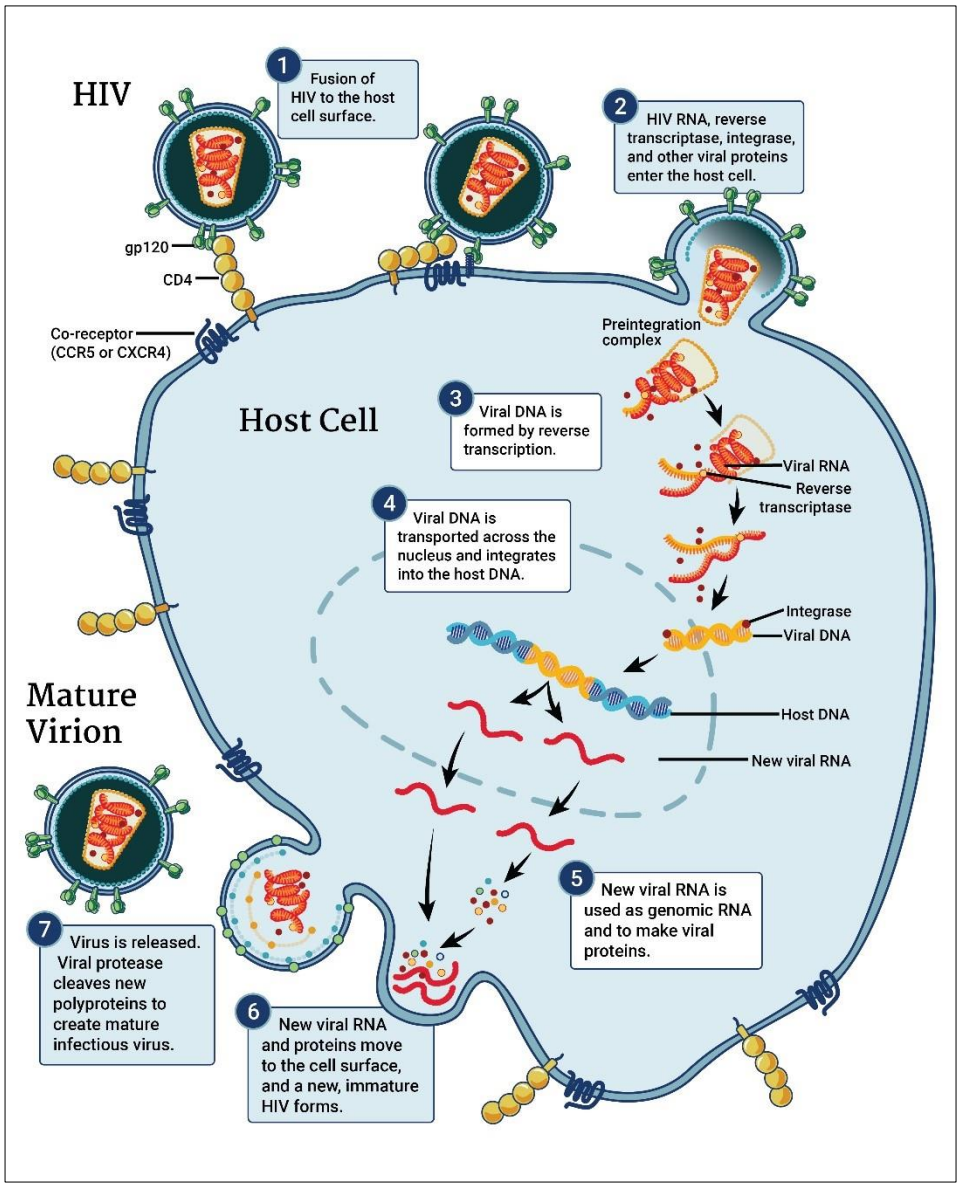
According to WHO, since the start of the HIV epidemic in the early 1980s, approximately 70 million have been infected with HIV and approximately 35 million

people have died [16, 18]. Since the mid-1990s, use of cART has dramatically improved prognosis and reduced risk of mortality and morbidity for PLWH. Effective cART has also been shown to eliminate the risk of HIV transmission [18]. However, access to HIV treatment is limited in some regions. As of 2019, approximately two-thirds of PLWH globally were accessing cART 67% (95% CI: 54-79 [7, 16, 18].

1.2.2 Cell-life cycle of HIV

The life cycle of the HIV virus consists of seven stages [19, 20] as shown in Figure 1.1. The first step is known as binding or attachment, which involves the interaction between gp120 (a protein found on the outer layer of the virus) and CD4 cell molecules [20, 21]. In detail, one or more proteins of the gp120 bind with chemokine receptors CCR5 receptor and CXCR4 co-receptor on CD4 cells [20, 21]. The second step is known as fusion, involves the joining of the HIV envelope and CD4 cell membrane allowing HIV to gain entry into the CD4 cell [21]. The third step, known as reverse transcription, occurs after HIV is inside the CD4 cell and releases the enzyme reverse transcriptase. The enzyme converts the HIV-RNA to HIV-DNA which is then able to enter inside the nucleus of the CD4 and combines with the host DNA [21, 22]. Integration is the fourth step, where HIV uses the enzyme integrase to integrate its viral DNA with the host DNA becoming provirus [20, 22]. The fifth step is replication. Once HIV-DNA is integrated with host DNA, the provirus is transcribed using host enzymes to form long chains of viral proteins in the cell nucleus [20, 21]. Following this step is assembly, the penultimate stage of the HIV-life cycle [20, 21]. The new HIV proteins and HIV-DNA migrate to the surface of the host cell and assemble into a non-infectious HIV [20]. The final stage called budding involves the newly formed non-infectious HIV containing long chains of protein which are cut into smaller pieces which are then combined within the host's cell membrane [20]. Following this step, the virus then buds out of the host cell to enter new CD4 cells [20]. Thousands of infectious HIV particles can be produced from a single CD4 cell [20].

Figure 1.1 Seven stages of HIV life cycle[20]



NIAID. HIV life cycle 2018. Available from: https://www.niaid.nih.gov/diseases-conditions/hiv-replication-cycle

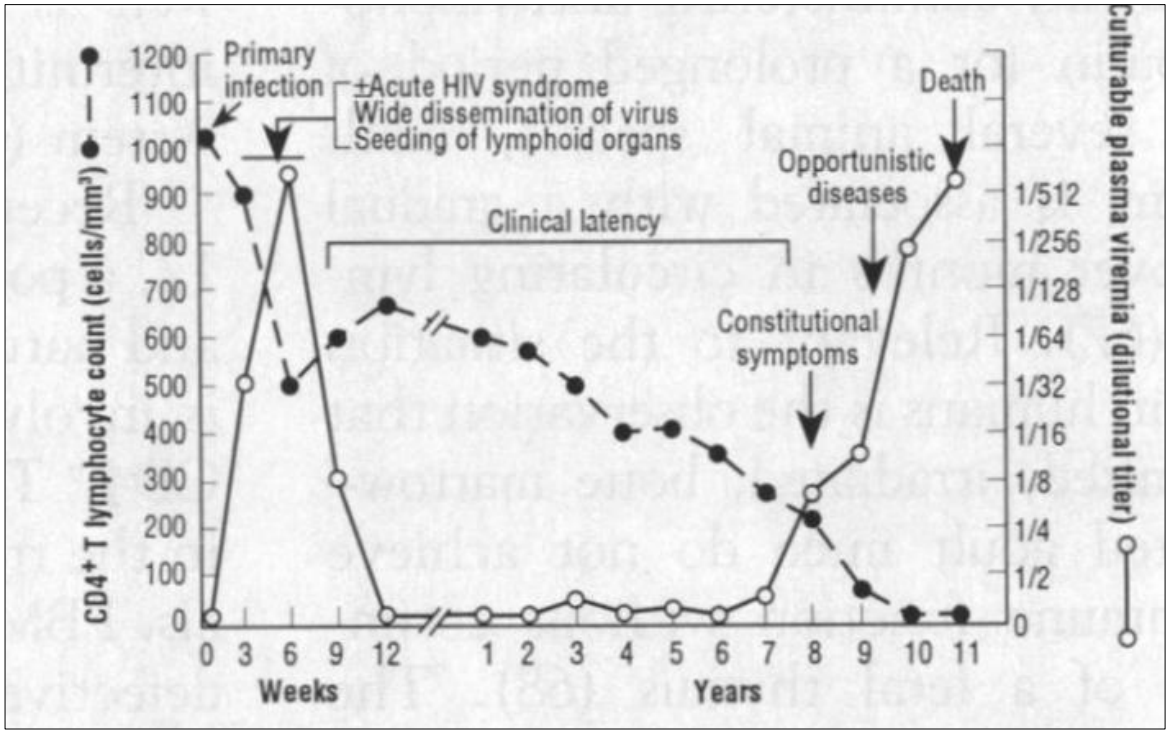
1.2.3 Natural history of HIV infection

The natural course of HIV infection in the absence of treatment is shown in Figure 1.2. There are three main clinical stages of HIV disease following infection. The primary infection phase usually lasting up to 12 weeks, the clinical latency phase, lasting a number of years, and finally the occurrence of opportunistic infections and eventually death [12].

Studies looking at individuals with HIV primary infection found high concentrations of HIV-RNA within the first two weeks from the time of infection followed by a noticeable decrease in viral load [23]. Although the virus is not eliminated from the body, the immune response suppresses the virus [24]. This phase also categorized as acute HIV infection syndrome, is typically characterised by individuals presenting with flu-like symptoms or illnesses, rashes, fever, fatigue, headache or swollen lymph nodes lasting 2-6 weeks [25-28].

In the clinical latency phase, individuals are asymptomatic with moderately low levels of CD4 and stable levels of HIV-RNA [12]. This phase lasts months to years as HIV continues to replicate, eventually leading to a decrease in CD4 cell count and HIV remains transmissible [12]. This intermediate stage is characterized by CD4 cell count <500cells/mm^3, and occurrence of infections such as fungal infections, vaginal candidiasis, cold sores or warts [29]. When CD4 cell count falls below <200cells/mm^3, opportunistic infections and cancers develop. So, if HIV infection is left untreated it eventually leads to severe AIDS/death [12, 29].

Figure 1.2 Pathogenesis of HIV disease following infection [12]



Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. Science (New York, NY). 1993;262(5136):1011-8.

1.2.4 Mode of HIV transmission

Mode of HIV transmission is mainly through bodily fluids such as semen, blood, and breast milk as well as mother to child transmissions during pregnancy and birth [16]. A meta-analysis study of cohorts looked at the risk per exposure of acquiring HIV from an infected person. The highest risk was seen in blood transfusion from an infected person estimated with a probability of 93% (95% CI: 89-96). Risk of HIV infection was also high in mother to child transmission 23% (95% CI: 17-29) if there are no preventative measures in place. Injecting drug use estimated transmission risk was 0.63% (95% CI: 0.41-0.92). Risk of HIV transmission via sexual exposure via anal intercourse estimated to be 1.4% (95% CI: 1-18). A major advance in understanding HIV transmission has occurred in the past decade. Studies have shown that in couples where one person is HIV-positive

and is on cART with undetectable HIV-RNA ≤200 copies/ml and the other person is HIV-negative, there is effectively no risk of HIV transmission during sex [30-32]. One of the most significant studies that contributed to this knowledge was the Partners of People on cART-A New Evaluation of the Risks (PARTNER) observational study. The study enrolled 1,166 sero-discordant couples (an HIV-positive person with an HIV-negative partner) who reported having condomless sex, and in which the HIV-positive partner was on suppressive cART. The study reported no episodes of HIV transmission between couples during the follow-up period (upper 95% CI = 0.30 per 100 couple years of follow-up [31]. The only infections registered in the study occurred outside of the sero-discordant couple under examination. Other studies have also found similar findings [33, 34]. There has now been an endorsement from UNAIDS stating that a person on cART with undetectable HIV-RNA ≤50 copies/ml has no chance of transmitting HIV[32]. Therefore, with regular monitoring of HIV-RNA to ensure treatment is suppressing the virus [33, 34] a person is effectively non-infectious.

1.2.5 HIV therapy

The main aim of the HIV therapy is to suppress the replication of HIV virus [34] this will then allow for recovery of CD4 and immune function. Viral suppression means reducing the level of viral replication such that HIV is no longer detectable in plasma using standard assays. Common limits of detectability of assays are <50 copies/mL or <200 copies/mL[35]. To achieve this suppression, there are an increasing number of HIV drugs available, classified into drug classes depending on the role of the drug on the HIV virus.

Typically, HIV treatment consists of a combination of three ART drugs, usually from two or more classes. The main drug class and how these relate to each of the stages in the HIV cycle mentioned in Section 1.2.2 and shown in Figure 1.1[36] are as follows:

- Nucleoside reverse transcriptase inhibitors (NRTIs) – These drugs interfere with HIV replication (step 5 in Figure 1.1) and are referred to as the backbone of HIV therapy[37]
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs)- these drugs interfere with the reverse transcription process step 3 in Figure 1.1[37].
- Protease Inhibitors (PIs) – These drugs interfere with step 7 in Figure 1.1 which involves the breakdown of larger proteins needed to generate new HIV particles[37]. In recent years, PIs are typically given with a booster drugs so that the duration of this process is maximised [37].
- Integrase strand transfer inhibitors (INSTIs) – These stop the virus from entering into human DNA in step 1 and 2 in Figure 1.1[37].
- Entry Inhibitors (Fusion Inhibitors and CCR5 inhibitors) – These stop the HIV entering the human cells in steps 1 and 2 in Figure 1.1 [37]

According to the most recent European AIDS Clinical society (EACS) guidelines published in 2019, cART is recommended for individuals infected with HIV regardless of CD4 [38]. The Strategic Timing of Antiretroviral Treatment (START) randomised trial evaluated the effect of two different strategies of cART initiation in HIV-positive individuals with a CD4>500 cells/mm^3 [39]. The trial randomly assigned individuals with CD4>500 cells/mm^3 to either immediate start of cART or deferred cART initiation until CD4 fell below 350 cells/mm^3 or the individual developed an AIDS defining condition [39]. The primary endpoints of interest were; any serious AIDS-related event, serious non-AIDS related event or death [39]. The study involved 4,685 HIV-positive individuals with a median CD4 of 651 cells/mm^3 and median HIV-RNA of 12,759 copies/ml at study entry [39]. The trial was interrupted early as the results of the interim analysis showed a hazard ratio of the primary endpoint for intervention versus control group of 0.43 (95% CI: 0.30 – 0.62, p<0.001) [39]. Therefore, the strategy of immediate cART resulted in lower risk of mortality and major morbidity. In light of these findings from the START trial, EACS have recommended initiating cART regardless of CD4 cell count. Importantly, assessing HIV-positive individuals' readiness to start and adhere to cART, is paramount to ensure virological suppression is achieved and maintained and that

the risk of transmission is minimised [38]. The commonly used ARV drugs and the regimens currently recommended in Italy for adults initiating cART for the first time (a population defined as cART-naïve) are shown below: [38]

Commonly used antiretroviral drugs by drug class

NRTIs - Abacavir (ABC), Lamivudine (3TC), Tenofovir (TDF-Disoproxil /TAF-Alafenamide), Emtricitabine (FTC)

NNRTIs - Efavirenz (EFV), Rilpivirine (RIL)

[1]PI/rs - Lopinavir/r(LPV/ritonavir), Darunavir (DRV/ritonavir), Atazanavir (ATV/ritonavir)

INSTIs - Raltegravir (RAL), Dolutegravir (DTG), Elvitegravir (ELV)

COBICISTAT (COBI) – acts as an enhancer for certain PIs and INSTIs

[1]Ritonavir enhances the metabolic process of the drug thus allowing for maximum impact.

Recommended regimens in Italy for starting cART as of 2016 [40]

2 NRTIs + INSTI

- ABC/3TC + DTG or ABC/3TC/DTG
- TAF/FTC + DTG or TDF/FTC + DTG
- TAF/FTC/EVG/COBI + RAL or TDF/FTC + RAL

2 NRTIs + NNRTI

- TAF/FTC/RPV or TDF/FTC/RPV

2 NRTIs + PI/r or PI/c

- TAF/FTC+ ATV/r or TAF/FTC + DRV/r
- TAF/FTC+ATV/COBI or TAF/FTC + DRV/COBI

1.2.6 Global epidemiology of HIV

According to WHO, in 2019 there were 38 million [31.6 - 44.5] PLWH and 1.7 [1.2 - 2.2] million people were estimated to be newly infected with HIV [16]. Number of deaths related to HIV in 2019 were estimated at 690,000 [500,000 – 970,000] [16,

[41]. New HIV infections have certainly declined, falling by 23% worldwide since 2010, marking an indication of improved HIV treatment as well HIV prevention tools [42]. According to UNAIDS, HIV infection rates are still high and could be reduced even further by increasing access to treatment, and reducing stigmatisation and discrimination. In addition social inequalities also play a role [41, 42].

Table 1.1 shows a summary of prevalence of HIV, estimated number of new HIV infections and treatment coverage among PLWH as of 2019 worldwide and by region. Low and middle-income countries have the highest prevalence of HIV. In Africa the estimated number of people living with HIV was 25.7 million ([22.3–29.3]), accounting for about 70% of all HIV cases worldwide [43]. The region also saw a decline in new infections since 2019, declining from 1,000,000 to 930,000 [43]. Specifically, east and southern region of Africa has the highest HIV prevalence and the most prevalent mode of HIV transmission is sexual transmission mostly through sex workers, PWID and men who have sex with men (MSM) [43].

In South-East Asia and Western Pacific the estimated number of PLWH was 5.8 million as of 2019 [43]. This accounts for 15% of the global population of PLWH. The most prevalent mode of HIV transmission is through sex workers, PWID and MSM [41].

In 2019, the estimated number of PLWH in Europe was 2.5 million, accounting for 7% of the global prevalence of HIV infections. In a more detailed report of HIV/AIDS surveillance in Europe, in the period between 2009 to 2018 newly diagnosed HIV infections increased by 14% (from 14.2 per 100,000 to 16.2 per 100,000) [44]. The most common reported mode of HIV transmission in Europe was heterosexual contact, accounting for 50% among newly diagnosed individuals with HIV[44]. The second most common reported mode of HIV transmission was MSM accounting for 23% [44] followed by injecting drug use accounting for 12% [44]. However, there is a variation in the most common mode of HIV transmission dependent on region. For instance in eastern Europe, heterosexual contact and injecting drug use are the most common accounting for 72% and 23% respectively

of new HIV diagnoses[44]. In western Europe, MSM transmission accounts for 52%, followed by heterosexual contact accounting for 43% and PWID accounting for 3% of new HIV infections[44].

In the Americas, the estimated number of PLWH was 3.7 million, accounting for 10% of the global prevalence of HIV infections. New HIV infections are most common among young people within key populations[45]. In particular the Caribbean has the second highest prevalent cases of HIV infections following sub Saharan Africa[45].

As of 2019, around 67% (range: 54-79) of PLWH had access to HIV treatment. This means that approximately 12 million people are still without access to treatment[7]. As shown in Table 1.1, Europe has the most coverage with 83% people receiving cART among PLWH [7].

Targets for 2020 were set by UNAIDS in terms of the HIV CoC. The HIV CoC consists of steps a person with HIV takes, from diagnosis to treatment. Targets have been set regarding these steps, specifically that 90% of people infected with HIV should be aware of their HIV-positive status, 90% of people who know their HIV status should be receiving cART and finally 90% of people receiving cART should be virally suppressed. However, globally as of 2019, these targets were not met. Among PLWH, 81% [68-95] knew their status. Among PLWH who knew their status, 67%[54-79] were accessing treatment and finally 59% [49-69] of people receiving cART were virally suppressed [42, 46]. However, these percentages vary considerably across regions and settings [46]. Reassuringly, the latest statistics for 2020 show an improvement. Among PLWH, 84% [67->98] knew their status. Among PLWH who knew their status, 73% [56-88] were accessing treatment and finally 66% [53-79] of people receiving cART were virally suppressed [42, 46].

Table 1.1 A summary of global and regional estimates of HIV prevalence, new infections and treatment coverage among people living with HIV as of 2019 [7]

Region	HIV prevalence (millions) [range]	HIV new infections (millions) [range]	Treatment coverage (%) among PLWH [range]
Global	38 [31.6 – 44.5]	1.7 [1.20 – 2.20]	67 [54 – 79]
Africa	25.7 [22.3 – 29.3]	0.97 [0.73 – 1.30]	70 [58 – 80]
Americas	3.7 [2.7 – 4.7]	0.17 [0.11 – 0.24]	67 [47 – 86]
South-east Asia	3.7 [2.8 – 4.6]	0.16 [0.11 – 0.21]	60 [44 – 75]
Western Pacific	1.9 [1.3 – 2.4]	0.11 [0.07 – 0.15]	65 [44 – 83]
Europe	2.6 [2.2 – 3.0]	0.19 [0.16 – 0.24]	83 [68 – 97]
East Mediterranean	0.4 [0.3 – 0.6]	0.44 [0.33 – 0.67]	24 [18 – 36]

UNAIDS. Global HIV & AIDS statistics — 2020 fact sheet 2020[7].

1.2.7 HIV epidemic in Italy

As this thesis includes data from HIV-positive people accessing care in Italy, this section gives an overview of the HIV epidemic in Italy.

Italy can be found in Southern Europe and has a population of approximately 60 million [47]. As of 2019, the estimated number of adults aged 15 and over living with HIV was 130,000 [71,000 – 210,000] [48-50] making up 0.3% of the global prevalence of PLWH [51]. New infections were estimated at 2,500 [<1000 – 7800] in 2019 equivalent to an estimated incidence of 0.09 [0.02 – 0.25] per 1000 individuals [48, 51]. There has been a general decline in HIV incidence from the start of 2004 (when the reporting of HIV surveillance began) [44].

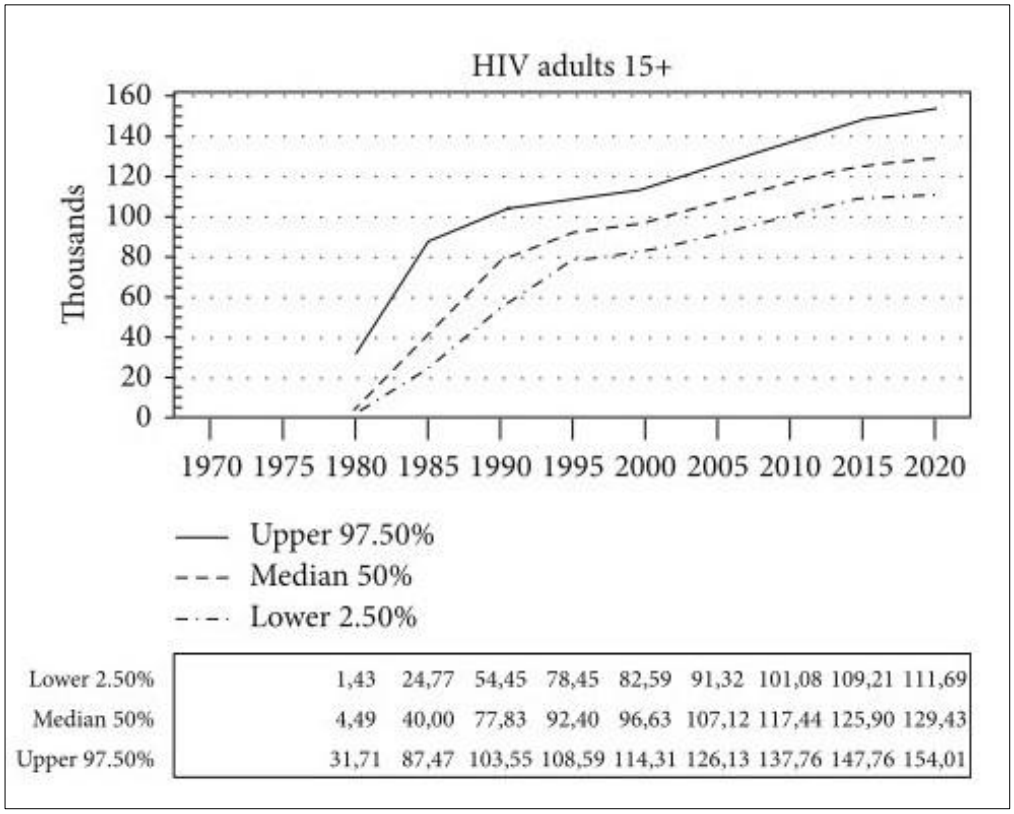
Figure 1.3 shows the projections of number of PLWH in Italy by 2020 according to *Camoni et al* and demonstrates that despite falling incidence there are increasing numbers of PLWH due to increases in life expectancy [2]. This is consistent with

latest estimates of HIV prevalence in Italy in 2020, estimated at 140,000 [120,000 – 150,000] highlighting the ever increasing burden of HIV and importance of achieving the 90-90-90 goals. The method used by the authors to create these projections were based on indicators relating to prevalence, incidence of HIV, death caused by AIDS, access to ARV drugs and their impact on PLWH living longer [2].

The distribution of mode of HIV transmission has changed over time. Among the 94,146 PLWH in Italy in 2012, the distribution was as follows – 38% Heterosexual, 28% - PWID, 28% MSM and 7% - Unknown [51]. Although high compared with countries in Northern Europe, the proportion of PWID used to be higher in Italy in the earlier years of the epidemic but has declined over time[51]. This could be explained by improvement in needle sharing prevention strategies.

In 2018 Italy had achieved the first 90 target set by UNAIDS two years prior to the year 2020 and had nearly reached 90 for the other two targets. Ninety-two percent of PLWH knew their HIV status. Eighty-seven percent of PLWH who knew their status were on treatment and 87% of PLWH on treatment were virally suppressed [46]. Recent data analysis of the Icona cohort including 8,241 HIV-positive people who achieved undetectable status (defined as ≤200copies/ml for more than 6 months) showed approximately 97% of PLWH remained virally suppressed over a 10-year period [52].

Figure 1.3 Projections of number of people living with HIV by 2020 in Italy [2]



Lower 2.50%	1,43	24,77	54,45	78,45	82,59	91,32	101,08	109,21	111,69
Median 50%	4,49	40,00	77,83	92,40	96,63	107,12	117,44	125,90	129,43
Upper 97.50%	31,71	87,47	103,55	108,59	114,31	126,13	137,76	147,76	154,01

Camoni L, Regine V, Stanecki K, Salfa MC, Raimondo M, Suligoi B. Estimates of the number of people living with HIV in Italy. BioMed research international. 2014;2014:209619.

1.3 Hepatitis C Virus

1.3.1 A brief overview

The hepatitis C virus is a flavivirus, *that infects the liver causing inflammation*[8]. There are two forms of infection: acute hepatitis C is when the infection lasts for a few weeks only: chronic hepatitis C is when the infection develops into a life-long infection which is the most common situation [8]. Chronic infection can lead to liver failure, cirrhosis (scarring of the liver tissue), decompensation and hepatocellular carcinoma (liver cancer)[9]. In most cases, people are unaware of having an infection and symptoms may not show immediately but instead may show decades later when physiological signs begin to show liver damage. HCV infection may also be detected during routine medical blood tests [9].

The first step in the diagnosis of HCV involves an antibody test for HCV (HCVAb). This assesses whether the person has been exposed to the virus, as having HCV antibodies indicates previous infection [53]. Following on from detection of HCVAb, another test is done to see if the individual has an active or recent HCV infection. Recent or active HCV infection is detected from hepatitis C ribonucleic acid (HCV-RNA) in serum or plasma using quantitative or qualitative molecular methods [53]. This test quantifies the amount of HCV-RNA in the blood [53]. An alternative test for HCV infection is the detection of HCV core antigen serum or plasma in the blood[54].

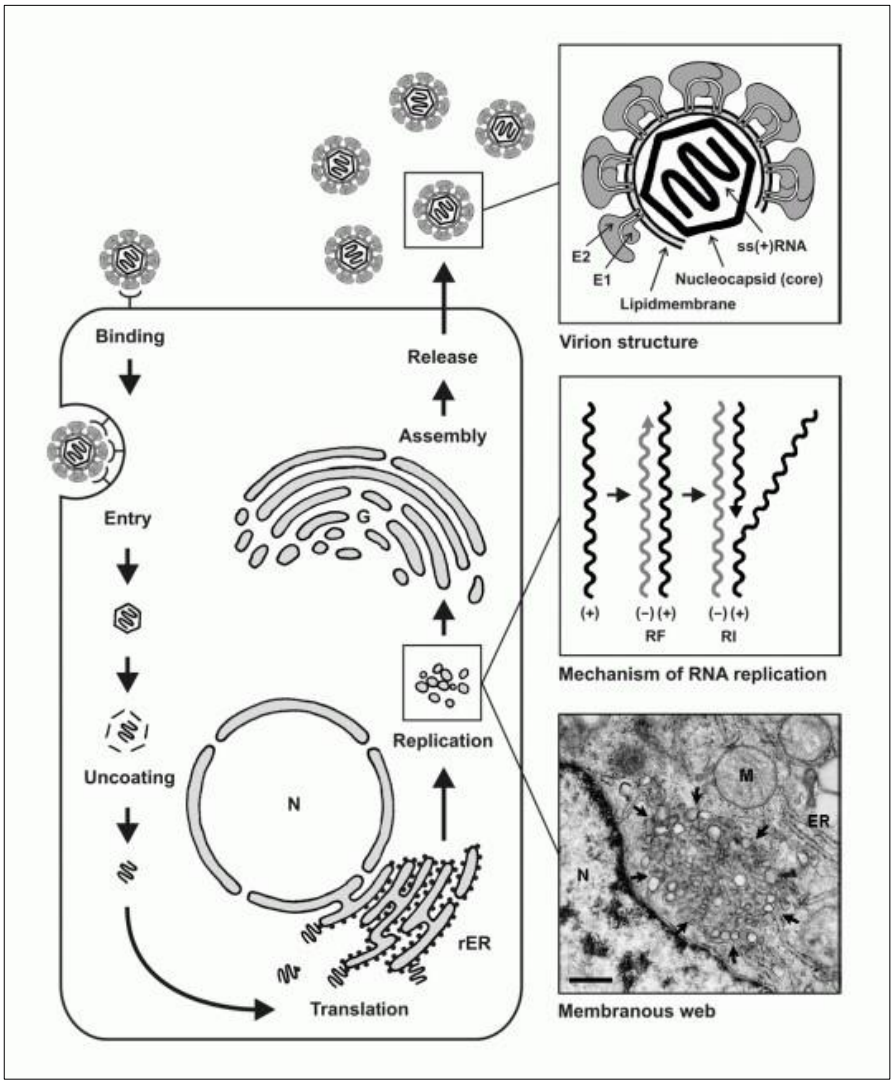
The strain of HCV virus an individual is exposed to when infected is termed as genotype, this is due to the ability of the HCV to mutate [55]. This was important pre-DAA era as knowing which HCV genotype one is infected with determined which treatment was received as genotype influenced response to treatment – more details are given in section 1.3.5

1.3.2 Cell-life cycle of HCV

The life cycle of HCV consists of eight stages [56, 57]. In the first stage during primary infection, HCV particles are transported through the blood stream to eventually make contact with liver cells called hepatocytes, see Figure 1.4 [57]. A coating surrounds the virus containing specific proteins which attach to a receptor on the surface of the liver cell (binding step in Figure 1.4). In detail, the initial viral attachment is facilitated by a substance called heparan sulphate proteoglycans, found on the surface of the hepatocyte, [58]. The second step involves the virus entering the liver cell by interacting with specific receptors, which assist in viral entry by inducing changes of the viral particle or signalling pathways for entry (entry step in Figure 1.4) [57]. Once the virus is inside the liver cell, the third step involves the coating of the virus breaking down leading to the release of viral RNA (uncoating step in Figure 1.4) [57]. The fourth step involves reproduction of the virus's RNA. The virus copies the liver's cell's RNA, thus leading to the liver not functioning as normal (translation step in Figure 1.4) [57]. The fifth step known as the replication step is where the virus is cloned repeatedly forming new viruses (replication step in Figure 1.4) [57]. The sixth step involves the production of the coating of the virus. The seventh step is the assembly involving the formation of new particles from the viral RNA coming together (assembly in Figure 1.4) [57]. The final step involves the release of the virus out of the liver cell to continue the same process in another liver cell (release step in Figure 1.4) [57]. The virus is protected by coating before it heads out of the liver cell.

It is worth noting that HCV does not get into the host DNA. That is the main reason why HCV can be eradicated by DAA drugs, while HIV cannot be eradicated by antivirals [57].

Figure 1.4 HCV cell replication and assembly cycle [8]



Chevaliez S, Pawlotsky J. Chapter 1: HCV Genome and Life Cycle Norfolk(UK): Horizon Bioscience; 2006.

1.3.3 Natural History of HCV infection

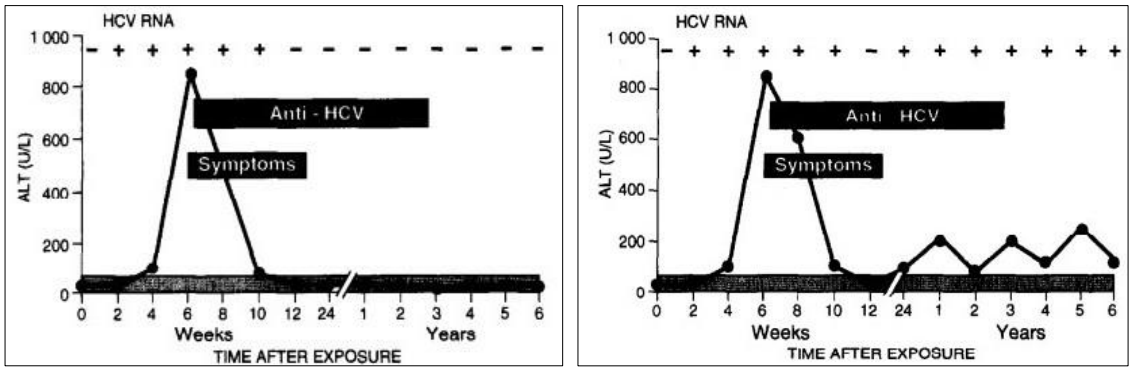
Acute hepatitis C

Following exposure, acute hepatitis can be detected within the first few weeks using serum HCV-RNA, which can be as high as 10^7 IU/ml. This occurs just before an increase in Alanine Aminotransferase (ALT) levels. ALT is a liver enzyme used as a marker to assess liver damage. When the liver is damaged, ALT leaks out into the blood stream and levels are elevated [10] (Figure 1.5). Common symptoms that

can be observed within 3-12 weeks of exposure include; malaise, weakness, jaundice, anorexia, nausea, and dark urine [59] [60]. Detection of HCVAb happens within 3 months of the start of symptoms. This initial three month period is known as the serologically silent period, thus limiting diagnosis of primary infection to HCV-RNA [60].

Symptoms can last several weeks but can lessen with a decrease in HCV-RNA and levels of ALT [10] [9]. In people with self-limited hepatitis, levels of ALT can return to normal. Although HCVAbs eventually decrease they can remain detectable for many years Figure 1.5 [60]. However, recovery from acute hepatitis C does not happen in approximately 75%-85% of people who progress to develop chronic infection, see Figure 1.5 [9]. In the 15-25% of people who experience clearance of HCV-RNA, *Grebely et al*, reported being female, having IL28 CC genotype an genotype 1 were independently associated with spontaneous clearance[61].

Figure 1.5 Time course of HCV markers for acute and chronic hepatitis c [10]



Marcellin P. Hepatitis C: the clinical spectrum of the disease. Journal of hepatology.

Chronic hepatitis C

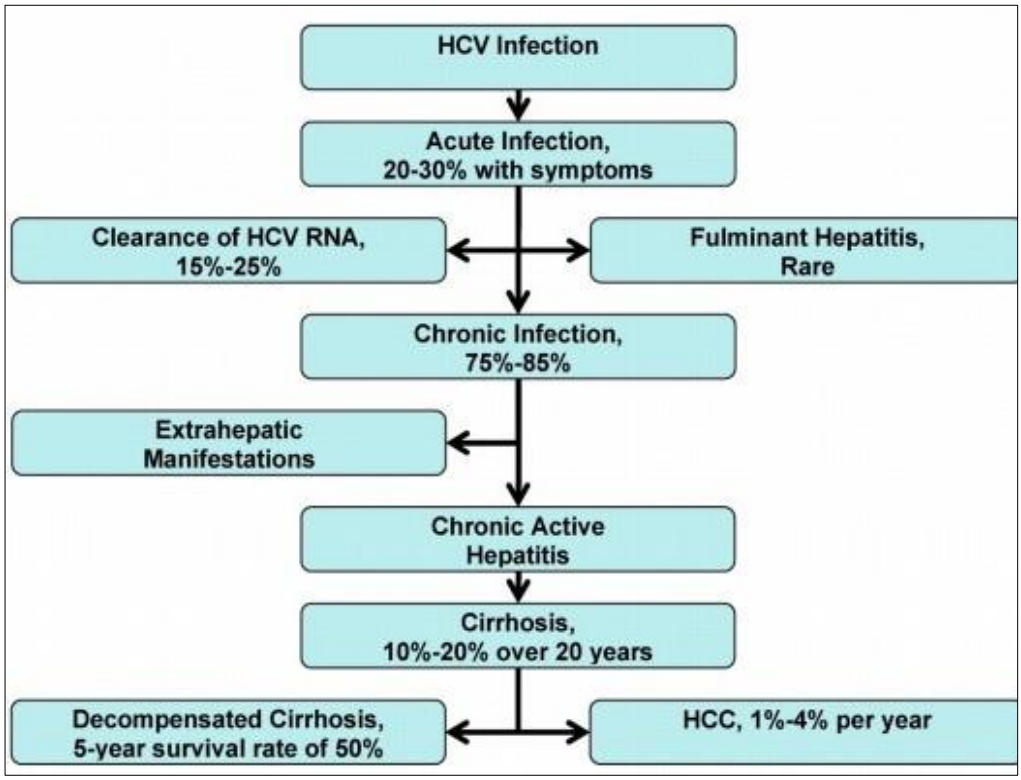
The presence of chronic hepatitis C is inferred from persistent levels of HCV-RNA from six months after the start of acute hepatitis infection, with fluctuating levels of HCV-RNA over time (Figure 1.5) [60] [10].

The extent of liver damage, known as fibrosis, determines whether the individual has mild, moderate or severe chronic hepatitis, which will affect disease prognosis [10]. Risk factors leading to severe liver scarring include: being older than forty years of age at time of HCV infection, male sex, white ethnicity, HIV-positive and immune suppression, alcohol consumption, HIV or HBV coinfection and presence of comorbidities [62] [9]. In chapter 4, I specifically evaluated the role of HCV as a possible effect modifier of the association between alcohol consumption and the risk of developing SLD.

Disease progression of chronic hepatitis C can lead to cirrhosis (severe scarring of the liver tissue), hepatocellular carcinoma (HCC) and death [10] [9]. Cirrhosis has been shown to develop in 10-20% of people and is often asymptomatic until people present with end-stage liver disease or HCC (Figure 1.6) [9]. Chronic HCV infection usually develops into severe disease within 20-30 years after infection (Figure 1.6) [9] [10] [62]. Some of the clinical complications include; ascites, upper gastrointestinal bleeding, secondary to varices portal hypertensive gastrophy and hepatic encephalopathy, fatigue, muscle weakness and wasting [9] [10].

HCC occurs in people with cirrhosis with incidence ranging from 1-4% per year (Figure 1.6) [10]. Median time to diagnosis of HCC has been estimated in post transfusion patients with HCV infection and estimated at around 30 years after infection [9, 63].

Figure 1.6 Flow diagram of the natural history of hepatitis C [9]



Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. Int J Med Sci. 2006;3(2):47-52.

1.3.4 Mode of HCV Transmission

Hepatitis C virus is mainly transmitted through blood to blood contact with an infected person [64]. Percutaneous (via skin) transmission may occur through blood transfusion, transplantation, injecting drug use or needle stick injuries in health care settings). HCV can also be transmitted via mucosal exposures (e.g. sexual contact) [64].

Blood transfusion

Prior to implementing blood donor screening procedures in 1991 to test for HCV antibodies or biomarkers, the most efficient known means of HCV transmission was through blood transfusion [65]. Retrospective studies in the USA, Europe and Asia (mostly Japan) in people who had undergone blood transfusion have shown

that >50% of these individuals seroconverted to HCV [65-70]. In contrast, in the UK, less than 2% of HCV infections were due to blood transfusion. A possible explanation was due to the introduction of self-exclusion of donors at risk of HIV infection in 1983[71].

The impact of blood donor screening has been significant in reducing the number of HCV infections due to blood transfusion. In Japan, among people receiving less than 10 units of blood, incidence rates declined from 4.9% prior to screening to 1.9% after screening [72]. In the US, incidence rates of post transfusion hepatitis C declined from 3.8% before 1990 to 0.6% after 1990 (the introduction of HCV screening) [73].

HCV infection may still occur after blood transfusion due to serological limitations for viral screening, as units of blood collected during the donor's serological window period may be infected while HCV is unable to be detected [64]. Current estimates of residual risk stand at 1:250,000 blood units transfused for hepatitis C virus [74]. Despite the introduction of blood donor screening, not all regions in the world screen blood donors. In sub-Saharan Africa residual risk estimates stand at 2.5 per 1,000 transfused units equivalent to more than 6,000 HCV infections per year due to blood transfusion [75]. Some explanation could be lack of or limited resources and health care infrastructure [76].

Injecting drug use

People who inject drugs are at increased risk of HCV infection, mostly through sharing non-sterile injection equipment's or drug use practices that facilitate efficient HCV transmission, such as drug preparation environments/equipment and drug cookers [77] [78]. In a systematic review investigating the global prevalence of HCV among PWIDs, the pooled estimate was 52.3% (range: 42.4-62.1) [79] [80]. In Italy the corresponding estimates was, 57.9% (range: 52.5-63.3) [81] [82]. In contrast to a global increasing burden of HCV infection in PWIDs, a decline in incidence of HCV infection has been reported in high income countries. This is due to decreased syringe borrowing and drug cessation intervention programs [83].

However, in low income countries, PWIDs continue to be at high risk of HCV infection due to drug preparation methods[77, 80, 84-86]. Various studies have investigated factors associated with increased risk of acquiring HCV among PWIDs. In observational studies, needle sharing, longer duration of injection drug use, concomitant HIV infection, older age and greater frequency of drug use have all been found to be correlated with the risk of HCV transmission [85] [87] [88] [79] [89].

Sexual transmission

Transmission of HCV through sexual contact remains a controversial issue [90] [91]. Risk of transmission seems to vary depending on types of sexual contact or behaviour [92]. Occurrence of HCV transmissions in HIV and HCV discordant couples in monogamous heterosexual partnerships has been shown to be rare with transmission rates estimated to be between zero and three percent[92-94].
A recent study of 500 couples in long term relationships with 12 sexual contacts reported in the first month of the relationship were followed up for three years with one spouse being HCV infected. The authors reported an incidence of HCV infection 3.6 per 10,000 person-years (95%CI: 0.0-7.7) in the negative partners[95]. In another study in Italy with longer follow-up of 10 years, involving 895 heterosexual partners in monogamous relationships, an incidence rate of 0.4% (0.37 cases per 1000 person years) was observed. A further evaluation of possible modes of HCV transmission in these few individuals who acquired HCV found that transmission risk was not due to sexual contacts [96].

In contrast, having multiple partners or sexual contact with people who have sexually transmitted infections increases the risk of HCV transmission [97] [98] [99]. In a small case-control study of 43 HCV-positive individuals and 172 HCV-negative individuals, reporting to have two or more partners in the past was found to be associated with an almost 3-fold higher risk of acquiring HCV infection adjusted OR=2.81 (95% CI: 1.14-6.89)] compared to having a single partner [99]. These findings were consistent with data from a large surveillance study of acute viral hepatitis during hospitalization carried out in Italy, indicating multiple sexual partners as one of the major risk factors for HCV infection aOR = 2.2 (95% CI: 1.6-

3.0)] [97]. In a review of HCV infection through sexual contact, HIV was found to be associated with HCV infection with an increased risk of coinfection shown to be in the range 3.3-3.9 [92] [98].

Risk of transmission of HCV infection through homosexual activity appears to be dependent on HIV infection status in some studies [100] [101] [102].However remains comparable between MSMs in the PREP era in the both HIV positive and HIV negative individuals[103]. This is explained by increased risk of sexual practices of MSM regardless of HIV status [103].. In a prospective cohort study of HIV-negative MSM, aiming to identify factors associated with the risk of HIV infection which enrolled 1,054 HCV-negative people, only one person acquired HCV infection in follow-up, corresponding to an incidence of 0.038 per 100 person-years (95% CI: 0.001-0.210). This case occurred in an individual reporting injecting drug use [104]. Similar findings in Amsterdam were reported in HIV-negative men followed up for almost 20 years, with an estimated incidence rate of HCV infection of zero, 0/7807 (95% CI: 0-0.05), 0 per 100 person-years [101]. However, a higher incidence of 0.11 per 100 person-years (95% CI: 0.03-0.26) was reported in another cohort of HIV-negative MSM, the majority of whom had reported sexual contacts with HIV-positive partners [102].

In the case of HIV-positive MSM, prevalence rates of HCV infection are reported to be much higher [100] [92] [105]. A study of 5,310 individuals followed up from the start of the HIV epidemic in 1984 to 2011 compared the incidence of HCV in HIV-positive men and HIV-negative men[100]. They found 115 incident cases of HCV infections translating to 2.08/1000 person years (95% CI: 1.73-2.49/1000); HCV infection was 8.5 times higher in HIV-positive men than in HIV-negative men (4.22 vs 0.5/1000 person years respectively) [100]. Sexual exposure to more than one male partner was also strongly associated with the risk of HCV infection [adjusted incidence risk ratio (aIRR) = 3.37 (95% CI: 1.69-6.74)] [100]. In a more recent case–control study conducted in Belgium, sexual intercourse with HIV-positive men was reported to be independently associated with HCV infection aOR=5.51 (95% CI: 1.87-16.20) [105]. *Nijmeijer et al*, reported even higher HCV incidence rates through

sexual transmission in HIV-positive MSM estimated at 1.8 per 100 person years in 2014 [106].

The topic of re-infection of HCV is beyond the scope of this thesis but worth mentioning, as it is also another concern in tackling a reduction in HCV transmission. Re-infection rates of HCV in HIV-positive MSM is on the increase, reported to be 10 times higher than primary infections [107]. Data from Austria, France, Germany and UK reported re-infection incidence rates 7.3/100 person-years in HIV-positive MSM who had cleared their HCV spontaneously[107]. Thus HIV-negative MSM are at risk of acquiring HCV through sexual transmission from their HIV-positive partners [107].

1.3.5 HCV therapy

Following infection with HCV, the main goal of therapy is to eradicate the virus[54]. This usually means having undetectable HCV-RNA and resolution of liver disease 12 or 24 (12/24) weeks after finishing treatment in individuals with mild or moderate liver disease [108]. However, for individuals with cirrhosis who clear HCV infection, risk of HCC or liver-related mortality is reduced but not eliminated [54]. In chapter 7, I used sustained virological response (SVR) at 12/24 weeks post end of HCV treatment (SVR12/24) as an outcome when assessing regional differences in terms of HCV continuum of care (CoC).

Prior to 2011, treatment for HCV was limited to Pegylated Interferon (PegIFN) and Ribavirin (RBV)[109, 110]. Following which the first generation of HCV therapy (DAA) drugs were developed. These were Boceprevir and Telaprevir used in people infected with HCV genotype 1[111-114]. Then in 2012 clinical trials of second generation DAAs treatment began[115]. From 2014 onwards effective DAAs which were interferon and ribavirin-free were approved. These were; Sofosbuvir, Simeprevir, Daclatasvir, Sofosbuvir/ledispasvir, Paritaprevir/ombitasvir/ritonavir and Dasabuvir [108, 115]. Direct acting antivirals are now recommend for use in clinical practice for all genotypes in the treatment of HCV [54]. To date, treatment for

HCV continues to advance rapidly such that as of 2020, the following are now recommended DAAs for HCV therapy in Europe; Sofosbuvir, Sofosbuvir/velpatasvir, Sofosbuvir/velpatasvir/voxileprevir, Glaceprevir/pibrentasvir an Grazoprevir/elbasvir[54].

The data analysis relating to DAA initiation (chapters 6 and 7) included in this thesis may not include the most recent recommended DAA drugs as data was locked prior to this date.

•

Pegylated Interferon and Ribavirin

In brief, human interferons are natural chemicals produced by the immune system that respond to infections in the body [116]. The natural form is called interferon alpha (IFNα) and is mostly used to fight flu infections. When used as treatment for HCV this is administered via injection through the skin [116]. However, the main limitation of IFNα is lack of viral suppression because once the drug is eliminated from the body, it is no longer present to kill the HCV[117] [109]. Toxicity is also an issue as many people are unable to tolerate treatment [118, 119]. A series of randomised controlled trials (RCTs) assessed the efficacy of IFNα alone in people infected with chronic hepatitis C [120, 121]. In the intervention group, more than 50% of individuals treated with IFNα had a complete response defined as low serum aminotransferase [120] [121]. However, six to twelve months post treatment, serum aminotransferase levels would revert to abnormal levels [120] [121]. This led to the development of PegIFN, which has an improved half-life compared to IFNα. This

was achieved by a process called Pegylation which involved attachment of a biological compound (Polyethylene glycol (PEG)) to interferon to make it last in the bloodstream longer [117] [109]. Pegylated Interferon refers to a drug class consisting of Peginterferon alfa-2a and Peginterferon alfa-2b [122]. RCTs comparing PegIFNα with IFNα found that SVR was greatly improved following 48 weeks of treatment with SVR ranging from 36% to 69% in the in the PegIFNα group [110] [123] [124].

Further advancements in the treatment of HCV infection led to the combination of PegIFN with another antiviral medication called Ribavirin. When used in combination, PegIFN and RBV enhances the effectiveness of the treatment [118, 119]. Ribavirin, a synthetic nucleoside initially used to treat HIV but found to be ineffective, was found to have antiviral activity against flaviviruses and was therefore also evaluated as a monotherapy in people with chronic HCV [125] [126]. In a randomised study comparing treatment with RBV vs Placebo in individuals with chronic HCV, low serum ALT levels were observed in 55% vs 5% ($p<0.001$) of individuals randomised to RBV and placebo respectively. Interestingly, after 24 weeks of stopping RBV, individuals would revert to abnormal levels of ALT and did not lower levels of HCV-RNA [126]. Another RCT comparing PegIFN and RBV vs IFN alone, showed that after 24 weeks of completing treatment, SVR was achieved in 54% vs 47% in people treated with IFN alone [118] [119]. Coupled with low SVR, IFN and RBV are both associated with adverse effects, limiting their efficacy [118, 119].

It is worth mentioning that the effectiveness of PegIFN and RBV were also assessed in genotype subgroups. Thus, achieved SVRs when treated with PegIFN and RBV varied by genotype. In people with genotype 1 infection, SVRs rates were between 33-46% compared to genotype 2 or 3 with much higher SVRs rates between 76%-82%) [119] [118]. In addition, in the RCTs mentioned previously, treatment duration also varied according to genotype, i.e. 24 weeks treatment duration in genotype 2 or 3 and 48 weeks for genotype 1 [127].

Direct acting anti-viral drugs

Similar to HIV therapy, DAAs can be divided into three classes dependent on the region of the HCV virus that is being targeted [115]. There are three non-structural regions on the HCV virus that play a role in HCV replication[115]. These drug classes can be grouped into Protease Inhibitors, Non-structural protein 5A inhibitors and Polymerase inhibitors [128].

First generation of DAA drugs

The first generation DAA approved in 2011, namely Boceprevir and Telaprevir both known as protease inhibitors, target the NS3-NS4A regions of the hepatitis virus and act by inhibiting the viral replication process of HCV[113].

Randomised controlled studies comparing standard therapy of PegIFN and RBV with/without Boceprevir or Telaprevir saw SVR rates of 60% in people with HCV genotype 1 compared to those treatment with PegIFN only [112, 114, 129]. Among individuals who were previous non-responders to PegIFN and Ribavirin, SVR rates were lower than 50% [112, 114, 129].

Effectiveness of Boceprevir and Telaprevir in HIV/HCV coinfected, HCV treatment naïve individuals have been evaluated in small phase II studies [130]. In people with undetectable HIV-RNA, an SVR of 70% was observed [130] [131]. Although these first generation DAAs showed an improvement in cure rates, the triple therapy regimens were still not well tolerated [111] [108].

Second generation of DAA drugs

A second wave of new DAAs targeting NS3A-NS4A and NS5A were approved in 2014 with a shorter treatment duration of 12-24 weeks, namely Simeprevir, Sofosbuvir, Sofosbuvir/ledipasvir, Daclatasvir, Paritaprevir/Ombitasvir/ritonavir and Dasabuvir [108].

Triple therapy of DAAs showed SVR rates of 80% in treatment naïve individuals with HCV genotype 1 [132] [133] [115, 134]. In treatment experienced people with HCV genotype 1, SVRs were slightly lower [115, 134] [135] [136] [137]. In HIV/HCV coinfected

individuals, high SVRs rates of >70% were also reported [138]. Apart from people with HCV genotype 1, effectiveness of these new DAAs has also been evaluated in people with other, easier to treat, HCV genotypes. Cure rates as high as 90% were observed in treatment naïve individuals treated with Sofosbuvir with PegIFN and RBV [139]. In studies with Sofosbuvir and RBV less than 80% cure rates were observed [140]. Similarly in HIV/HCV coinfected individuals, in a small study of 63 participants comparing treatment duration of 12/24 weeks of Abbvie 3D with RBV, SVRs of >90% were reported in both groups [141].

Treatment prioritization of HCV infection has also evolved over time. From 2018, treatment for HCV has been recommended for all HCV-positive individuals [142]. Before this, treatment was recommended for specific groups. In 2015 for example, the recommendations were to prioritise HCV-positive people with advanced liver disease. People with HIV/HCV coinfection were also prioritized for HCV treatment [108]. However, prior to 2018 options for DAA were limited in people with HIV/HCV coinfection because of drug-drug interactions between ARV drug and DAAs[108, 142]. For example, Simeprevir and Paritaprevir/Ombitasvir/ritonavir cannot be administered with NNRTIs or some of the PIs [108]. In Italy, *Monforte et al* looked at access to and initiating DAA in HIV/HCV coinfected people naïve to DAA as of January 2013 [143]. In 2,607 HIV/HCV coinfected people, 35% (n=920) had started DAA and the following factors were associated with DAA initiation; HIV-RNA <50copies/ml, higher CD4 and HCV genotype 3 [143]. The authors reported that for 90% (829/920) of individuals there was data on whether there was a change in cART, three months prior to DAA initiation. The authors found that 28% (230/829) of those had modified the third drug in the cART regimen prior to starting DAA though information about which specific modifications were done was not given. [143]

The challenges for drug-drug interactions in HIV/HCV coinfected individuals continues to a lesser extent even with the newer more effective DAAs[54].

1.3.6 Evaluation of liver disease severity

According to the EASL guidelines, the extent of liver damage needs to be assessed among people with HCV infection before HCV therapy begins. The fibrosis stage is initially assessed using non-invasive methods, in particular biomarkers [54]. Further details are given below.

Scoring systems have been developed to classify the grade and stage of hepatic disease [144] [145]. The most widely used scoring systems include: METAVIR, Batts and Ludwig and International Association for Study of the Liver (IASL) [146] [147] [148]. METAVIR (based on liver biopsy) scoring is commonly reported and thus I focused on this scoring system in this thesis. Liver fibrosis is staged on a F0-F4 scale according to the METAVIR scoring system, as follows; F0=no fibrosis, F1=portal fibrosis without septa, F2=portal fibrosis with rare septa, F3=numerous septa without cirrhosis and F4=cirrhosis). F≥2 is considered significant fibrosis [147] [149].

Invasive methods

Liver Biopsy

Liver biopsy is an invasive procedure that involves obtaining tissue samples from the liver which are then assessed for the degree of inflammation and stage of liver disease [150] [145] [151]. Liver biopsy is used in cases where there are unexpected aetiologies [54]. The procedure has several potential complications such as possible pain, bleeding and perforation of other organs; some may even be life threatening[151]. In addition, the procedure is subject to sampling error and thus in some people may not reflect the true extent of liver damage. Another issue is that variation in pathologists assessment of the extent of liver damage is also possible [151] [145] [152].

Hence, the recommendation of non-invasive markers mentioned previously as the first point of evaluation of stage of liver disease [153].

Non-invasive markers

They are several non-invasive markers used to predict stages of liver disease. These are; Fibrotest, Acoustic radiation force impulse (ARFI), Aixplorer, Aspartate Aminotransferase-to-Platelet ratio index (APRI), Fibroscan and Fibrosis-4 (FIB-4) [54]. The main measures of stage liver disease that I used in this thesis also used to define prospective clinical endpoints are Fibroscan and FIB-4.

Fibrosis-4 index (FIB-4)

The FIB-4 index measures the stage of liver disease combining biochemical values; (platelets (PLT), ALT, aspartate aminotransferase (AST)) and age of the individual (see formula in Table 1.2) [154]. A cut-off of ≤1.45 (METAVIR ≤F2) represents moderate fibrosis and >3.25 (METAVIR ≥F3) represents advanced fibrosis [155]. Studies including HCV mono-infected individuals have been

Table 1.2 Non-invasive methods for the evaluations of liver fibrosis

performed to validate FIB-4 and found correct classification of people with advanced liver disease and cirrhosis [155]. However, FIB-4 has its own limitation of not being able to fully discriminate people with values between 1.45 and 3.25 [155]. This is possibly explained by old age and low platelet count. The sensitivity and specificity for the cut-offs of: ≤1.45 are 90% and 58% and >3.25 are 55% and 92% respectively [54].

Aspartate Aminotransferase-to-Platelet ratio index (APRI)

The APRI also measures stage of liver disease calculated using biochemical values (PLT and AST) (Table 1.2). This index to predict liver fibrosis was developed and validated in treatment naïve chronic hepatitis C individuals [156]. A cut-off of ≤0.50 (METAVIR F0) represents no significant fibrosis, a cut-off of >1.00 (METAVIR F≥2) represents significant fibrosis and a cut-off of >2.00 (METAVIR F4) represents cirrhosis [156]. The sensitivity and specificity for the cut-offs of >1.00 and >2.00 are 48% and 94% and 77% and 75% respectively. The main

advantages of using the APRI score, is its simplicity [54]. Although simple to use, a limitation to the APRI score is its performance in people with moderate fibrosis [156].

Name	Formula/components	Cut-offs
Fibrosis 4 index [154, 155]	$FIB4 = \frac{Age(years) * AST(U/L)}{Platelet\ count\ (10^9/L) * \sqrt{ALT(U/L)}}$	≤1.45 = Mild fibrosis 1.45-3.25 = Moderate fibrosis >3.25 = Advanced cirrhosis
Aspartate Aminotransferase -to-Platelet ratio index [156]	$APRI = \frac{\frac{AST\ level}{AST(Upper\ Limit\ Normal\ [40IU/L])}}{Platelet\ count\ (10^9/L)} * 100$	≤0.50 = Absence of significant fibrosis >1.50 = Presence of significant fibrosis ≤1.00 = Absence of cirrhosis >2.00 = Presence of cirrhosis

Transient Ultrasound Elastography (FibroScan)

Fibroscan is an ultrasound based procedure used to assess liver fibrosis by measuring liver stiffness [157]. Liver stiffness (elasticity) values typically range from 2.5 to 75 kPa. A cut-off of ≤7.1 (METAVIR F<2) represents mild fibrosis, a cut-off of >7.1 – 9.5 (METAVIR F=2) represents significant fibrosis and a cut-off of >9.5 (METAVIR F≥3) represents cirrhosis [157]. The sensitivity and specificity for the cut-offs of ≤9.5 and >9.5 are 72% and 80% and 72-77% and 85-90% respectively [54]. The procedure is painless and easy to perform and therefore is a good non-invasive alternative in detecting presence of cirrhosis. Limitations include difficulties in individuals who are obese, as liver stiffness measurements may be challenging to obtain [157].

1.3.7 Global epidemiology of HCV

According to the Global Hepatitis Report, as of 2017 there were 71 million people infected with chronic HCV infection [158, 159]. The East Mediterranean and European regions were reported as having the highest HCV prevalence contributing 2.3% (uncertainty interval: 1.9-2.4) and 1.5% (uncertainty interval: 1.2-1.5) of the total global population with HCV infection respectively [158]. In 2015, incidence rate of new HCV infections were estimated as 23.7/100,000 population globally [160]. The

number of people infected with HCV in Europe is estimated at 14 million and HCV incidence rate is estimated as 62/100,000 population [160]. In terms of proportion of people who have been treated approximately 20% (14 million/71 million) of individuals living with HCV infection, were aware of HCV diagnosis and of these only 38% (5 million) were treated [158].

1.3.8 HCV epidemic in Italy

In a European report of HCV prevalence in 2018, relative to other European countries studied, Italy stands as the country with highest HCV prevalence of 5.9%. This compared to, 3.5% in Romania, 2% in Spain, and ~1% in Belgium, France, Bulgaria, Poland, Turkey Germany and UK [161] [162]. It was noted that a possible explanation of high incidence could have been due to an increase in HCV transmission from transfusion with infected blood [163] [164]. The historical burden of HCV in Italy was high in the mid-80s, mostly in PWID's and older age groups because this is when HCV symptoms were likely to show [163, 164]. A decrease in HCV incidence over time can be explained by improvements in health care systems and awareness of sterilization for blood transfusion procedures [165] [161].

Epidemiological data on HCV infections is limited in Italy as most studies are either based in the 1990s or performed at local levels [166]. A study accumulating regional level data showed high prevalence of HCV infections in the southern region compared to the northern region with estimates of 16% and 4% of the national HCV prevalence in the southern and the northern regions respectively [166] [167].

Gardini et al conducted a study to quantify HCV-positive patients in hospitals in Italy representative of each region (south, centre, north). The cross-sectional study carried out between September 2017 and January 2018 included 2,860 HCV-positive people of whom 54% (n=1,548) were HCV viremic and the 46%(n=1312) reported being cured. The proportion who were HCV viremic among HCV-positive individuals across the regions were as follows; south - 62% (509/824), centre - 43% (260/600) and north - 54% (779/1436) [167]. This is possibly explained by more

socio-economically deprived areas in the south compared to other regions which may impact on access to health services, diagnosis and treatment. There was a higher prevalence of HCV viremia in rural areas, where access to health care services were limited. Interestingly in the same study, prevalence of PWIDs among those with HCV was highest in the southern regions; south – 99% (816/824), centre – 58% (349/600) and north – 80% (1151/1436) [167]. Estimates of HIV/HCV coinfected individuals were also presented across regions as follows; south – 0%, centre - 4% (24/600) and north - 13% (183/1436)[167]. The authors did acknowledge the lack of the data and that their estimate may therefore not accurately represent the number of HIV/HCV coinfected individuals attending hospital. Data on infection route and other demographic factors were collected using questionnaires via face to face interviews. One difficulty is that the HIV/HCV coinfected populations also include people from different risk groups considered 'hard to reach' such as PWIDs and prisoners making it challenging to obtain accurate data[167]. Thus, one of the strengths of the Icona and Hepaicona cohorts is that epidemiological data is collected from people seen for routine clinical care across the country, and therefore likely to give a representative picture of HIV/HCV coinfected individuals in Italy.

In chapter 7, I investigated this issue of possible regional differences in the HCV care pathway in Italy. As highlighted by the above studies, potential regional disparities in access to diagnosis and treatment is also another challenge in tackling HCV eradication among HIV/HCV coinfected individuals.

Distribution of genotypes in Italy

Briefly, in a more recent analysis of the Icona cohort looking at the prevalence of HCV genotype of patients who entered into care between 1997 and 2015, included 12,135 individuals. Of whom 3,407 (28%) were found to be HIV/HCV coinfected and 40% (n=1349) had data on HCV genotype. The distribution of HCV genotype was as follows: G1 – 49%, G2 – 3%, G3 – 36% and G4 – 11%. Interestingly the authors reported, younger age, MSM receiving care from central region and

enrolled in more recent years were all associated with being infected with HCV genotype 1 [168].

1.4 HIV/HCV coinfection

1.4.1 Global prevalence of HIV/HCV coinfection

According to a systematic review carried out by *Platt et al* in 2015, the overall burden of HIV/HCV coinfection was estimated to be approximately 2.3 million HIV-positive people worldwide equating to 6.2% (range:3.4 – 11.69) of the general HIV-positive population [169] [170] [3]. As HIV and HCV have common routes of transmission, i.e. injecting drug use, sexual contact, this has led to varying prevalence estimates of HIV/HCV coinfection in different geographical regions worldwide due to the differing prevalence of persons at risk [169] [171] [172].
In the USA where the main risk factors for HIV/HCV coinfection are injecting drug use and haemophilia, prevalence estimate is 16.1% (95% CI; 14.3–17.8) in HIV infected individuals [173].

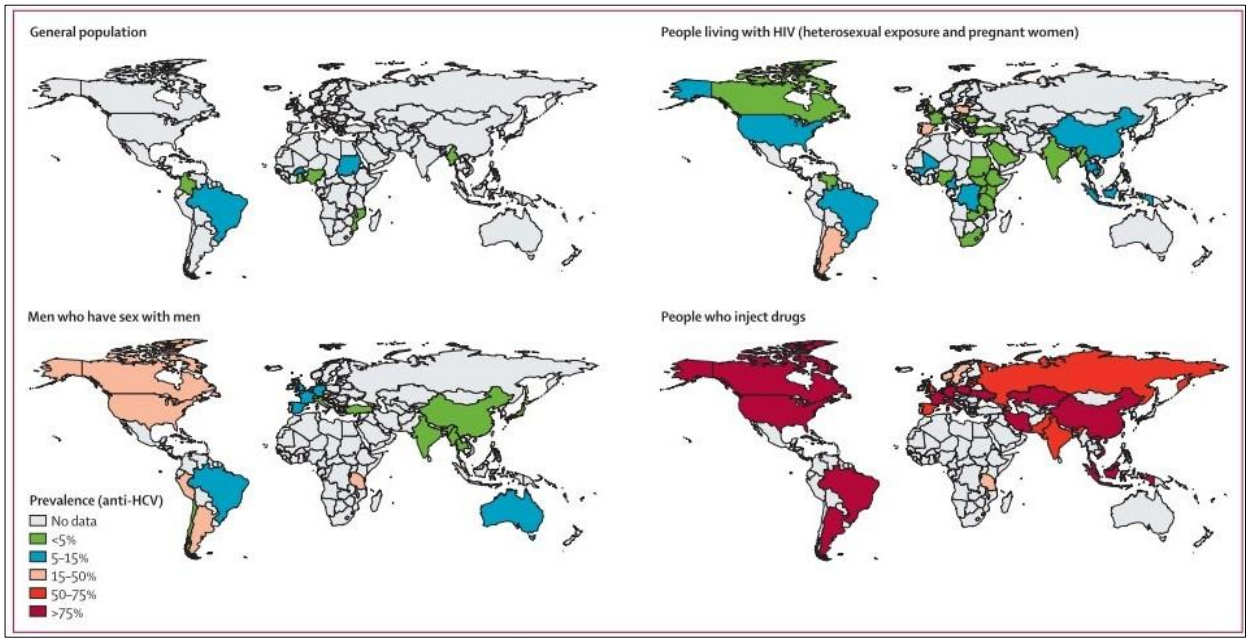
In Europe, the distribution of HIV/HCV coinfection also varies geographically (Figure 1.7). Among PLWH HIV/HCV coinfection is most commonly reported in Eastern and Southern Europe with a prevalence of 58% and 29% respectively; areas in which HIV is mostly acquired through injecting drug use [174] [175]. In Italy the current estimate of HCV infection amongst PLWH is approximately 35% [176]. In Northern and Western Europe, estimates are 17% and 20% respectively mostly acquired through sexual transmission among MSM [174] [175] [177].

In Asia, HIV/HCV coinfections are most common in PWIDs with >80% of PWIDs individuals coinfected [178] [179] [180] [181]. In contrast, lower prevalence estimates of <50% of HIV/HCV coinfection rates were observed in other risk groups such as sexual contact and HCV infected blood donors [182] [183] [184].

In sub-saharan Africa overall prevalence HCV infections among HIV-positive individuals are estimated at 5.7% (95% CI: 4.9 – 6.6) [185] [186, 187]. In terms of this region, HIV/HCV coinfections are most prevalent in West Africa and are estimated at 6.7% (95% CI 6.0-7.6) of PLWH, followed by southeast Africa 4.3% (95% CI: 3.6-4.7) [185] [186, 187]. The differing estimates are possibly explained by variation in prevalence of risk groups in different regions of Africa. Most common risk factors reported for the HIV/HCV coinfections were through blood transfusions, sexual contact and unsafe medical practices, especially in pregnant women [188] [189] [190, 191].

In North Africa, although generally low prevalence estimates of HIV/HCV coinfections are present in the general population, prevalence in the PWID population is fairly high, with PWID comprising of 80% of all HIV/HCV coinfected individuals in the region [192] [193].

Figure 1.7 Estimates of prevalence of HIV/HCV coinfection among general population, PLWH, MSM and PWID; January 2002 to January 2015 in 88 countries [3]



Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, et al. Prevalence and burden of HCV coinfection in people living with HIV: a global systematic review and meta-analysis. The Lancet Infectious diseases. 2016;16(7):797-808.

1.4.2 HIV and its effect on HCV infection

HIV infection has been shown to worsen the outcome of chronic HCV infection, by increasing HCV-RNA, increasing risk of cirrhosis and liver failure, and compromising the response to interferon therapy [194].

HIV and HCV disease progression among HCV-positive people

Disease progression of HCV leads to liver-related death if left untreated[54]. As mentioned in section 1.3.3 HCV is initially asymptomatic and symptoms may not show for years. The prevalence estimate of SLD was assessed in a meta-analysis study of 17 studies comprising of 3,567 HIV/HCV coinfected individuals, 21% (95% CI: 16-28%) and 49% (95% CI: 40-59) had cirrhosis at 20 and 30 years respectively after HCV infection [195].

More so, higher rates of fibrosis progression were observed in HIV/HCV coinfected individuals compared with HCV mono-infected individuals [196]. This highlights the importance of managing both HIV and HCV infections. To confirm this, strong evidence comes from a meta-analysis of eight studies carried out between January 1996 and June 1999) examining the role of HIV/HCV coinfection on the risk of progressive liver disease among people with HCV. The authors reported a combined relative risk of 2.92 (95% CI: 1.70-5.01) associated with coinfection vs. HCV mono-infection [196]. These findings were consistent with a EuroSIDA cohort study comprising of HIV/HCV coinfected individuals carried out prior to the DAA era[197]. The authors assessed the association between fibrosis and liver-related mortality. Advanced liver fibrosis (METAVIR F>3 was associated with 6-fold (sHR 6.25, 95% CI 4.08–9.58, P<0.0001) increased hazard compared to METAVIR F≤1, using sub-distribution (estimates of HR in the presence of competing events) [197].

HIV and HCV-RNA among HIV/HCV coinfected people

HIV/HCV coinfection is associated with high levels of HCV-RNA; this evidence comes mostly from data collected from serum samples [198] [199] [200]. Despite

studies, showing evidence of increased HCV-RNA in HIV/HCV coinfected individuals, according to a recent study this is not the case in individuals receiving cART. A prospective cohort study of 1,541 HIV/HCV coinfected people conducted in Europe, in which individuals were followed-up for a median of 5 years showed that HCV-RNA increased on average by 27.9% per year (95% CI: 6.1 – 53.5) in HIV/HCV coinfected individuals not receiving cART compared to only 2.6% per year (95% CI: -1.1-6.5) in HIV/HCV coinfected individuals receiving cART [201]. Further, the study also showed that a log unit increase in HIV-RNA was associated with 11% increase in HCV-RNA [201].

Impact of antiretroviral therapy on outcomes for HCV infection among HIV/HCV coinfected people

The impact of cART on liver–related outcomes remains a controversial issue. The use of cART has been shown to be associated with reduced liver fibrosis progression as well reduced liver-related mortality in HIV/HCV coinfected individuals in a number of studies [202] [203] [204] [205] [206] [207]. For example, *Benhamou et al* evaluated the role of cART comparing 182 untreated and treated HIV/HCV coinfected individuals from January 1995 to March 2000, of whom 35% (n=63) were treated with PIs. Over a 25-year period of observation lack of cART was associated with a four-fold risk advanced liver fibrosis progression RR=4.74 (95% CI: 1.3-16.7) compared to treated individuals [207]. However, in other studies, no effect of cART on liver-related outcomes has been found comparing HIV/HCV coinfected with HIV mono-infected individuals. These differing findings are likely to be explained by a number of factors including the specific cohorts studied, sample size, lengths of follow-ups, type of cART initiated and potential confounding factors and varying adjustment strategies used [208] [203] [205].

In a study of HIV/HCV coinfected individuals (the majority of whom had haemophilia) followed up for an average of 12 years, the authors showed liver related mortality rates of 0.45/100 person-years follow-up in people treated with cART compared to 1.70/person-years follow-up in those without cART [204]. Treatment with cART was found to be associated with a reduction in liver-related

mortality OR = 0.11 (0.02-0.56, p=0.018) [204]. However, discussions have surrounded the findings of this study alluding to the possible bias being introduced in the analysis. Specifically, a large number of individuals in the study appeared to be a selected population of people who had survived more than 10 years to receive cART which may explain the better outcome observed [209]. Consistently, *Marine-Barjoan et al* found large differences between HIV/HCV coinfected and HCV mono-infected individuals with advanced liver fibrosis (26% compared with 7%), suggesting that starting cART earlier was associated with slower liver fibrosis progression due to controlled HIV disease [202].

A prospective study of 472 participants of whom 54% (n=256) were HIV/HCV coinfected, documented a total of 134 deaths over 8,343 patient-months of follow-up of which 41% were attributable to liver-related mortality. Having suppressed HIV-RNA and being on cART were associated with reduced risk of liver mortality [206].

However, in a large cohort study of 23,441 HIV-positive individuals, no effect of cART was found in HIV/HCV coinfected individuals. There was no association between duration of cART and risk of liver-related deaths, RR=1.00 (0.93-1.07) [208]. In contrast, *Lo Re V 3rd et al* assessed incidence of hepatic decompensation in cART treated HIV/HCV coinfected individuals compared with HCV infected [210]. They found frequent occurrence of hepatic decompensation in HIV/HCV coinfected individuals with an increased risk of 83% aHR[95%CI] = 1.83 [1.54 – 2.18][210].

The literature has highlighted conflicting findings regarding the relationship between cART and progression of HCV infection. One potential reason for a mixed impact of cART on liver disease progression is the link between liver disease and cART toxicity. Additionally, the stage of HIV disease progression and duration of HIV infection could affect risk of hepatic decompensation among HIV/HCV coinfected individuals. There is some suggestion that liver disease may impact on use and tolerability of cART in terms of adverse effects. As mentioned in section 1.1, at the time of the analyses, this was an important clinical question and therefore required more research, especially with more modern therapies. In

chapter 5, I look at the role of HCV infection on the discontinuation of specific cART regimens. I also looked at reasons for discontinuation and discuss the literature on this issue in more detail. However, treatment for HCV has greatly advanced since then and this is no longer such a relevant issue.

1.4.3 HCV and its effect on HIV infection

HCV and HIV disease progression among HIV-positive people

Following the introduction of effective cART, the effect of HCV on HIV has been more controversial with mixed evidence from a number of studies in the earlier cART era [211] [212]. For example, in a Swiss cohort study of HIV-positive individuals starting cART between June 1996 and May 1999, 3,111 individuals were included, of whom 37% (n=1157) were HIV/HCV coinfected, the majority 87% (1006/1157) of whom had a history of injecting drug use. In this analysis, being HCV-positive was independently associated with the risk of developing a new defining AIDS clinical event or death (HR=1.70 (95% CI: 1.26-2.30, p<0.05) compared to HCV-negative, adjusted for gender, age, CD4, HIV-RNA, AIDS, IDU and on NRTIs [213]. Similarly, in Italy, *De Luca A et al* using the data from the Icona cohort evaluated 1,320 treatment naïve HIV-positive individuals starting cART of whom 45% (n=600) were HCV-positive. The authors found being HCV-positive was associated with the risk of HIV clinical progression (HR=1.57 95% CI: 1.01-2.61, p=0.04) independent of gender, age, current HIV-RNA, CD4, IDU and cART [214]. However, these findings were not confirmed in a study conducted in the USA between January 1995 and January 2001, also a prospective HIV cohort of 1,955 individuals of whom 45% (n=873) were HIV/HCV coinfected with 85% (n=742) having a history of injecting drug use. In particular, they found no differences in risk of acquiring AIDS (RH=1.03 (95% CI: 0.86-1.23, p>0.05) or death (adjusted RH=1.01 (95% CI: 0.65-1.56, p>0.05)) when comparing HIV/HCV coinfected with HIV mono-infected individuals, adjusted for age, CD4, HIV-RNA and years on cART [215]. Similarly in an analysis of the EuroSIDA cohort of people enrolled between May 1994 and November 2003 included 5,957 participants of whom 33% (n=1960) were HCV-positive. The authors found a lack of association with the incident risk of

AIDS/death after adjusting for other prognostic factors such as gender, age, ethnicity, geographical region, mode of HIV transmission, CD4, HIV-RNA, and cART (aIRR=0.97 (95% CI: 0.81-1.16, p =0.072) [216].

In the pre-cART era, similar findings were also observed. For example in a study of 652 HIV-positive women enrolled between December 1989 and 1995 of whom 29% (n=190) had HCV and 42% (n=261) reported drug use, HCV infection was found not to be associated with the risk of occurrence of an AIDS defining event/death (RH=0.75 (95% CI:0.37-1.53, p=0.433) [217]. A larger international retrospective study of 1,649 participants of whom 16% (n=264) were HCV infected, also found no differences in HIV disease progression between HIV/HCV coinfected and HCV mono-infected individuals [218]. Although most participants had, CD4 <250 cells/mm^3, there was no difference in the risk of AIDS/death events (13% vs. 11%) comparing HCV mono-infected and HIV/HCV coinfected participants [218].

Therefore, in summary, the literature has highlighted a possible association of HCV infection with HIV disease progression, however these findings may be dependent on factors adjusted for and possibly the calendar period being analysed. In addition, differences could also be attributable to the HIV populations studied.

Impact of HCV infection on all-cause mortality in HIV-positive people

Although the impact of HIV/HCV coinfection on progression of HIV is controversial, among HIV-positive people, HIV/HCV coinfection typically leads to more rapid progression of liver disease and plays a major role in liver-related morbidity and all-cause mortality [219] [220] [221].

A large study in Spain of 5,914 HIV-positive individuals studied over a 10-year period (1997 to 2008) compared overall mortality rates and liver-related deaths with those observed for the general population. Standardized mortality ratios of people with HIV were 11.5 (95% CI: 9.9-13.4) in those with HCV infection and 2.4 (95% CI: 1.9-3.1) in those without HCV[219]. The risk of liver-related deaths was found to be 10 times higher in the cohort of HIV-positive individuals than in the

general population and 22 times higher in subset of HIV/HCV coinfected compared to the general population [219]. In a much larger cohort of 23,441 HIV-positive individuals 66% of whom also had HCV infection, 5.3% (n=1246) of individuals died during follow-up and 14.6% of these were from liver-related causes[208]. An examination of the factors predicting liver-related deaths showed older age, lower CD4, injecting drugs, HCV infection and HBV infection were all independently associated [208]. An increase in mortality rates due to non-AIDS related causes have been demonstrated widely in the HIV-positive population. A study in Canada with 1,987 HIV-positive individuals showed that the risk of non-AIDS related deaths increased from 7% to 32% over the years 1984–2003 and, again, higher risk of death was associated with HIV/HCV coinfection, injecting drugs and older age [220].

In a meta-analysis of 37 studies looking at the effect of HCV on HIV disease, HIV disease progression and overall mortality rates were compared before (pre January 1996) and after (post January 1996) the advent of cART [221]. In the pre-cART era, HIV/HCV coinfection seemed to protect against the risk of death with a relative risk for overall mortality comparing HIV/HCV coinfected individuals with HIV mono-infected of 0.69 (95% CI: 0.54-0.88) [221]. In contrast, in the cART era the direction of this same relative risk of death was inverted favouring the HIV mono-infected population 1.35 (95% CI: 1.11-1.63) [221]. This is partly explained by the fact that HIV-positive people are now living longer on cART and that liver disease is especially emerging among the HIV/HCV coinfected individuals.

Although most studies showed an increase over time in mortality rates due to liver–related disease in the HIV-positive population, a more recent study showed a decrease in liver-related deaths [222]. Briefly, a large study followed up 49,731 HIV-positive individuals between the years 1999 and 2011 [222]. There were 3,909 deaths observed and 13% of these were found to be liver-related. The rate of death was shown to decrease over time with a rate of 17.5 in years between 1999-2000 followed by a rate of 9.1 in the years between 2009-2011 while liver-related deaths also decreased from 2.7 to 0.9 over the same time-span [222].

Impact of HCV on CD4 cell count in HIV-positive people

The effect of HCV infection on HIV has been studied in terms of CD4 cell recovery in HIV-positive individuals after starting cART. *Antonucci et al,* again using the data of the Icona cohort, evaluated 1,219 HIV/HCV coinfected people, of whom 23% (n=284) were HCV viremic. They found that the chance of achieving CD4 cell count recovery of >100cells/mm^3 on cART from pre-cART levels was poorer in HCV viremic individuals than non-HCV infected individuals aRH=0.82 (95% CI: 0.66-1.01, p=0.06) [223]. This was an important result as a similar analysis from the same group have previously found no statistical evidence despite in the probability of CD4 recovery on cART when comparing individuals grouped according to their HCVAb-test results alone a aHR of 0.72 (95% CI; 0.43-1.12, p>0.05) for HCVAb-positive versus negative [214]. In a Danish study of HIV-positive individuals starting cART, HCV-positive individuals were found to have lower CD4 compared to individuals without HCV infection [224]. In contrast, a prospective study of 4,208 HIV-positive individuals with stable HIV-RNA after starting cART and 12,492 person years follow-up found no evidence for a difference in annual CD4 change between HCV-positive (35.5 cells/ml (95% CI: 27.2-43.9)) and HCV-negative individuals (38.3 cells/ml (95% CI:34.8-41.9, p=0.17) [225]. Similarly, a number of other studies of HIV-positive individuals initiating cART also found no association between HCV infection and CD4 recovery [226] [227]. One of the possible reasons for these discrepancies is that not all analyses were restricted to HCV viremic people, i.e. those with chronic HCV.

1.5 Previous research on HIV/HCV coinfection using Icona and Hepaicona cohorts and rationale of research questions in my thesis

First of all, HIV/HCV coinfection is prevalent worldwide. Estimates presented previously show that about 2.3 million of HIV-positive people also have HCV infection [3, 228]. It has also been shown that for PLWH in the cART era, liver disease is a leading cause of death if left untreated [158]. Fortunately, the approval of DAAs for the treatment of HCV in 2014 gives the potential for HCV eradication rates of more than 90% [142]. In addition, eradication rates are similar in both HCV

mono-infected and HIV/HCV coinfected people [38]. This breakthrough in, and effectives of, DAA treatment has led to programs for HCV elimination in the HIV/HCV coinfected population. One of this is led by the Icona Network and has the ultimate objective of HCV elimination by the end of 2023 [229]. More so, WHO has now set global elimination targets to reduce the burden of disease by 2030 [158].

Some chapters in the thesis (chapter 4 to 6) include analyses based on data prior to the DAA era. At the time of the analyses there were different clinical questions and treatment strategies that were relevant to that pre-DAA era but which in the DAA era may no longer be an issue. With the advent of DAA treatments I focused my later chapter (7) specifically on the recent period (2015). Even in the DAA era, there are a number of remaining challenges in managing HCV and working towards eradication amongst PLWH[5].

My research question relating to the relationship between alcohol consumption and SLD outcome among PLWH, and the impact of HCV on this (chapter 4), has not previously been looked at in either the Icona and Hepaicona cohorts.
As mentioned in section 1.1, although data on alcohol are collected in these cohorts, these have been previously used only as descriptive variables to characterise the cohorts. Therefore, my work on mapping physicians' assessments of patients' alcohol consumption to those used in national drinking guidelines from NIFN in Italy, assessing how useful these physician recorded alcohol data are, investigating whether the measure of alcohol can predict risk of SLD, and assessing how the relationship differs according to HCV coinfection, can be considered as completely novel. Addressing these questions is important in informing clinical care and management of PLWH and in gaining understanding of the role of alcohol, as a potentially modifiable lifestyle factor, in serious outcomes among PLWH with and without HCV coinfection. The analysis is important also because few other HIV cohorts routinely collect information on alcohol and there are no widely adopted standardised alcohol measurement tools in studies of PLWH

or in clinical care. Therefore evidence on the utility of this physician assessed measure of alcohol consumption can help to inform future work.

Another historical challenge addressed in the thesis was the role of HCV in the discontinuation of ARV drugs, due to HCV potentially interfering with elimination of ARV drugs by the liver, leading to toxicity, and this is the issue addressed in my research in chapter 5. Previous research in Icona relating to discontinuation of cART has focused on older cART regimens and at the time, results were showing toxicity to as the main reason of cART discontinuation [230]. More recently, *Di Biagio et al* assessed the rate and predictors of cART discontinuation between January 2008 and October 2014 [231]. The study focused on three main outcomes; toxicity, intolerance and simplification. The main finding of this analysis was that simplification was the main reason of drug discontinuation[232]. My work relating to cART discontinuation, specifically focused on the role of HCV infection in HIV-positive individuals enrolled up to June 2016. I evaluated HIV drugs that are more modern and used a different statistical approach when assessing risk of discontinuation of each cART separately. However, with new DAA treatments for HCV there are now treatment options available for HIV-positive people regardless of cART regimen.

The HCV CoC pathway is a way of characterising the journey of HCV-positive individuals through the care continuum from diagnosis all the way to treatment. Examining the proportions moving from each stage to the next can help to shed light on barriers at different stages of care. For example, screening of HCV may limited in some settings because of availability of health care resources [158]. Timing of HCV diagnosis is a challenge as some people are still diagnosed in the late stages of HCV disease [158, 159]. It is known that HCV is initially asymptomatic, therefore, there are many people who are unaware of their HCV infection until they develop severe symptoms [12]. In line with the WHO global target of HCV elimination, a further question in my thesis relates to late HCV presentation, and its association with all-cause mortality and HCV treatment initiation (chapter 6). This has not been done previously in Icona and I specifically focus on people with

recent HIV diagnosis because these group of people are also likely to be recently tested for HCV. As mentioned in section 1.1, this will add to understanding of the HCV care pathway and whether they are people still presenting late into clinical care with HCV.

Previous research in Icona by *d'Arminio Monforte A et al* looked at access and response to DAA in HIV/HCV coinfected individuals who were naïve to DAA as of January 2013 as mentioned in section 1.3.5[143]. My thesis takes this further by examining the HCV care pathway in chapter 7 and assessing whether region of care is associated with each of the outcomes: HCV testing, initiation of DAAs and achieving SVR, adjusting for potential confounders. This analysis broadly includes HIV/HCV coinfected people enrolled in Icona and Hepaicona from January 2015.

The thesis questions are important because they address clinical questions using real-world setting of unselected HIV-positive people seen for routine clinical care with or without HCV. Understanding gained could help to inform clinical practice in terms of how both diseases are managed. With Italy having the highest prevalent cases of HCV infection, now is an even more important time to investigate potential challenges of HCV infection among HIV infected people. As WHO have set targets towards the global elimination of HCV, Icona and Hepaicona cohorts are rich epidemiological data sets with both historical and current data which is well suited to investigate potential ongoing challenges in managing both HIV and HCV diseases.

The latter chapters (6 and 7) of my thesis have adopted modern and robust statistical methods to address the research questions. Specifically, I improved my analyses relating to model building by using Direct Acyclic graphs (DAGs) to identify potential confounders in terms of assessing factors associated with the outcomes of interest.

In these various analyses, my thesis will provide information relating to ongoing challenges in managing HCV in HIV-positive people seen in routine clinical care in

Italy using real-world data. Clinical and methodological implications of the findings are discussed in chapter 8.

CHAPTER 2

2 DATA AND METHODOLOGY

2.1 Aim

The aim of this chapter is to describe methodology for the literature reviews and the studies used in this thesis, including details of methods of data collection, data management, populations included in each analysis and finally statistical methodology used to address specific research questions in the each of the results chapters.

2.2 Introduction

To address the aims set out in each chapter, this thesis utilized data from two large observational prospective cohort studies; Icona and Hepaicona. Both cohorts include PLWH seen for care at a number of Infectious diseases clinics across Italy (more details in sections 2.4.1 and 2.4.2). In each of the results chapters, I carried out a series of analyses using a subset of participants selected from these cohorts that fulfilled a defined inclusion criterion. Each of the results chapters (4 to 7) include: the aims and specific objectives; an introduction; a literature review; explanation of which cohort was used in the analysis; definition of the outcome and exposures; description of inclusion criteria of the population in the analysis; specific statistical analysis methodology for chapter; results; discussion and conclusions.

2.3 Methodology for literature reviews

For each of the individual results chapters (4 to 7), I carried out a literature review of relevant publications surrounding the research question(s). The date range of each literature search is specified in Table 2.1 and in the relevant chapters.

The aim of the literature reviews was to gain comprehensive information on what relevant research has previously been published each the specific topics. I

identified gaps in the literature relating to the specific research question(s) and this guided the development and planning of my analyses.

I searched for published articles using key terms specific to each chapter using the PubMed database. PubMed is freely available on the internet with over 25 million records available. A summary of the specific search terms used for each of the research questions is shown in . I also read relevant publications referenced within each published article as well looking at related citations. The process of synthesising the information initially involved screening titles and abstracts. After selecting possible relevant articles, I read each paper and summarised the objectives and the key findings, as well as the relevance to my specific research question.

Table 2.1 Literature review questions and search terms used in chapters 4, 5, 6 and 7

Chapter	Research question	Search terms	Inclusion criteria
4	What is the role of HCV coinfection on the association between alcohol and liver disease in PLWH?	((((HIV[Title]) OR human immunodeficiency virus[Title])) AND ((HCV[Title]) OR HEPATITIS[Title])) AND alcohol[Title] (N=43) Search ((((HIV[Title]) OR human immunodeficiency virus[Title])) AND ((HCV[Title]) OR HEPATITIS[Title])) AND liver[MeSH Terms] Filters: Publication date from 2000/01/01; Humans Search (((((HIV[Title]) OR human immunodeficiency virus[Title])) AND ((HCV[Title]) OR HEPATITIS[Title])) AND alcohol[Title]) AND (((((liver[Title]) OR fibrosis[Title]) OR cirrhosis[Title]) OR hepatocellular[Title]) AND ("2000/01/01"[PDat] : "3000/12/31"[PDat])) Filters: Publication date from 2000/01/01 (using different possible terms for liver disease) **First search: up to July 2018** **Second search: up to January 2021**	Humans, English
5	What is the role of HCV coinfection on discontinuation of specific antiretroviral drugs in PLWH?	Search (((((Antiretroviral therapy) OR ART) OR CART) OR Highly Active Antiretroviral therapy)) AND ((Stopping) OR discontinuation) Sort by: PublicationDate Filters: Humans **First search: up to October 2016** Search ((((((ART[MeSH Terms]) OR Antiretroviral[MeSH Terms]) OR CART[MeSH Terms]) OR highly active antiretroviral therapy[MeSH Terms]) AND Humans[Mesh])) AND (((discontinuation[Title]) OR stopping[Title]) AND Humans[Mesh]) Search (((((Antiretroviral therapy) OR ART) OR CART) OR Highly Active Antiretroviral therapy)) AND ((Stopping) OR discontinuation) Filters: Humans **Second search: up to December 2017** **Third search: up to January 2021**	Humans, English
6	What is the role of late HCV presentation on all-cause mortality	((((((HCV[MeSH Terms]) OR hepatitis c virus[MeSH Terms])) AND (((((screening[MeSH Terms]) OR diagnosis[MeSH Terms]) OR	Humans, English

Chapter	Research question	Search terms	Inclusion criteria
	and HCV treatment initiation among newly diagnosed coinfected HIV individuals seen for routine clinical care in Italy?	detection[MeSH Terms]) OR testing[MeSH Terms]) AND Humans[Mesh])) AND Humans[Mesh])) AND (((coinfection[MeSH Terms]) OR coinfection[MeSH Terms]) AND Humans[Mesh]) **First search: up to April 2019** **Second search: up to February 2021**	
7	Are there regional differences in terms of the continuum of care for HCV among HIV/HCV coinfected individuals seen for routine clinical care in Italy since January 2015?	Search (((HCV[MeSH Terms]) OR hepatitis c[MeSH Terms])) AND ((continuum of care) OR cascade of care) **First search: up to January 2019** **Second search: up to February 2021**	Humans, English

2.4 Data used in this thesis

This thesis uses two cohorts established from the Icona Foundation Network, which started in 1997: the Icona and the Hepaicona cohorts. These studies are prospective observational research studies which means that there was no intervention by the researchers, and the studies are concerned with what happens in the usual course of the individual's care and management. Therefore, the findings in this thesis should reflect outcomes in usual medical care. The Icona and Hepaicona cohorts contain routine clinical information and results of tests which are conducted as part of routine care. Both cohorts include PLWH residing and seen for care in a number of Infectious disease participating clinics in Italy and include longitudinal information on the participants as they are followed up within routine care. Each cohort is described in the sections below.

2.4.1 Overview of the Icona Foundation Study cohort

Icona is an ongoing prospective multicentre observational cohort study which began recruiting HIV-positive individuals in 1997 with the only inclusion criteria being that the individuals are naïve to cART at recruitment. Participants who are cART-naïve are defined as "participants *who have never undergone antiretroviral therapies regardless of the clinical stage of their disease, the degree of immunological impairment, or the motivation for not having previously started therapy".* At the time of the analyses of this thesis the number of participating centres in Icona was 40. As of January 2021, there were more than 18,000 antiretroviral naïve HIV-positive individuals enrolled in Icona. Participating centres have also increased to 56.

The main objective of Icona is to evaluate the experiences, prognosis, management and outcomes of PLWH enrolled in routine clinical care. In addition, the Icona data has information about the natural history of HIV and hepatitis virus infections from data available from participants prior to HIV treatment. The main

strength of Icona is that the cohort is able to provide data on a range of epidemiological, clinical, biological and behavioural parameters and changes in these during treatment and care. The cohort design allows research questions to be addressed prospectively (because information on an exposure is measured prior to occurrence of the outcome of interest), meaning temporality (i.e. the effect occurs after the cause) is well defined.

Other important strengths of the Icona dataset to address the research questions include the following features:

- Long duration of follow-up. The study has now been running for more than 20 years and has follow-up for more than 18,000 HIV-positive individuals. This enables the study of long-term outcomes, and of changes over calendar time
- Inclusion of an unselected and diverse population of HIV-positive individuals across Italy with a significant proportion of women (approx. 25% of the cohort) and good representation of different mode of HIV transmission groups
- Collection of socio-economical factors (maximum level of education and employment status at enrolment)
- Collection of repeated information on lifestyle factors such as smoking and alcohol consumption. Both this and the previous feature are unusual in routine HIV clinical databases.
- Collection of information on long-term non-AIDS clinical outcomes such as severe liver disease (SLD).
- More than 50,000 biological samples and more than 15,000 cell tissue and full blood samples stored in a repository linked with the clinical data

The high prevalence of HCV in Italy means that an Italian cohort is well placed to examine questions related to HIV/HCV coinfection. Historically Icona has provided an important contribution on the potential impact of HCV on the natural history of HIV. It remains important to address questions relating to HIV/HCV coinfection, ranging from pathogenesis to clinical outcomes and response to treatment. Very

recently the focus in HIV/HCV coinfection research has shifted towards the response to new DAA in real-world clinical practice.

In terms of the questions addressed in this thesis, Icona can provide useful information on the usual clinical management of HIV/HCV coinfection. For example, in chapter 6 I assessed the prevalence of late HCV diagnosis and its association with HCV treatment initiation which gives some insights into access to health care. Additionally, the Icona cohort provides long term follow-up data for PLWH that is particularly useful in addressing questions related to long-terms outcomes (such as SLD, in chapter 4) and discontinuation of HIV therapy and its interplay with HCV infection (addressed in chapter 5). Finally, the inclusion of information on HCV-negative individuals as well as HIV-positive individuals allows comparative questions to be addressed, as in chapters 4 and 5.

In terms of international presence, Icona collaborates internationally with other HIV cohort studies and plays an important role in identifying overall needs of HIV-positive individuals and the impact of HIV therapy. Icona has published extensively and research has been presented at a large number of conferences. During the course of this PhD study I contributed to its publication history as chapters 4 and 5 have been published in peer review journals. More information about the history of the study can be found at the Icona web page and a description of the cohort in chapter 3.

2.4.2 Overview of the Hepaicona cohort

Hepaicona is a cohort created subsequently to the parental Icona cohort and now runs in parallel with it, using the clinical sites network and recruitment structure already in place for Icona. Hepaicona is the most comprehensive observational cohort study of HIV/HCV coinfection ever conducted in Italy. Enrolment started in the autumn of 2013 and the key inclusion criteria for the study is for participants to be HIV-positive have a detectable HCV-RNA at inclusion and to be naive to DAA. With regards to HIV treatment, individuals are predominantly cART-experienced as

the cART-naïve individuals are prioritized for enrolment into Icona. Participants can only be enrolled in one cohort and not both and remain in the enrolled cohort. The main goal of Hepaicona is to provide information necessary to inform therapeutic strategies and clinical management of HIV/HCV coinfection with special focus on the rate of access to and response to modern DAA treatment.
Indeed, over the past decade Hepaicona has already provided a clear epidemiological picture of the characteristics and outcomes of the population of HIV/HCV coinfected individuals in Italy. More information can be found at the Hepaicona web page and a description of the cohort in chapter 3. As of January 2021, there were more than 3,000 participants enrolled in Hepaicona.

The use of the Hepaicona cohort in addition to Icona in my thesis is a key strength in terms of questions related to management of coinfection (chapters 6 and 7). Hepaicona can provide reliable data relating to DAA treatment uptake among HIV/HCV coinfected individuals across Italy. For example, in chapter 7 one of the objectives was to estimate the proportion of DAA treatment uptake as part of the HCV CoC by geographical region. Hepaicona was particularly suited for this aim as most of the data were collected after January 2015, the date in which DAA became universally accessible in Italy. Hepaicona was also crucial for the CoC step of estimating SVR for which the data in Icona were less accurate.

2.4.3 Recruiting sites for Icona and Hepaicona

There are more than 56 participating sites currently recruiting for both the Icona and Hepaicona cohorts across Italy which can be grouped according to their geographical location in the country as; north, centre and south sites. As mentioned above, a large proportion of the HCV chronically infected HIV-positive participants seen at these sites who were not already in Icona because they were not cART-naive were subsequently included in Hepaicona starting from January 2013.

2.4.4 Organisational structure of Icona and Hepaicona

The organisational structure of both cohorts includes board of directors, presidential committee, scientific secretary, scientific committee, statistical and data monitoring team and finally the biological bank committee.
Icona was initially funded with a 10-year grant (1997-2007) from Glaxo-Wellcome although interest and funding somewhat waned by the end of that period which resulted in a decline of new enrolments. After 2007 the cohorts have been sponsored by multiple funding sources including both EU grants and industry, and Icona was again able to achieve the target enrolments of approximately a 1,000 new cART-naïve participants per year.

2.4.5 Participant enrolment and data collection

In Icona and Hepaicona, PLWH who are aged 18 or over meeting the inclusion criteria are enrolled into the specific cohort when they have given their informed consent for their routine clinical visit data to be collected. For the purposes of anonymity, participants are assigned a random identifier number which does not contain sensitive information such as city of residency, national health number, etc. Participants who satisfy the eligibility criteria and consent are enrolled consecutively from each site. This consecutive enrolment aims to ensure an unselected sample including all eligible participants regardless of possible concerns of the recruiters over participant regularity of follow-up or extent of adherence to therapy.

All participating centres are connected to a network where clinical epidemiological data are recorded on a secure database found at (www.Icona.org) by health care providers. Table 2.2 gives a description of the variables collected. Since data is collected on electronic case report forms (eCRF), there is reduced errors in terms of inserting non-plausible data. For example, some fields have drop down options to select appropriate answers, therefore no room to enter unrecognisable data. An example of the Icona eCRFs are reported in the Appendix.

Some fields are free text fields which are routinely checked, coded and cleaned. Other free text fields have been coded for specific analyses. For example, in chapter 4 I went through the free text of reported alcohol use to construct the exposure variable for use in the analysis. Another example is the recording of reasons for hospitalization in terms of ICD9 codes. I went through the IC9 codes and classified any reasons for hospitalization relating to liver disease as this one on my outcomes in some of the analyses.
Despite the entry checks built into the data collection, it is always possible to have outliers for continuous parameters (i.e. measurements outside the normal range).

According to the study protocols, data is collected at enrolment, at any change of cART or HCV therapy, in the event of a clinical event comprising of AIDS and non-AIDS related diseases, hospitalisation and death and at least every six months. Hepatitis serology markers (e.g. HCVAb test results), biomarkers (e.g. ALT, etc.) are also typically collected at least twice per year while HCV-RNA values are typically more frequently collected around the episode of HCV treatment. HIV laboratory data such as CD4 or HIV-RNA are collected every 6 months as a minimum but even more frequently for the sites of the Network that can provide these data electronically direct from their local laboratories (in which case all available information is included). The frequency of monitoring has slightly changed over the course of this thesis but all data used in the analyses in this thesis are prior to the partial disruption of HIV care due to the COVID-19 pandemic.

2.4.6 Data management

The data are released by the data managers to the statistical team at regular intervals of at least 6 months or more often (‘on demand’) if necessarily via secure means and the data are password protected. For both the Icona and Hepaicona databases, the data are received as separate excel or access spread sheets and each data table containing a unique anonymised participant identifier number, which is used to merge all the data tables together. Data managers also prepare

codebooks describing which variables are recorded in each table, the description of these variables (format, units, range, meaning) and whether the data tables are in long (repeated rows for each participant) or wide (one row for each participant) format as shown in Table 2.2. If there is a change in the structure of these tables, this is communicated by the data managers prior to sending the new data sets to the statisticians. Any new variable prospectively generated is also added to the database and ready for future tables downloads.

The full set of data tables and their content is shown in Table 2.2. In brief, data items such as demographics, socioeconomics, mode of HIV transmission, HCV infection status, history of previous HCV therapy (mostly relating to pre-DAA treatment), are initially collected at study entry. Alcohol consumption status, smoking status, biochemical and haematology blood test results are collected prospectively at follow-up clinical visits. Other data collected prospectively at clinical visits includes hepatitis infection (A, B, C or Delta) serology test results, HIV markers (CD4, HIV RNA), any new clinical diagnoses, hospitalisations, the date of start and stop of HIV therapies, HCV therapies and other concomitant therapies.

In the central database, tables with repeated measures for the same individual are collected and stored in a long file format (each line relates to one clinical visit date). The data that are in long file format are transposed into a wide format in preparation for the analysis, so that for each participant there are multiple columns for each repeated measurement of a variable, with a suffix number indicating each of the visit dates. Therefore, wide format data files can be merged by individual participant. Once all tables are merged, this becomes the master data set for analysis corresponding to the specific data lock used (thus for example I mentioned in chapter 1 that the data lock used for chapter 4 was 30th June 2016 and this may vary by chapter).

As an example of data transposition, say one participant had five visits including the date of enrolment in the original long data set (i.e. 5 rows of data points). Once the data are transposed the participant would have five date columns, one for each

visit date (date1, date2, date3, date4 and date5). When writing SAS code I would refer to this format of the data being in form of arrays. Suppose then that the maximum number of visits for a person is 286 and that I wanted to select the first ever HCVAb-positive serology test result (first HCVAb positive test following study entry) for each participant; to select this visit my SAS code uses arrays and an iterative looping process similar to this shown below (commentaries are shown in green):

```
*Array of clinical visits and HCV tests;

array fol(286) fol1-fol286; *variables for clinical visits (format dd/mm/yy)
array hcv(286) hcv1-hcv286; *variables for HCVAb test result indicator (0=Negative, 1= Positive)

*Obtaining the first ever HCVAb+ test result;

i=286; *start from the latest available clinical visit date

do until (i=0); *to the first clinical visit

if fol[i]>. & hcv[i]=1 then do; *if date of clinical visit is not missing and HCVAb test results equals 1 (i.e. it is a positive test), then do the following

d_hcvab=fol[i]; *create a variable called 'd_hcvab' corresponding to the clinical visit date in which the test results was positive for the first time
end;

i=i-1; end; *a counter to finish this iterative process
```

This generic iterative process is typically used when there are repeated measurements over time for a variable and there is the need to select only one of these values. For example, assuming that the closest value recorded prior to a specific date is requested (say the date of starting cART), the SAS loop would look similar to the one shown above but selecting the most recent date prior or equal to the date of cART initiation. Commentary is also a very important part of SAS coding as it improves reproducibility as it is important that a description is provided so that the programming and definitions can be understood and used again in the future by a different user [233].

Of note once this wide master data set is created, it is always possible to back-transpose it to long format if needed. This is often the case for analysis involving

the calculation of person years of follow-up or the creation of weights to estimate marginal structural models.

Table 2.2 Summary description of datasets downloaded at every data lock

Excel data set	**Brief description**
Participant enrolment data (data in wide form) This data set gives information on number participant enrolled as of the specific date of data lock.	Contains data collected at enrolment. Date of consent, name of site where participant was enrolled, Demographics (height, gender, age at first visit, year of birth, ethnicity, employment status, nationality) Mode of HIV transmission (heterosexual, homosexual, MSM, PWID, other) Date of first HCVAb positive test, first HCVAb positive test (Y/N), previous HCV therapy (Y/N), date of first HCV treatment, outcome of other first HCV treatment, RBV status (Y/N), PegIFN (Y/N), IFN (Y/N),
Participant history data (data in wide form)	Education status, smoking status. Contains date of first CD4 and corresponding CD4, nadir CD4, date of first HIV-RNA and corresponding value, date of first positive HIV test, date of first negative HIV test.
Clinical visits (data in long form)	Contains date of each six-monthly clinical visit, hepatitis status (hepatitis A, B, C or D). More specifically for HCV (an indicator variable to indicate absence or presence of HCVAb at each visit if measured, alcohol consumption status (assessed by treating physician), also data on frequency, type of beverage consumed. Smoking status, biochemical and haematology blood samples (e.g. AST, ALT, PLT - I use these to calculate fibrosis score at each clinical visit where data is available)
Withdrawal from the study (data in wide form)	Contains date of withdrawal, reason for withdrawal, date of death and cause of death is reported.

Excel data set	Brief description
Hepatitis C markers	Contains more detailed data on HCV status collected at clinical visits, date of HCV test, method used, outcome of HCV test (positive or negative), HCV-RNA and corresponding units, HCV genotype, type of test (qualitative/quantitative)
Hepatitis B markers (data in long form)	Contains more detailed data on HBV status collected at clinical visits, date of HBV test, method used, outcome of HCV test (positive or negative), HBV-RNA and corresponding units, HBV genotype, type of test (qualitative/quantitative)
HIV markers (data in long form)	Contains more detailed data on HIV test collected at clinical visits. Contains date of HIV test, lymphocyte count, CD4, CD8 count, HIV-RNA, method used for the test.
Hospitalisation data (data in long form)	Contains date of admission and date of discharge, reason for hospitalisation, ICD9 codes
Clinical diagnosis (data in long form)	Contains data on disease specific diagnosis including; AIDS, non-AIDS, Cardiovascular, Tumours and date of diagnosis for each of these conditions
Clinical diagnosis of liver disease (data in long form)	Contains date of liver disease diagnosis, an indication of type of diagnosis (i.e. acute, chronic, fibrosis, HCC, ascites, compensated liver disease, hepatic encephalopathy)
HIV therapy (data in long form)	Contains data on HIV therapies received, include date started, date stopped (blank if ongoing), name of regimen (e.g. Combivir (AZT/3TC), name of drug class (e.g. NRTI)) reasons for starting ARV, reason for discontinuation of ARV, an indicator of whether it's a booster treatment (Y/N).
Other therapies (data in long form)	Contains data on other therapies received, more specifically contains data on HCV therapy received

Excel data set	Brief description
	(inclusive of name of pre-DAA and DAA treatment received), date started, date stopped (blank if ongoing),
Liver stiffness	Contains date of liver stiffness measure, liver stiffness value

2.4.7 Data checking and coding

In general, the data management team completes routine data cleaning and checking. Prior to any statistical analysis, I carried out further data checks on the distribution of continuous variables and presence of outliers. Any data issues found I reported back to the data managers team. Any changes in the dataset made as a result of these checks are retained in the master tables and are included in the following data downloads. Additional SAS coding, to read the data in and derive new variables, was done for each of the new analyses.

Missing data are not uncommon in observational studies. In analyses involving the use of categorical factors (e.g. level of alcohol consumption), for the purpose of including all the subjects, an additional category of people with 'unknown level of consumption' is often created (the so called 'missing indicator method', see section 2.8 and chapter 8 for more on this). For example, CD4 at cART initiation, could be categorised as <200, ≥200 cells/mm^3 and unknown (which indicates that CD4 was not recorded at that point in time). I have used this method for variables with a significant proportion of missing data, in order to include all subjects in analyses and to ensure that the same denominator was used when comparing the unadjusted and adjusted parameter estimates of nested regression models so that results could be correctly interpreted.

2.4.8 Overview of variables used in the thesis

Table 2.3 shows the main variables considered in this thesis with their broad categorisation. For all variables which are time-varying the exact value used in the analysis and at which time point is defined in each chapter.

Table 2.3 Overview of variables used in the thesis

	Variables	Type of data	Classification
Demographics	Age (years)	Continuous	-
	Gender	Binary	Male, female
	Nationality	Binary	Italian, non-Italian
	Italy's Geographical region of recruiting site in Italy	Categorical	North, centre and south
	Mode of HIV Transmission (only one classification is recorded) e.g. PWID and MSM not captured	Categorical	PWID, MSM, heterosexual or other/unknown
	Calendar year enrolled[a]	Categorical	1997-2002, 2003-2008, 2008-2012, 2013-2018 (e.g. chapter 6)
HIV related factors	[td]AIDS diagnosis	Binary	No, yes
	[td]CD4 cell count [a,b] (cells/mm^3)	Continuous and categorical	≤200, >200, unknown (e.g. chapter 6)
	[td]HIV-RNA [a,b,c] (copies/ml)	Continuous and categorical	≤50, 51 – 1000, >1000, unknown (e.g chapter 3)
Lifestyle	[td]Alcohol consumption[d]	Categorical	Abstain, moderate, hazardous, unknown
	[td]Smoking status	Categorical	No, yes, unknown
Social-economic factors	Education (age range)	Categorical	Primary (<11), secondary (11-16), college (16-18), university (18+), other/unknown
	Employment status	Categorical	Employed, unemployed, other, unknown
cART	[td]ARV status	Binary	Naive, non-naive
	[td]ARV regimen drug class	Categorical	NRTI, NNRTI, PI, INI (e.g. chapter 5)

	Variables	Type of data	Classification
Hepatitis	[td]Hepatitis B	Categorical	Positive negative, not tested
	[td]HCV status	Categorical	Positive negative, not tested
	[td]HCV-RNA (IU/mL)	Continuous	-
	[td]HCV Genotype status	Categorical	1, 2, 3, 4 and other (in HCV-RNA+)
Liver disease	[td]Fibrosis score [a]	Categorical	<1.45 (Mild), 1.45-3.25 (Moderate), >3.25 (Advanced)

[a] This varies depending on baseline date and data of data lock
[b,c] In some chapters these have been the categorisations
[d] Derived variable (see chapter 4)
[td] time updated variables (i.e. collected prospectively)
PWID = person who inject drugs
MSM = men who has sex with men
NRTI = Nucleoside reverse transcriptase inhibitors
NNRTI = Non - Nucleoside reverse transcriptase inhibitors
PI = Protease Inhibitors
INI = Integrase inhibitors

2.4.9 Number of participants included in each results chapter

Table 2.4 shows a summary of which cohort was used in each of the analyses, the date of data lock, and the number of participants included as well as a brief description of the inclusion criteria. Note that the number of participants included in the various analysis changed depending on data lock and inclusion criteria.

Table 2.4 Detailed summary of data included for analyses in each results chapter in the thesis

Research question (cohorts)	**Chapter**	**Date of data locked**	**N included (n included in Icona + n included in Hepaicona)**	**Brief description of inclusion criteria**
Prevalence of HCV at Icona enrolment and characteristics of Icona and Hepaicona study participants according to HCV status (Icona and Hepaicona)	3	30th June 2016	16116 (14532 + 1584)	All HIV-positive individuals with/ without HCV
		30th June 2016	3025 (1441 + 1584)	All HIV/HCV coinfected individuals with detectable HCV-RNA and naïve to DAA
What is the role of HCV coinfection on the association between alcohol and liver disease in PLWH? (Icona and Hepaicona)	4	30th June 2016	9542 (8876 + 666)	All HIV-positive individuals with/without HCV who were free from severe liver disease at enrolment. Participants enrolled prior to 1st January 2002 were excluded from the main analysis

Research question (cohorts)	Chapter	Date of data locked	N included (n included in Icona + n included in Hepaicona)	Brief description of inclusion criteria
				because alcohol use was not collected in a standardised way.
What is the role of HCV coinfection on discontinuation of specific antiretroviral drug in PLWH? (Icona)	5	30th June 2016	10637	All HIV/HCV coinfected individuals who started cART defined as at least three antiretroviral drugs of any drug class were included. Specifically, separate study populations have been used for each of the ARV drug evaluated by including people who started a cART combination including the drug of interest when people were naïve to this particular drug.
What is the impact of late HCV presentation on all-cause mortality and treatment initiation among newly diagnosed HIV/HCV coinfected individuals seen for routine clinical care in Italy? (Icona)	6	31st January 2018	768	All HIV/HCV coinfected individuals with at least one month of follow-up and with HIV diagnosis within six months of enrolling in the Icona cohort
Are there regional differences in terms of continuum of care for HCV among	7	31st January 2018	3417 (940 + 2477)	All HIV/HCV coinfected individuals alive as of January

Research question (cohorts)	Chapter	Date of data locked	N included (n included in Icona + n included in Hepaicona)	Brief description of inclusion criteria
HIV/HCV coinfected individuals seen for routine clinical care in Italy since January 2015? (Icona and Hepaicona)				2015 and in active follow-up defined as having their date last clinical visit after 01st January 2014

2.5 Basic statistical methods

The following section describes the epidemiological terms and basic statistical methods used in this thesis. Analyses specific to a particular results chapter are addressed in the individual chapter.

2.5.1 Measures of disease

When evaluating the frequency of occurrence of a disease or condition of interest, there are two main measures used in epidemiology: prevalence and incidence (more details below).

Prevalence

The prevalence of a disease measures the burden of a disease or condition at a specific time point. Prevalence measures are useful in assessing the burden of chronic conditions because the state of these diseases is relatively stable over time. The prevalence is a proportion, calculated as the number of individuals with the disease at a specific defined time-point divided by the total number of people in the population at that time. It is usually expressed as a percentage.
For example, in chapter 4 I presented the prevalence of alcohol consumption at study enrolment for the sample population included in the analysis.

Incidence: rate and risk

The incidence rate of a disease or condition is defined as the number of new cases of the disease occurring in a specified period in the population of interest divided by the person-time at risk.
For example, the HCV incidence rate is defined as the number of new HCV infections observed in the target population in a specified time period divided by the sum of the time spent by each individual under observation while they were free from the disease of interest and remained at risk and under follow-up. Of note, this accounts for participants' drop-out, so only cumulative time at risk for developing disease is used. Incidence may be expressed as number of cases per 100,000 person-years.

It is also worth mentioning that for incidence calculations, usually once an individual has been classified as a new case, they are no longer at risk of becoming a new case and they would not further contribute to the person years at risk. For some outcomes though, individuals may have multiple events of the outcome of interest. In this case, incidence may be restricted to the first event, or may include multiple events. In the latter case an individual would continue to be at risk and contribute to person-time at risk after the first event.

Typically, incidence rates are compared between a group who is exposed to a risk factor and those who are not, or in treated vs. untreated. A number of 'estimands' for the treatment/exposure effect are typically used such as the difference in incidence rates or the rate ratio; these give a measure comparing incidence in the exposed group versus unexposed group. The rate ratio is perhaps the most commonly used estimand and it is defined as the incidence rate in exposed divided by incidence rate in unexposed group. For example, in chapter 5 I presented incidence rate ratios of specific ARV discontinuation comparing the HIV/HCV coinfected group to the HCV-negative group.

In some studies, person years at risk cannot be calculated and all is known is whether the person has experienced or not experienced this event of interest within a specific period. In this situation, it is not possible to calculate a rate and a risk is instead calculated. The term 'risk' is defined as the number of events in the period divided by the number of people in the study population at the start of the study period.

Again generally the interest lies in comparing the risks of an event between two different groups using the risk ratio (RR). The RR is calculated as the risk of the event in exposed group divided by the risk of the event in the unexposed group. Below is an illustration of how this particular estimand is calculated using a 2x2 table for exposure and outcome.

	Event occurs during follow-up period	Event does not occur during follow-up period	Total
Exposed	a	b	a+b
Not exposed	c	d	c+d
Total	a+c	b+d	a+b+c+d=N

Risk of event in the exposed $P\ (exposed) = \frac{a}{a+b}$

Risk of event in the unexposed $P\ (unexposed) = \frac{c}{c+d}$

Risk ratio (RR) = $\frac{P\ (exposed)}{P(unexposed)} = \frac{\frac{a}{a+b}}{\frac{c}{c+d}} = \frac{a(c+d)}{c(a+b)}$

For rate ratios, risk ratios and odds ratios

- If the ratio=1 then the rate (risk or odds) of disease is the same in both exposed and unexposed groups
- If the ratio<1 then the rate (risk or odds) of disease for the exposed group is smaller than the risk of disease in the unexposed group.
- If the ratio >1 the rate (risk or odds) of disease for the exposed group is larger than the risk of disease in the unexposed group.

Odds

Alternatively, measures of probability of acquiring the disease can be expressed in terms of 'odds'. The odds are defined as the probability of the event of interest occurring divided by probability of the event not occurring. When comparing the odds of an event between groups this would be expressed as an odds ratio (OR): the odds in the exposed group divided by the odds in the unexposed group. Using the 2 x 2 table above this can be expressed as;

$$Odds\ ratio = \frac{ad}{bc}$$

In the case were the sample size is large, and the event is rare, the OR and RR are similar. The terms relative risk (RR) is often reported in literature to indicate comparisons between exposed and unexposed group regardless of the actual measure used (e.g. rates, risks, odds); however in this thesis the specific measure used will be indicated.

95% confidence intervals

Estimates of the above disease measures are presented together with their 95% confidence intervals (95% CI). The 95% CI gives a range in which the true value of the measure in the population is likely to lie (with 95% confidence). This reflects the fact that the analysis is based on a sample of the population (which is assumed to be representative of the population of interest) and there is uncertainty around the estimate.

2.5.2 Descriptive statistics

The distribution of each of the variables were checked to determine what summary statistic was most appropriate. Continuous variables such as age, CD4, HIV-RNA are reported as a median value with interquartile range (IQR). Mean and standard deviation (SD) are instead reported if the distribution is symmetrical. The median is a more accurate central measure than the mean for variables with a skewed distribution and is less influenced by outliers. For binary data such as gender (male, female), mode of HIV transmission (PWID, MSM, heterosexual contacts, other- I show frequency (n) and percentage (%) within each binary or categorical group.

2.5.3 Statistical tests to compare groups

To compare groups in univariable analysis, I used a Wilcoxon signed rank sum test to compare the distribution of the ranks for continuous variables between two groups. If more than two groups were compared, I used the Kruskall Wallis test.

Both these tests are non-parametric tests which are appropriate for skewed data, under the assumption that the data are not Normally distributed.
I used a Pearson chi-squared test to compare proportions between groups and the Fisher exact test if in 2 x 2 tables the expected frequency count in at least one of the cells was <5.

All statistical tests in the thesis are two-sided results are presented as p values. The p-value is defined as the probability of observing the difference (or one more extreme) under the assumption that the null hypothesis (of no difference between groups in the population) is true. I used the standard threshold for hypothesis testing with a p value of <0.05 taken to denote statistical significance, unless reported otherwise. A p-value <0.05 means that there is less than 5% chance to observe the difference seen (or a more extreme result) if the null hypothesis (H_0) were true, which represents strong evidence against the null hypothesis.

2.6 Statistical models

A statistical model expresses a mathematical relationship between variables that can be fitted to the observed data. The models are used to assess if a set of covariates predict the outcome of interest. In particular, when considering the association of an exposure with an outcome, a statistical model may be used to ‘adjust’ the association for other factors – the other covariates included in the model. Therefore, such models (multivariable analysis) are used to attempt to control for potentially confounding factors. The type of outcome (e.g. binary or continuous) determines which type of model is fitted.

Estimates of the effect of the covariate of interest obtained from the fitted models were presented in the results chapters together with 95% confidence intervals and corresponding p-values.

2.6.1 Logistic regression

When modelling relationships between an outcome which is a binary (e.g. yes or no) variable and other variables of interest (continuous, categorical or binary), a logistic regression model can be used. For example, in chapter 5, I assessed the determinants of late HCV presentation at enrolment in Icona. A binary response variable was created for late HCV presentation. As mentioned previously, we could compare the proportion of individuals who were diagnosed late with HCV across groups e.g. across mode of HIV transmission group using a chi-squared test and the same test is used when fitting univariable logistic regression models. However, in observational data confounding cannot be ruled out by other factors so the analysis never stops at the univariable comparisons. In fact, a multivariable logistic regression model is used to estimate the effect of the exposure after controlling for potential confounding factors.

Notation of the logistic regression model

The outcome variable is described as Y_i (where i represents an observation). $Y_i=1$ represents an individual who has the event of interest with probability p_i and $Y_i=0$ represents an individual who does not have the event of interest with probability 1-p_i. Equation 1 shows how this probability can be modelled as a linear function of the vector of covariates (β_1, β_2...β_k). In logistic regression, the logit transformation of this probability is used, which takes the form of Equation 2. The log(odds) of the p_i is what is modelled as a linear combination of the covariates (Equation 3).

Equation 1

$$transformation\ (p_i) = \beta_0 + \beta_1 x_1\ \beta_2 x_2 + \cdots + \beta_k x_k$$

Where, β_k are the estimate coefficients and x_k are covariates.

Equation 2 - The logit transformation

$$logit\ (p) = \frac{\log(p)}{\log(1-p)}$$

Where, p is the probability of the event of interest

Equation 3 - The logistic regression model

$$logit\,(p) = (\beta_0) + \beta_1 x_1\, \beta_2 x_2 + \cdots + \beta_k x_k$$

Where, β_k are the estimate coefficients and x_k are covariates.

Odds ratios can therefore be estimated from the logistic regression model, by exponentiating the coefficients. These are presented with 95%CIs. If the 95%CI for an odds ratio does not contain one this would be consistent with a statistically significant result (p-value<0.05).

For example, using the outcome of late HCV presentation, ORs associated with the categorical variable, mode of HIV transmission, shows whether the odds for late HCV presentation are higher or lower, say, in PWID compared to MSM (if MSM is set as the comparator group). In the case of a continuous covariate instead, say age (in years), the OR would give the increase in odds of the outcome per one year older in age.

The logistic regression has the following assumptions:

- Observations are independent of each other (from different individuals)
- Independent variables should not be highly correlated with each other
- The logistic regression assumes linearity of independent variables on the log odds scale

2.6.2 Survival analysis

Survival analysis is a set of methods that account not only for whether an event occurs but how quickly it occurs. Survival models model the time to the event of interest to occur. In survival analysis, the time at risk is defined as the time interval from a chosen time zero to the date of when the event occurs or to the date in which individuals are no longer at risk of the outcome. For example, in chapter 4, I looked at time to occurrence of SLD from date of enrolment in the Icona cohort. For individuals who did not experience SLD in the study observation period, their

follow-up was ended at the date they were last seen (i.e., date of their last clinical visit or the date of death).
Of note, this type of analysis can also accommodate individuals who may have been lost to follow-up or withdrew or did not experience the event during the period of analysis.
The terminology used for individuals who do not experience the event is called censoring. 'Right censoring' occurs when:

- The Individual did not experience the event of interest in the analysis period
- The individual is lost to follow-up during the study period
- An individual withdraws from the study

A key assumption in survival analysis is that censoring is not informative (i.e., there are no common causes of censoring and of the outcome of interest). There are sophisticated methods accounting for informative censoring so that this assumption can be relaxed. In chapter 5, for example I used inverse probability of censoring weight to retain exchangeability of participant in follow-up conditioned to the covariates that were used for the construction of the weights (more details in chapter 5).

Notation used in survival analysis
Let *T* denote the response variable (time to event), T≥0
Let *F(t)* denote the cumulative probability failure time at time t.
Let *S(t)* denote the survivor function, Equation 4.

Equation 4 - Survival function

$$S(t) = \Pr(T > t) = 1 - F(t)$$

A simple way of computing the survival function over time is the Kaplan Meier (KM) estimator. The KM plot can give a visual representation of whether a given event occurs more frequently in the early time periods of follow-up than later on the

observation period and can be used to compare survival between groups. The estimates account for the number of individuals still at risk at any given time-point.

The following assumptions are necessary:

- Individuals who are censored have the same survival function as individuals who continue to be followed-up
- Survival probabilities are the same for all individuals regardless of when they entered the study
- The event happens at one specified time

Assuming that every individual follows the same survival function, S(t) can be estimated using the KM estimator: this is a non-parametric estimator.
The survival probability at any time point can be calculated as;

Equation 5 Kaplan-Meier estimator function

$$\hat{S}(t) = \prod_{t_j \leq t} \frac{n_j - e_j}{n_j}$$

Where $n_j =$
$number\ of\ individuals\ at\ risk\ of\ the\ event\ at\ start\ of\ the\ anlaysis\ period$ and $e_j = the\ cumulatve\ number$ of events at time t_j.

When comparing survival functions of categorical variables, the log rank non-parametric test is commonly used to assess if one group has a higher probability of the event of interest occurring in the follow-up period. For example, in chapter 4, I presented KM plots stratified by alcohol consumption categories to assess if the probability of developing SLD was higher in individuals with a history of abstaining from alcohol or of hazardous drinking, compared to moderate drinkers. In chapter 5, I also presented KM plots to assess if the probability of all-cause mortality is higher in individuals with late HCV presentation compared to individuals not presenting late with HCV.

Cox regression

The KM method only deals with the relationship of one predictor at a time with the outcome of interest, however when looking at multiple predictors and their relationships with the outcome of interest, Cox proportional hazard model (another type of regression model) is more suitable.

The coefficients in the Cox regression model relate to the hazard. A hazard at a time t *(h(t))* is the risk of the individual experiencing the event of interest at given time. It is also known as the instantaneous event rate for an individual who has not experienced that event of interest up to time t. The way the hazard changes over time is called the hazard rate. When comparing groups, the hazard ratio can be calculated (obtained from exponentiating the coefficients from the Cox model).

In Cox proportional hazards model, the assumption is that the hazard between exposed and unexposed groups remains proportionately constant over time, an assumption that needs to be checked. For example, this can be done by graphing the survival function against survival time and if the curves by exposure group are parallel then the assumption is satisfied. In contrast, no shape is assumed for baseline hazard which is a nuisance parameter in this semi-parametric model.

For an example in chapter 4, I fitted a Cox regression model to evaluate the association between alcohol consumption and the risk of severe liver disease after adjusting for potential confounders.

The Cox model is expressed as;

$h(t) = h_0(t)\ (exp^{(\beta_1 x_1\ \beta_2 x_2 + \cdots + \beta_k x_k)})$

Where $h(t)$ = hazard function,

Where β_k are the estimated coefficients and x_k are covariates.

The exponentiated coefficients are estimates of the hazard ratio associated with that covariate

2.6.3 Poisson regression

In a Poisson regression analysis, the outcome is expressed as number of events per person years of follow-up. The model is used for analysing rates.
The Poisson model is expressed as a logarithmic link function as below;

$$\log[E[d_i]] = \log[n_i] + \alpha + \beta_1 x_1 \beta_2 x_2 + \cdots + \beta_k x_k$$

Where d_i is the incidence rate or number of events observed in n_i person years of follow-up in group i. Where β_k are the estimated coefficients and x_k are covariates.

In addition, $\log[n_i]$ is known as the offset and represents a measure of the length of exposure. The offset forms part of the covariates in the model and is useful in situations where each individual may have varying time lengths of exposure to the event of interest.

The rate ratio can be estimated from model coefficients as
$\widehat{RR} = exp^{\widehat{\beta}}$

In chapter 5 I looked at discontinuation rates of specific cART regimens expressed as number of discontinuations per person years of follow-up. I assumed a Poisson distribution for the number of discontinuations in follow-up. Incidence rate ratios of discontinuation were presented stratified by HCV infection, to evaluate whether there was an excess in risk of stopping specific antiretroviral drugs that could be attributable to coinfection with HCV.

All model assumptions were checked by appropriate graphs and formal statistical tests.

2.6.4 Methods for model building

In each of the results chapter, I give details of the multivariable model building strategy used. It is common practice in the literature to perform univariable analyses and then to include in the multivariable models only variables that are statistically significant at a chosen level (e.g. 10% or 20%) at the univariable stage. However, this strategy is not widely recommended outside of pure prediction models as it carries the risk of excluding key confounding factors. A confounder is a variable which is a common cause of exposure and outcome and is not on the causal pathway between exposure and outcome. A model that fails to control for confounding is likely to provide a biased estimate of the association between the exposure of interest and the risk of outcome.

I have not used a statistical significance-based approach to model building in my thesis. In the initial chapters of this thesis, my general approach to construct the multivariable model was first to consider and identify all possible confounding factors for the association of interest and then fit the model sequentially, by controlling for an increasing number of potential confounders and showing the results of the association with the exposure of interest at each step. A footnote to the table of results is also added indicating what was exactly included in each model step adjustment. The sequential models followed this general order;

Model#1: unadjusted
Model#2: model#1 + demographic factors
Model#3: model#2 + HIV-related factors
Model#4: model#3 + any other factors

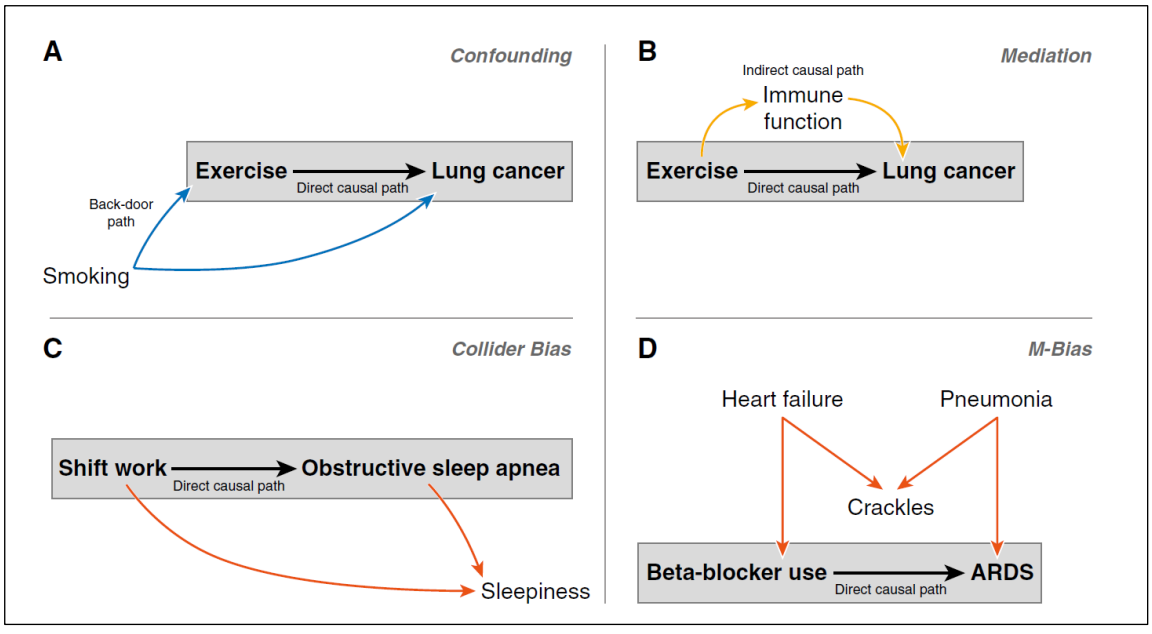
For the later chapters 6 and 7, I used a more rigorous procedure to identify potential confounders to be included in the multivariable model. Specifically, postulated relationships between factors were described, and confounding factors identified, through the visual aid of causal graphs (DAGs).

2.6.5 Methods to identify potential confounders for inclusion in the statistical model

Directed Acyclic Graphs (DAGs)

A key problem in the analysis of real-world data is how to correctly identify and account for confounding. In chapters 6 and 7 I used the causal language and DAGs to illustrate assumptions on confounding, mediation and collision for these analyses. The use of DAGs is gaining more recognition and popularity in recent years [234, 235]. In a DAG, all variables in the graph are connected by arrows and these are designed to represent causation rather than just association of the underlying relationships. DAGs are a good way to graphically describe the causal assumptions by explicitly showing the relationships between variables (both measured and unmeasured) [234, 236].

Figure 2.1 Four key junctions in a directed acyclic graph [1]



Lederer DJ, Bell SC, Branson RD, Chalmers JD, Marshall R, Maslove DM, et al. Control of Confounding and Reporting of Results in Causal Inference Studies. Guidance for Authors from Editors of Respiratory, Sleep, and Critical Care Journals. Annals of the American Thoracic Society. 2019;16(1):22-8.

When making inference in epidemiological studies they are four main types of biases that may be present and can affect the association between exposure and outcome. I will give more details below, but in brief these are the following:

- confounding – if there is a variable which is not on the causal pathway but is a common cause of both outcome and exposure;
- mediation – if there is a variable lying on the causal pathway between exposure and outcome;
- collision – if there is a variable which is the effect of exposure and outcome
- m-bias – another case of collision bias but in this case neither exposure or outcome is a direct cause of the collider variable [(1)].

The illustration of terminologies to identify confounding/mediation/collision using DAGs were taken from *Lederer et al* (Figure 2.1). In a DAG, two variables (nodes) are connected by a set of arrows (regardless of arrow direction) and this connection is termed a 'path'. If there are more than two variables (nodes) these are called 'junctions' and Lederer describes the four key junctions that regulates causal associations between variables (Figure 2.1).

Confounding

In epidemiology a main interest is often to evaluate whether there is a causal link between exposure and outcome, rather than just an association (i.e. Figure 2.1-A: exercise → lung cancer). This is because if a cause of lung cancer is clearly identified efforts can be made to remove such a cause to reduce morbidity and mortality. Any other alternative path other than the direct path connecting exposure and outcome is known as a 'back-door path' (i.e. Figure 2.1-A: exercise ← smoking → lung cancer) so the path from exercise to lung cancer can also be through the back door of smoking. The presence of an 'open' back-door path between exposure and outcome means there is confounding. In this case, smoking is confounder of the causal association between exercise and lung cancer. Therefore confounders by definition in terms of DAGs are variables that naturally open back-

door paths. So, if there is an open path, an association will be observed between exposure and outcome. To close the back-door path, means controlling for the confounder i.e. adjusting for smoking, or removing the association between smoking and exercise. Also, if there are many back-door paths, the task is to identify a minimum set of variables which are sufficient to close all these paths.

Mediation

Figure 2.1-B, shows another type of path called 'indirect causal path' and includes a mediator i.e. immune function which is mediating the causal effect between exercise and lung cancer (Figure 2.1-B: exercise → immune function → lung cancer). This is also sometimes called a 'chain'. Controlling for a mediator will close the indirect path which will bias the estimate of the association between exposure and outcome. Therefore, the advantage of using a DAG in this situation is that it allows the identification of mediators to avoid controlling for them as they should be not be treated like confounders.

Collision

Figure 2.1-C, in contrast, shows a collider i.e. 'sleepiness' which blocks the back door path of the association between shift work and obstructive sleep apnea (Figure 2.1-C: shift work → sleepiness ← obstructive sleep). Of note, the collider variable 'sleepiness' is a consequence of both exposure and outcome. Controlling for a collider typically generates an inverse correlation between exposure and outcome which does not exist. For example, suppose we found a sleepy person with sleep apnea, the presence of sleep apnea is the likely cause of them being sleepy and less likely to be a shift worker. Likewise, if we found a sleepy shift worker, it could be that their sleepiness is likely to be due to them working shifts rather than sleep apnea. Therefore, sleep apnea would be less common among shift workers and we will conclude that there is an inverse association when in reality the two variables are not linked. Therefore, controlling for a collider will naturally open a back door path and as a consequence of this, bias is introduced

[1]. In addition, controlling for a collider can further introduce bias if there is an unmeasured confounder U which is a common cause of the collider and the outcome (as it opens a back-door path to outcome through U, not shown in the Figure).

M-bias

There is another case of collider bias where neither exposure nor outcome is a direct cause of the collider variable (Figure 2.1-D: beta blocker ← heart failure → crackles ← pneumonia → ARDS) this is termed as m-bias. In this rare although possible scenario, controlling for crackles will introduce confounding as the back door path from beta blocker to ARDS will have been opened [1]. Of note, the m-bias graph is often used also to criticise the standard definition of confounder in epidemiology. Indeed, crackels are both associated with exposure (beta-blocker) and outcome (ARDS) and therefore satisfy the classic definition of a confounder, but it is not actually a confounder.

Essentially DAGs show whether exposure and outcome share one of more of the four junctions described above. For example, when dealing with confounding it is important to understand the relationship between the exposure, outcome and confounder so as to avoid adjusting for colliders or mediators [237]. Thus, under the assumption of correct model specification, if there is no unblocked path from exposure to outcome then there is no confounding of the total effect of exposure to outcome [234]. Of course, there are situations in which it is impossible to remove all the confounding bias in the observational setting as there are key unmeasured confounders and it is impossible to find a variable that can be used as an instrument. In this case, researchers should be intellectually honest and declare that a casual link cannot be established.

In chapters 6 and 7, I used the DAGitty software [238] to visualize the assumed causal relationships that I had postulated and to decide which variables were considered minimally sufficient to remove confounding. The variables and the

relationship between variables included in the DAG were selected based on hypothesis, results of previous research studies and other axiomatic knowledge. It is possible that more than one set of these potential confounding variables are able to block all confounding paths. If this was the case, I reported in these chapters the results of all separate adjustments suggested from the DAG.

2.7 Dealing with time varying covariates and the use of inverse probability weighting

Time-varying covariates are defined as variables that change over time. Most of the research questions in this thesis focused on long term effects of potential risk factors measured at time zero of the analysis (baseline) on the outcome of interest. In these analyses, it is normally sufficient to control for confounding of baseline factors and this can be done using conventional methods modelling the risk conditioned on individuals' vector of baseline covariates such as a multivariable Cox regression model. The relevance to this thesis is that in chapter 5 there was a clear potential issue with informative censoring as the probability of discontinuing an ARV drug because of, say, toxicity could not be assumed to be independent of stopping because of say, failure. Indeed, the analysis did identify factors which appeared to increase the chance of discontinuing a drug because of toxicity but also because of failure. Therefore, in chapter 5, bias due to informative censoring has been minimised by using inverse probability weighting of censoring weights. In practice, this method is based on building a logistic regression model to obtain an estimate of the probability of being censored given individual's history of covariates and use this probability to construct the weights. The weights are then used to re-establish exchangeability between the exposed and not exposed group which was lost due to the fact that participants dropped out of the study for stopping the drug for a reason different from the one of interest. More specific details about how this analysis was performed are shown in the methods section in chapter 5 [239].

2.8 Dealing with missing data

There are a number of different methods to handle missing data. One possible way is to use a complete case analysis after excluding participants who have missing values. This can however introduce selection bias; the extent of this can be assessed by comparing the distribution of the remaining characteristics between included and excluded participants. Instead, as mentioned previously, I used the so called 'missing indicator method' which consists in creating an additional category to indicate the group with missing data for that variable. This approach, which retains a complete data set for all variables, enables fair comparisons between the results of nested regression models. For example, in chapter 4 the level 'unknown consumption' was created for the exposure alcohol consumption in people for whom the information was not available. However, this simple approach does not guarantee unbiased estimates and has issues regarding the interpretation of the results if, for example, the 'unknown consumption' group shows a very different risk compared to the other categories. Therefore, I investigated an alternative approach as outlined below.

Data can be either 'missing completely at random (MCAR)' or 'missing at random (MAR)'. MCAR exists when missing data is observed randomly across all observations. Instead, in the MAR missing data mechanism, unobserved values are distributed randomly in some subgroups and differences between missing values and observed values can be explained by the differences in the observed data [240]. Under the MAR assumption, a common approach to handle missing data is to carry out multiple imputations (MI) of missing values.

Besides the missing indicator method, in chapter 4, I also used MI to impute alcohol consumption data for participants with missing data under MAR assumption. MI is a method that creates several imputed data sets which are then combined to obtain results from each of those datasets (more details in chapter 4). It is worth noting that even when using MI, misclassification of the exposure can still occur [241]. An alternative approach to better tackle mis-classifications of the

exposure due to missing data, which however was not used in this thesis, is to carry out an external validation and then check if results are similar (also known as calibration) [241].

2.9 Statistical software used

All statistical analyses were performed using SAS 9.4 (Statistical Analysis Software, Cary NC, USA).

CHAPTER 3

3 PREVALENCE OF HCV IN ICONA AT ENROLMENT AND CHARACTERISTICS OF ICONA AND HEPAICONA STUDY PARTICIPANTS ACCORDING TO HCV-RELATED FACTORS

3.1 Aim and objectives

The aim of this chapter is to describe prevalence of HCV at enrolment among Icona participants and to assess the characteristics of the study populations of Icona and Hepaicona cohorts according to HCV-related factors in order to set the scene for and inform the analyses carried out in the following chapters 4 to 7.

The specific objectives are:

1. To describe trends of recruitment over time in the number of participants enrolled in Icona and their distribution in terms of geographical region, mode of HIV transmission and HCV prevalence
2. To describe participants' characteristics (demographics, HIV-related, socio-economic and lifestyle factors) in Icona and Hepaicona
    - Stratified by HCVAb status at enrolment (Icona only)
    - Stratified by HCV-RNA status amongst those who were HCVAb positive at enrolment (Icona and Hepaicona)
3. To describe participants' characteristics (demographics, HIV-related, socio-economic and lifestyle factors) stratified by stage of liver disease among HIV/HCV coinfected individuals in Icona and Hepaicona
4. To briefly describe the findings of an analysis using the Icona cohort data performed previous to my involvement with Icona and external to this thesis that evaluated incidence of new HCV infections in Icona
5. To identify individuals who, by 30th June 2016, were under active follow-up and still naïve to direct acting antivirals (DAAs) in Icona and Hepaicona and

to describe their main characteristics (demographics, HIV-related, socio-economic and lifestyle factors).

3.2 Introduction

All analyses presented in this chapter (and those in chapter 4 and 5) were performed using the data collected up to 30th June 2016. The number of participants included in the tables in this chapter may differ from the number of participants included in the analyses in subsequent chapters because the latter were typically based on specific inclusion criteria. Furthermore, the analyses in chapters 6 and 7 were performed on updated datasets from both databases, locked on 31st January 2018 (see Table 2.4 in chapter 2).

This chapter aims to describe the prevalence of HIV/HCV coinfection and the characteristics of HIV/HCV coinfected individuals compared to those not infected with HCV to provide a representative picture of the burden and correlates of HIV/HCV coinfection among PLWH who were seen for care in Italy. In particular, objective 5 above was prompted by the fact that around the time of this analysis Agenzia Italiana Farmaco Industria (AIFA) was greatly interested in estimating the number of HIV/HCV coinfected individuals eligible for immediate DAA initiation. In addition, examination of patterns of missing data for some key variables over time and the factors associated with HIV/HCV coinfection and other HCV-related measures informs analyses in subsequent results chapters. Participant characteristics considered included: demographics, HIV-related factors, socio-economic and lifestyle factors at the time of enrolment.

In investigating the burden of HIV/HCV coinfection, it is necessary to consider whether the person was tested for HCVAb or HCV-RNA, and if tested for both, whether they tested positive for HCVAb only or additionally for HCV-RNA. A positive result for both would indicate chronic infection. Alternatively, a person could be HCVAb positive but HCV-RNA negative either because they had

spontaneously cleared the infection or because they had been successfully treated.

3.3 Methods

3.3.1 Inclusion criteria of participants included in the descriptive analyses

Analyses include PLWH with/without HCV infection enrolled Icona and PLWH with HCV enrolled in Hepaicona.
The descriptive analysis stratified by HCV-RNA status was restricted to those who were HCVAb positive. The descriptive analysis stratified by stage of liver disease, was restricted to HIV/HCV coinfected individuals who had a measure of FIB-4 score at enrolment in the cohorts.
In the descriptive analysis estimating HIV/HCV coinfected individuals eligible for immediate DAA initiation, the population sample was restricted to participants who were HCV-RNA positive and naïve to DAA as of January 2015.

3.3.2 Data

Participants' HCV infection status was determined at enrolment. HCV infection status was established either from the serology test (a positive HCVAb test result) or, if serology was not available, from an HCV-RNA positive (quantitative or qualitative) test result or from the availability of HCV genotype.
Stage of liver disease at enrolment was determined using participants FIB-4 score calculated using age, ALT, AST and PLT (see chapter 1, Table 1.3 for formula) and was categorised as mild (FIB-4<1.45), moderate (FIB-4: 1.45-3.25) and advanced (FIB-4>3.25) liver fibrosis.

As mentioned in chapter 2 (Table 2.2), all factors used in this chapter were determined at enrolment. Demographic factors (age, gender, geographical region, calendar year enrolled) were collected from the participants' enrolment data. HIV-related factors (CD4 and HIV-RNA) were collected from the data table of HIV

markers and were considered as time-fixed variables at enrolment. Socio-economic factors (education and employment) were collected from participants' history data. Lifestyle factors (smoking and alcohol consumption) were collected from clinical visits and treated as time-fixed variables at enrolment.
The categories for each of the factors considered in this analysis were presented in chapter 2 Table 2.2.

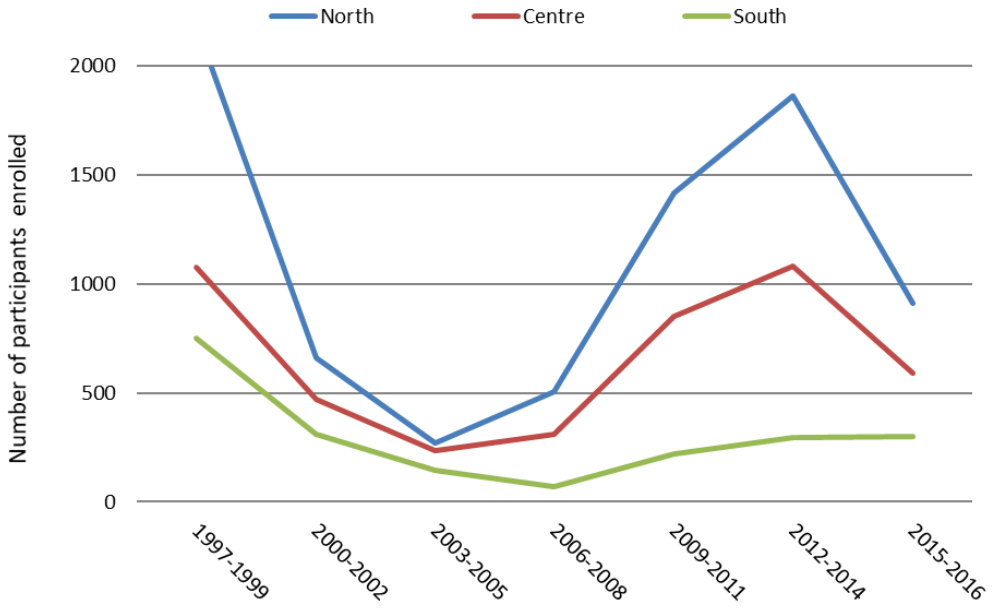
3.4 Results

3.4.1 Icona

3.4.1.1 Summary of recruitment in the Icona cohort from 1997 to 2016

As of 30th June 2016, Icona had enrolled 14,532 HIV-positive individuals (median calendar year of enrolment was 2009). Figure 3.1 shows trends over time in the number of people enrolled in the cohort by geographical region of the enrolling site. The largest infectious disease units are in the north (mainly from Lombardy region) and centre (mainly from Lazio region) of Italy which is reflected in the larger numbers enrolled from the sites located in these regions. Historically, there were fewer sites from the south in intermediate years although new sites in the south have been recently opened to enrich representativeness of that area.

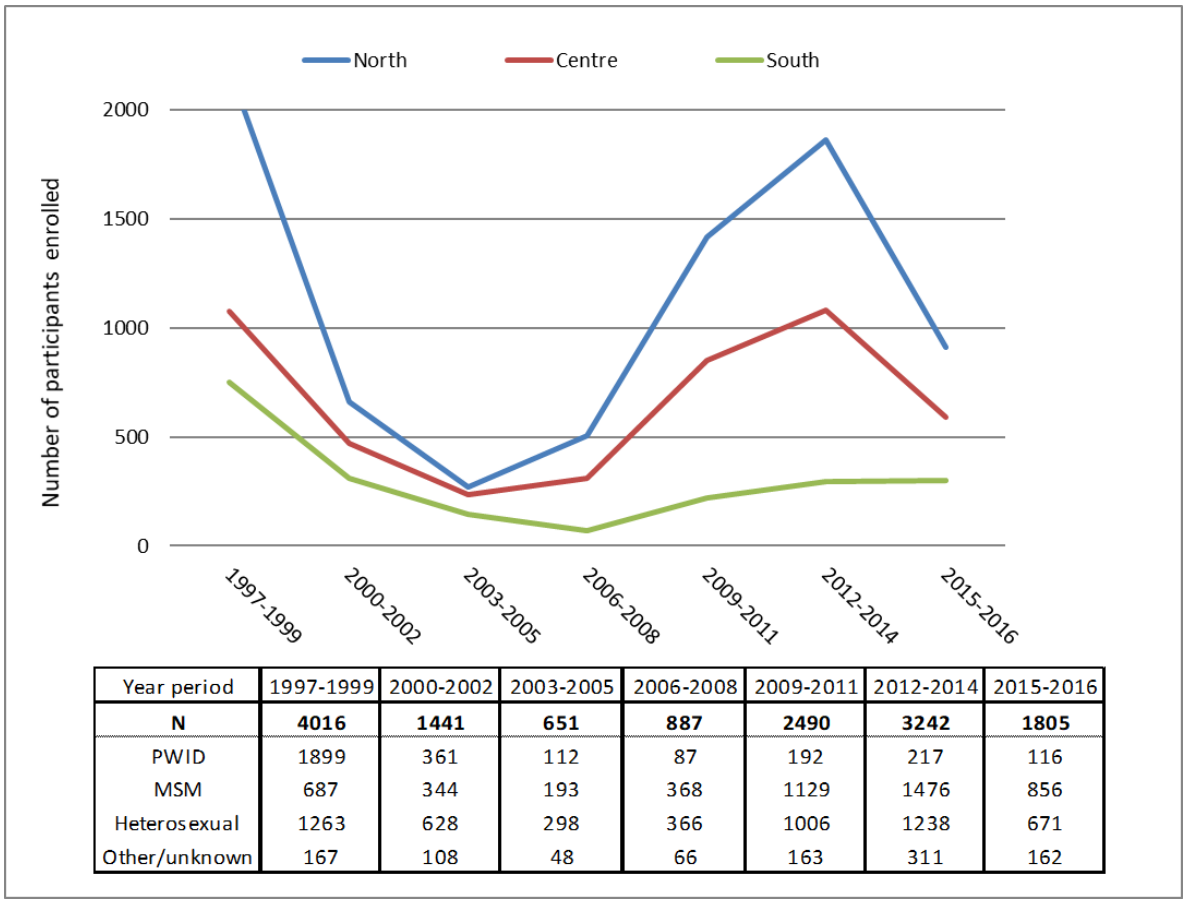
Of interest, the demographics of the Italian epidemic have changed over time. While most people diagnosed with HIV were PWIDs in the early years, this shifted to a much larger prevalence of MSM and heterosexual individuals in more recent years.



Year period	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	2015-2016
N	**4016**	**1441**	**651**	**887**	**2490**	**3242**	**1805**
PWID	1899	361	112	87	192	217	116
MSM	687	344	193	368	1129	1476	856
Heterosexual	1263	628	298	366	1006	1238	671
Other/unknown	167	108	48	66	163	311	162

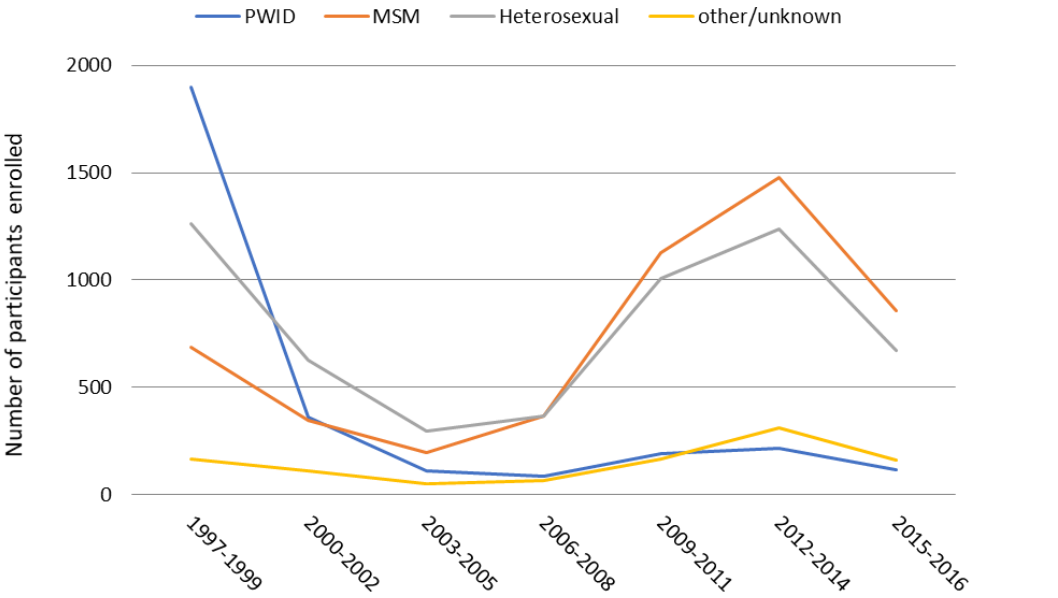
Figure 3.2 shows the number of Icona participants over time by calendar period of enrolment stratified by mode of HIV transmission. In both graphs, the drop in enrolment in very recent years simply reflected a delay in reporting for the year 2016 which is the year in which the analysis was conducted. The drop in enrolment between the years 2003 to 2008 was due to a temporary shortage of funds for the cohort.

Figure 3.1 Number of participants enrolled in Icona between 1997 to 2016 by geographical region of enrolling site



Year period	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	2015-2016
N	**4016**	**1441**	**651**	**887**	**2490**	**3242**	**1805**
PWID	1899	361	112	87	192	217	116
MSM	687	344	193	368	1129	1476	856
Heterosexual	1263	628	298	366	1006	1238	671
Other/unknown	167	108	48	66	163	311	162

Figure 3.2 Number of participants enrolled in Icona between 1997 to 2016 by mode of HIV transmission



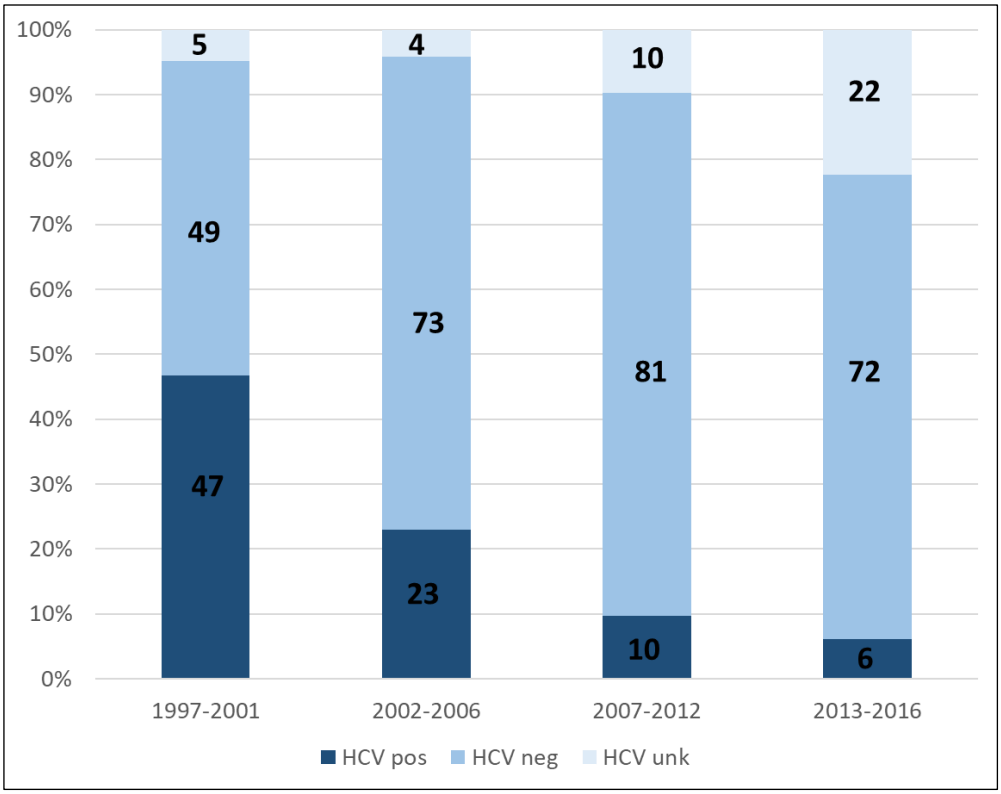
Year period	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	2015-2016
N	**4016**	**1441**	**651**	**887**	**2490**	**3242**	**1805**
PWID	1899	361	112	87	192	217	116
MSM	687	344	193	368	1129	1476	856
Heterosexual	1263	628	298	366	1006	1238	671
Other/unknown	167	108	48	66	163	311	162

3.4.1.2 Prevalence of HCV coinfection at enrolment in Icona

Twenty three percent (n=3,279) of Icona participants were HIV/HCV coinfected at enrolment. Figure 3.3 shows the prevalence of HCV infection status (measured using serology tests alone) at enrolment over time with a prevalence of HCV coinfection in earlier years followed by a decrease over time. In the early period (1997-2001) almost half of Icona participants had HCV coinfection; this halved in 2002-2006 and continued to fall such that in the latest period (2013 to 2016) only 6% had HCV coinfection. This high proportion in the early years was mainly driven by coinfection among PWID. In more recent years the Icona population mainly

comprised individuals reporting acquiring HIV through MSM or heterosexual contact in whom HCV prevalence was lower. On average, there was approximately 10% (n=1,596) of the cohort who were not ever tested for HCVAb, or for whom the results of the test were not recorded in the database (Table 3.1, Figure 3.3). This proportion increased over time to 22% in the most recent period. Missing HCV status could be due to the fact that some people enrolled in Icona had been in routine clinical care for some time and were not re-tested at the time of entry in the cohort but there were also a proportion of people who were genuinely tested late for HCV or remained untested. In chapter 6 I investigate the prevalence and correlates of presenting late with HCV, in newly diagnosed HIV individuals enrolled in Icona up to January 2018 and investigate the excess risk in mortality associated with presenting late with HCV.

Figure 3.3 HCV prevalence at enrolment in Icona between 1997 to 2016



3.4.1.3 Demographic, HIV-related factors, socio-economic and lifestyle factors at enrolment in Icona stratified by HCVAb status

Demographic factors by HCVAb status

Characteristics of individuals at enrolment overall and stratified by HCVAb status are shown in Table 3.1. Overall, the PLWH enrolled in Icona consists predominantly of males, making up three-quarters of the cohort and median age of the cohort is 36 years (IQR: 31-44). In most cases, HIV was acquired through heterosexual contacts (38%) or MSM (34%). The three HCVAb status groups (negative, positive unknown) differed for most baseline characteristics except for gender and age. As expected, the most marked difference was that HCVAb positive individuals were much more likely to be PWIDs (75%) than HCV negative or unknown (3% and 14% respectively). Individuals who were HCVAb positive were more prevalent in Icona sites from the southern region compared to HCVAb negative or unknown. Regional differences in terms of access to care as part of the HCV continuum of care have been investigated in greater detail in chapter 7. HCVAb positive individuals were more likely to be Italian, and more likely to have been enrolled prior to 2001.

A cross tabulation of mode of HIV transmission by calendar year (not shown) and Figure 3.2 shows that most participants enrolled in Icona in the earlier years were PWID and this largely explains why only 5% of participants had not been tested for HCV (Figure 3.3). Table 3.1 and Figure 3.3 also show that a larger proportion of individuals not tested for HCV in the most recent periods, possibly explained by delayed reporting (i.e. data not available at the time of data lock). Additionally, in more recent years participants were more like to have acquired HIV through MSM and heterosexual contact and may have been considered to be at low risk of HCV infection and therefore not frequently tested. Such substantial differences in the prevalence of HIV/HCV coinfection by calendar year between groups have the potential to cause bias in any comparisons between positive and negative HCVAb

status. Thus, in the analyses in this thesis in which HCV was the exposure of interest I have performed a multivariable analysis after controlling for calendar year at baseline or after restricting to participants who were enrolled after a certain calendar date. Additionally associations or lack of them may reflect changes in alcohol consumption after HCV diagnosis.

Table 3.1 Participants' demographic factors in Icona at enrolment, stratified by HCVAb status

	HCVAb negative N= 9657	**HCVAb positive** N= 3279	**HCVAb unknown** N= 1596	**Total** N= 14532
Gender, n(%)				
Male	7320 (75.8)	2430 (74.1)	1265 (79.3)	11015 (75.8)
Female	2337 (24.2)	849 (25.9)	331 (20.7)	3517 (24.2)
Age, years				
Median (IQR)	37 (30, 45)	36 (32, 40)	37 (31, 46)	36 (31, 44)
Region, n(%)				
North	5124 (53.1)	1769 (53.9)	932 (58.4)	7825 (53.8)
South	1219 (12.6)	638 (19.5)	231 (14.5)	2088 (14.4)
Centre	3314 (34.3)	872 (26.6)	433 (27.1)	4619 (31.8)
Nationality, n(%)				
Italian	7855 (81.3)	3105 (94.7)	1274 (79.8)	12234 (84.2)
Non-Italian	1802 (18.7)	174 (5.3)	322 (20.2)	2298 (15.8)
Mode of HIV transmission, n(%)				
PWID	299 (3.1)	2456 (74.9)	229 (14.3)	2984 (20.5)
MSM	4157 (43.0)	280 (8.5)	616 (38.6)	5053 (34.8)
Heterosexual	4442 (46.0)	447 (13.6)	581 (36.4)	5470 (37.6)
Other/Unknown	759 (7.9)	96 (2.9)	170 (10.7)	1025 (7.1)
Calendar year enrolled, n(%)				
1997 – 2001	2423 (25.1)	2328 (71.0)	239 (15.0)	4990 (34.3)
2002 – 2006	950 (9.8)	300 (9.1)	54 (3.4)	1304 (9.0)
2007 – 2012	3455 (35.8)	412 (12.6)	417 (26.1)	4284 (29.5)
2013 – 2016	2829 (29.3)	239 (7.3)	886 (55.5)	3954 (27.2)

HIV-related factors by HCVAb status

Figure 3.4 shows that levels of CD4 at enrolment was above 350 cells/mm^3 in at least 60% of the cohort, with a median of 425 cells/mm^3 (IQR: 241-607). Median CD4 slightly decreased over calendar time and remained stable over time, which may in part reflect the fact that rate of HIV testing has not increased in recent years in Italy [(242)] and there has not been a reduction in the prevalence of late presenters at entry in the cohort (Figure 3.4). This is despite the fact that guidelines recommend earlier initiation of treatment regardless of CD4 starting from the year 2015. A small proportion (6%) of participants had undetectable (<50 copies/ml) HIV-RNA at baseline while high (>1,000 copies/ml) HIV-RNA was observed in at least 65% of participants.

Median CD4 was similar across the HCVAb status groups. Individuals not tested for HCV were also more likely not to have CD4 or HIV-RNA measured compared to the HCVAb positive or HCVAb negative. It is possible that the reason for concomitant missing data is a delay in reporting which affects both the laboratory and clinical data in a number of Icona sites.

Figure 3.4 Median CD4 (cells/mm^3) in Icona for participants enrolled between 1997 and 2016

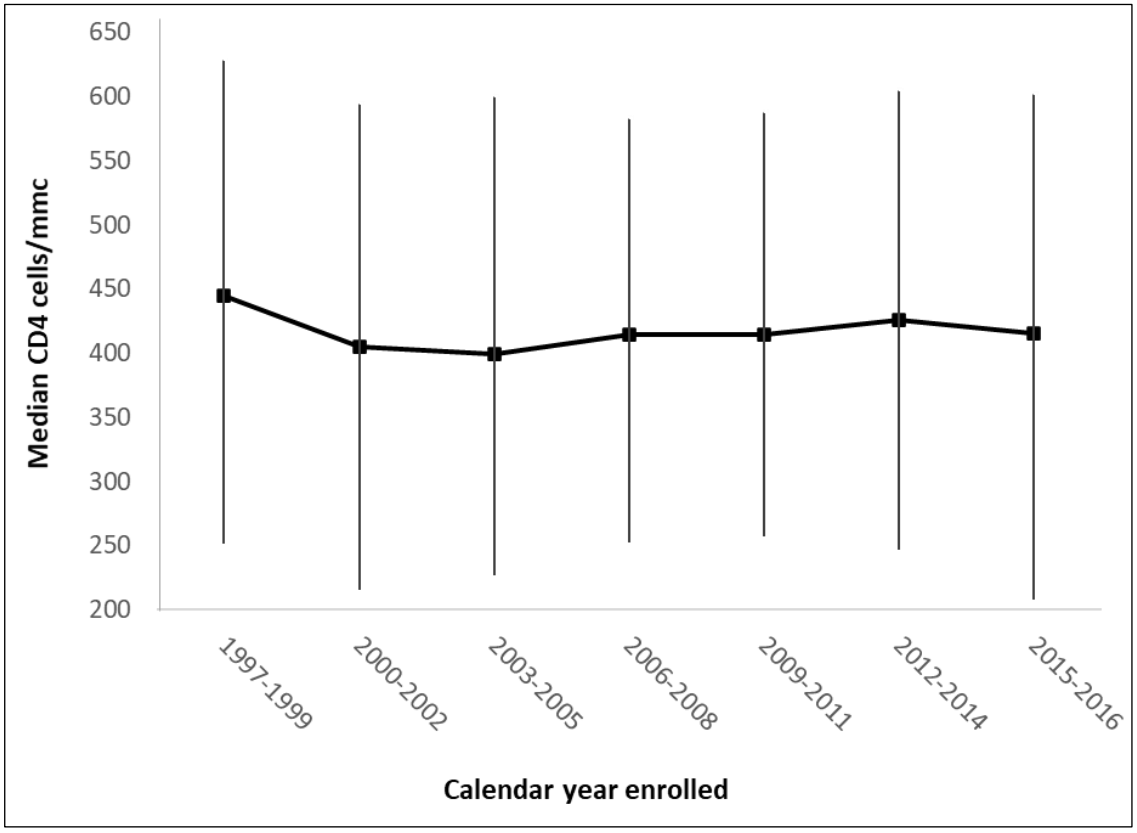


Table 3.2 Participants' HIV-related factors at enrolment in Icona stratified by HCVAb status

	HCVAb negative	**HCVAb positive**	**HCVAb unknown**	**Total**
	N= 9657	N= 3279	N= 1596	N= 14532
CD4 cell count, cells/mm^3				
Median(IQR)	426 (241, 608)	423 (236, 603)	427 (250, 603)	425 (241, 607)
CD4 cells/mm, n(%)				
0-200	1893 (19.6)	685 (20.9)	261 (16.4)	2839 (19.5)
201-350	1667 (17.3)	578 (17.6)	250 (15.7)	2495 (17.2)
>350	5595 (57.9)	1944 (59.3)	814 (51.0)	8353 (57.5)
unknown	502 (5.2)	72 (2.2)	271 (17.0)	845 (5.8)

	HCVAb negative	HCVAb positive	HCVAb unknown	Total
	N= 9657	N= 3279	N= 1596	N= 14532
HIV-RNA, log10 cps/ml				
Median (IQR)	3.96 (2.73, 4.76)	4.00 (2.97, 4.79)	3.73 (2.50, 4.69)	3.95 (2.77, 4.76)
HIV-RNA, cps/ml, n(%)				
0-50	629 (6.5)	105 (3.2)	147 (9.2)	881 (6.1)
51-1000	2034 (21.1)	693 (21.1)	311 (19.6)	3038 (20.9)
>1000	6328 (65.6)	2291 (69.9)	840 (52.6)	9459 (65.1)
unknown	657 (6.8)	189 (5.8)	297 (18.6)	1143 (7.9)

Socio-economic and lifestyle factors by HCVAb status

Socio-economic and lifestyle factors are shown in Table 3.3. There were some differences in terms of maximum achieved level of education. HCVAb positive individuals were more likely to have lower levels of education and more likely to be unemployed compared to HCVAb positive and HCVAb unknown individuals. Also, people with unknown HCVAb status were more likely to have missing data for education, employment and smoking status. This clustering of missing data is an important observation which affected how some of the analyses have been approached later in the thesis.

Alcohol use was reported by the treating physician who asked the participants about frequency/quantity of alcohol consumed regularly. The quality and utility of the data collected using this method are further investigated in chapter 4 which focusses on the role of HCV on the association between alcohol consumption and risk of developing severe liver disease (SLD). Overall, over half of participants had missing data for alcohol consumption. The proportion with missing data was especially high for early years of enrolment in the cohort (see Figure 3.5 and chapter 4 section 4.3.1), therefore the analysis in chapter 4 was restricted to participants enrolled after a certain calendar date.

Figure 3.5 shows the trend over time of missing data for variables that have >20% missing data overall. A larger proportion of participants had missing data for HCV-RNA, education, alcohol and smoking status in earlier years. As mentioned in

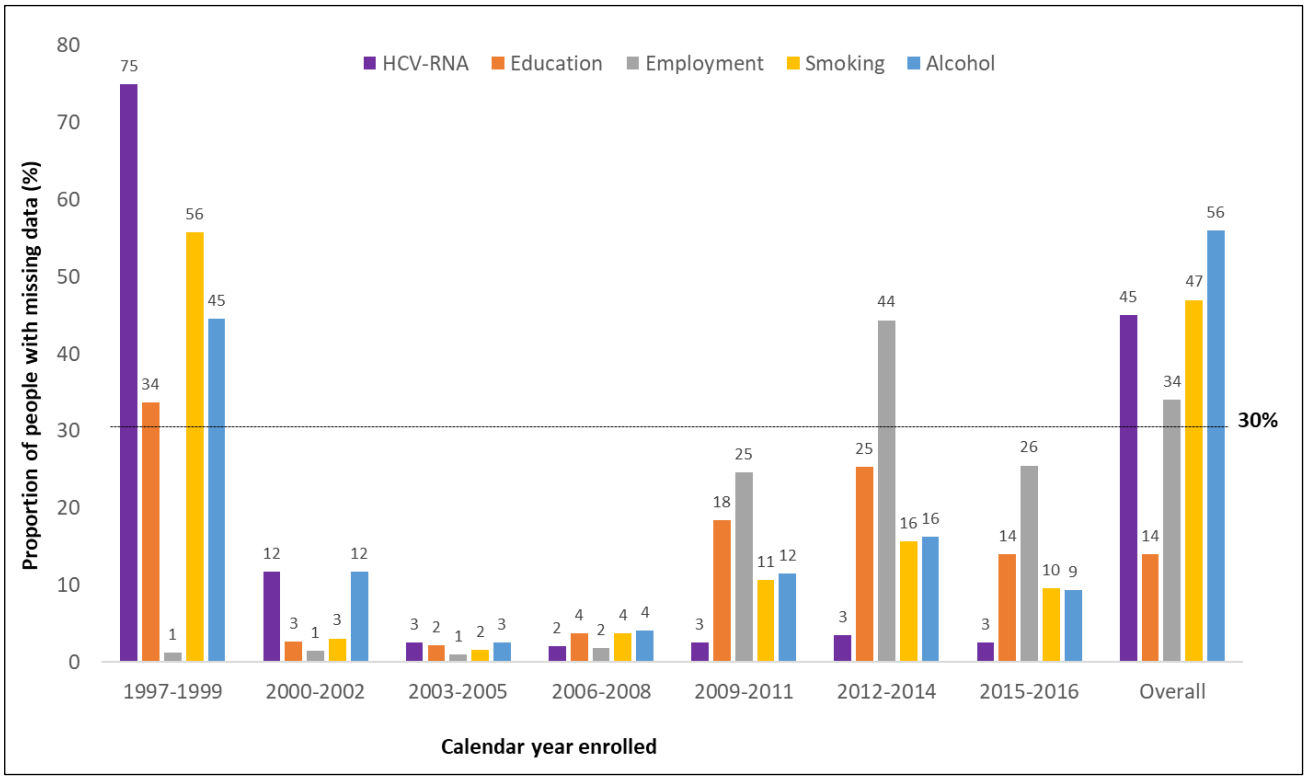
section 3.4.1.1, the drop in proportion of people with missing could possibly be explained by a drop in recruitment together with change in the demographic composition of participants. However, starting from 2006 the proportion of participants with missing data tended again to increase for all variables. Except for HCV-RNA where levels of missing remained low over time as routine measurement of HCV-RNA possibly started after 2002.

Table 3.3 Participants socio-economic and lifestyle factors at enrolment in Icona stratified by HCVAb status

	HCVAb negative N= 9657	**HCVAb positive** N= 3279	**HCVAb unknown** N= 1596	**Total** N= 14532
Education, n(%)				
Primary school (<11)	670 (6.9)	303 (9.2)	57 (3.6)	1030 (7.1)
Middle school (11-16)	2017 (20.9)	1184 (36.1)	200 (12.5)	3401 (23.4)
Secondary school (16-18)	2980 (30.9)	556 (17.0)	336 (21.1)	3872 (26.6)
University (18+)	1036 (10.7)	72 (2.2)	140 (8.8)	1248 (8.6)
Other/unknown	2954 (30.6)	1164 (35.5)	863 (54.1)	4981 (34.3)
Employment, n(%)				
Employed	6068 (62.8)	1909 (58.2)	777 (48.7)	8754 (60.2)
Unemployed	1142 (11.8)	1045 (31.9)	243 (15.2)	2430 (16.7)
Other	1032 (10.7)	186 (5.7)	135 (8.5)	1353 (9.3)
Unknown	1415 (14.7)	139 (4.2)	441 (27.6)	1995 (13.7)
Smoking, n(%)				
No	3298 (34.2)	282 (8.6)	377 (23.6)	3957 (27.2)
Yes	2535 (26.3)	839 (25.6)	372 (23.3)	3746 (25.8)
Unknown	3824 (39.6)	2158 (65.8)	847 (53.1)	6829 (47.0)
Alcohol consumption, n(%)				
Abstain	2800 (29.0)	525 (16.0)	272 (17.0)	3597 (24.8)
Moderate	1627 (16.8)	210 (6.4)	237 (14.8)	2074 (14.3)
Hazardous[1]	453 (4.7)	148 (4.5)	60 (3.8)	661 (4.5)
Unknown	4777 (49.5)	2396 (73.1)	1027 (64.3)	8200 (56.4)

[1] (See chapter 4 for more details) Defined using the Italian National Institute for Food and Nutrition of >3 drinks/day ≥5 drinks per occasion for men or and >2drinks/day or ≥4 drinks per occasion for women

Figure 3.5 Proportion of participants with missing data for variables with >20% missing data by calendar period



30% cut-off for missing data, i.e people enrolled pre-2000 were excluded in the chapter 4 main analysis

3.4.1.4 Demographic, HIV-related factors, socio-economic and lifestyle factors at enrolment in Icona stratified by HCV-RNA status amongst HCVAb positive people

An HCV-RNA test result was available at enrolment for 55% (n=1,807) of a total of 3,279 HCVAb positive participants; of these, 85% (n=1,540) were HCV-RNA positive and 15% (n=267) were HCV-RNA negative.

Demographic factors by HCV-RNA status

Demographics of people positive at the HCV serology test stratified by HCV-RNA test results are shown in Table 3.4. Participants who were not tested for HCV-RNA were more likely to be younger, more likely to be enrolled from the southern region

and more likely to be enrolled in earlier years. The lack of HCV-RNA measures in early years of enrolment is due to the fact that the test has been routinely introduced in the clinics only after 2002. The issue of the rate of HCVAb serology and HCV-RNA testing has been investigated in further detail in chapter 6 and 7. Specifically, in chapter 6, I provided an estimate of the rate of HCV testing in Icona participants with an HIV diagnosis within six months of enrolment. In chapter 7, I evaluated whether geographical region was a factor associated with the rate of HCV testing (both serology and HCV-RNA) in the cohorts.

HIV-related factors by HCV-RNA status

Table 3.5 shows that HIV-related factors had similar distributions across HCV-RNA status groups. Interestingly, among people with unknown HCV-RNA status making up 45% (1472/3279) of people HCVAb positive individuals, CD4 and HIV-RNA were measured in the majority of participants with <10% of missing data in each case. This reflects the fact that HCV-RNA has been collected in the cohort database relatively recently while the other HIV immuno-virological markers are regularly reported by the sites.

Table 3.4 Participants demographic factors in Icona and at enrolment amongst HCVAb positive individuals stratified by HCV-RNA status

	HCV-RNA negative	**HCV-RNA positive**	**HCV-RNA unknown**	**Total**
	N= 267	N= 1540	N= 1472	N= 3279
Gender, n(%)				
Male	187 (70.0)	1147 (74.5)	1096 (74.5)	2430 (74.1)
Female	80 (30.0)	393 (25.5)	376 (25.5)	849 (25.9)
Age, years				
Median (IQR)	38 (33, 44)	37 (33, 41)	35 (32, 39)	36 (32, 40)
Site geographical position, n(%)				
North	146 (54.7)	855 (55.5)	768 (52.2)	1769 (53.9)
South	30 (11.2)	251 (16.3)	357 (24.3)	638 (19.5)
Center	91 (34.1)	434 (28.2)	347 (23.6)	872 (26.6)

	HCV-RNA negative	HCV-RNA positive	HCV-RNA unknown	Total
Nationality, n(%)				
Italian	236 (88.4)	1460 (94.8)	1409 (95.7)	3105 (94.7)
Non-Italian	31 (11.6)	80 (5.2)	63 (4.3)	174 (5.3)
Mode of HIV Transmission, n(%)				
PWID	158 (59.2)	1122 (72.9)	1176 (79.9)	2456 (74.9)
MSM	49 (18.4)	142 (9.2)	89 (6.0)	280 (8.5)
Heterosexual	47 (17.6)	222 (14.4)	178 (12.1)	447 (13.6)
Other/unknown	13 (4.9)	54 (3.5)	29 (2.0)	96 (2.9)
Calendar year enrolled, n(%)				
1997 – 2001	125 (46.8)	959 (62.2)	1244 (84.5)	2328 (71)
2002 – 2006	36 (13.5)	182 (11.8)	82 (5.6)	300 (9.1)
2007 – 2012	68 (25.5)	267 (17.4)	77 (5.2)	412 (12.6)
2013 – 2016	38 (14.2)	132 (8.6)	69 (4.7)	239 (7.3)

Table 3.5 Participants HIV-related factors in Icona and enrolment amongst HCVAb positive individuals stratified by HCV-RNA status

	HCV-RNA negative N= 267	HCV-RNA positive N= 1540	HCV-RNA unknown N= 1472	Total N= 3279
CD4 cells/mm^3				
Median(IQR)	416 (239, 619)	415 (229, 596)	389 (192, 582)	405 (212, 592)
CD4 cells/mm^3, n(%)				
0-200	51 (19.1)	298 (19.4)	336 (22.8)	685 (20.9)
201-350	42 (15.7)	269 (17.5)	267 (18.1)	578 (17.6)
>350	171 (64.0)	932 (60.5)	841 (57.1)	1944 (59.3)
unknown	3 (1.1)	41 (2.7)	28 (1.9)	233 (2.2)
HIV-RNA, log10 cps/ml				

	HCV-RNA negative	HCV-RNA positive	HCV-RNA unknown	Total
Median(IQR)	4.35 (3.48, 4.94)	4.38 (3.63, 5.03)	4.43 (3.60, 5.04)	4.40 (3.60, 5.02)
HIV-RNA, cps/ml, n(%)				
0-50	18 (6.7)	51 (3.3)	36 (2.5)	105 (3.2)
51-1000	64 (24.0)	347 (22.5)	282 (19.2)	338 (10.4)
>1000	177 (66.3)	1049 (68.1)	1065 (72.4)	2291 (77.0)
unknown	8 (3.0)	92 (6.0)	(6.1)	189 (5.8)

Socio-economic and lifestyle factors by HCV-RNA status

Table 3.6 shows socio-economic and lifestyle factors stratified by HCV-RNA status amongst HCVAb positive individuals. Similarly to what was found with the serology data, participants who were HCV-RNA positive were more likely to have lower levels of education and more likely to be unemployed compared to HCVAb negative individuals. Interestingly, people not HCV-RNA tested were more likely to be unemployed and more likely also to have missing data on smoking status or alcohol consumption.

3.4.1.5 Descriptive summary statistics for HCV-RNA and HCV genotype at enrolment in Icona amongst HCV-RNA positive individuals

In a subset of 1,382 out of 1,540 HCV-RNA positive individuals, HCV genotype was assessed. The breakdown of participants according to HCV-RNA categories was as follows: <500k IU/L 594 (39%), 500k-1M IU/L 230 (15%) and >1M IU/L 558 (36%) and not measured 158 (10%). The distribution of genotypes (GT) were GT1- 607 (39%), GT2- 37 (2%), GT3- 440 (29%), GT4- 120 (8%), other/unknown- 336 (22%). As mentioned in chapter 1, a possible explanation of a higher prevalence of GT1 could be infections obtained through blood transfusions or needle sharing. This is consistent with the Icona study population being enriched with PWIDs in earlier years of enrolment. This was important to know in the pre-DAA era as the

outcome of treatment was affected by HCV-genotype. In DAA era, there are now more options available to treat different genotypes.

Table 3.6 Participants socio-economic and lifestyle factors amongst HCVAb positive individuals enrolled in Icona stratified by HCV-RNA status

	HCV-RNA negative	**HCV-RNA positive**	**HCV-RNA unknown**	**Total**
	N= 267	N= 1540	N= 1472	N= 3279
Education, n(%)				
Primary school (<11)	25 (9.4)	156 (10.1)	122 (8.3)	303 (9.2)
Middle school (11-16)	99 (37.1)	681 (44.2)	404 (27.4)	1184 (36.1)
Secondary school (16-18)	74 (27.7)	323 (21.0)	159 (10.8)	556 (17.0)
University (18+)	12 (4.5)	44 (2.9)	16 (1.1)	72 (2.2)
Other/unknown	57 (21.3)	336 (21.8)	771 (52.4)	1164 (35.5)
Employment, n(%)				
Employed	169 (63.3)	976 (63.4)	764 (51.9)	1909 (58.2)
Unemployed	48 (18.0)	415 (26.9)	582 (39.5)	1045 (31.9)
Other	24 (9.0)	75 (4.9)	87 (5.9)	186 (5.7)
unknown	26 (9.7)	74 (4.8)	39 (2.6)	139 (4.2)
Smoking, n(%)				
No	30 (11.2)	170 (11.0)	82 (5.6)	282 (8.6)
Yes	106 (39.7)	475 (30.8)	258 (17.5)	839 (25.6)
Unknown	131 (49.1)	895 (58.1)	1132 (76.9)	2158 (65.8)
Alcohol consumption, n(%)				
Abstain	62 (23.2)	313 (20.3)	150 (10.2)	525 (16.0)
Moderate	30 (11.2)	118 (7.7)	62 (4.2)	210 (6.4)
Hazardous[1]	21 (7.9)	103 (6.7)	24 (1.6)	148 (4.5)
Unknown	154 (57.7)	1006 (65.3)	1236 (84.0)	2396 (73.1)

[1](See chapter 4 for more details) Defined using the Italian National Institute for Food and Nutrition of >3 drinks/day ≥5 drinks per occasion for men or and >2drinks/day pr ≥ 4 drinks per occasion for women

3.4.1.6 Demographic, HIV-related, socio-economic and lifestyle factors at enrolment in Icona stratified by stage of liver disease amongst HIV/HCV coinfected individuals

Among the HCVAb positive individuals, stage of liver disease was measured in 99% of participants (3234/3279). In particular, 52% (n=1,712) of HCV-positive people presented with mild, 32% (n=1,044) with moderate liver fibrosis and 15% (n=478) with advanced liver fibrosis at entry. The variables needed to calculate a FIB-4 were complete at entry for HCVAb positive individuals with only 1% (n=45/3279) of participants for whom FIB-4 could not be calculated.
HIV/HCV coinfection often leads to SLD and this is the primary clinical outcome of some of the analysis presented in chapters 4 and 6. Of course in these chapters, participants with SLD at entry in the cohort were excluded from the analysis because they had already developed the event of interest. Thus, the table included here is important as it shows the prevalence of SLD in the cohort before these exclusions were operated. Of note, a diagnosis of SLD at entry might have been caused by delayed HCV testing (covered in more detail in chapter 6).

Demographic factors by stage of liver disease

Individuals with advanced fibrosis, were on average, slightly older (median age 38 years (IQR: 35-43) than those with mild or moderate liver fibrosis (35 (IQR: 31-38) and 37 (IQR: 34-42) years respectively and more likely to be male and Italian. Among participants with advanced stage of liver disease (FIB-4 >3.25) PWID were more prevalent (83%) than in those with mild or moderate liver fibrosis (79% and 70%) respectively. There were no clear differences by geographical region or calendar period.

Table 3.7 Participant demographics in Icona amongst HCVAb positive individuals stratified by stage of liver disease

	FIB-4				Total
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	
	N=1712	N=1044	N=478	N=45	N=3279
Gender, n(%)					
Male	1186 (69.3)	822 (78.7)	389 (81.4)	33 (73.3)	2430 (74.1)
Female	526 (30.7)	222 (21.3)	89 (18.6)	12 (26.7)	849 (25.9)
Age, years					
Median (IQR)	35 (31, 38)	37 (34, 42)	38 (35, 43)	34 (31, 39)	36 (32, 40)
Region, n(%)					
North	941 (55.0)	543 (52.0)	258 (54.0)	27 (60.0)	1769 (53.9)
Center	301 (17.6)	231 (22.1)	101 (21.1)	5 (11.1)	638 (19.5)
South	470 (27.5)	270 (25.9)	119 (24.9)	13 (28.9)	872 (26.6)
Nationality, n(%)					
Italian	1599 (93.4)	999 (95.7)	464 (97.1)	43 (95.6)	3105 (94.7)
Non-Italian	113 (6.6)	45 (4.3)	14 (2.9)	2 (4.4)	174 (5.3)
Mode of HIV Transmission, n(%)					
PWID	1194 (69.7)	828 (79.3)	397 (83.1)	37 (82.2)	2456 (74.9)
MSM	198 (11.6)	63 (6.0)	18 (3.8)	1 (2.2)	280 (8.5)
Heterosexual	269 (15.7)	121 (11.6)	50 (10.5)	7 (15.6)	447 (13.6)
Other/unknown	51 (3.0)	32 (3.1)	13 (2.7)	0 (0.0)	96 (2.9)
Calendar year enrolled, n(%)					
1997 – 2001	1177 (68.8)	773 (74.0)	352 (73.6)	26 (57.8)	2328 (71)
2002 – 2006	174 (10.2)	86 (8.2)	39 (8.1)	1 (2.2)	300 (9.1)
2007 – 2012	225 (13.1)	122 (11.7)	55 (11.5)	10 (22.2)	412 (12.6)
2013 – 2016	136 (7.9)	63 (6.0)	32 (6.7)	8 (17.8)	239 (7.3)

HIV-related factors by stage of liver disease

Table 3.8 shows HIV-related factors stratified by stage of liver disease. There is some suggestion in the literature that levels of CD4 are correlated with stage of liver disease [197]. This appeared to be confirmed in this analysis as HIV-positive

individuals with advanced liver disease were more likely to have lower median CD4 of 293 cells/mm^3 (IQR: 128-475) compared to HIV-positive individuals with mild or moderate liver disease, with median CD4 cell counts of 450 cells/mm^3 (IQR: 284 - 638) and 378 cells/mm^3 (IQR: 128 – 475) respectively. These results also suggest that HIV/HCV coinfected individuals presenting late for HIV care with a low CD4 may also be presenting late with HCV. Again, I investigated this further in chapter 6 by looking at determinants of late HCV diagnosis.

Table 3.8 Participants HIV-related factors in Icona among HCVAb positive individuals stratified by stage of liver disease

	FIB-4				Total
	<1.45	**1.45-3.25**	**>3.25**	**Unknown**	
	N= 1712	N= 1044	N= 478	N= 45	N= 3279
CD4 cells/mm^3					
Median(IQR)	450 (284, 638)	378 (172, 567)	293 (128, 475)	362 (219, 571)	405 (212, 592)
CD4 cells/mm^3, n(%)					
0-200	254 (14.8)	264 (25.3)	157 (32.8)	10 (22.2)	685 (20.9)
201-350	276 (16.1)	196 (18.8)	97 (20.3)	9 (20.0)	578 (17.6)
>350	1141 (66.5)	566 (54.2)	214 (44.8)	23 (51.1)	1944 (59.3)
unknown	41 (2.4)	18 (1.7)	10 (2.1)	3 (6.7)	72 (2.2)
HIV-RNA, log10 cps/ml					
Median(IQR)	4.30 (3.51, 4.93)	4.48 (3.71, 5.08)	4.58 (3.72, 5.28)	4.99 (4.49, 5.37)	4.40 (3.60, 5.02)
HIV-RNA, cps/ml n (%)					
0-50	61 (3.6)	23 (2.2)	21 (1.5)	0 (0.0)	105 (3.2)
51-500	356 (20.8)	218 (20.9)	116 (24.3)	3 (6.7)	693 (21.1)
>1000	1194 (69.7)	756 (72.4)	307 (64.2)	32 (75.6)	2291 (69.9)
Unknown	101 (5.9)	47 (4.5)	33 (6.9)	8 (17.8)	189 (5.8)

Socio-economic and lifestyle factors by stage of liver disease

Socio economic and lifestyle factors stratified by stage of liver disease are presented in Table 3.9. Those with advanced liver disease (FIB-4>3.25) tended to have lower levels of educational and were somewhat more likely to be

unemployed. They were also somewhat more likely to be current smokers and report hazardous alcohol consumption, although the large amount of missing data for these factors in the study population overall means these small differences are difficult to interpret.

For this reason, as shown in Figure 3.5, the analyses in chapter 4 assessing association between alcohol consumption and risk of severe liver disease used a restricted calendar period (Table 4.2), with an additional analysis using multiple imputation methods to account for missing alcohol data.

Table 3.9 Participants socio-economic and lifestyle factors by stage of liver disease

	FIB-4				
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	**Total**
	N= 1712	N= 1044	N= 478	N= 45	N= 3279
Education, n(%)					
Primary school (<11)	156 (9.1)	99 (9.5)	43 (9.0)	5 (11.1)	303 (9.2)
Middle school (11-16)	609 (35.6)	388 (37.2)	176 (36.8)	11 (24.4)	1184 (36.1)
Secondary school (16-18)	322 (18.8)	163 (15.6)	69 (14.4)	2 (4.4)	556 (17.0)
University (18+)	53 (3.1)	15 (1.4)	4 (0.8)	0 (0.0)	72 (2.2)
Other/unknown	572 (33.4)	379 (36.3)	186 (38.9)	27 (60.0)	1164 (35.5)
Employment, n(%)					
Employed	1006 (58.8)	616 (59.0)	270 (56.5)	17 (37.8)	1909 (58.2)
Unemployed	516 (30.1)	332 (31.8)	172 (36.0)	25 (55.6)	1045 (31.9)
Other	108 (6.3)	55 (5.3)	22 (4.6)	1 (2.2)	186 (5.7)
Unknown	82 (4.8)	41 (3.9)	14 (2.9)	2 (4.4)	139 (4.2)
Smoking, n(%)					
No	163 (9.5)	86 (8.2)	31 (6.5)	2 (4.4)	282 (8.6)
Yes	452 (26.4)	252 (24.1)	122 (25.5)	13 (28.9)	839 (25.6)
Unknown	1097 (64.1)	706 (67.6)	325 (68.0)	30 (66.7)	2158 (65.8)
Alcohol consumption, n(%)					
Abstain	307 (17.9)	140 (13.4)	72 (15.1)	6 (13.3)	525 (16.0)
Moderate	129 (7.5)	58 (5.6)	20 (4.2)	3 (6.7)	210 (6.4)
Hazardous	62 (3.6)	48 (4.6)	35 (7.3)	3 (6.7)	148 (4.5)

	FIB-4				
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	**Total**
	N= 1712	N= 1044	N= 478	N= 45	N= 3279
Unknown	1214 (70.9)	798 (76.4)	351 (73.4)	33 (73.3)	2396 (73.1)

3.4.2 Demographics of Icona compared with PLWH in Italy

Table 3.10 shows a summary of gender, age mode of HIV transmission in Icona and PLWH in Italy. This is based on HIV surveillance data of new HIV diagnoses on people who test positive for HIV for the first time[243]. In terms of gender and age, the Icona cohort is a fair representation of PLWH in Italy.

Table 3.10 Summary of demographics in Icona compared to the PLWH population in Italy

	Icona	[1]PLWH in Italy[243]
Gender, n(%)		
Male	75.8	79.9
Female	24.2	20.1
Age, years		
Median	36	40
Mode of HIV transmission, n(%)		
PWID	20.5	3
MSM	34.8	45.7
Heterosexual	37.6	42.4
Other/Unknown	7.1	8.9

[1]Data based on surveillance of new HIV diagnoses on people who test positive for HIV for the first time[243]

3.4.3 A summary of findings of a previous analysis using Icona data that evaluated incidence of new HCV infections

In this thesis evaluating HCV as a risk factor for specific outcome (chapters 4 and 6) it is crucial to provide not only an estimate of the prevalence of HCV infection in the cohort but also the rate of acquiring HCV over follow-up. Repeated HCVAb tests are available in the Icona database. This longitudinal testing data have been used in a previous Icona study external to this thesis, the analysis of which was not conducted by me, to obtain a crude estimate of the rate of HCV seroconversion over time in the cohort of those who initially tested HCVAb negative. This analysis was conducted in 2016 and included 4,059 HCVAb negative participants in Icona [(244)]. Over a total of 28,867 person years of follow-up (PYFU), 185 HCV seroconversions were recorded with an incidence rate of 0.6/100 PYFU (95 %CI: 0.5-0.7). Incidence rate trends over time were also calculated and, overall, a decrease from 1.6/100 PYFU in 1997-2000 (95%CI: 1.3-2.0) to 0.4/100 PYFU in 2013-2016 (95%CI: 0.3-0.6) was found [(244)]. After stratification by mode of HIV transmission, incidence rate of HCV seroconversion was much higher among PWID at 7.2/100 PYFU (95%CI: 5.4-9.6) compared to other groups, (0.7/100 PYFU (95%CI: 0.6-0.9), 0.3/100 PYFU (95%CI: 0.2-0.4) in MSM and heterosexual contact groups, respectively [(244)]. The higher incidence of HCV in the earlier calendar period is likely to reflect the higher proportion of PLWH who were PWID in this period (see Figure 3.2). Because, at the time of drafting this chapter, this incident analysis had already been recently performed. I have used this existing background data rather than performing an update of the analysis.

3.4.4 Hepaicona

Hepaicona by study design only enrols HIV/HCV coinfected persons, specifically HIV-positive/HCV-RNA positive individuals who at study entry are naïve to DAA treatment. Enrolment began in October 2013 and data collected on people enrolled up to 30th June 2016 are included here. As of 30th June 2016, Hepaicona had

enrolled 1,584 HIV/HCV coinfected individuals with calendar year of enrolment median (min-max) 2015 (2013 – 2016).

3.4.4.1 Demographic factors, HIV-related, socio-economic and lifestyle factors at enrolment in Hepaicona stratified by stage of liver disease

Characteristics of HIV/HCV coinfected individuals enrolled in Hepaicona are shown stratified by stage of liver disease in Table 3.11 .Similarly, to HIV/HCV coinfected participants in Icona, Hepaicona predominantly includes male participants. However, the Hepaicona coinfected population is older than that included in Icona, with a median age of 48 years (IQR: 43 – 52) vs. 36 (IQR: 32 – 40). Of the 1520/1584 for whom there a was an available measure of FIB-4, the stage of liver disease was distributed as follows; mild 34% (n=519), moderate 40% (n=613) and advanced 26% (n=388).

Liver disease of HCV-RNA positive people in Hepaicona was, on average, more advanced than for HIV/HCV coinfected people enrolled in Icona since the former were coinfected for a longer period of time. Within Hepaicona, (participants with advanced disease were older), but there were no marked differences by stage of liver disease by gender, region, nationality or mode of HIV transmission.

Table 3.11 Participant demographics at enrolment in Hepaicona stratified by stage of liver disease

	FIB-4				Total
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	
	N= 519	N= 613	N= 388	N= 64	N= 1584
Gender, n(%)					
Male	361 (69.6)	454 (74.1)	302 (77.8)	49 (76.6)	1166 (73.6)
Female	158 (30.4)	159 (25.9)	86 (22.2)	15 (23.4)	418 (26.4)
Age, years					
Median (IQR)	45 (40, 49)	49 (45, 53)	51 (46, 54)	48 (45, 52)	48 (43, 52)

	FIB-4				Total
	<1.45	1.45 - 3.25	>3.25	Unknown	
	N= 519	N= 613	N= 388	N= 64	N= 1584
Region, n(%)					
North	297 (57.2)	351 (57.3)	218 (56.2)	62 (96.9)	928 (58.6)
Center	87 (16.8)	86 (14.0)	42 (10.8)	1 (1.6)	216 (13.6)
South	135 (26.0)	176 (28.7)	128 (33.0)	1 (1.6)	440 (27.8)
Nationality, n(%)					
Italian	492 (94.8)	589 (96.1)	374 (96.4)	63 (98.4)	1518 (95.8)
Non-Italian	27 (5.2)	24 (3.9)	14 (3.6)	1 (1.6)	66 (4.2)
Mode of HIV Transmission, n(%)					
PWID	378 (72.8)	479 (78.1)	303 (78.1)	50 (78.1)	1210 (76.4)
MSM	39 (7.5)	20 (3.3)	10 (2.6)	5 (7.8)	74 (4.7)
Heterosexual	62 (11.9)	67 (10.9)	32 (8.2)	2 (3.1)	163 (10.3)
Other/Unknown	40 (7.7)	47 (7.7)	43 (11.1)	7 (10.9)	137 (8.6)
Calendar year enrolled, n(%)					
2013 - 2016	519 (100)	613 (100)	388 (100)	64 (100)	1584 (100)

HIV-related factors by stage of liver disease

Table 3.12 shows HIV-related factors stratified by stage of liver disease. Overall, at enrolment in Hepaicona, high median CD4 581 cells/mm^3 (IQR: 378-809) and undetectable HIV-RNA were observed indicating that the majority of participants entered the study when they were already cART experienced. As a result, CD4 at entry was higher than that shown for HIV/HCV coinfected in Icona (median CD4 405 cells/mm^3 (IQR: 212 – 592)). A similar correlation between low CD4 and stage of liver disease is also seen here. CD4 was the lowest in participants with advanced liver fibrosis with a median of 406 cells/mm^3 (IQR: 238-617) compared to participants with mild and moderate liver fibrosis who had median CD4 of 645 cells/mm^3 (IQR: 468-886) and 597 cells/mm^3 (IQR: 400-827), respectively.

The distribution of genotypes (GT) were GT1- 49% (n=772), GT2- 2% (n=33), GT3- 23% (n=352), GT4- 15% (n=241) and other/unknown- 11% (168) with no marked difference according to severity of liver disease. The proportion of HCV-RNA

positive participants with a genotypic test result available was higher than that observed in the HIV/HCV coinfected population in Icona, although the overall distribution of genotypes is similar.

Table 3.12 HIV-related factors and HCV genotype at enrolment in Hepaicona stratified by stage of liver disease

	FIB-4				Total
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	
	N= 519	N= 613	N= 388	N= 64	N= 1584
CD4 cells/mm³					
Median(IQR)	645 (467, 898)	602 (399, 831)	406 (238, 617)	620 (468, 755)	581 (378, 809)
CD4 cells/mm³					
0-200	14 (2.7)	31 (5.1)	62 (16.0)	1 (1.6)	108 (6.8)
201-350	37 (7.1)	61 (10.0)	74 (19.1)	7 (10.9)	179 (11.3)
>350	397 (76.5)	437 (71.3)	190 (49.0)	41 (64.1)	1065 (67.2)
unknown	71 (13.7)	84 (13.7)	62 (16.0)	15 (23.4)	232 (14.6)
HIV-RNA, log10 cps/ml					
Median(IQR)	1.38 (1.28, 1.60)	1.32 (0.48, 1.60)	1.40 (1.04, 1.60)	1.28 (1.28, 1.36)	1.38 (1.04, 1.60)
HIV-RNA, cps/ml, n(%)					
0-500	333 (64.2)	397 (64.8)	253 (65.2)	37 (57.8)	1020 (64.4)
>500-1000	20 (3.8)	29 (4.7)	21 (5.4)	0 (0.0)	70 (4.4)
Unknown	166 (32.0)	187 (30.5)	114 (29.4)	27 (42.2)	494 (31.2)
HCV genotype					
1	266 (51.3)	294 (48.0)	178 (45.9)	34 (53.1)	772 (48.7)
2	14 (2.7)	9 (1.5)	9 (2.3)	1 (1.6)	33 (2.2)
3	78 (15.0)	147 (24.0)	113 (29.1)	14 (28.9)	352 (22.2)
4	86 (16.6)	104(17.0)	47 (12.1)	4 (6.3)	241 (15.2)
Other/unknown	75 (14.4)	59 (9.4)	41 (10.5)	11 (17.2)	186(11.7)

Socio-economic and lifestyle factors by stage of liver disease

Table 3.13 shows socio-economic and lifestyle factors stratified by stage of liver disease. Over half of the participants had missing data for smoking and alcohol consumption across all stages of liver disease, and about a third were missing for employment status. There was no clear differences between the groups.

Table 3.13 Socio-economic and lifestyle factors at enrolment in Hepaicona stratified by stage of liver disease

	FIB-4				Total
	<1.45	**1.45 - 3.25**	**>3.25**	**Unknown**	
	N= 519	N= 613	N= 388	N= 64	N= 1584
Employment, n(%)					
Employed	257 (49.5)	255 (41.6)	152 (39.2)	20 (31.3)	684 (43.2)
Unemployed	68 (13.1)	100 (16.3)	59 (15.2)	16 (25.0)	243 (15.3)
Other	34 (6.6)	44 (7.2)	28 (7.2)	2 (3.1)	108 (6.8)
Unknown	160 (30.8)	214 (34.9)	149 (38.4)	26 (40.6)	549 (34.7)
Smoking, n(%)					
No	93 (17.9)	91 (14.8)	56 (14.4)	4 (6.3)	244 (15.4)
Yes	170 (32.8)	166 (27.1)	109 (28.1)	8 (12.5)	453 (28.6)
Unknown	256 (49.3)	356 (58.1)	223 (57.5)	52 (81.3)	887 (56.0)
Alcohol use, n(%)					
Abstain	143 (27.6)	142 (23.2)	91 (23.5)	9 (14.1)	385 (24.3)
Moderate	61 (11.8)	57 (9.3)	32 (8.2)	3 (4.7)	153 (9.7)
Hazardous	33 (6.4)	34 (5.5)	27 (7.0)	0 (0.0)	94 (5.9)
Unknown	282 (54.3)	380 (62.0)	238 (61.3)	52 (81.3)	952 (60.1)

3.4.4.2 HIV/HCV coinfected participants eligible for immediate start of DAA in Icona and Hepaicona stratified by cohort

At the time of drafting this chapter, the Agenzia Italiana Farmaco Industria (AIFA) (the Italian equivalent to European Medical Association (EMA) in Europe) was in need to acquire a reliable estimate of the burden of HIV/HCV coinfected individuals

seen for care in Italy and eligible for immediate start of DAA treatment. Importantly, before 2015 in Italy as mentioned in chapter 1, (section 1.3.5), in the HIV/HCV coinfected population HCV treatment was prioritized on the basis of liver disease severity. Thus, it was important to provide an estimate of those with severe disease who were entitled to immediate start with DAA. This gap in knowledge motivated this final section of this chapter. For this purpose, the data of the Icona and Hepaicona cohorts were merged together. All individuals in Hepaicona were included, however in individuals selected from Icona were those participants who were HCV-RNA positive and naïve to DAA which is the inclusion criteria to be enrolled in Hepaicona. The analysis showed that, as of January 2015, a total of 3,025 participants were eligible for DAA treatment and of these 583 (19%) with advanced liver disease were eligible for immediate treatment.

One problem with the data shown in previous sections is the fact that inclusion criteria for the two cohorts are quite different and therefore a direct comparison between the characteristics of participants enrolled in the two studies is likely to biased by these selections. In this section I formally compare the characteristics of participants in the two cohorts by restricting to patients eligible for DAA treatment in both cohorts. Specifically, this was done by including only Icona participants who approximately satisfied the inclusion criteria for Hepaicona (i.e. at any point during follow-up found to have detectable HCV-RNA and no prior exposure to DAA).

Table 3.14 shows characteristics as presented in previous sections but merging together the data of the two cohorts and stratifying by cohort (Icona - N = 1441 and Hepaicona - N = 1584). Despite the homogeneous criteria for inclusion in the analysis, except for gender and nationality, there were still differences between cohorts. The differences observed in previous tables (e.g. a higher proportion of HIV/HCV coinfected participants with advanced stage of liver disease in Hepaicona) also persisted in this comparison. Individuals enrolled in Hepaicona were more likely to have HCV genotype 1 infection, to be of older age, and somewhat to report PWID as mode of HIV transmission, rather than MSM or heterosexual contact, Hepaicona participants had a much higher median CD4 and

lower prevalence of immunosuppression. The majority of Hepaicona participants had viral suppression compared to a low proportion in Icona, reflecting the higher proportion of ART-treated participants

Table 3.14 Participants' characteristics for HIV/HCV coinfected individuals in Icona and Hepaicona who eligible for DAA initiation stratified by cohort

	Icona	**Hepaicona**	**Total**	***p-value**
	N= 1441	N= 1584	N= 3025	
Stage of liver disease, n(%)				<.001
FIB-4(<1.45)	755 (52.4)	519 (32.8)	1274 (42.1)	
FIB-4(1.45-3.25)	481 (33.4)	613 (38.7)	1094 (36.2)	
FIB-4(>3.25)	195 (13.5)	388 (24.5)	583 (19.3)	
Unknown	10 (0.7)	64 (4.0)	74 (2.4)	
Genotype, n(%)				<.001
1	556 (38.6)	772 (48.7)	1328 (43.9)	
2	33 (2.3)	33 (2.1)	66 (2.2)	
3	415 (28.8)	352 (22.2)	767 (25.4)	
4	114 (7.9)	241 (15.2)	355 (11.7)	
Other/unknown	323 (22.4)	186 (11.7)	509 (16.8)	
Gender, n(%)				0.688
Male	1070 (74.3)	1166 (73.6)	2236 (73.9)	
Female	371 (25.7)	418 (26.4)	789 (26.1)	
Age, years				
Median (IQR)	36 (33, 41)	48 (43, 52)	42 (36, 49)	<.001
Region, n(%)				0.021
North	790 (54.8)	928 (58.6)	1718 (56.8)	
South	246 (17.1)	216 (13.6)	462 (15.3)	
Center	405 (28.1)	440 (27.8)	845 (27.9)	
Nationality, n(%)				0.275
Italian	1369 (95.0)	1518 (95.8)	2887 (95.4)	
Non-Italian	72 (5.0)	66 (4.2)	138 (4.6)	
Mode of HIV Transmission, n(%)				<.001

	Icona	Hepaicona	Total	*p-value
	N= 1441	N= 1584	N= 3025	
PWID	1053 (73.1)	1210 (76.4)	2263 (74.8)	
MSM	134 (9.3)	74 (4.7)	208 (6.9)	
Heterosexual	203 (14.1)	163 (10.3)	366 (12.1)	
Other/Unknown	51 (3.5)	137 (8.6)	188 (6.2)	
CD4 cells/mm^3				<.001
Median(IQR)	422 (234, 600)	581 (378, 809)	490 (306, 709)	
CD4 cells/mm^3				<.001
0-200	280 (19.4)	108 (6.8)	388 (12.8)	
201-350	252 (17.5)	179 (11.3)	431 (14.2)	
>350	782 (54.3)	1065 (67.2)	1847 (61.1)	
unknown	127 (8.8)	232 (14.6)	359 (11.9)	
HIV-RNA, log10 cps/ml				<.001
Median(IQR)	4.36 (3.62, 5.01)	1.38 (1.04, 1.60)	2.88 (1.40, 4.50)	
HIV-RNA, cps/ml				<.001
0-500	110 (7.6)	1020 (64.4)	1130 (37.4)	
>500	1129 (78.3)	70 (4.4)	1199 (39.7)	
Unknown	202 (14.0)	494 (31.2)	696 (23.0)	
[+]Education, n(%)				
Primary school (<11)	148 (10.3)	-	148 (10.3)	
Middle school (11-16)	639 (44.3)	-	639 (44.3)	
Secondary school (16-18)	295 (20.5)	-	295 (20.5)	
University (18+)	41 (2.8)	-	41 (2.8)	
Other/Unknown	318 (22.1)	-	318 (22.1)	
Employment, n(%)				<.001
Employed	902 (62.6)	684 (43.2)	1586 (52.4)	
Unemployed	399 (27.7)	243 (15.3)	642 (21.2)	
Other	72 (5.0)	108 (6.8)	180 (6.0)	
Unknown	68 (4.7)	549 (34.7)	617 (20.4)	
Smoking, n(%)				<.001
No	156 (10.8)	244 (15.4)	400 (13.2)	
Yes	441 (30.6)	453 (28.6)	894 (29.6)	
Unknown	844 (58.6)	887 (56.0)	1731 (57.2)	

	Icona	Hepaicona	Total	*p-value
	N= 1441	N= 1584	N= 3025	
Alcohol consumption, n(%)				<.001
Abstain	284 (19.7)	385 (24.3)	669 (22.1)	
Moderate	108 (7.5)	153 (9.7)	261 (8.6)	
Hazardous	92 (6.4)	94 (5.9)	186 (6.1)	
Unknown	957 (66.4)	952 (60.1)	1909 (63.1)	

*Kruskall Wallis and chi-squared tests. ⁺Education level is not collected in Hepaicona

3.4.5 Summary

This descriptive analysis of the data of the two cohorts has shown that the prevalence of HCV at enrolment was 23% in Icona over the whole calendar period of enrolment and was much higher among PWID and in the early calendar periods. It was also higher among native Italians and the southern region. This is an important finding as for example the epidemic in other countries such as the UK is more prevalent among migrants [245]. Among those HCVAb positive with an HCV-RNA test available, 85% were HCV-RNA positive. Individuals in Hepaicona were an older population presenting with advanced liver disease compared to individuals enrolled in Icona, although the Hepaicona participants had more favourable HIV-related markers (CD4 and HIV-RNA) as this population was not cART naïve at study enrolment.

Another important finding from this chapter was the fact that for potentially important confounding variables, such as alcohol consumption, for an important proportion of the participants enrolled in the cohorts (up to 50%) the data was missing at enrolment. Clear clustering in missing data was apparent, meaning that participants who had missing values for one variable were much more likely to have missing values for other factors. For the following variables in Icona, HCV-RNA, alcohol and smoking status, the proportion of missing values was much greater in the early calendar period and decreased after that with a tendency to increase again after 2006. This is likely to be due to a delay in implementation of

data collection e.g. HCV-RNA was only started in 2002. These results had implications for the approach to analysis in some of the subsequent chapters.

The pattern of missing data observed informed for some factors subsequent analysis in my thesis. I have often used the 'missing indicator method' (the 'unknown' category) in subsequent chapters of this thesis to ensure a complete dataset for all variables. Furthermore, the analysis in chapter 4 was restricted to participants enrolled after the year 2002 due to large proportion of missing data for alcohol consumption in people enrolled in earlier years. Because of the significant proportion of missing values for alcohol, I also used multiple imputation assuming that the data were missing at random (MAR).

Additionally, potential confounders have also been identified in relation to the association between factors which were the exposures of interest in subsequent chapters and clinical outcomes. For example, alcohol consumption was identified as an important confounder and potential effect measure modifier for the association between HCV status and liver disease.

As PLWH are living longer, several issues related to the management, treatment and prognosis of these individuals remains to be further addressed. Icona and Hepaicona are rich data sets with long-term follow-up in a setting of historically high prevalence of HIV/HCV coinfection. These clinical cohort studies therefore represent an ideal setting in which to investigate my thesis objectives.

Particular strengths of the cohorts include the large sample sizes and, in theory the routine collection of socio-economic factors and lifestyle factors including alcohol. Such measures are potentially key factors in questions related to HIV/HCV diagnosis and prognosis, but they are rarely routinely available in HIV clinic databases. However, the large number of missing values for these factors suggests that the systems for collection of such information in the studies have not been entirely successful, particularly in the early years of Icona. Some of my work

was pivotal to set the pathway to data cleaning and improve the future collection of some of these factors.

The Icona dataset allows comparison of HCV positive and HCV negative individuals which forms a key part of analyses in chapters 4 to 6. The addition of Hepaicona data is particularly important, as by definition it includes HIV/HCV coinfected individuals with previous history of cART treatment and provides more accurate data for HCV-RNA testing, especially around the date of HCV treatment initiation as measuring HCV-RNA is a requirement for study entry.

CHAPTER 4

4 WHAT IS THE ROLE OF HCV COINFECTION IN THE ASSOCIATION BETWEEN ALCOHOL AND LIVER DISEASE IN PLWH?

4.1 Aim and objectives

The aim of this chapter is to investigate whether HCV was an effect measure modifier for the relationship between alcohol consumption and risk of severe liver disease (SLD). This was firstly done by assessing the value of alcohol consumption data collected in the Icona and Hepaicona cohorts by physician assessment in predicting the risk of SLD in PLWH with/without HCV.

The specific objectives are:

1. To classify participants drinking behaviour from the information collected by physician assessment at enrolment to derive an alcohol consumption variable in line with the Italian National Institute for Food and Nutrition (NIFN) national guidelines
2. To assess the association between the alcohol consumption variable and risk of SLD in PLWH
3. To formally evaluate whether HCV infection is a potential effect measure modifier for the association between alcohol consumption and risk of SLD in PLWH (in the Icona data only)
4. To explore the extent of under reporting of alcohol consumption and repeat the analysis (objectives 2 and 3) using statistical methods to handle missing data

4.1 Introduction

In the era of effective cART, there has been a significant decrease in AIDS-related mortality [246]. As PLWH are now living longer, liver disease is emerging as the major cause of morbidity and mortality in HIV-positive individuals with death rates

ranging from (13 - 18%) [247, 248]. Severe liver disease can include a spectrum of concomitant infections and conditions such as; chronic hepatitis C, chronic hepatitis B, abnormal liver function tests, liver decompensation, clinical diagnosis of liver cancer such as hepatocellular carcinoma or even liver disease related to alcohol abuse [246, 249]. Globally, alcohol is one of the top three priority public health areas of WHO and is the leading cause of ill health and premature death [250]. The temporal trend in Italy among the general population indicates a decrease in prevalence of alcohol use [251]. However, in Italy 4% of deaths in the general population are currently attributable to alcohol consumption [252].

Alcohol consumption is a risk factor for liver disease. A systematic review carried out in 2019, looking at the relationship between alcohol consumption and risk of liver cirrhosis compared alcohol drinkers to abstainers in the general population and involved almost 3 million participants with 5,505 having liver cirrhosis. The systematic review included seven cohorts and two case-control studies from United States, Italy, China and the United Kingdom. In comparison to long term abstainers, the authors reported that drinking >5 drinks per days was associated with a higher risk of liver cirrhosis in both women and men (RR = 12.4 (95% CI: 6.7 - 23.3) and RR = 3.8 (95% CI: 0.85 - 17.0)) respectively [253]. Interestingly the two case-control studies included in the systematic review were conducted in Italy. In one study, drinking >5 drinks per day showed a higher risk of liver cirrhosis in both women RR = 7.5 (95% CI: 3.5 - 16.3) and men RR = 9.10 (95% CI: 2.9 - 28.1) respectively in comparison to life time abstainers [253]. The wide confidence intervals in these studies is a consequence of the small sample size, possibly recall and confounding bias which cannot be ruled out [253].

In the context of liver disease, alcohol and HCV are both risk factors for liver disease [254]. Therefore, investigating whether the effect of alcohol on risk of SLD varies by HCV status is an important question. Evidence for an interaction between excessive alcohol consumption and hepatitis C has been previously reported [255-258] [259]. However, debate continues in the literature about the presence of interaction between alcohol and hepatitis [254, 260, 261]. For example, some studies

have demonstrated an additive effect of alcohol and HCV in relation to risk of liver disease. Other studies have demonstrated a synergistic interaction. In other words the combined effect of alcohol consumption and HCV on risk of liver disease is greater than the sum of the effects from the two individual factors [254, 262]. *Ashwani et al* suggests that the different mechanism of HCV and alcohol consumption both leading to liver damage are different [262]. For example, *Ashwani et al* further explains that alcohol inhibits the adaptive immune response responsible for HCV clearance. Thus contributing to increased prevalence of HCV[262]. Additionally PWID are known to engage in hazardous drinking, and thus likely increasing risk of HCV infection through unsafe needle sharing practices[262].

Assessment of alcohol intake in PLWH and understanding the relationship with SLD is a necessity for targeting diagnosis and optimal clinical management of HIV-positive individuals with/without HCV infection. At the time in which this analysis was conducted this was an important question as, while it was not possible to eradicate HCV from an individual, alcohol consumption is a modifiable risk factor.

The Icona and Hepaicona cohorts collects data on alcohol consumption via patients' interview conducted by the treating physician and for the first time in both cohorts, the value of collecting these data is evaluated in this chapter by means of testing its correlation with the risk of long-term clinical progression. Additionally, the Icona cohort provides data to enable the assessment of whether an interaction between HCV and alcohol consumption exists in prediction of SLD.

4.2 Literature review

The literature review in this section presents evidence from studies that estimated the prevalence of alcohol consumption in HIV-positive individuals with/without HCV infection. I briefly mention some common tools of assessment of alcohol consumption in HIV cohorts. The literature on the role of alcohol consumption for predicting risk of liver disease is reviewed with special focus on papers that evaluated the interaction between alcohol and HCV in HIV-positive cohorts. This is

followed by further discussion of accuracy of measurement of alcohol consumption in HIV cohort studies. The literature search was first done up to May 2016 and subsequently updated up to January 2021 (see Table 2.1 in chapter 2 for details for search terms).

4.2.1 Alcohol use in HIV cohorts with/without HCV infection

Studies dating back to the mid-1990's, show that, as in the general population, alcohol consumption was prevalent in PLWH with estimates around 40% [263-265]. The literature suggests that alcohol use remains common in HIV cohorts also in more recent years, [266-269] [270] with an important minority fulfilling criteria for harmful or hazardous alcohol consumption. *Galvan et al* used brief questionnaires based on frequency and quantity of alcohol consumed over a period of 30 days adopting the definition of hazardous drinking which is described in the USA national drinking guidelines. The study (HIV Cost and Services Utilization Study) enrolled 2,864 individuals of whom 53% reported any alcohol consumption in the preceding month [269]. In this analysis, 8% of PLWH were classified as hazardous drinkers defined as consuming ≥5 alcoholic drinks or drinking for ≥4 days during the previous 4 weeks [269].

In the SWISS HIV cohort study in Switzerland, *Conen et al* found that approximately half of the participants in the cohort declared consuming alcohol [267]. The study asked two questions, the first on drinking status (yes/no) and the second on average daily consumption [267]. The study enrolled 6,323 HIV-positive individuals of whom 52% reported consuming alcohol less than once a week, 40% were classified as light drinkers (daily drinking: women <20g and men<40g) and 8% were classified as moderate/severe drinkers (daily drinking: women >40g and men >60g) [267]. *Chander et al* in 2006 assessed alcohol consumption based on drinks consumed per day or per week and using cut-offs defined by the USA drinking guidelines [271]. Specifically, hazardous drinking was defined as >7 drinks/week or >3 drinks/occasion for women and >14 drinks/week or >14 drinks/occasion for men [271]. The study involved 1,957 HIV-positive individuals of

whom 46% reported any alcohol use and 11% reported hazardous rates of drinking [271]. This high rate of hazardous drinking could possibly be explained by the fact that half of the sample were PWIDs who may have different alcohol consumption patterns to other groups of PLWH [271].

Other studies of HIV-positive individuals that included PWIDs show a high prevalence of alcohol use [265, 272]. *Crum et al* studied 188 HIV-positive PWIDs and found 76% reporting any alcohol use and 34% were classified as hazardous drinkers defined as consuming >21 drinks per week [265]. In another study of 220 HIV-positive individuals reporting drug use, 84% reported consumption of alcohol [272]. Using a different definition of hazardous drinking (>4 drinks/day), 63% of the individuals could be classified as heavy drinkers [272]. In addition, even higher estimates of heavy drinking were found in HIV-positive individuals reporting previous alcohol problems [255]. In the HIV Alcohol Longitudinal Cohort study of HIV-positive individuals with current or past problems (*Samet J H et al),* 30% reporting heavy drinking [273].

Focusing on HIV/HCV coinfected individuals, prevalence of alcohol consumption can also be high [255] [257]. *Cheng et al* assessed HIV disease progression in viremic individuals and found that of the 396 HIV-positive individuals in their study, 50% were HCV-RNA positive, of whom 29% reported heavy alcohol consumption [255]. Interestingly, in a study looking at the impact of being informed of the HCV infection status, awareness of HCV diagnosis was associated with participants being less likely to consume alcohol [274]. In another study, *Chaudhry et al*, assessed a cohort of 1,358 individuals of whom 49% were HIV/HCV coinfected. Of these, 70% reported no alcohol consumption, supporting the idea that consumption might be reduced in the HIV/HCV coinfected population [275]. However, there are still a significant proportion of HIV/HCV coinfected individuals who continue to report alcohol consumption including, hazardous drinking [257, 267, 275-277]. In the same study, 11% of the HIV/HCV coinfected individuals reported hazardous drinking [275]. A lower prevalence of alcohol consumption among HIV/HCV coinfected compared to HIV mono-infected was found in the SWISS HIV cohort

study in which 7% (442/6323) of participants were coinfected of whom 5% reported heavy drinking [267]. In the New Orleans Alcohol Use in HIV-positive individuals (NOAH) study, out of a total of 353 participants, 16% (n=53) were HIV/HCV coinfected individuals. Lifetime alcohol use was defined as consuming >600kg of alcohol in the individual's lifetime [270]. In terms of differences in prevalence of alcohol use, they found 27% vs. 14% of lifetime alcohol use in HIV/HCV coinfected compared to HIV mono-infected respectively (p=0.019) [270]. Similar estimates have also been reported by cohorts of HIV/HCV coinfected individuals in France [277, 278].

4.2.2 Assessment of alcohol consumption

Studies assessing alcohol consumption in HIV-positive individuals with/without HCV have used different ways of collecting information. An exploratory exercise looking at how alcohol consumption was evaluated in a number of selected European HIV cohorts is shown in Table 4.1. This demonstrates the varied approaches to collection of information on alcohol, and the fact that such information is collected in only a limited number of HIV cohort studies.

In Icona and Hepaicona, alcohol assessment is based on patients' interview by the treating physician who asks three questions about the frequency and quantity of alcohol consumed on a daily basis. Physician or trained medical professional assessment of alcohol use in HIV cohorts is not uncommon [257, 276, 277, 279]. For an example, *Fuster et al*, assessed lifetime drinking history in a process where participants underwent a structured interview to recall patterns of alcohol consumption over time [257].

Roux et al assessed agreement of reported alcohol consumption between self-administered questionnaires and face to face medical interviews. They found that of the 544 HIV/HCV coinfected individuals 14% (n=76) under-reported their level of alcohol consumption during the face to face medical interviews. This suggests the possibility of under reporting alcohol use during face to face interviews [277].

Besides structured or unstructured patient-interviews, there are other ways of assessing alcohol use. The literature has highlighted that the most common approach involves the use of standardized questionnaires such as the Alcohol Use Disorder Identification Test (AUDIT) or the Cut-down, Guilty, Annoyed and Eye-opener (CAGE) [280-282]. These may be self-administered, are typically used in for screening and may be targeted at populations who are already suspected to have problems with alcohol. The AUDIT questionnaire was developed by WHO and is used for screening unhealthy alcohol consumers or people at risk of heavy alcohol use in the general population [283]. The questionnaire has 10 Likert scale type questions and responses to each of the questions are given a score in the range 0-4 [283]. The first three questions relate to hazardous alcohol use captured from questions on frequency of use/quantity consumed while the remaining seven questions relate to dependence and harmful alcohol use[283]. A total score resulting from the sum of all the responses is calculated with a possible range 0-40, with high scores indicating unhealthy alcohol use [283]. A shortened and identified as (AUDIT-C) uses only the first three questions on frequency and quantity of alcohol and has a possible range of 0-12 [283].

The CAGE questionnaire was also designed as a screening tool for use in people with suspected alcohol problems, but the questions differ from the frequency or quantity approach and relate to indicators of alcohol abuse [284]. For each positive response, a score of one is assigned; a total score of two or more positive indicates possible alcohol dependency [285]. The CAGE questionnaire has been assessed both in the general population and in HIV-positive individuals [266]

Other means of assessing alcohol consumption involve the use of biomarkers [286, 287]. However, one of the limitations of the use of biomarkers to assess alcohol consumption is the limited duration of alcohol in the blood (typically lasts up to 12 hours) [288].

There is no consensus on what is considered the gold standard for alcohol assessment [289]. In a review of alcohol measures in Europe, the authors

recommendations for researchers assessing alcohol use are to employ a tool which includes core items such as: alcohol drinking status, average quantity of alcohol consumption, frequency and amount of heavy episodic drinking [280, 281].

Table 4.1 Selected cohort studies in some European countries collecting data on alcohol consumption [List is not exhaustive]

Country Standard drink (Units in g)	Cohort	Questions on alcohol consumption
Denmark 12g	DANISH HIV[290]	1) How much do you drink a week/day
Europe 12g	EuroSIDA[291]	1)Current alcohol abuse (Yes no unknown) 2)Past alcohol abuse (Yes no unknown)
French 10g	ANRS CO4 French Hospital Database for HIV (FHDH)[292]	1) Consumption (Yes, no, unknown) 2) Consumption of glasses a day(<4, 4-8, >8, Unknown)
	ANRS COPANQ[292]	1)alcohol Yes/no; if yes: 2)number of glasses per day/week or /month
	ANRS CO6 PRIMO[293]	1)alcohol Yes/no; if yes: 2)number of glasses per day/week or /month
	ANRS CO8 COPILOTE[294]	1)Frequency of consumption 2)quantity consumed daily"
	ANRS CO13 HEPAVIH[295]	1)Alcohol consumption (g/day)
Germany 10g	German Competence Network for HIV/AIDS (KompNET)[296]	1)Consumption of alcohol, amount and frequency (drinks/day)
Spain 10g	Athens Multicentre AIDS Cohort Study AMACS) [297]	If patient is abusing alcohol without specifying thresholds
Switzerland 10g	Swiss HIV Cohort Study [298]	The questionnaire captures information on the 1)Frequency 2) quantity, (grams) 3) Pattern of alcohol consumption. For this analysis, self-reported alcohol consumption was categorized into; abstention or very low (1 g/d), low (1–9

Country Standard drink (Units in g)	Cohort	Questions on alcohol consumption
		g/d), moderate (10–29 g/d in women and 10–39 g/d in men), and high alcohol intake (>39 g/d).
United Kingdom 8g	Royal Free Cohort[299]	a. Has the patient ever been a regular heavy drinker (on average > 6 units per day) over a period of years? b. If Yes: **Number of years:** ..... c. If Yes, is patient currently a regular heavy drinker? d. Has patient ever received treatment for an alcohol problem from a physician or treatment programme?
United Kingdom 8g	European Collaborative Study (ECS) [300]	1)Current alcohol use (Yes/No) 2)Number of units per week

4.2.3 The role of alcohol consumption on risk of severe liver disease in HIV-positive individuals with/without HCV infection

There are conflicting results regarding the association between alcohol consumption and risk of liver disease in the literature. Some studies have found an association between level of alcohol consumption and liver disease both in HIV mono-infected and HIV/HCV coinfected populations but the majority appeared to have found no association. For instance, *Muga et al* assessed the role of alcohol consumption in the progression of liver disease in HIV/HCV coinfected drug users (N=244) and found that alcohol consumption was not associated with higher FIB-4 (p=0.695) compared to HCV mono-infected [(301)].

Some studies suggest that there could be a highly increased risk of liver disease in HIV/HCV coinfected individuals who also consume unhealthy levels of alcohol compared to HIV mono-infected, reporting a significant interaction between HCV infection and alcohol consumption. For example, in the Veterans Aging Cohort Study (VACS) in the USA, which enrols HIV-positive individuals, the researchers used the standardized AUDIT-C questionnaire to investigate the association between alcohol consumption and risk of liver disease [(302)]. Interestingly, hazardous drinking was found not to be associated with advanced fibrosis (FIB-4 >3.25) aOR=1.26, (95% CI: 0.87 - 1.82), adjusted for sex, ethnicity, diabetes mellitus, BMI, HBV status, HCV status, CD4 and HIV-RNA [(302)]. However, in the same multivariable logistic model, chronic HCV infection (defined by being HCV-RNA positive) was found to be strongly associated with the risk of advanced liver disease aOR=5.03 (95% CI: 3.62 - 6.97) [(302)]. When the interaction between the HCV infection and alcohol use was investigated, the study found that in HIV/HCV coinfected individuals who were also classified as hazardous drinkers, the estimated increase in risk of liver disease was extremely high: aOR =25.2 (95% CI: 10.6 - 59.7) compared to HCV uninfected [(302)]. This suggests that HCV may have synergistic effect with alcohol on the risk of liver disease. However, another study by *Bilal et al* that assessed the interaction between HCV and hazardous alcohol consumption, found higher levels of FIB-4 in HIV/HCV coinfected regardless of

alcohol consumption compared to individuals not infected with HCV. Alcohol consumption for individuals was classified as hazardous drinking when >7/14 drinks per week for women and men were reported, respectively. Therefore authors suggested that the association of HCV with SLD was more likely be explained by the effect of HCV-viremia instead of the level of alcohol consumption on the risk of liver fibrosis [303]. Another possible explanation was the fact that alcohol consumption in HIV/HCV coinfected individuals had been under-reported [303].

In contrast, *Ferguson et al* in a cross-section study evaluated the interaction between HCV and lifetime alcohol use in predicting liver disease in HIV/HCV coinfected individuals and found no significant interaction. They did however observe that HIV/HCV coinfected individuals who reported drinking <4 drinks/day over 10 years showed an increased risk of liver disease compared to HIV mono-infected people who drank this amount but the result did not reach statistical significance, after adjusting for age, sex, HBV, smoking status, HIV-RNA and current drinking status [aOR =2.7 (95% CI: 0.7 - 10.5)] [270]. A possible explanation for this finding is reverse causality if HIV/HCV coinfected participants had to stop current alcohol use due to health concerns [270].

A possible explanation of conflicting findings summarising the association between alcohol consumption and risk of liver disease could be due to how alcohol consumption was measured in the various studies. *Fuster D et al* assessed the association between alcohol use and absence of liver fibrosis in HIV-positive individuals with/without HCV. In this analysis, the exposure alcohol consumption was assessed in three different ways [257]. The first was lifetime alcohol use, measured as <2 standard drinks/day (28g/d) for 14 years or >4 standard drinks/day for 28 years. The other measures were based on number of years of heavy drinking (e.g. ≥5 drinks on one occasion and current heavy use in the previous month). Also, the definitions varied according to age and gender: >14 drinks/ week or ≥5 drinks on 1 occasion for men ≤65 years of age, and >7 drinks/week or ≥4 drinks on 1 occasion for all women and for men >65 years of age) [257]. Almost half

of the study participants reported lifetime alcohol consumption of >4 drinks/day for 28 years, 69% reported >9 years of heavy drinking, and 33% reported current heavy use [257]. Regardless of which measure was used, the authors did not find an association between alcohol use and absence of liver fibrosis [257]. The authors noted that lifetime alcohol exposures would have been a more appropriate measure, owing to the rigorous methodological approach, and they were surprised not to find an association [257]. A possible explanation for the lack of an association could be attributable to HCV infection acting as a competing risk in this setting, thus placing less importance on the impact of alcohol use and liver damage [257].

Tsui et al, investigated the association between risky drinking and levels of serum aminotransferase (AST and ALT, markers for liver disease). The cohort study included HIV-positive individuals with current or past alcohol problems with/without HCV infection. The study found an association of risky drinking with increased AST and ALT values in coinfected individuals but not among HIV mono-infected [304].

In contrast, *Chaudhry et al* assessed 1,358 HIV-positive individuals and categorized hazardous consumption for men reporting >14 drinks/week or >4 drinks per occasion and for women reporting >7 drinks/week and >3 drinks per occasion [275]. Using this definition, 10% of the study population were categorized as hazardous drinkers. In a subgroup analysis of 662 HIV/HCV coinfected individuals, 11% reported hazardous drinking and no association was found between alcohol consumption and the risk of liver fibrosis [275]. However, due to the small sample size of HIV/HCV coinfected individuals, it is possible that the analysis was underpowered [275]. Of note, when restricting the analysis to HIV mono-infected individuals, hazardous drinking was associated with an increased risk of liver fibrosis [275].

Interestingly, in a more recent study of the Women's Interagency Study (WIHS), moderate drinking was found not to be associated with fibrosis progression (defined as change in FIB-4 units per year) among HIV/HCV coinfected women (n=686) but heavy drinking was associated. When compared to abstainers,

moderating drinking (defined as <14 drinks/week) showed no association with FIB-4 unit change, mean/year= 0.006 (95% CI: - 0.18 - 0.19), however heavy use (defined as >14 drinks/week) was associated with a FIB-4 acceleration mean/year= 0.25 (95% CI: 0.01 - 0.49) [305].

In summary, the role of alcohol consumption in PLWH with/without HCV in the risk of SLD is conflicting in the literature. A major reason for this may relate to inaccuracy of measurement and lack of validity of measurement tools to capture actual alcohol consumption (see section 4.2.4 below).

Some other possible reasons to consider as mentioned by *Rehm et al* relate to; (i) the measure of alcohol consumption (dose-response or categorical), (ii) type of outcome used (surrogate markers, morbidity or mortality or both) and possibly (iii) gender [259]. Confounding bias is also an issue, although most of the literature reviewed have adjusted for possible confounding variables, but with variation in the variables adjusted for which may impact considerably on results. However, it is evident from the literature reviewed that the sample sizes of HIV mono-infected and HIV/HCV coinfected included in the various analyses are not large and analyses have been performed in specific subsets, further limiting the power of the analyses.

4.2.4 Under-reporting of alcohol consumption in HIV cohort studies

In general, non-response or under reporting of alcohol consumption is likely to be common [306]. The method used to assess alcohol consumption is likely to influence response. In the case of HIV-positive individuals, under-reporting of alcohol consumption may occur for reasons such as social desirability or fear that it may affect their access to cART, or to other treatments [277, 285-287]. Few studies have assessed the extent of under-reporting by comparing self-reported alcohol use or interviews carried out by medical professionals with the more objective results of blood tests [277, 286, 287]. Furthermore, there may be important differences between interview-based assessments of alcohol use and self-report. As mentioned in

section 4.2.2, *Roux et al* assessed the extent of under-reporting of alcohol consumption by comparing self-reported alcohol with face to face interviews in HIV/HCV coinfected individuals [277]. Of the 544 HIV/HCV coinfected individuals enrolled, 34% were identified as alcohol abusers through self-reports, however 14% under-reported alcohol consumption in face to face interviews [277]. The researchers noted that self-reporting of alcohol use in HIV/HCV coinfected individuals was considered more reliable and a better estimate of the true alcohol consumption [277]. One of the possible reasons is that individuals may experience stigmatization or possible discrimination if reporting alcohol use face to face, were as in self-report they may not feel the need to restrict disclosure of alcohol use. As mentioned previously, another reason that may be relevant is the fear that admitting to high alcohol consumption may compromise treatment and care. The researchers in the above study noted that individuals not yet receiving treatment for HCV were at higher risk of under-reporting during the face to face interviews [277].

Other studies compared alcohol levels from blood tests with self-reported alcohol consumption [286, 287]. *Asiimwe et al* compared self-reported alcohol use with levels of alcohol in the blood using a biomarker called Phosphatidylethanol (PEth) [286]. In a study of 209 HIV-positive individuals with a history of risk drinking behaviour over the past 12 months, using quantity-frequency measures of self-reported alcohol consumption, 19% reported abstaining from alcohol in the previous 3 months. However when using PEth, 25% of these were found to be positive for PEth, revealing alcohol use in the previous three months [286]. On the other hand, using another a biomarker called carbohydrate deficient transferim (CDT), in a study of 163 HIV-positive individuals initiating cART, 74% (120/163) consuming alcohol more than 30 days prior to cART initiation [287]. However, only 8/120 (6.7% (95%CI: 2.9 - 12.7)) were tested positive with CDT i.e. they had alcohol in the plasma [287]. This substantial difference in estimate may relate to the sensitivity of this biomarker test. The authors noted that false negatives were possible as the test was only able to detect alcohol consumed up to one month prior testing. Further, in HCV-positive individuals, it is possible to have elevated levels of CDT which may not necessarily

be due to alcohol consumption. These results highlight the difficulty of assessment of alcohol; that observable and measurable data for alcohol consumption may have serious limitations [307]. Overall, although both studies promote the benefits of using objective methods to measure alcohol consumption. These biomarkers have important limitations as discussed as well as being and not easily accessible in terms of cost and technology. Therefore self-completed measures remain a key tool and efforts should focus on trying to improve the accuracy of these self-reported alcohol consumption tools [286].

Another issue that may be particularly relevant to routine collection of alcohol in the clinical setting is missing data, which may be due to the information not being regarded as a clinical priority in a time-constrained setting, or perceived sensitivities in asking questions on alcohol. As described in chapter 3, there were large amounts of missing data for socio-economic and lifestyle factors including alcohol consumption in both Icona and Hepaicona. The analysis in this chapter considers different methods for handlings the issue of missing data for alcohol consumption.

4.2.5 Summary

The above review of the literature showed that alcohol use is common amongst HIV-positive individuals regardless of the mode of alcohol assessment. Prevalence estimates of alcohol use varied depending on the exact population included in the analyses, definition of standard drinks in different countries and cut-offs used to define moderate or hazardous drinking. The review demonstrated that variations in the prevalence of hazardous use were to some extent dependent on which risk groups were included in the studies with a with a high prevalence typically reported in PWID.

There seemed to be some evidence of synergistic interaction between alcohol consumption and HCV. On the other hand, some studies observed no interaction. Again, particularly in a formal test of interaction, it is unclear whether negative

results were genuine findings or they might be explained by small sample size and lack of power as well as other factors such as mode of alcohol assessment or other reasons.

A major difficulty with assessing the validity of alcohol measures is the lack of gold standard. Those assessing levels of alcohol use from biomarkers and comparing these with the prevalence of self-reported alcohol use, found difficulties in interpretation of disagreement between the methods, and some concluded self-report may be preferable. Even with self-reported assessment, under-reporting of alcohol consumption may be common. Authors have suggested this could be due to fear of individuals' hindrance to HIV/HCV therapy or social desirability. Investigation of the usefulness of alcohol data in predicting serious outcomes gives important evidence of the validity of the measures. There was conflicting evidence on the possible impact of alcohol use on the risk of liver disease with some studies reporting an association and others not.

In the two cohorts of HIV-positive individuals analysed in this thesis, data on alcohol use has been collected from the outset and with increased accuracy in Icona starting from 2002. Answers to the three questions in the Icona Network eCRFs was used to classify participants' alcohol consumption following the standard drinking guidelines specific to Italy [308]. This enabled me to carry out analyses to estimate the prevalence of hazardous and other alcohol consumption at study entry in both HIV-positive and HIV/HCV coinfected individuals, to investigate it's relationship with SLD and assess whether there is an interaction between HCV status and alcohol consumption on the risk of clinical outcome.

In addition, alcohol consumption is a potential cause of other exposure variables such as mode of HIV transmission (sexual contacts vs. PWID) and treatment initiation (heavy drinkers are potentially less likely to access care/treatment) as well as of liver-related outcomes and therefore likely to be an important confounder in most of the analyses in this thesis. It is therefore important that alcohol use is

measured in all participants and in the most accurate way to reduce the risk of having residual confounding in the analyses.

4.3 Methods

4.3.1 Inclusion criteria and missing data patterns

This analysis included all HIV-positive individuals with/without HCV enrolled in Icona and the Hepaicona cohorts up to 30th June 2016 who were free from SLD (see section 4.3.2) at enrolment. Individuals enrolled prior to 1st January 2002 were excluded from this analysis as more than 70% of individuals did not have sufficient data on alcohol use (Table 4.2). After 1st January 2002, the percentage with missing information in Icona dropped to a more acceptable level of approximately 30% (Table 4.2).

Table 4.2 Distribution of participants enrolled and frequencies of missing data on alcohol use

Year enrolled	All	Number not missing	Number Missing	Percentage Missing	Percentage not Missing
	13174	**6762**	**6412**		
1997*	1873	187	1686	90.0	10.0
1998*	864	83	781	90.4	9.6
1999*	104	3	101	97.1	2.9
2000*	630	106	524	83.2	16.8
2001*	161	45	116	72.0	28.0
2002	406	297	109	26.8	73.2
2003	238	160	78	32.8	67.2
2004	197	155	42	21.3	78.7
2005	151	121	30	19.9	80.1
2006	162	122	40	24.7	75.3
2007	208	173	35	16.8	83.2
2008	427	243	184	43.1	56.9
2009	600	401	199	33.2	66.8
2010	594	405	189	31.8	68.2
2011	1102	780	322	29.2	70.8
2012	1031	665	366	35.5	64.5

Year enrolled	All	Number not missing	Number Missing	Percentage Missing	Percentage not Missing
	13174	**6762**	**6412**		
2013	1031	637	394	38.2	61.8
2014	1304	843	461	35.4	64.6
2015	1471	956	515	35.0	65.0
2016	620	380	240	38.7	61.3

*Excluded from the main analysis

4.3.2 Data

Alcohol consumption

Information relating to alcohol consumption is collected in Icona by participant' interview conducted by the treating physician at study enrolment and at subsequent clinical visits (at least every 6 months) during follow-up. This analysis only included assessments carried out at baseline (enrolment) prior to cART initiation. Exact questions in the participants' interview (with possible responses) were as follows;

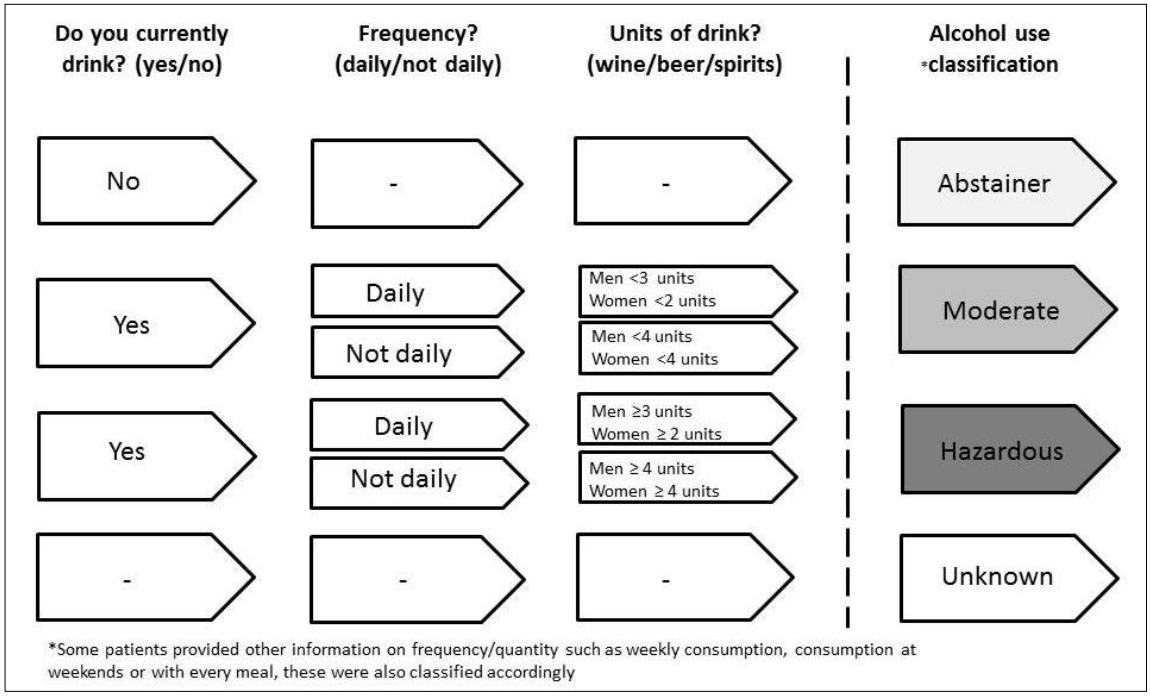
1) Do you currently drink alcohol? (Yes/No/Do not know);
2) How frequently do you drink alcohol? (Daily/ Less than daily/Do not know);
3) How many units of (Wine/Beer/Spirits) do you consume per day?

Frequency and quantity consumed was translated into drinking categories by mapping the data to the definitions described in the NIFN guidelines. At the time of this analysis, a unit of alcohol in Italy was defined as containing 12g of pure alcohol which corresponds to 125ml of wine, 330ml of a can of beer and 40ml of liquor [308].

Alcohol consumption at baseline was categorised into four groups (abstainer, moderate drinking, hazardous drinking and unknown) as shown in Figure 4.1 Hazardous drinking was defined as ≥3 units/day for men and for women ≥2 units/day. In cases were drinking was reported as ' less frequently than daily' WHO guidelines were used which state that more than 4 drinks per occasion is considered hazardous drinking [309]. People were classified as moderate drinkers if they consumed a non-zero amount below the hazardous drinking thresholds.

Abstainers were people who reported not drinking alcohol at all. In some individuals who reported more than one type of drink, the drink with the highest quantity of alcohol was used in the classification process. In instances were information of alcohol was given in other metrics e.g. ml, this was converted to units/day (e.g. 500ml of wine a day equated to 500ml/125ml = 4 units/day). In a few instances information regarding alcohol consumption was constructed from free text. For example a participant reporting 'having had a drink with a meal', this was assumed to be 2 units/day to take into account 2 meals a day would be classified as a moderate drinker.

Figure 4.1 Classification of alcohol consumption



Other definitions (HIV/HCV coinfection and severe liver disease event)

HCV-positive infection status was based on HCV antibody (HCVAb) test results or HCV-RNA>615 IU/mL or a positive HCV-RNA qualitative test or a genotype test result being reported (in patients without HCVAb test).

Time from enrolment to development of SLD in follow-up was the primary endpoint. This was a composite endpoint defined at the time of first experiencing one of the

following events (first of these occurring):

(i) A FIB-4>3.25 (where FIB-4 was calculated using the formula in and assessed at each clinical visit [310]

(ii) A clinical diagnosis of liver disease from medical records (ascites, decompensated cirrhosis, hepatocellular carcinoma, hepatic encephalopathy, oesophageal varices)

(iii) Liver-related death. Cause of death was classified using CoDE methodology.

4.3.3 Statistical analyses

Baseline was defined as the date of enrolment which ranged between 1st January 2002 and 30th June 2016 in Icona and between 1st October 2013 and 30th June 2016 for people in Hepaicona. Individuals were followed up until the date of experiencing the composite endpoint or their follow-up time was censored at the date of their clinical visit at which they were last seen free from SLD or last date of data-lock, 30th June 2016. Summary statistics were used to describe the study participants overall and after stratification by alcohol consumption category at baseline. Formal comparisons between alcohol consumption categories were performed using chi-squared tests.

Methods for assessing the association of alcohol consumption with risk of severe liver disease

Time to SLD was summarised using the standard KM method overall and after stratifying by baseline alcohol consumption category. Univariable and multivariable Cox regression models were fitted to estimate hazard ratios of the risk of SLD associated with levels of alcohol consumption. In the Cox regression model, only time-fixed covariates (confounders) measured at baseline were included. Potential confounders measured at baseline were considered in separate multivariable models and fitted sequentially as follows:

- Model #1 controlling for demographic factors (gender, age, nationality, geographical region, calendar year of enrolment)

- Model #2 (model #1 plus previous AIDS diagnosis, CD4, HIV-RNA and HBV infection status)
- Model #3 (model #2 plus mode of HIV transmission and HCV infection status)
- Model #4 (model #3 plus smoking status).

Results from the multivariable models were presented as adjusted hazard ratios (aHRs) with 95%CIs. The 'moderate drinking' category was used as the reference category, because previous literature suggested this may be preferable to using the 'abstainers' category [311]. It is possible that a proportion of abstainers may be individuals who have given up alcohol due to health problems or severe alcohol addiction in the past.

Methods to investigate the interaction between alcohol consumption and HCV on risk of severe liver disease

Only the participants of the Icona cohort were used for this part of the analysis as Hepaicona includes only HIV/HCV coinfected individuals so no HIV-mono infected group was available for comparison. Multivariable Cox regression models were fitted to estimate crude hazard ratios of the risk of SLD associated with HCV ignoring alcohol consumption. Then a model including both alcohol and HCV and an interaction term between HCV and alcohol consumption was fitted thereby formally testing for an interaction on a multiplicative. My a priori hypothesis was that the impact of HCV on risk of SLD might vary by level of alcohol consumption was fitted thereby. This hypothesis was formally tested by adding an interaction term to the Cox regression Model #4.

The estimates of unadjusted and adjusted HRs (95% CIs) of the risk of SLD associated with alcohol consumption were shown by means of both forest plots after stratification by HCV infection status and stratification by alcohol status together with a p-value for the interaction test. This was repeated in the multiple imputation (MI) analysis (more details regarding MI below).

Methods for handling missing data for alcohol consumption

Missing data, if not taken into account may introduce bias. Generally, it is important to understand why data are missing as the risk of bias depends on reasons as to why the information is missing. In the dataset used in this analysis chapter, even after restricting to people enrolled after 2002, approximately 30% of participants included had missing data for alcohol consumption which was considered acceptable to continue the analysis although still too high to ignore.

A first method, used in this analysis to handle missing data is called the 'missing indicator method' (see also chapter 2 section 2.8) which consists in using a categorical variable for alcohol consumption, one of the categories being the 'missing value'. This method has the advantage that none of participants are excluded but it is also prone to bias [312].

Other methods involve replacing missing values with values imputed from the observed data. These methods can still lead to bias or to lack of precision in the final estimates but are generally considered to be superior to the missing indicator method. Specifically (as mentioned in chapter 2 section 2.10) in this chapter analysis I have also handled missing data for alcohol consumption using MI. MI was carried out to re-classify people with missing data on alcohol consumption into either; 'Abstainer', 'Moderate' or 'Hazardous' drinker.

One of the main untestable assumptions underlying this approach is that data is deemed to be missing at random (MAR) i.e. differences between missing values and observed values can be explained by the differences in the observed data [240]. MI is a method that creates several imputed data sets which are then combined to obtain results from each of those datasets. This technique can be summarized in three steps; imputation, analysis and pooling of the analyses from the imputed datasets. Thus, imputation involves filling in missing data points as many times as is required or specified and results in the number of datasets that was required or specified. The next step involves the analysis, i.e. performing separate analysis for

each of the imputed data sets. Then the final step involves pooling the analyses together into a final result [313, 314]. It is worth noting that although MI can improve the validity of the results, the method requires modelling the distribution of alcohol consumption in terms of the observed data i.e. covariates that may be associated with missing data on alcohol consumption.

The imputation analysis then begins with identifying baseline characteristics associated with missing data on alcohol consumption. Essentially, a comparison of the characteristics of people who reported and those who did not report alcohol use is carried out, using formal statistical comparison tests such as chi-squared for proportions, non-parametric tests for non-normally distributed variables. Iterative methods for doing MI involves joint modelling which is based on the assumption that the variables being modelled all follow a normal distribution. However, in cases where this is not fulfilled, this iterative process may not be appropriate. Therefore a more flexible method used to handle different types of variables is one called the Fully Conditional Specification (FCS), which specifies the imputation model for each variable (i.e. linear regression for continuous variables and logistic regression for categorical variables) [314]. In this analysis because alcohol consumption is a categorical variable, the FCS imputation algorithm was implemented [314]. More specifically, the DISCRIM method in SAS was used to impute the missing alcohol categorical variable. The number of imputations chosen was arbitrary and it was assumed that ten imputes with 100 iterations was sufficient for the purpose of re-classification. Variables considered predictors of unreported alcohol use included in the MI model were: age, mode of HIV transmission, nationality, AIDS diagnosis, CD4, HIV-RNA, HCV, SLD and calendar year of enrolment.

The third step involved fitting separate univariable and multivariable Cox models to each of the imputed datasets and the final step pooled the results together to obtain an overall estimate of the relative hazard of SLD associated with levels of alcohol consumption using the Rubin's combination rules [315]**.** This method combines estimates from imputed datasets to estimate standard errors, confidence intervals, and p-values to produce an overall estimate of the HR for the imputed

datasets. The command PROC MIANALYZE in SAS v9.4 was used to combine results across imputed datasets. Multiple imputation diagnostics were ran which assessed the stability of the trace plots for continuous variables.

Sensitivity analysis

I also carried out a sensitivity analysis of the primary analysis (Cox regression analysis) using the data of the whole cohort and compared the results.

4.4 Results

4.4.1 Classification of alcohol use

The analysis included 9,542 HIV-positive individuals who satisfied the inclusion criteria (n=8,876 from Icona and n=666 from Hepaicona). When mapping the questions on the eCRFs to the NIFN guidelines, the distribution of participants according to baseline level of alcohol consumption was as follows: abstainers 3,422 (36%; 95% CI (35 - 37)), moderate users 2,279 (23%; 95% CI (23 - 25)), hazardous drinkers 637 (7%; 95% CI (6 - 7)), and unknown alcohol status 3,204 (34%; 95% CI (33 - 35)) Table 4.3). The same frequency distribution after restricting to participants with available data on alcohol consumption (subset of n=6,338) was the following; abstainers (54%; 95% CI (53 - 55)), moderate users (36%; 95% CI (35-37)) and hazardous drinkers (10%; 95% CI (9 - 11).

4.4.2 Baseline characteristics stratified by alcohol consumption status

Baseline characteristics of HIV-positive individuals stratified by alcohol consumption status is shown in Table 4.3. The majority of individuals were HCV negative (60%), male (78%); median age [IQR] 38 (31-47) years, acquired HIV through MSM or heterosexual contact (79%), were attending clinic in the northern region (54%), enrolled in recent years (57%), with all enrollment necessarily after 2002 as per inclusion criteria. Compared to moderate drinkers, hazardous drinkers were more likely to be HIV/HCV coinfected ($p<0.001$), male ($p<0.001$), of older age ($p<0.001$), to be PWID and not MSM acquired ($p<0.001$), attending clinic in the northern region ($p<0.001$) and to be smokers ($p<0.001$). Those with missing alcohol data were more likely to have missing data for other variables, as discussed in chapter 3.

Table 4.3 Characteristics of HIV-positive individuals stratified by alcohol consumption at enrolment

Baseline characteristics	Moderate (N=2279)	Abstainer (N=3422)	Hazardous (N=637)	Unknown (N=3204)	Total (N=9542)
HCV infection, n(%)					
Negative	1549 (68.0)	2366 (69.1)	409 (64.2)	1374 (42.9)	5698 (59.7)
Positive	250 (11.0)	410 (12.0)	119 (18.7)	439 (13.7)	1218 (12.8)
Not tested	480 (21.1)	646 (18.9)	109 (17.1)	1391 (43.4)	2626 (27.5)
Gender, n(%)					
Male	1954 (85.7)	2363 (69.1)	567 (89.0)	2584 (80.6)	7468 (78.3)
Female	325 (14.3)	1059 (30.9)	70 (11.0)	620 (19.4)	2074 (21.7)
Age, years					
Median (IQR)	37 (30, 45)	38 (31, 47)	41 (34, 49)	39 (31, 48)	38 (31, 47)
Mode of HIV Transmission, n(%)					
PWID	250 (11.0)	367 (10.7)	114 (17.9)	431 (13.5)	1162 (12.2)
MSM	1124 (49.3)	1317 (38.5)	222 (34.9)	1276 (39.8)	3939 (41.3)
Heterosexual contact	757 (33.2)	1517 (44.3)	269 (42.2)	1094 (34.1)	3637 (38.1)
Other	148 (6.5)	221 (6.5)	32 (5.0)	403 (12.6)	804 (8.4)
Nationality, n(%)					
Italian	1915 (84.0)	2572 (75.2)	516 (81.0)	2625 (81.9)	7628 (79.9)
Region, n(%)					
North	1144 (50.2)	1577 (46.1)	382 (60.0)	2078 (64.9)	5181 (54.3)
Center	866 (38.0)	1366 (39.9)	217 (34.1)	973 (30.4)	3422 (35.9)
South	269 (11.8)	479 (14.0)	38 (6.0)	153 (4.8)	939 (9.8)
AIDS diagnosis, n(%)					
Yes	156 (6.8)	336 (9.8)	43 (6.8)	266 (8.3)	801 (8.4)
CD4 cells/mm^3, n(%)					
≤300	580 (25.4)	1156 (33.8)	172 (27.0)	839 (26.2)	2747 (28.8)
301-500	593 (26.0)	810 (23.7)	166 (26.1)	697 (21.8)	2266 (23.7)
≥501	861 (37.8)	1035 (30.2)	216 (33.9)	884 (27.6)	2996 (31.4)
Unknown	245 (10.8)	421 (12.3)	83 (13.0)	784 (24.5)	1533 (16.1)
HIV-RNA, n(%)					
≤5000	389 (17.1)	605 (17.7)	114 (17.9)	633 (19.8)	1741 (18.2)
5001-10000	172 (7.5)	208 (6.1)	48 (7.5)	177 (5.5)	605 (6.3)

Baseline characteristics	Moderate (N=2279)	Abstainer (N=3422)	Hazardous (N=637)	Unknown (N=3204)	Total (N=9542)
10001-100000	922 (40.5)	1222 (35.7)	231 (36.3)	997 (31.1)	3372 (35.3)
≥100001	567 (24.9)	1025 (30.0)	179 (28.1)	744 (23.2)	2515 (26.4)
Unknown	229 (10.0)	362 (10.6)	65 (10.2)	653 (20.4)	1309 (13.7)
Smoking, n(%)					
No	924 (40.5)	2201 (64.3)	188 (29.5)	424 (13.2)	3737 (39.2)
Yes	1268 (55.6)	1092 (31.9)	413 (64.8)	480 (15.0)	3253 (34.1)
Unknown	87 (3.8)	129 (3.8)	36 (5.7)	2300 (71.8)	2552 (26.7)
Hepatitis B, n(%)					
Yes	59 (2.6)	107 (3.1)	25 (3.9)	64 (2.0)	255 (2.7)
Calendar year, n(%)					
2002-2006	313 (13.7)	473 (13.8)	69 (10.8)	299 (9.3)	1154 (12.1)
2007-2012	671 (29.4)	1113 (32.5)	218 (34.2)	929 (29.0)	2931 (30.7)
2013-2016	1295 (56.8)	1836 (53.7)	350 (54.9)	1976 (61.7)	5457 (57.2)
Follow-up (months)					
Median (IQR)	23.4 (4.8, 53.5)	26.5 (7.4, 57.1)	25.6 (5.6, 54.8)	23.9 (6.6, 51.8)	24.7 (6.3, 54.4)

4.4.3 Alcohol consumption and risk of severe liver disease

HIV-positive individuals included in this analysis were followed-up for a median [IQR] of 24.7 months [6.3 - 54.4]. A total of 7% (n=617) participants experienced the composite SLD outcome (n=506 FIB-4>3.25, n=110 clinical diagnosis of liver disease, n=1 liver-related death. Figure 4.2 shows the KM estimates of time to SLD event according to baseline alcohol consumption level for 9,542 HIV-positive individuals included in the analysis. Alcohol consumption level was associated with risk of SLD, with higher risk for hazardous drinking and for the missing alcohol category. The estimated cumulative risk of experiencing SLD by 60 months (95% CI) from baseline in abstainers, moderate, hazardous or unknown alcohol use were 8.4% (7.1 - 9.7), 7.9% (6.3 - 9.5), 10.7% (7.4 - 14.1) and 11.4% (9.9 - 12.9), respectively [log rank p<0.001].

Figure 4.2 Cumulative risk of severe liver disease stratified by alcohol use

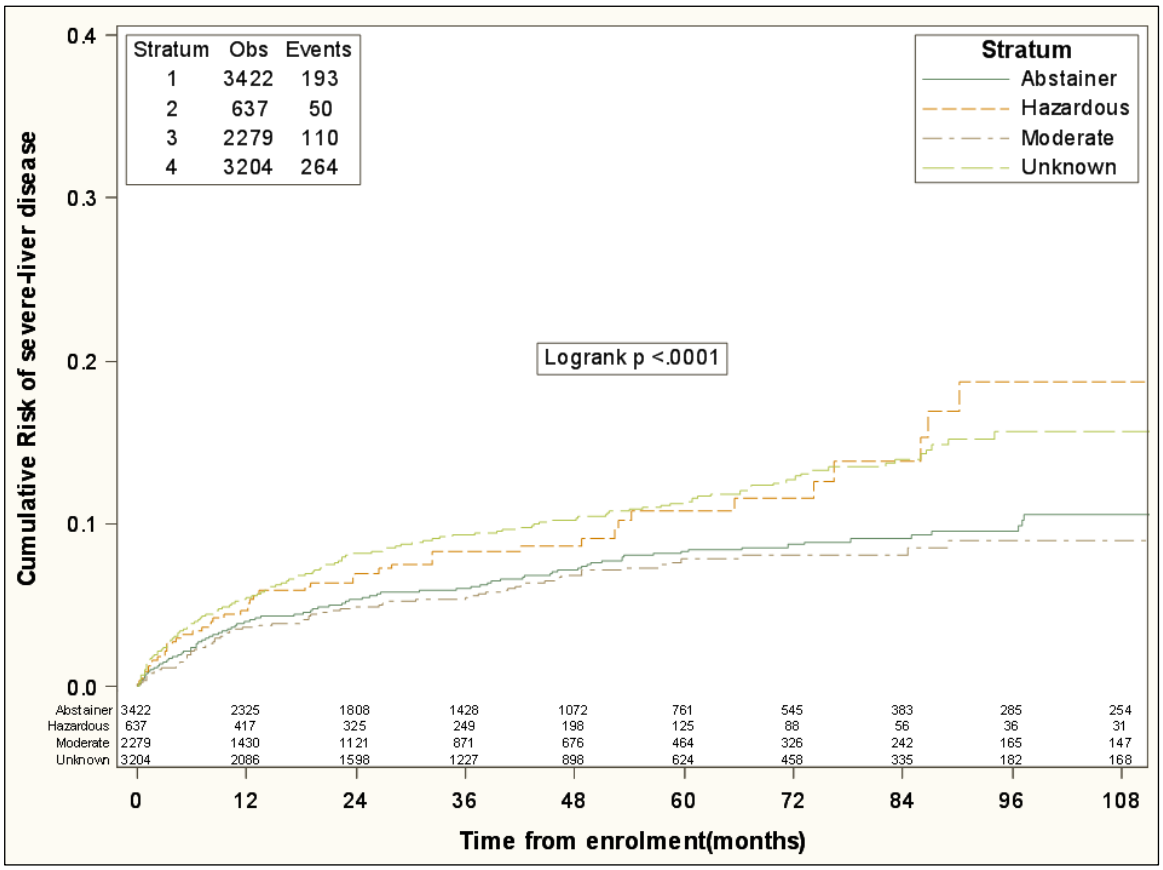


Table 4.4 shows unadjusted and adjusted estimates from the Cox regression model. In the univariable with moderate drinkers as the comparator group, hazardous drinking and unknown alcohol status were strongly associated with increased risk of SLD unadjusted HR = 1.61 (95% CI: 1.16 - 2.26); p=0.005 and 1.67 (95% CI: 1.34 – 2.09); p<0.001 respectively. In contrast, there was no evidence for a difference between abstaining and moderate consumption unadjusted HR = 1.09 (95% CI: 0.87-1.38); p=0.446. There was an extremely strong association of positive HCV status with risk of SLD HR=7.10 (95% CI: 5.89-8.55); p<0.001, with missing status also associated with increased risk HR=1.67 (95% CI: 1.37-2.05); p<0.001. Older vs younger age, Italian vs non-Italian, the middle calendar period vs the initial and final, AIDS diagnosis vs non, missing CD4, missing HIV-RNA, PWID route of transmission, HBV infection, smoking or

unknown smoking status were also associated with risk of SLD. In higher vs lower HIV-RNA was associated with reduced risk of SLD.

After controlling for age, gender, nationality, region, calendar year enrolled, HIV related factors and HBV, alcohol consumption remained associated with the risk of SLD with some attenuation of risk. Still using the moderate consumption as the comparator, adjusted HR (95% CI) for hazardous drinking and unknown alcohol consumption were [aHR=1.45 (1.03 - 2.03; p=0.031) and aHR=1.37 (1.09 - 1.72; p=0.007)] respectively.

However, after additionally adjusting for mode of HIV transmission and HCV infection, the effect of hazardous drinking was attenuated further (aHR = 1.30 (95% CI: 0.92 – 1.82); p = 0.129) but unknown alcohol consumption remained associated with risk of SLD (aHR = 1.43 (95% CI: 1.13 – 1.80); p = 0.003). After further adjustment for smoking status, alcohol consumption was no longer significantly associated with risk of SLD; global p=0.446) . Positive HCV status remained associated with SLD in the final adjusted model HR=2.94 (95%CI: 2.24 – 3.86); p<0.001.

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Table 4.4 Univariable and Multivariable Cox regression models for severe liver disease

	Unadjusted RH (95% CI)	p-value	global p-value	Model 1 RH (95% CI)	p-value	global p-value	Model 2 RH (95% CI)	p-value	global p-value	Model 3 RH (95% CI)	p-value	global p-value	Model 4 RH (95% CI)	p-value	global p-value
Alcohol use															
Abstainer	1.09 (0.87, 1.38)	0.446	<.001	1.08 (0.85, 1.37)	0.506	<.001	1.06 (0.84, 1.34)	0.640	0.007	1.10 (0.87, 1.40)	0.413	0.009	1.09 (0.86, 1.38)	0.498	0.446
Moderate	1.00			1.00			1.00			1.00			1.00		
Hazardous	1.61 (1.16, 2.26)	0.005		1.45 (1.04, 2.04)	0.028		1.45 (1.03, 2.03)	0.031		1.30 (0.95, 1.86)	0.129		1.32 (0.94, 1.85)	0.107	
Unknown	1.67 (1.34, 2.09)	<.001		1.56 (1.24, 1.95)	<.001		1.37 (1.09, 1.72)	0.007		1.43 (1.13, 1.80)	0.003		1.12 (1.86, 1.46)	0.408	
Gender															
Male vs Female	1.17 (0.96, 1.43)	0.117	0.117	1.12 (0.91, 1.38)	0.286	0.286	1.13 (0.92, 1.39)	0.259	0.259	1.15 (0.92, 1.43)	0.213	0.213	1.15 (0.92, 1.43)	0.217	0.217
Age, years															
per 10 years older	1.60 (1.50, 1.71)	<.001	<.001	1.56 (1.45, 1.67)	<.001	<.001	1.48 (1.37, 1.59)	<.001	<.001	1.47 (1.36, 1.59)	<.001	<.001	1.46 (1.34, 1.58)	<.001	<.001
Nationality															
Italian vs Non-Italian	1.75 (1.36, 2.25)	<.001	<.001	1.26 (0.97, 1.65)	0.081	0.081	1.20 (0.92, 1.56)	0.184	0.184	0.97 (0.74, 1.27)	0.829	0.829	0.98 (0.74, 1.28)	0.877	0.877
Region															
North	1.00		0.096	1.00		0.341	1.00		0.242	1.00		0.391	1.00		0.353
Center	0.83 (0.69, 0.98)	0.031		0.90 (0.75, 1.07)	0.226		0.88 (0.74, 1.05)	0.157		0.94 (0.79, 1.13)	0.553		0.95 (0.79, 1.14)	0.596	
South	0.95 (0.72, 1.27)	0.739		1.08 (0.81, 1.44)	0.601		1.09 (0.81, 1.46)	0.584		1.16 (0.87, 1.58)	0.291		1.19 (0.89, 1.60)	0.240	
Calendar year enrolled															
2002 - 2006	1.00		<.001	1.00		<.001	1.00		<.001	1.00		<.001	1.00		<.001
2007 - 2012	0.57 (0.45, 0.71)	<.001		0.52 (0.41, 0.66)	<.001		0.51 (0.40, 0.65)	<.001		0.63 (0.49, 0.79)	<.001		0.59 (0.46, 0.75)	<.001	
2012 - 2016	1.08 (0.86, 1.35)	0.511		0.88 (0.70, 1.11)	0.269		0.67 (0.53, 0.86)	0.001		0.69 (0.54, 0.89)	0.005		0.64 (0.49, 0.82)	<.001	
AIDS Diagnosis															
Yes vs, No	1.64 (1.30, 2.07)	<.001	<.001	-			1.34 (1.05, 1.73)	0.021	0.021	1.40 (1.09, 1.79)	0.008	0.008	1.40 (1.09, 1.80)	0.008	0.008
CD4															
≤300	1.00		<.001	-			1.00		<.001	1.00		<.001	1.00		<.001
301-500	0.69 (0.54, 0.90)	0.005		-			0.86 (0.65, 1.13)	0.280		0.91 (0.68, 1.19)	0.487		0.91 (0.69, 1.19)	0.491	
>500	0.96 (0.77, 1.20)	0.724		-			1.22 (0.95, 1.56)	0.113		1.39 (1.07, 1.78)	0.010		1.39 (1.08, 1.80)	0.008	
Unknown	3.36 (2.72, 4.16)	<.001		-			4.93 (3.60, 6.75)	<.001		2.62 (1.86, 3.70)	<.001		2.43 (1.72, 3.44)	<.001	
Viral load, copies/mL															
≤5000	1.00		<.001	-			1.00		<.001	1.00		0.012	1.00		0.010
5000-10000	0.64 (0.44, 0.93)	0.018		-			1.09 (0.73, 1.62)	0.670		1.53 (1.02, 2.30)	0.041		1.55 (1.03, 2.33)	0.034	
10000-100000	0.63 (0.50, 0.79)	<.001		-			1.12 (0.86, 1.45)	0.410		1.57 (1.19, 2.07)	0.001		1.59 (1.21, 2.10)	0.001	
>100000	0.71 (0.56, 0.90)	0.005		-			1.08 (0.82, 1.43)	0.566		1.63 (1.21, 2.19)	0.001		1.64 (1.22, 2.22)	0.001	
Unknown	1.43 (1.12, 1.83)	0.005		-			0.57 (0.44, 0.76)	<.001		1.05 (0.79, 1.38)	0.752		1.06 (0.80, 1.40)	0.652	
HBV infection															
Yes vs, No	1.91 (1.33, 2.74)	<.001	<.001	-			1.92 (1.34, 2.77)	<.001	<.001	1.97 (1.37, 2.84)	<.001	<.001	2.03 (1.41, 2.93)	<.001	<.001
Mode of HIV Transmission															
PWID	1.00		<.001	-			-			1.00		<.001	1.00		<.001
MSM	0.15 (0.13, 0.19)	<.001		-			-			0.45 (0.34, 0.59)	<.001		0.44 (0.33, 0.58)	<.001	
Heterosexual contacts	0.18 (0.15, 0.22)	<.001		-			-			0.44 (0.33, 0.58)	<.001		0.43 (0.32, 0.57)	<.001	
Other	0.32 (0.24, 0.43)	<.001		-			-			0.64 (0.47, 0.89)	0.008		0.62 (0.45, 0.86)	0.004	
HCV infection status															
Negative	1.00		<.001	-			-			1.00		<.001	1.00		<.001
Positive	7.10 (5.89, 8.55)	<.001		-			-			2.91 (2.22, 3.82)	<.001		2.94 (2.24, 3.86)	<.001	

	Unadjusted RH (95% CI)	p-value	global p-value	Model 1 RH (95% CI)	p-value	global p-value	Model 2 RH (95% CI)	p-value	global p-value	Model 3 RH (95% CI)	p-value	global p-value	Model 4 RH (95% CI)	p-value	global p-value
Unknown	1.67 (1.37, 2.05)	<.001		-			-			1.36 (1.10, 1.69)	0.005		1.34 (1.08, 1.67)	0.007	
Smoking status															
No	1.00		<.001	-			-			-			1.00		0.001
Yes	1.39 (1.13, 1.70)	0.001		-			-			-			0.91 (0.73, 1.13)	0.405	
Unknown	2.30 (1.89, 2.80)	<.001		-			-			-			1.46 (1.12, 1.91)	0.006	

4.4.4 Effect of alcohol consumption on risk of severe liver disease by HCV status in Icona

Figure 4.3 shows stratified results from the unadjusted and adjusted (model 4) Cox regression models including an interaction term between HCV and alcohol consumption. In the unadjusted model there was some evidence of an interaction between alcohol and HCV (p=0.064). The HRs for hazardous drinking compared to moderate was much larger in the HCV-positive and HCV-unknown subgroups, as opposed to the HCV-negative stratum, suggesting a more adverse effect of alcohol on SLD in those with HCV. In the adjusted model, the test for this interaction was not significant, indicating that the association between level of alcohol consumption and risk of SLD did not differ by HCV status (p = 0.740)

In Figure 4.4, in which the stratification was reversed (showing the HR associated with HCV status, stratified by alcohol consumption), although the magnitude of the HR for HCV varied across the alcohol categories, high HRs were observed for HCV-positive vs. HCV negative regardless of alcohol consumption status in both unadjusted and adjusted models.

Figure 4.3 Cox regression unadjusted and adjusted RHs stratified by HCV status and alcohol status for risk of severe liver disease

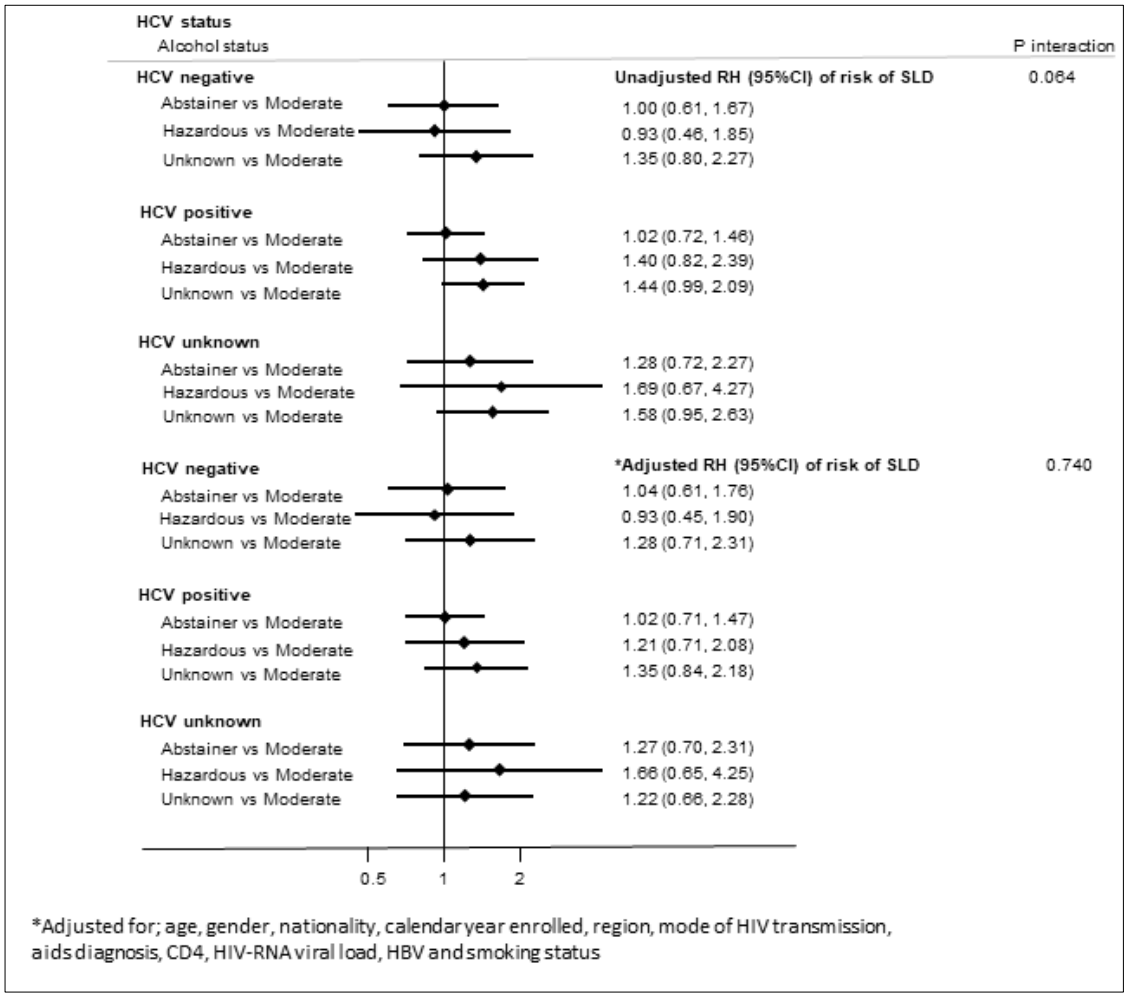
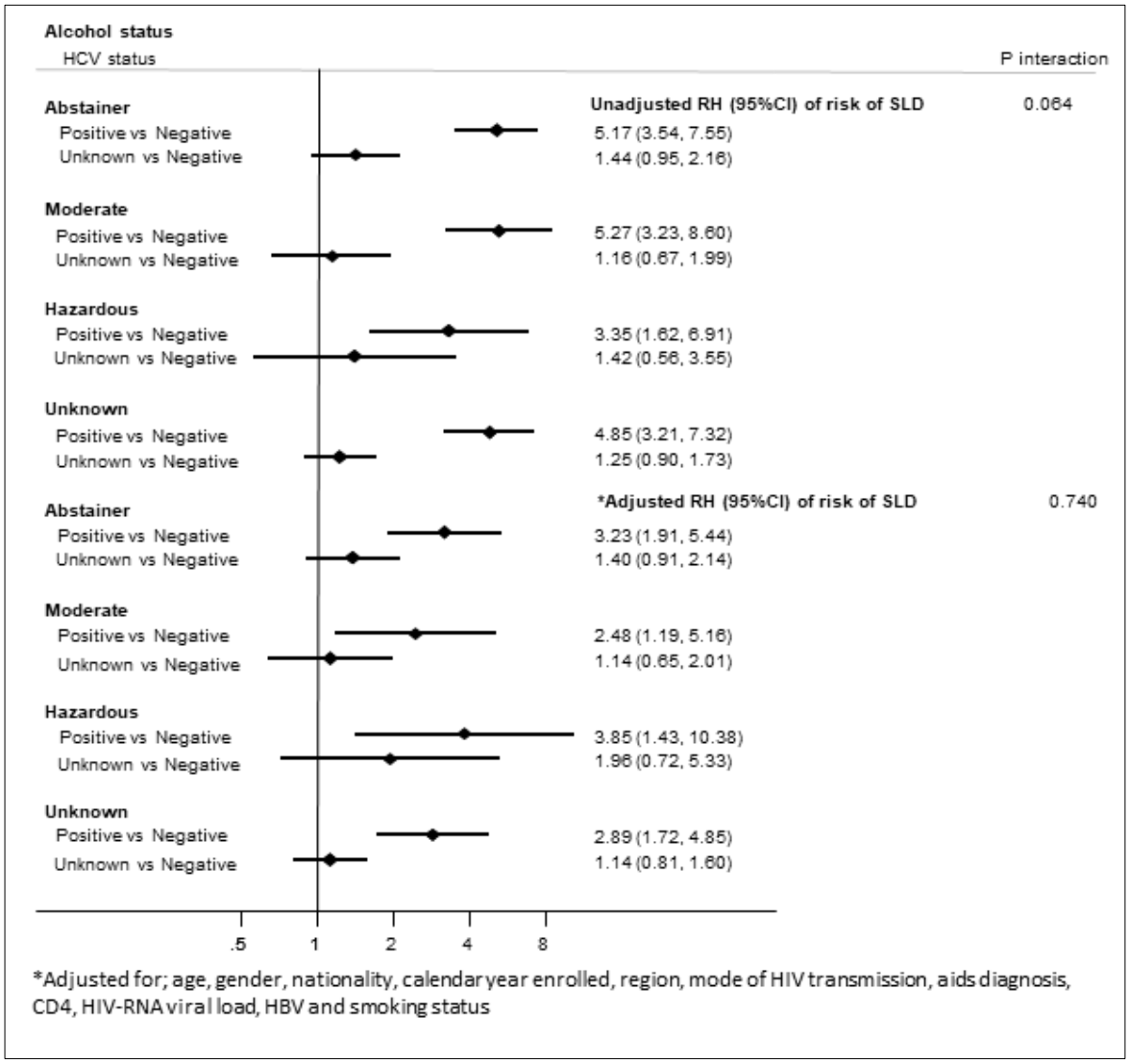


Figure 4.4 Cox regression unadjusted and adjusted RHs stratified by Alcohol and HCV status for risk of severe liver disease



4.4.5 Analysis using multiple imputation for missing alcohol

Reclassification of alcohol use using multiple imputation

Table 4.5 shows baseline characteristics stratified by completeness of data on alcohol use (reported vs. not-reported). Compared to those with complete data on

alcohol consumption, individuals with missing data were more likely to be male (p<0.001), of older age (p<0.001), Italian nationality (p<0.001), enrolled in the northern region (p<0.001), be PWID (p<0.001), to have missing data also for smoking status (p<0.001), missing HCVAb test result (p<0.001) and to be enrolled in later calendar years (p<0.001).

After using MI to reclassify people with missing data, the overall distribution of alcohol consumption (vs. the prevalence in the missing indicator analysis) was as follows: hazardous use (10% vs. 7%), moderate use (37% vs. 23%) and abstainers (53% vs. 36%).

Table 4.5 Characteristics of HIV-positive individuals stratified by reported and non-reported alcohol use

Characteristics	**Reported** N= 6338	**Non-reported** N= 3204	**p-value**
Gender, n (%)			<.001
Male	4884 (77.1)	2584 (80.6)	
Female	1454 (22.9)	620 (19.4)	
Age, years			<.001
Median (IQR)	38 (31, 46)	39 (31, 48)	
Mode of HIV Transmission, n (%)			<.001
PWID	731 (11.5)	431 (13.5)	
MSM	2663 (42.0)	1276 (39.8)	
Heterosexual contacts	2543 (40.1)	1094 (34.1)	
Other/Unknown	401 (6.3)	403 (12.6)	
Nationality, n (%)			<.001
Italian	5003 (78.9)	2625 (81.9)	
Region, n (%)			<.001
North	3103 (49.0)	2078 (64.9)	
Center	2449 (38.6)	973 (30.4)	
South	786 (12.4)	153 (4.8)	
AIDS diagnosis, n (%)			
Yes	535 (8.4)	266 (8.3)	0.817
CD4 cells/mm^3, n (%)			<.001
<300	1908 (30.1)	839 (26.2)	
301-500	1569 (24.8)	697 (21.8)	

Characteristics	Reported N= 6338	Non-reported N= 3204	p-value
≥501	2112 (33.3)	884 (27.6)	
Unknown	749 (11.8)	784 (24.5)	
HIV-RNA, n (%)			<.001
<5000	1108 (17.5)	633 (19.8)	
5001-10000	428 (6.8)	177 (5.5)	
10001-100000	2375 (37.5)	997 (31.1)	
≥100001	1771 (27.9)	744 (23.2)	
Unknown	656 (10.4)	653 (20.4)	
Smoking, n(%)			<.001
No	3313 (52.3)	424 (13.2)	
Yes	2773 (43.8)	480 (15.0)	
Unknown	252 (4.0)	2300 (71.8)	
Hepatitis B, n (%)			
Yes	191 (3.0)	64 (2.0)	0.004
HCV Infection, n (%)			<.001
Negative	4324 (68.2)	1374 (42.9)	
Positive	779 (12.3)	439 (13.7)	
Not tested	1235 (19.5)	1391 (43.4)	
Calendar year enrolled, n (%)			<.001
2002-2006	855 (13.5)	299 (9.3)	
2007-2012	2002 (31.6)	929 (29.0)	
2013-2016	3481 (54.9)	1976 (61.7)	
Follow-up (months)			
Median (IQR)	25.2 (6.1, 55.6)	23.9 (6.6, 51.8)	0.002

Cox regression analysis with multiple imputation

In the Cox regression analyses, after combining MI estimates from separate multivariable models, results were similar to those of the main analysis. Hazardous drinking was associated with the risk of SLD (unadjusted HR=1.70 (95% CI: 1.24 – 2.34]; p=0.002 and abstaining was not associated with risk SLD compared to moderate drinking (unadjusted HR=1.15 (95% CI: 0.90 – 1.47); p=0.261) Table 4.6. However, as like in the main analysis, after adjusting for potential confounders the association was attenuated. In particular after including mode of HIV transmission and HCV infection, hazardous drinking was also no longer significantly associated with risk of SLD (aHR = 1.29 (95% CI: 0.93 – 1.78); p =

0.120) suggesting the risk associated with hazardous drinking was not independent of PWID risk group and HCV status. After further adjustment for smoking status, alcohol consumption was even less associated with risk of SLD (global p=0.724) Table 4.6

In the MI analysis, the test for this interaction between alcohol and HCV was not significant in unadjusted or adjusted results, indicating that the association between level of alcohol consumption and risk of SLD did not differ by HCV status (p = 0.522) (Figure 4.6, Figure 4.5).

Figure 4.5 Cox regression unadjusted and adjusted RHs with multiple imputation stratified by HCV and alcohol status for risk of severe liver disease

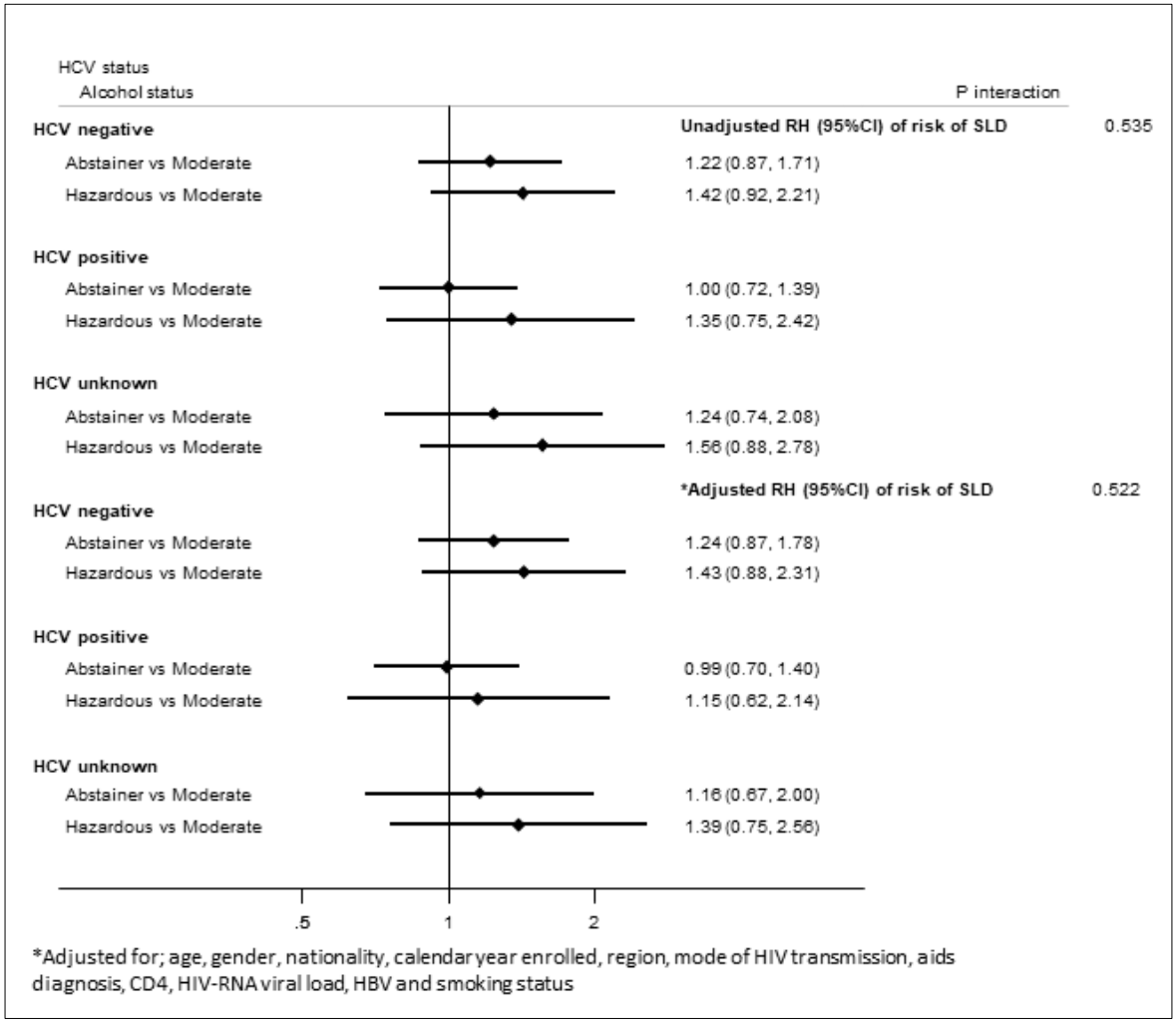


Figure 4.6 Cox regression unadjusted and adjusted RHs with multiple imputation stratified by alcohol and HCV status for risk of severe liver disease

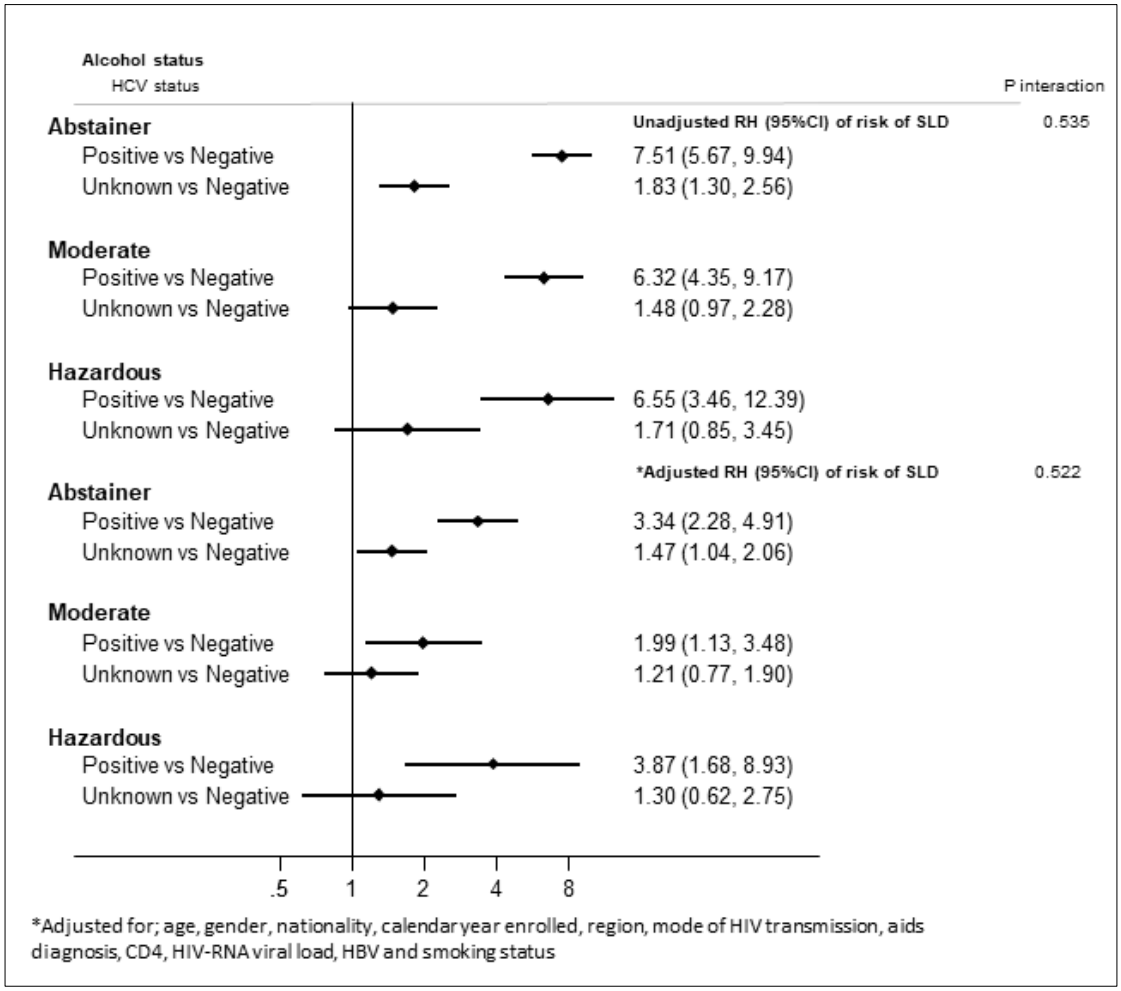


Table 4.6 Univariable and multivariable Cox regression models for severe liver disease with multiple imputation

	Unadjusted RH[95% CI]	p-value	Model 1 RH[95% CI]	p-value	Model 2 RH[95% CI]	p-value	Model 3 RH[95% CI]	p-value	Model 4 RH[95% CI]	p-value	Global p
Alcohol use											0.724
Abstainer	1.15 (0.90, 1.47)	0.261	1.13 (0.88, 1.45)	0.339	1.11 (0.87, 1.42)	0.394	1.13 (0.89, 1.44)	0.315	1.13 (0.88, 1.45)	0.353	
Moderate	1.00		1.00		1.00		1.00		1.00		
Hazardous	1.70 (1.24, 2.34)	0.002	1.48 (1.07, 2.05)	0.020	1.43 (1.03, 1.99)	0.037	1.29 (0.93, 1.78)	0.120	1.30 (0.94, 1.81)	0.114	

1. Age, gender, ethnicity, geographical region, calendar year enrolled; 2. Model 1 + HIV-related factors + HBV; 3. Model 2 + Mode of HIV transmission and HCV infection; 4.Model 3 + Smoking status

4.4.6 Sensitivity analysis - additional analysis including all HIV-positive individuals enrolled from 1997

In an additional analysis which included all HIV-positive individuals enrolled from after 1997 (N=13,174), a total of 1,488 (11%) people experienced SLD events (n=1,177 FIB-4>3.25, n=308 clinical diagnosis of liver disease, n=3 liver-related mortality).

In this analysis, after controlling for potential confounders (model#4) alcohol consumption remained independently associated with the risk of SLD. Compared with moderate drinkers, hazardous drinking was associated with risk of SLD (aRH = 1.42 [1.04 - 1.93]; p = 0.029). However, after additionally adjusting for smoking status, the association with alcohol consumption was attenuated (Table 4.7 Univariable and Multivariable Cox regression models for severe liver disease including all HIV-positive individuals enrolled in early years

Table 5.1 List of antiretroviral drugs included in the analysisTable 4.7). Thus, compared to the analysis restricted to people enrolled in the cohort after 2002, this analysis has a larger number of events and greater statistical power but confounding by smoking was equally important.

Table 4.7 Univariable and Multivariable Cox regression models for severe liver disease including all HIV-positive individuals enrolled in early years

	Unadjusted RH[95% CI]	p-value	Global p-value	Model 1 RH[95% CI]	p-value	Global p-value	Model 2 RH[95% CI]	p-value	Global p-value	Model 3 RH[95% CI]	p-value	Global p-value	Model 4 RH[95% CI]	p-value	Global p-value
Alcohol use			<.001			<.001			0.002			0.021			0.203
Moderate	1.00			1.00			1.00			1.00			1.00		
Abstainer	1.28 (1.03, 1.58)	0.024		1.27 (1.03, 1.58)	0.027		1.23 (0.99, 1.53)	0.060		1.18 (0.95, 1.47)	0.126		1.12 (0.89, 1.39)	0.326	
Hazardous	1.77 (1.30, 2.41)	<.001		1.68 (1.23, 2.29)	0.001		1.66 (1.21, 2.26)	0.002		1.42 (1.04, 1.93)	0.029		1.43 (1.04, 1.95)	0.026	
Unknown	2.19 (1.81, 2.65)	<.001		1.80 (1.48, 2.19)	<.001		1.71 (1.40, 2.08)	<.001		1.51 (1.24, 1.84)	<.001		1.14 (0.92, 1.43)	0.236	

1. Age, gender, ethnicity, geographical region, calendar year enrolled;
2. Model 1 + HIV-related factors + HBV;
3. Model 2 + Mode of HIV transmission and HCV infection;
4. Model 3 + Smoking status

4.6 Discussion

For the first time in the Icona and Hepaicona cohorts, this analysis set out to investigate the value of data collected on alcohol consumption via physician assessment in predicting the risk of developing SLD. Additionally using this newly created alcohol variable, I assessed whether there was an interaction between HCV and alcohol consumption on the risk of SLD. Classification of alcohol consumption was obtained from mapping the responses to the questions as reported in the eCRF to drinking thresholds defined by the NIFN guidelines. After applying the inclusion criteria of enrolment post 2002, out of a total 9,542 eligible PLWH 6,338 (66%) had available data on alcohol consumption. Among individuals with data on alcohol consumption, using the MI method, the overall estimate of the current prevalence of alcohol consumption was 46%: 10% of individuals were classified as hazardous drinkers, 36% as moderate drinkers and 54% as abstainers. Other HIV studies in which alcohol consumption was measured with similar questionnaires have reported prevalence of current use ranging in the window [30-45%] [255, 257, 265, 267, 271, 275, 316, 317]. Our estimate of hazardous use of 10% was similar to that reported in other studies ranging in the window [8-12%] [255, 269, 271, 275, 285] despite the differing assessment tools used. Other studies using standardised questionnaires such as AUDIT or CAGE reported higher estimates of hazardous alcohol consumption/alcohol dependency which may be because these [318] [277] are validated tools are more sensitive in identifying hazardous alcohol use than our three simple questions [266, 277, 319].

The somewhat lower prevalence of hazardous drinking found in our analysis compared to those showing a prevalence of >10% may also be due the fact that alcohol consumption was collected via face to face interview rather than anonymously. Additionally the case mix of individuals included in the analyses might have also played a role as other studies included a large number of PWIDs who are known to consume more alcohol. Patterns and levels of alcohol use are also likely to vary geographically and between countries. Finally, differences in prevalence estimates of alcohol consumption could also be explained by

differences in definitions of units of alcohol. In Italy, for example, twelve grams of alcohol is equivalent to a standard drink which is different from what is used in other countries.

This analysis also set out to investigate whether alcohol consumption assessed via participant' interview conducted by the treating physicians was associated with the risk of developing SLD. Seven percent of the study population experienced SLD over follow-up. Although the risk of SLD appeared marginally lower for moderate drinkers compared with abstainers, this difference was not statistically significant. A lower risk in moderate drinkers compared to abstainers has been previously documented and a possible explanation for this finding is that patients who are currently abstaining may include individuals who were never drinkers as well as those who previously were heavy drinkers and currently abstain due to medical or other reasons [320]. Unfortunately, in this analysis it was not possible to separate these groups.

In the multivariable analysis adjusting for baseline demographic factors and HBV infection, hazard drinking was independently associated with increased risk of SLD. However, after further adjusting for mode of HIV transmission, HCV infection and smoking status the association was largely attenuated. Nevertheless, it is worth noting that the magnitude and direction of risk of SLD remained similar and the results cannot rule out with 95% probability an 85% increase in risk of SLD for hazardous vs. moderate drinkers. Alcohol intake was associated strongly with PWID risk group and was also associated with HCV positive status, and this multivariable analysis suggested that these three factors are not independent predictors of SLD risk, with HCV being the overriding factor determining risk. *Lim et al*, in 2,111 HIV-positive individuals showed a similar risk of advanced fibrosis associated with hazardous alcohol use, after adjusting for potential confounders including HCV infection [302]. In another study including 308 HIV-positive individuals in which heavy alcohol use was defined as >2 drinks/day or ≥5 drinks per occasion and >1 drink per day or ≥4 drinks per occasion for men and women respectively reported that overall 10% developed liver fibrosis. Consistent

with our results, the authors found no significant association between heavy alcohol use and risk of advanced liver fibrosis, after controlling for age, sex, HCV-RNA and CD4 [274]. In contrast *Chaudhry et al,* in a study conducted in 2009 did find an association between alcohol use and risk of liver fibrosis (measured using the APRI score) after adjusting for potential confounders including HCV infection [275].

I formally evaluated whether HCV was an effect measure modifier for the association between alcohol and risk of SLD. There was some suggestion of this in the unadjusted analysis, but in the adjusted analysis there was no evidence that the association between alcohol consumption and the risk of SLD varied by HCV infection status (p=0.740) and this remained consistent even in the MI analysis. Of interest, also in the analysis by *Chaudhry et al* as well as in another study, there was no evidence that the association between alcohol consumption and the risk of SLD varied by HCV infection status [275] [270]. In contrast other studies have found a synergistic effect between alcohol consumption and HCV on SLD risk in HIV/HCV coinfected populations. However, these latter studies were mostly cross-sectional or case control studies and included participants with excessive alcohol intake and populations enriched with PWIDs [254].

This significant proportion of missing data identified in the analysis of the whole Icona cohort highlights the challenges of collecting complete data on alcohol consumption as part of routine clinical care of PLWH. Even after restricting to people enrolled after 2002 and using all data including free text on alcohol consumptionapproximately 30% of individuals did not have measures of alcohol use at study enrolment. Reasons for under-reporting are unclear. Missing data may have occurred because the physician failed to ask the questions or because the individual was unwilling to give information for other unknown reasons. However, the prevalence of people with missing information was generally consistent with that seen in other HIV cohort studies showing estimates of under-reporting ranging in the window [7-41%] [267, 277, 319, 321].

As previously mentioned, possible reasons for under-reporting of alcohol use include social desirability and fear of the impact on antiretroviral therapy initiation [277, 285, 286]. Of note, in Italy to have a drink with a meal is considered normal and this might explain the low percentage of people reporting heavy drinking. Some studies have assessed the extent of under-reporting by comparing self-reported alcohol consumption with the results of blood tests or biomarkers, or interviews carried out by professionals and found a lack of agreement between these measures [277, 286, 287]. The main limitation of using blood tests is that, there is a time limit after which you can no longer detect alcohol consumption in the blood. This depends on the exact test done. For some of the tests described in the introduction of this chapter this is acceptable as it can be as long as one month. Importantly, physician assessment like that implemented in Icona are likely to measure alcohol consumption even less accurately than self-administered questionnaires[322].

The results of the analysis evaluating the association between alcohol consumption and the risk of SLD after the MI reclassification, were similar to those of the main analysis. This may imply that missing data genuinely did not introduce significant bias in this analysis or, equally likely, that MI was unable to rectify an untestable inherently existing bias. Of note, in a study in which the MI approach was used to investigate the impact of self-reported abstinence on the probability of cART initiation consistency between the main findings and the MI results was also observed. [321]. Overall, although statistical methods exist to handle missing data, the reasons for missingness are unknown and it is not possible to exclude that these are linked with the outcome chosen for the analysis. Indeed, alcohol intake itself could be a possible reason for not reporting alcohol consumption leading to violation of the MAR assumption. It is established that if the MAR assumption does not hold, standard MI methods are unable to control for the bias [312]. Also, as mentioned in chapter 2, alternative methods such a regression calibration would have been more suitable in a situation of possible mis-classification of the exposure [241].

One of the cautions in using the MI approach is ensuring that the amount of missing data is not too large otherwise MI with a limited number of imputed datasets such as those used here may not be appropriate. For this reason, people enrolled in the early years, i.e. pre 2002 were excluded as there was >70% missing data on alcohol consumption. To assess the impact of excluding these individuals, a sensitivity analysis was done including the whole cohort, with broadly similar results.

4.7 Limitations

There are some limitations that should be addressed. In all analyses individuals were classified according to the alcohol consumption reported at entry in the cohort, which assumes that alcohol consumption behaviour remained constant over time. However, it is possible that drinking habits (and patterns of missing data) changed over follow-up potentially leading to a dilution of the association. Use of baseline values was done mainly to simplify the analysis as mechanisms of time-dependent confounding in this context are largely unexplored and potentially difficult to address by means of a standard Cox regression analysis.

Secondly, as typical in the observational setting, it is not possible to rule out the presence of residual or unmeasured confounding. For example, data collected on mode of HIV transmission in Icona and Hepaicona do not allow to distinguish between ex-PWID and current PWID, leading to potential residual confounding due to misclassification. Since PWID was strongly associated with alcohol use and SLD, this could impact the results. In addition, because of the large proportion of people with missing data, selection bias cannot be entirely ruled out. Indeed, people lacking information on alcohol use were different from those with complete data for a number of factors known to be associated with the risk of SLD (Table 4.5). However, the amount of missing data observed in our cohorts is consistent with that found in other HIV cohorts and the results of the analysis of the MI dataset was very similar to those of the analysis retaining the missing alcohol data group. Importantly, as stated above, the analysis relies on the assumption that data

are MAR, given the other measured covariates in the model. Finally, it is possible that the physicians may not have asked the questions on alcohol use in standardised fashion in accordance with the format on the eCRF, again leading to measurement error.

Despite these limitations, data on alcohol consumption in PLWH are seldom collected in the context of HIV observational studies so the Icona Network studies represent a promising exception. Furthermore, certainly at the time in which this analysis was conducted, there remained conflicting evidence regarding the association of level of alcohol consumption and risk of developing SLD. Additionally the role of HCV as an effect modifier on risk of SLD had not been thoroughly evaluated in studies of the size of Icona. Therefore, this analysis provided an important contribution to the literature on these topics.

4.8 Conclusion

In conclusion, I evaluated the value of alcohol consumption data obtained by brief physician interview of PLWH to predict their future risk of occurrence of SLD. The association between hazardous alcohol consumption and risk of SLD that was evident in unadjusted analysis was largely explained by differences in HCV status and mode of HIV transmission, as well as smoking status. Therefore there was no strong evidence that hazardous drinking as assessed in these cohorts was predictive of SLD independently of HCV status and other factors. HCV was very strongly associated with risk of SLD, but there was no evidence for an interaction between HCV and alcohol consumption on the risk of SLD in adjusted analysis. This finding remained consistent even after performing MI to account for missing alcohol consumption data.

At the time in which this analysis was conducted this was an important question as it was not possible to eradicate HCV from an individual, while alcohol consumption is a modifiable risk factor.

Findings from this data analysis indicate that data collection on historical alcohol consumption including items which could allow to distinguish between people who had currently stopped drinking from those who never drank would be useful for future studies.

4.9 Further work

Following on from this work, which has been published in the BMC Public Health Journal in 2019 [14] improvement of data collected on alcohol consumption was planned in Icona using a more standardized screening questionnaire such as the AUDIT-C. Other resources could include the use of plasma (as described in section 2.4.1, Icona has a repository biobank linked to clinical data). Although only plasma and full blood samples are collected in Icona these could be retrospectively used to measure levels of alcohol consumption. However success rate is limited by how long alcohol can be detected in the samples. Icona and Hepaicona are observational studies with alcohol consumption collected routinely every six months, so trends or changes of alcohol consumption over time could also be assessed.

Although we adjusted for potential confounders in the Cox regression analysis, it is possible that a different approach to analysis may give additional insights. Mediation analysis could be used to investigate if the association between level of alcohol consumption and risk of SLD was mediated by some other time-dependent factors. The use of a DAG (which was introduced only for the analyses included in the following chapters 6 and 7) would have been useful to depict the complicated relationship between alcohol consumption and risk of SLD involving key confounders factors such as HCV infection and smoking more transparently.

CHAPTER 5

5 WHAT IS THE ROLE OF HCV COINFECTION ON DISCONTINUATION OF SPECIFIC ANTIRETROVIRAL DRUGS IN PEOPLE LIVING WITH HIV?

5.1 Aim and objectives

The aim of this chapter is to assess the impact of HIV/HCV coinfection on stopping specific ARVs in the real world setting of a cohort of HIV-positive individuals seen for care in Italy. This chapter also aims to identify which ARV drugs have the highest risk of discontinuation in HIV/HCV coinfected individuals.

The specific objectives are:

1. To assess the association between HCVAb status and the risk of stopping cART for any reason by drug class
2. Among, HCVAb positive individuals, to assess the association between HCV-RNA status and the risk of stopping cART for any reason by drug class
3. To assess the association between HCVAb status and the risk of stopping specific ARV drugs
4. Among, HCVAb positive individuals, to assess the association between HCV-RNA status and the risk of stopping specific ARV drugs
5. To carry out a sensitivity analysis of the association between HCVAb status and the risk of stopping specific ARV drugs using a narrower definition of cART discontinuation

5.2 Introduction

Treatment of HIV with cART has resulted in increased survival rates and a reduction in morbidity and opportunistic infections among HIV-positive individuals. Furthermore, viral suppression on cART eliminates the risk of sexual transmission of HIV [31]. Recognition of these benefits of cART treatment has resulted in guidelines recommending initiation of cART to all HIV-positive individuals

irrespective of levels of CD4 [38]. Antiretroviral treatment is for life and one of the major challenges is ensuring adherence to cART.

Discontinuation of cART is associated with increased risk of AIDS and non-AIDS events [323]. Sustainability of cART in HIV-positive individuals is key to ensure targets set by UNAIDS for the year 2030 (90% diagnosed, 90% treated, 90% achieving viral suppression) is reached [41]. For individuals with HIV/HCV coinfection, ARV drug regimens recommended are the same as those recommended for HIV mono-infected individuals [38]. However, adherence to ARVs may differ in HIV/HCV coinfected individuals for a number of reasons, and in particular discontinuation of ARVs may be linked to specific conditions such as liver disease [38].

There are many known risk factors associated with discontinuation of specific ARV drugs (regardless of whether the decision to stop was made by either the individual or treating physician). It has been hypothesised that coinfection with HCV can lead to a higher rate of specific ARV drugs discontinuation because an enzyme known as cytochrome P450 (CYP450) found in the liver, appears to play a role in lowering concentration levels of specific drugs in the blood stream. If there are insufficient levels of CYP450 caused by liver disease, concentration of ARV drugs may build up in the blood to toxic levels which may lead to drug discontinuations (more in section 5.3.1). Therefore, the main rationale for hypothesizing that the rate of specific ARV drug discontinuation might differ between HCVAb positive and HCVAb negative is the fact that specific drug levels might be higher in HIV/HCV coinfected as compared to HIV mono-infected individuals causing more intolerance.

As mentioned in chapter 1 section 1.2.5, the specific ARVs recommended for use in cART naïve HIV-positive individuals considered in this analysis are: Tenofovir Disoproxil Fumarate (TDF) or Tenofovir Alafenamide (TAF - a novel prodrug of TDF), Emtricitabine (FTC) and Lamivudine (3TC), Efavirenz (EFV), Rilpivirine

(RPV), Atazanavir (ATV),Lopinavir (LPV/r), Darunavir (DRV/r), Raltegravir (RAL), Elvitegravir (ELV) and Dolutegravir (DLG).
At the time in which this analysis was conducted, HCV eradication was not possible in many HIV/HCV coinfected individuals, and therefore it was important to evaluate the impact of HCV infection on the risk of specific ARV discontinuation so that cART could be better tailored to individuals according to their current HCV infection status.

It is worth mentioning, that there might be several other reasons as to why HCV status might impact on drug discontinuation. PLWH with HCV coinfection may differ from mono-infected in terms of demographic factors such as age, race, mode of transmission; they may also differ in terms of, psychosocial factors such as depression or substance or alcohol abuse [324, 325]. In chapter 3, the descriptive analyses of both cohorts indicated a high prevalence of PWID in HIV/HCV coinfected individuals who were also more likely to consume alcohol than other risk groups. Such factors may be lead to lower ARV drugs tolerance, reduced adherence and ART discontinuation [324, 325].

In this analysis, after accounting for possible differences in characteristics between HCV uninfected and HIV/HCV coinfected, the focus was to assess whether there were biological effects of the drugs metabolisation in the liver over and above any differences in possible confounding factors associated with specific modern ARV drugs discontinuation. Additionally, the role of HCV viremia was also assessed to determine if active HCV infection had even greater impact on cART discontinuation.

5.3 Literature review

Prior to the literature review, I give a brief background on the pharmacokinetics of the specific ARVs included in the analyses shown in this chapter. The literature review focused on studies reporting the role of HIV/HCV coinfection on the risk of specific ARV drug discontinuation for any reason. First, I review the previous

research conducted in Icona on ART discontinuation. Then I present results from the literature search of articles relating to HCV and cART discontinuation which was originally done up to December 2017 and then updated in January 2021. See Table 2.1 for details of literature review search.

5.3.1 Brief background on Antiretroviral kinetics

All ARV drugs entering the body must be eventually metabolized (drug metabolism is process by which the body breaks down the drug into active chemical substances to be absorbed in the body into) [326] [36] [327]. The main site for this drug metabolism is in the liver where a group of enzymes known as CYP450 play a major role in this process [326] [36] [327]. Cytochrome P450 also plays a role in lowering concentration levels of drugs in the blood stream [327] [326] [36]. Thus, for individuals with liver disease, the process of drug metabolism maybe compromised due to insufficient levels of CYP450 enzymes. Therefore, concentration of drugs may build up in the blood to toxic levels which might lead to drug discontinuation, with individuals likely to present with signs and symptoms indicative of toxicity [36] [326] [327].

Nucleoside Reverse Transcriptase Inhibitors

Some of the characteristics that influence whether Nucleoside Reverse Transcriptase Inhibitors (NRTIs) are susceptible to hepatic impairment is their low protein-binding ability, meaning that these drugs are relatively well absorbed and are largely eliminated through the kidneys [326] [36]. Concerning the specific NRTI drugs which I included in this analysis, TDF or TAF - a novel prodrug of TDF, FTC and 3TC are mainly excreted via the kidneys and therefore the pharmacokinetics of these drugs are not known to be affected by liver disease[36, 326] but care is needed when these drugs are used in people with renal disease. In contrast, ABC is metabolized in the liver and is therefore used with caution in people with moderate or severe hepatic impairment [36, 326, 328, 329].

Non-Nucleoside Reverse Transcriptase Inhibitors

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are metabolized in the liver by the CYP450 enzyme system, therefore presence of hepatic impairment is likely to affect the pharmacokinetics of these drugs[36, 326]. NNRTIs can also act as inducers or inhibitors meaning they increase the rate of metabolism by drug interactions with CYP450, resulting in the reduction of the effect of the medication. Also, if a medication is taken with an agent that inhibits its metabolism this can lead to therapeutic failure [330]. Concerning the specific drugs I included in this analysis; EFV is both an inhibitor and an inducer of the CYP450 enzyme system [36]. It can also alter the pharmacokinetics of other ARV drugs and therefore it needs to be used with caution in individuals with hepatic impairment [36, 331]. Rilpivirine is also metabolized in the liver and has inhibiting action like that of EFV [332]. Nevirapine has also been known to be associated with increased liver enzyme levels as the drug is known to induce its own metabolism. Therefore, liver impairment is expected to be associated with increased levels of hepatoxicity on NNRTIs [326].

Protease inhibitors

Most of the Protease Inhibitors (PIs) are metabolized in the liver by the CYP450 enzymes and because of this they have the potential to interact with other drugs [36, 326]. PIs are boosted with ritonavir (PI/r) which is a pharmacokinetic enhancer because it inhibits part of the CYP450 enzyme system (CYP3A), thus leading to increased concentrations of PIs [36, 326, 333]. Although, ritonavir can be well tolerated, it often causes side-effects and possible liver injury [333]. Therefore, hepatic impairment plays a role in the alteration of the pharmacokinetics of the PIs[326, 334-336]. Concerning the specific drugs I included in this analysis; ATV/r in combination with ritonavir, is metabolised and eliminated by the liver, therefore it is typically affected by liver disease [36, 334]. LPV/r and DRV/r are both used in combination with ritonavir are also similarly affected by hepatic impairment [36, 335].

Integrase inhibitors

The Integrase Inhibitors (INIs) is the drug class most recently introduced in the clinics. INIs examined in this analysis included: RAL, ELV and DLG [337]. They are

all used in combination with other ARV drugs. These drugs are all metabolized in the liver by a process called glucuronidation which involves the combination of drugs and glucuronic acid to form water soluble substances and eventually excreted via the kidneys [338]. There is minimal involvement with CYP450 enzymes system, thus drugs in this class are considered safe and well tolerated in HIV/HCV coinfected individuals [339, 340]. Although the mechanism of INIs on liver injury is still unknown, any effect observed may be due to the other drug regimens used in combination with INIs[338].

5.3.2 Previous analysis of cART discontinuation in the Icona cohort

Drug discontinuation is a topic that has been thoroughly investigated by the Icona Network group over the years, starting from 1997. This section summarises previous analyses of cART discontinuation in the Icona cohort.

The risk of specific cART discontinuation of first-line regimens was assessed in the Icona cohort in the early era of cART and in recent cART period [230, 341, 342]. A number of analyses conducted in the early cART era showed that the most common reason for specific cART discontinuation was due to toxicity/intolerance [230]. For example, in one of the first ever publications of the Icona cohort, in a sample population of 862 PLWH who were cART-naïve enrolled between March 1997 and March 1999, *d'Arminio Monforte A et al* reported the probability of first cART regimen discontinuation due to toxicity was 26% (95% CI: 21.9 – 28.9) after one year of starting cART [230]. In more detail, the frequency and reason of discontinuation of cART in this study was assessed among the 862 HIV-positive individuals initiating cART (mostly NNRTIs in combination with ritonavir, indinavir or saquinavir). The authors reported that after a median follow-up of 45, weeks, over a third of individuals (36.2%) had discontinued (including switched) ≥1 drug of the initial therapy. The most common reason for discontinuation was toxicity in 21.1% (n=182) followed by 7.1% (n=61) discontinuing because of failure [230]. The drug specific regimens showing the highest risk of discontinuing because of toxicity were those including 2 NRTIs plus PI/rs. When considering discontinuing for

toxicity or failure (immunological/virological) as the outcome, this analysis did not find an association between HIV/HCV coinfection and risk of discontinuing treatment. However, time spent on treatment was found to be an important predictor of discontinuation (the longer the duration on treatment the lower the risk of stopping cART) [230].
Fortunately, ARV drugs that are currently recommended and used in routine clinical care are much better tolerated and more efficacious than some of those evaluated in this old analysis [38] [343, 344].

In a more recent analysis of the Icona cohort, *Cicconi et al* analysed the data of 3,291 individuals, similarly finding that 36.1% (n=1,189) discontinued ≥1 drug in the first cART regimen over the first 12 months of cART. Overall, the main reasons for discontinuation were intolerance/toxicity in 58.5% (696/1189) and poor adherence in 24% (285/1189) [341]. In the analysis by *Cicconi et al* most of the HIV-positive individuals had started cART more recently than in the previous Icona study described above, i.e. 30% had started between 2000 and 2002 and 60% started after 2002 [341]. *Cicconi et al* found that individuals initiating cART in the period 2003 to 2005 were less likely to discontinue their treatment due to intolerance/toxicity compared to those starting before the year 2000 [341].
Discontinuation of specific ARVs may be for reasons unrelated to toxicity and adherence. The authors of the above study also found a higher risk of discontinuing for simplification in more recent periods (2003 – 2005) compared to earlier years, aHR = 15.3 (95% CI: 3.2 - 72.5) [341] likely reflecting the greater options for rationalising treatment regimens that became available in more recent years. When evaluating whether calendar period of starting cART was associated with incidence of cART discontinuation, other studies have shown that over time there was a reduction in rates of discontinuation due to toxicity [231, 341, 345]. This is possibly due to the increase in the rate of pro-active switching to newly available simplified single tablet regimens.

In this same analysis, the authors found that individuals starting PI-based regimens or three NRTI-based regimen combination were at an increased risk of

discontinuation of at least one drug compared to individuals who started with NNRTI-based regimens aHR = 1.63 (95% CI: 1.31 – 2.02) and ARH = 1.63 (95% CI: 1.22 – 2.18) respectively adjusted for age, gender, HCVAb status, CD4, HIV-RNA, type of cART regimen started [341]. In contrast to the earlier analysis, in this analysis, HIV/HCV coinfection was found to be associated with a moderately increased risk of cART discontinuation aHR = 1.18 (95% CI: 1.00 -1.41) compared to HIV mono-infected individuals adjusted for the same variables [341].

In an even more recent study in Icona with updated data involving 4,052 individuals, *Di Biagio et al* looked at reasons for ARV drug discontinuation of first-line regimens in the modern cART era defined as individuals starting cART after December 2017. Discontinuation was defined as stopping and or switching of at least one drug contained in the regimen. The main reason for discontinuation reported was stopping for simplification [231].

All these previous analyses have considered discontinuation or switching of any cART drug as the outcome. My analyses in this chapter differ from this in that they specifically investigate the role of HCV in the probability of discontinuation of specific modern ARV drugs. Also, while all these analyses are based on number of patients who started their first cART regimen from ART-naïve, in my analysis each drug included in the regimen was counted as a separate unit in the analysis. Thus for example a participant who started FTC+TAF+DRV/r contributed to three records (one for each of the drugs started).

5.3.3 Role of HCV infection in cART discontinuation

In this section, I summarise studies that looked at time to stopping or switching at least one drug but some looked at total discontinuation and the reasons and role of HCV may differ between these two endpoints.

Evidence on the role of HCV coinfection in specific cART discontinuation continues to be conflicting in the literature. In addition to the Icona analysis described above,

some other studies have identified HCV-infection as a risk factor for cART discontinuation while others found no evidence for an association [346-348]. For example, *Ripamonti et al* investigated risk factors for modification of initial cART first-line regimens within the first 12 months of treatment between November 1996 and December 2000 [346]. In this analysis, modification was defined as interruption of the cART regimen (i.e. stopping all drugs) for at least one month or change of at least one drug in the regimen. The study included 465 cART naive HIV-positive individuals of whom 45.6% (n=212) were HCV seropositive [346]. The majority of individuals, 79.1% (n=368) received old generation PI-based therapy (IDV, NFV, SQV and RTV), followed by 18.5% (n=86) receiving NNRTI-based therapy (EFV, NVP) and few individuals, 2.3% (n=11) received NRTI-based therapy [346]. A total of 40.2% (n=187) modified/discontinued their regimen by the end of first year of treatment. The most frequently cited reasons for modification were intolerance/toxicity 67.3% (126/187), clinical/virological failure 17.6% (33/187) and non-adherence 14.9% (28/187) [346]. HIV/HCV coinfection was shown to have a 43% higher risk of discontinuation/modification of cART compared to HIV mono-infection (RR = 1.43 (1.05 – 1.94, p=0.02) [346]. *Ripamonti et al* argued that this finding could be due to the fact that most individuals were HCV viremic with some degree of liver fibrosis which could explain reasons of intolerance leading to modification/discontinuation [346]. In addition, the majority of individuals were on PI-based regimens. These are known to be metabolised in the liver, thus any presence of liver damage is expected to influence the process of metabolism of these drugs. Also in this study, old ARV drugs were used which were worse tolerated than more modern ARVs.

Drug discontinuation was extensively examined also in one of the largest European HIV cohort, the EuroSIDA study. Similar findings of an association of HCV and drug discontinuation was reported by *Mocroft et al* who identified HCV as one of the factors associated with the risk of stopping any part of the cART regimen started due to treatment failure or toxicity and patient/physician choice in the EuroSIDA cohort. In EuroSIDA, as in the Icona cohort, treating physicians record a number of possible reasons for discontinuation and only the main reason is

typically used in the analysis and reported [347]. Because patient/physician choice is often hiding issues related to intolerance/toxicity of the drugs, the decision was made to collapse together all reported reasons in the analysis of EuroSIDA data. In this study involving 1,198 HIV-positive individuals initiating cART between 1999 and 2004 (defined as date of last follow-up), regimens started were NNRTI-based 49% (n=587), PI-based 26% (n=311) and dual PI-based 16% (n=192), all with a backbone of two NRTIs. After one year of initiating cART, 30% (n=359) had discontinued or switched at least one drug of these initial regimens [347]. Compared to HIV mono-infection, HCV infection was found to be associated with a higher risk of discontinuation due to toxicity or patient/physician choice adjusted IRR=1.48 (95% CI: 1.15-1.92, p =0.0025) adjusted for gender, calendar year of starting cART, time on cART, cART naïve status, CD4, type of cART and region [347]. When using single PI-based regimens as the comparator, dual PI regimens were found to be associated with a higher risk of discontinuation due to toxicity or patient/physician choice aIRR =1.61 (95% CI: 1.19 - 2.18, p = 0.0019) [347]. This result is expected as PIs are metabolised in the liver, so any liver damage will affect this process. In contrast, single NNRTI-based regimens were found to associated with a reduced risk of discontinuation due to toxicity or patient/physician choice aIRR = 0.77 (95% CI: 0.60 – 0.98, p = 0.035)[347] compared to single PI-based regimens. The authors speculated that this result could be explained by the different drugs used in combination with the NNRTI vs PI-based cART and also reported a decreased incidence of discontinuation due to toxicities in regimens started after 1999 [347]. The authors also acknowledge that the majority of individuals with HCV infection were PWIDs which may explain the excess of risk in discontinuation associated with HCV-infection.

In another EuroSIDA analysis including individuals initiating cART from 1999 to 2004, specially looking at drug regimens. *Mocroft et al* evaluated the role of HCV in discontinuation of any nucleoside pair or third drug in the cART regimen due to toxicities or patient/physician (TOXPC) choice in 4,929 HIV-positive individuals of whom 27.5% (n=1355) were HIV/HCV coinfected [348]. HCV-positive status was determined on the basis of a positive serology test results (HCVAb positive). The

main analysis could not identify specific components of the cART regimens used that were associated with poor tolerance in HIV/HCV coinfected individuals [348]. Regimens studied included two NRTIs pairs (ZDV/3TC, 3TC/d4T, ddi/d4T) with a third drug (ABC, NFV, IDV, NVP, EFV, LPV/r). They observed 2,141 discontinuations of NRTIs, contributed by 80.2% (n=1,386) unique individuals [348]. Overall, they found a lower proportion of HIV/HCV coinfected individuals discontinuing NRTIs compared to mono-infected (75.3% vs. 82.4%) [348]. In terms of discontinuation of the third drug, 2,501 discontinuations were observed and contributed by 72.9% (n=1,350) HIV-positive individuals [348]. Similarly, a lower proportion of HIV/HCV coinfected individuals discontinued the anchor drug compared to HIV mono-infected (69.1% vs. 74.7%) [348]. Overall, discontinuation was mostly due to patient choice. However, in terms of incidence of discontinuation due to TOXPC of both NRTI pairs and the third drug, incidence of stopping was higher in HIV/HCV coinfected than HIV mono-infected (19.1 per 100PYFU, 95% CI: 17.5 – 19.3 and 15.8 per 100PYFU, 95% CI: 15.0 – 16.6) respectively [348]. For the third drug, incidence discontinuation was higher in HIV/HCV coinfected than HIV mono-infected (22.4 per 100PYFU 95% CI: 20.9 – 23.9 and 18.4 per 100PYFU 95% CI: 17.5 – 19.3) [348]. The authors speculate that there might be residual confounding due to mode of HIV transmission as most HCV-infected participants were PWID who are known to have poorer adherence to cART. Possible interaction between cART and recreational drugs may also play a role in drug discontinuation [348].

When specific ARV drugs were investigated, the analysis showed high rates of discontinuation for a number of them (i.e. DDI, d4T, IDV and boosted PI regimens) but similar patterns of discontinuations were observed in both HIV/HCV coinfected and those HIV mono-infected [348]. It is worth mentioning that some of the ARV drugs examined in this analysis are no longer recommended by guidelines and used in the clinics today.

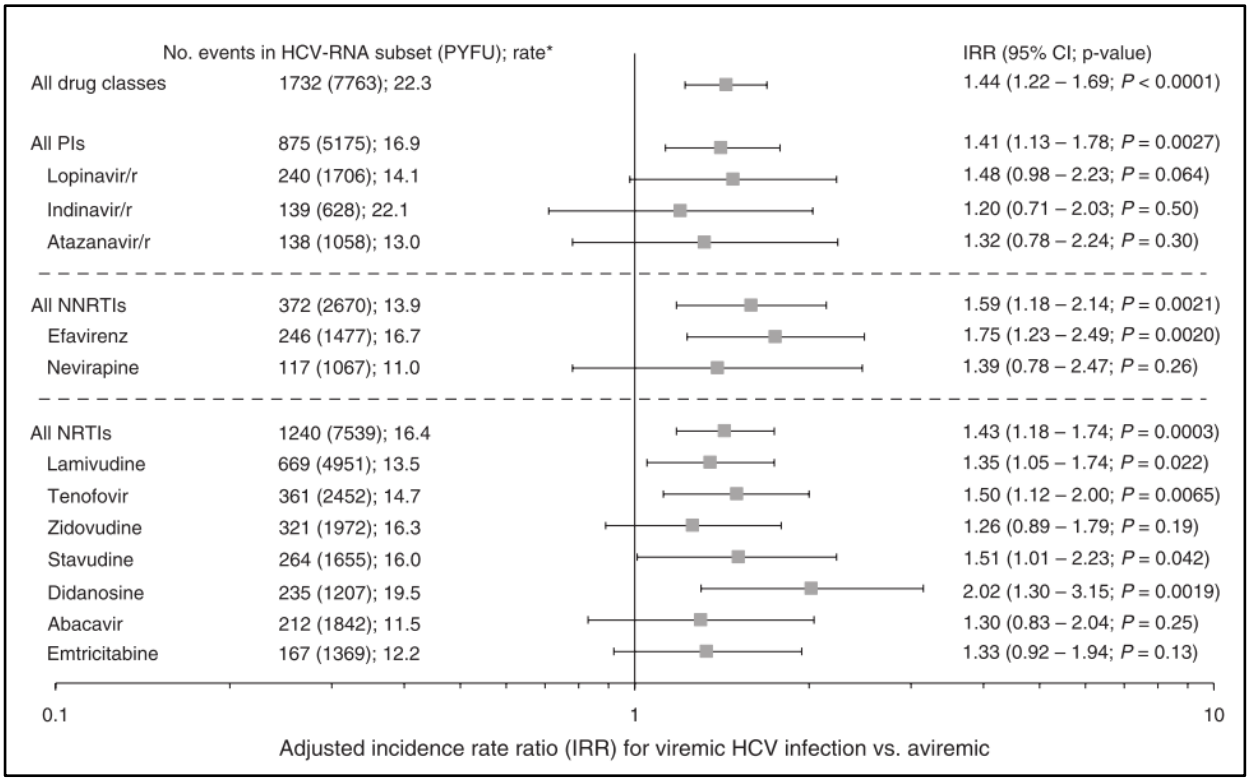
In a more recent analysis of the EuroSIDA data involving HIV/HCV coinfected individuals enrolled between 1999 and 2012, *Grint D et al* expanded the analysis by Mocroft et al by looking at the possible effect of chronic infection in people who

were HCVAb positive [349]. This was done after having filled the gap for HCV-RNA data using stored plasma samples. Discontinuation of cART was defined as stopping at least one of the drugs in the regimen. The study included 9,535 of whom 28.7% (n=2,744) were HIV/HCV coinfected of whom 1904 had HCV-RNA data available. Eighty-one percent (1538/1904) were viremic. The study found an increased risk of 44% of cART drug discontinuation for HCV viremic compared with aviremic individuals (aIRR = 1.44 (95% CI: 1.22 – 1.69) [349]. There was a higher incidence of cART drug discontinuation due to toxicity and patient/physician choice among individuals with viremic HCV coinfection vs. those who had cleared HCV-RNA[349]. In terms of drug classes, NNRTI showed the strongest association with a 59% higher risk of cART discontinuation in viremic individuals compared to aviremic individuals aIRR = 1.59 (95% CI: 1.18 – 2.14) [349].

Specific ARV drugs investigated included the following: (PIs: LPV/r, IDV ATV/r), (NNRTIs: EFV, NVP), (NRTIs: 3TC, TDF, ZDV, d4T, ddI, ABC, FTC). Adjusted rate ratios of stopping associated with being HCV-RNA viremic vs./ aviremic and stratified by specific drugs received obtained from fitting Poisson regression models, are shown in
Figure 5.1[349]. In summary, HCV viremia was associated with a higher incidence of PI discontinuation, aIRR = 1.41 (95% CI: 1.13 – 1.78, p=0.0027). In particular, viremia was associated with 48% higher risk of LPV/r discontinuation which was, however, marginally not significant (aIRR = 1.48 (95% CI: 0.98 – 2.23, p=0.064)[349]. In terms of specific NNRTIs, HCV viremia was linked to EFV discontinuation aIRR = 1.75 (95% CI: 1.23 – 2.49, p=0.0029) [349]. In terms of NRTIs, viremia was linked to discontinuation of a number of drugs were associated; 3TC (aIRR = 1.35 (95% CI: 1.05 – 1.74)), TDF (aIRR = 1.50 (95% CI: 1.12 – 2.00)), d4T (aIRR = 1.51 (95% CI: 1.01 – 2.23) and ddI (aIRR = 2.02 (95% CI: 1.30 – 3.15) [349]. The authors noted that the strong and significant association with chronic HCV infection seen for the risk of stopping 3TC and TDF could be possibly due to their use in combination with anchor drugs associated with hepatotoxicity [349]. For example, EFV was strongly associated with the risk of drug

discontinuation and because it is used in fixed combinations with TDF, this could have explained some of the effect seen for TDF [349].

Figure 5.1 Adjusted incidence rate ratios of specific ARV discontinuation for HCV viremic infection vs. aviremic among HIV-infected individuals receiving cART [349]



Grint D, Peters L, Rockstroh JK, de Wit S, Mitsura VM, Knysz B, et al. Increased incidence of antiretroviral drug discontinuation among patients with viremic hepatitis C virus coinfection and high hyaluronic acid, a marker of liver fibrosis. AIDS (London, England). 2014;28(4):577-87

In contrast, other studies did not find an association between HIV/HCV coinfection and the risk of cART discontinuation [231, 324, 350]. *Hooshyar et al*, estimated the effect of HIV/HCV coinfection on the risk of complete ART discontinuation in cART-naïve HIV-positive individuals [324] enrolled in the study between 2000 and 2003. Discontinuation was defined as stopping all ARV drugs in the regimen for longer than a two week interval. The study included 296 HIV-positive individuals of whom 22% (n=64) were HIV/HCV coinfected. In terms of specific regimens started, most had started regimens containing NNRTIs 40% (n=118) included EFV, followed by PIs 43% (n=127) which included NFV, IDV and LPV/r [324]. They found that the risk of complete cART discontinuation in HIV/HCV coinfected compared to mono-

infected to be somewhat higher: HR = 1.39 (95% CI: 0.95 – 2.03; p = 0.09) but did not reach statistical significance [324]. It is worth noting that in this study, HCV status was determined by HCV antibody testing alone [324].

In an analysis using the SWISS HIV cohort including 1,866 HIV-positive individuals, receiving ART between 2000 and 2005, of whom 17% (n=325) had active HCV infection, cART changes were evaluated 12 months after starting treatment [351]. Changes to treatment was defined as a replacement of at least one drug in the regimen or discontinuation of ≥1 drug for more than 2 weeks or addition of new drugs to an unchanged regimen. In this analysis, the risk of cART discontinuation was not associated with HIV/HCV coinfection aRRR = 1.47 (95% CI: 0.82 – 2.66, p=0.20) adjusted for age, gender, IDU, HBV infection, AIDS diagnosis, CD4, HIV-RNA, NRTI and third drug, [351].

From examining the literature of studies published on the topic, apart from HCV infection and type of cART started, other factors such as younger age, female gender, high HIV-RNA, low CD4, mode of HIV transmission (i.e. MSM or heterosexual vs. PWID), earlier years of initiating cART, longer time spent on cART, were also found to be risk factors for discontinuation/switching of cART drugs, as well as stage of liver disease for which an interaction with use of specific ARV drugs has been also found [230, 352-354]. In the START trial (a randomised trial in 2015 randomising individuals to either deferring cART initiation or immediate start of cART), *Bansi-Matharu et al* assessed factors associated with risk of cART interruption [355]. Of the 3,438 participants who had started cART for the first time 11% (n=378) overall had their treatment interrupted. Factors associated with the risk of cART interruption in both immediate arm and deferred arm were age (older people less like likely to interrupt their cART compared to younger people) and education (individuals with a post-graduate education were less likely to interrupt their cART compared to individuals educated to high-school level) [355]. This PhD chapter does not specifically evaluate the risk of cART discontinuation associated with these other factors besides HCV infection, however some of these variables

were included as potential confounders for the association between HCV and risk of ARV stop in the regression models used.

5.3.4 Limitations of published studies

In some of these studies, the point estimates for the RR indicated an increased risk of cART discontinuation for HIV/HCV coinfected individuals without however reaching statistical significance (e.g. the Swiss cohort study above). However, it is possible that the analyses were not sufficiently powered to detect a significant association and/or that key confounding factors such as lifestyle factors such as alcohol consumption status and mode of HIV transmission were not accounted for. In the analysis presented in this chapter I was particularly concerned about trying to minimise all possible bias due to measured confounding. Additionally, to find the biological effect of HCV, over and above its effect non-adherence.

Another explanation for the discrepancy in results between different studies could be the fact that different definitions of discontinuations were used which means it is not necessarily appropriate to directly compare results across studies. For example, some studies have defined discontinuation based on a time-frame e.g. having stopped a drug for at least one week, or one month. The exact definition of discontinuing a regimen also varies across studies, some focussing only on the anchor drug in the regimen, others counting stopping ≥1 of the drugs in the regimen or in some cases only total interruptions. This could have been dictated by the specific clinical question or by how the data have been collected in first place in the various cohorts. In defining discontinuations, there is also the challenge of dealing with modification of regimens or modification of formulation of the same regimen i.e. from individual drugs to fixed dose combinations. In recent years clinicians often recommend a pro-active switch to newly available combinations with a slightly better toxicity profile (i.e. the switch from TDF to TAF for renal toxicity). Some of these simplifications only amount to a change in formulation with no change in the actual drugs used so these are not normally counted in statistical analyses as events. A few have focused on discontinuation defined as stopping all

or any of the drugs in the regimen. Simplifications continue to be controversial as to whether they constitute discontinuations [347, 349, 351, 356].
For some changes the actual reason for the modification is difficult to classify. In most studies only one reason is reported on the CRFs, however reasons for discontinuation may be multifaceted and various different reasons may be grouped under physician or patient choice.

It is also important to note that there are instances in which the current regimen might not have led to toxicity so severe to require discontinuation so the risk of stopping a drug does not capture the full risk of experiencing adverse events on that drug. Overall, there is some evidence that HCV coinfection may play a role in discontinuation of cART drugs or regimens, although there are conflicting findings on the issue. These conflicting findings could be due to a number of reasons such as sample size, modality of data collection of the reasons for discontinuation (e.g. self-report, data collected retrospectively of prospectively, physician collecting data, etc.) or the definition of the main exposure HCV infection (simply positive for HCVAb or HCV-RNA positive individuals with evidence of chronic infection) or even including HBV-infected in the definition.

Some studies have only included specific drug regimens or regimens that are no-longer used in routine clinical care. Some studies only included specific risk group populations. Another limiting factor when trying to compare the results of these studies included a lack of methods in the statistical analysis to account for informative censoring and this likely to cause some bias.

5.3.5 Summary on literature review

The literature review highlighted that, even in recent years, discontinuation of one or more cART drugs remained relatively common among HIV-positive individuals with or without HCV infection, regardless of the drug specific regimen initiated. Regardless of the mode of data collection for the reason for cART discontinuation and whether data were collected retrospectively or prospectively, there was

agreement that the most common reason for discontinuation in the early cART era was toxicity/intolerance and in some cases immunological/virological failure. Other common reasons that emerged were non-adherence and patient/physician's decision which may be strongly linked to tolerability and toxicity. After the introduction of modern drugs, most HIV cohort studies demonstrated a decrease in discontinuation for toxicity/intolerance due to their improved toxicity profile. Additionally, there was some evidence to suggest that discontinuation for simplification has become more common with these modern regimens. This is due to the high efficacy of some of the new drugs (some showing excellent tolerability and potency profiles even when used in dual combinations in selected population with controlled viremia and at low risk of rebound), as well as convenience (less drugs or lower number of pills/day) and costs [38].

Due to conflicting evidence available at the time of performing this analysis, and the limitations in some previous studies, there was a need to further investigate the impact of HIV/HCV coinfection and HCV viremia on cART discontinuation particularly to look at specific currently used ART drugs. There are several important strengths of this analysis. First, it is based on a heterogeneous cohort, in which the modality of data collection of cART discontinuation has been carefully standardised across the recruiting sites. Second there is a long duration of follow-up spanning over a period from 1997 to 2017, allowing trends over time to be investigated. Third, HIV-positive individuals enrolled in the Icona cohort are routinely tested for both HCVAb and, when HCVAb positive, further tested for HCV-RNA, thus enabling a series of analysis involving both markers. Finally, this chapter uses sophisticated statistical methods to appropriately control for potential informative censoring (i.e. when participants' follow-up is truncated for reasons that are correlated with the outcome of interest).

5.4 Methods

5.4.1 Inclusion criteria of individuals included in the analysis

In this analysis, I evaluated the risk of stopping those specific ARV drugs which, at the time of this analysis in 2016, were recommended by guidelines and frequently used in clinical practice [38].

All HIV-positive individuals enrolled in Icona up to 30th June 2016 who started the specific cART regimens (defined as at least three antiretroviral drugs of any drug class were included) selected in this analysis. Specifically, for each of the ARV drug evaluated, a separate study population was created including everybody in the cohort who started a cART including the drug of interest for the first time (e.g. when studying ABC, I included everybody in the cohort who started an ABC-containing cART regimen for the first time when they were ABC-naïve, etc.). Baseline for each specific drug under evaluation was defined as the date of starting the specific cART regimen. Baseline summaries for the overall population were based on date of starting any cART regimen. As mentioned in section 5.3.1, see list of ARV drugs evaluated in Table 5.1 List of antiretroviral drugs included in the analysis

Table 5.2 Exposure and potential confounders (measured at baseline and time-dependent)Table 5.1 below.
Individuals with HBV infection were excluded from all study populations as HBV also affects the liver and I wanted to focus on the effects of HCV alone.

Table 5.1 List of antiretroviral drugs included in the analysis

Drug class	Antiretroviral drugs
NRTI	Abacavir (ABC), Lamivudine (3TC), Tenofovir (TDF), Emtricitabine (FTC)
NNRTI	Efavirenz (EFA), Rilpivirine (RIL)
PI/r	Lopinavir/r(LPV/r), Darunavir (DRV/r), Atazanavir (ATV/r)
INI	Raltegravir (RAL), Dolutegravir (DLG), Elvitegravir (ELV)

5.4.2 Data

Exposure and potential confounders (based on clinician suggestion and reviewing the literature) considered in this analysis are shown in Table 5.2 Exposure and potential confounders (measured at baseline and time-dependent)

Table 5.3 Number of HIV-positive individuals starting each drug and distribution of regimensTable 5.2. I aimed to establish whether the data carried evidence to support the hypothesis of a biological effect of HCV infection on the risk of cART discontinuation after accounting for possible sources of measured confounding. In the main analysis, all variables were fitted as time-fixed at baseline (defined as date of starting the drug of interest) including CD4, alcohol consumption and HCVAb status. In a sensitivity analyses, HCVAb, CD4 and HIV-RNA were fitted as time-dependent covariates using inverse probability of weights in order to control for time dependent confounding.

Table 5.4 Exposure and potential confounders (measured at baseline and time-dependent)

Factors	Name of Variables	Classification
Exposures	HCVAb status¥	HCVAb+, HCVAb-, HCVAb not tested
	HCVAb+/ HCV-RNA status	HCV-RNA+, HCRNA-, HCV-RNA not tested
Demographics	Gender	Male, Female
	Age (years)	Continuous (age per 10 years older)

Factors	Name of Variables	Classification
	Mode of HIV Transmission	PWID, MSM, Heterosexual, Other/unknown
	Nationality	Italian, non-Italian
	Calendar year of starting cART	Continuous (per year longer
	Recruiting site	North, South, Centre
Metabolic factors	Diabetes	No, Yes
	BMI kg/m^2	≤25, 25-30, >30, unknown
HIV-related factors	AIDS diagnosis	No, Yes
	CD4 cell count¥	≤200, 201-350, >350, unknown
	HIV-RNA¥	≤1000, 1001-10000, >10000, unknown
Previous drug history	Prior cART (i.e. prior to starting the specific drug in question	No, Yes
	Concomitant use of cART drugs	e.g. TDF/FTC, ABC/3TC (see specific footnotes in results tables of Poisson models)
Liver related factors	FIB-4 score¥	≤3.25, >3.25
	Alcohol use¥	Abstainer, moderate, hazardous, unknown
Concomitant cause of hepatoxicity*	Concomitant use of non HIV/HCV drugs	No, Yes

¥ Time-dependent in the sensitivity analyses
*All co-medications which have been previously shown to have potential hepatotoxicity (www.livertox.nih.gov).

5.4.3 Exposures and outcomes

Exposures

The main exposure for this analysis was HCVAb infection status at baseline classified as HCVAb negative (comparator), HCVAb positive and HCVAb not tested. HCVAb status was determined using all serology test results prior to cART as well as those recorded up to three months after cART initiation to minimize the frequency of people not tested for HCVAb.

In addition, I further stratified individuals who were HCVAb positive as follows; HCVAb+/HCV-RNA positive (i.e. the chronically infected), HCVAb+/HCV-RNA negative (the spontaneously cleared or cured) and HCVAb+/HCV-RNA not tested (still using HCVAb negative as the comparator group).

Main outcome

The main outcome in this analysis was specific ARV drug discontinuation. In the Icona database, when the participant discontinues a drug, regardless of whether they are switching or not, the treating physician reports reasons for the ARV drug discontinuation. At the time of this analysis, these were classified as follows: viral/immunological failure, toxicity/intolerability, simplification, non-adherence, other or unknown (i.e. reason for stopping not recorded).

A further categorisation of the reasons for stopping due to toxicity/intolerability included; liver, kidney, gastrointestinal tract, cardiovascular, central nervous system/peripheral nervous system (CNS/PNS), metabolism, haematology or other/unspecified toxicity.

The date of ARV drug discontinuation was defined as the first time in which the drug under evaluation was discontinued and the reason for stopping was defined using the main reason reported by the treating physicians in the eCRFs. Secondary reported reasons for stopping the drugs are also present in the database but have not been used in this analysis.

Stopping of a drug was counted as an event only when there was no date of re-starting the same drug within one month of the date of stopping. This was also true for all fixed dose combination regimens. For example, suppose ATRIPLA (FTC/TDF/EFV) was stopped and FTC/TDF was re-started within a month of the date of stopping, then this episode would count as a stop of EFV but not of TDF or FTC. Switch to fixed-dose combinations using exactly the same ARVs (e.g. was previously on Truvada-(TDF/FTC) + EFV and switched to Atripla- TDF/FTC/EFV), dosage adjustment and structured treatment interruptions were not counted as discontinuations.

5.4.4 Statistical analyses

Separate analyses were carried out for each ARV drug with time zero defined as the date of starting the cART combination containing the drug under evaluation when the included person was still naïve to this drug (i.e. baseline date and study

population was different in each of the specific drug analyses). However, for the general description of the cohort, I used a single study population of all individuals who were included in at least one of the specific drug analyses. Baseline date for this overall analysis was defined as date of starting any ARV drug under evaluation. Participants' characteristics at baseline were determined using all the information collected within three months prior and up to the date of cART initiation. Participant characteristics at baseline were stratified by HCVAb infection status at baseline.

For descriptive purposes, overall frequency (percentage) of each ARV drug discontinuation was calculated as well as the breakdown of reasons for discontinuation, with special focus on those due to toxicity/intolerance.
In the main analysis, an individual's follow-up time accrued from the date of starting the cART regimen containing the ARV drug under evaluation to the date of stopping this drug regardless of the reason (viral/immunological failure, toxicity/intolerability, simplification, non-adherence, other or unknown) or to the date last seen in the clinic or the date of death.

Incidence Rate (IR) of discontinuation for each ARV drug was expressed per 100 person-years of follow-up (PYFU) and 95% CIs. Note that an individual could contribute person-years and events to more than one ARV drug/model. For example, individuals who started the combination TDF/FTC/DTG contributed to three separate analyses, one for each of the drugs in this combination. The calculation of unadjusted incidence rates and rate ratios were obtained using the IRI command in STATA and PROC GENMOD in SAS respectively[357].

Primary analysis

For the primary analysis with HCVAb status at baseline as the exposure variable I fitted multivariable Poisson regression models sequentially by adding at various steps a number of identified potential confounders (all time fixed factors measured at baseline).

Secondary analysis

The secondary analysis was identical to the primary analysis but with HCVAb+/HCV-RNA status at baseline as the main exposure variable instead of using the serology test results alone.

Sensitivity analysis

In a sensitivity analysis, instead of including all discontinuations, regardless of the reason, I included discontinuations for all reasons except those for simplification or viral/immunological failure. The rationale behind this outcome modification was to restrict the analysis to discontinuation of events solely due to toxicity. Of note, stops due to other reasons such as patients or clinician choice were still counted as events as it was felt likely that the true underlying reason for the majority of discontinuations due to 'patients/physician choice' were likely to be related to intolerance/toxicity. Of note when using this alternative endpoint, the assumption that stopping for failure or simplification is independent of the risk of stopping due to all other reasons is unlikely to hold. Therefore, Inverse Probability Weighting (IPW) was used to control for informative censoring due to stopping for these reasons. This analysis used HCVAb status as a time-dependent exposure variable and also included time varying covariates in the model (CD4, HIV-RNA) as additional confounders to control for. As mentioned in chapter 2, section 2.7, it is ideal when possible to account for all possible sources of bias, including time dependent confounders.

As a post hoc analysis, upon observing a large imbalance of calendar year of starting Lamivudine-based cART by HCVAb status, I restricted the analysis to individuals initiating Lamivudine after 2002. Similar patterns were observed also for other ARV drugs but the sensitivity analyses were restricted to Lamivudine because it was the drug with the most discontinuations.

.

Model building strategy for all analyses

For each ARV drug considered, separate multivariable Poisson regression models were fitted to assess the impact of HCV infection status on risk of ARV drug

discontinuation due to any reasons (and in the sensitivity analysis with outcome stopping due to all reasons except viral or immunological failure/simplification). In order to ensure statistical power and narrow confidence intervals for the adjusted rate ratios, multivariable models were fitted only for drugs with >100 individuals discontinuing. For drugs with <100 individuals discontinuing, univariable results need to be interpreted with caution and in some cases, estimates were unobtainable or there was great uncertainty around the estimates.
As in chapter 4, I used a manual sequential strategy to build the multivariable Poisson regression model. The steps of this sequential adjustment are described below (see Table 5.2 for variable definitions and categorisation):

Unadjusted: Exposure variable only (HCVAb or HCVAb/HCV-RNA status)
Model 1: Exposure variable + Demographics + metabolic factors (BMI, diabetes)
Model 2*: Model 1 + HIV-related factors + previous cART use + concomitant ART use
Model 3: Model 2 + liver disease related factors
*Included time-dependent variables in the sensitivity analysis

For the main analysis, baseline values were used for all factors. For the sensitivity analysis; HCV status, FIB-4, alcohol use, CD4 and HIV-RNA were used as time updated factors.
Note, model 3 included FIB-4 which is a factor that might be on the causal pathway between exposure and outcome.

Inverse probability weighting method used in the sensitivity analyses
In this alternative analysis, I assessed the effect associated with current HCV status which might differ from that recorded prior to starting cART used in the main analysis. Also in the main analysis assessing the association between HCV infection and risk of cART discontinuation, confounding was controlled for by regression adjustment. However, standard statistical methods may not be able to appropriately adjust for confounding and selection bias when there are measured time-varying confounders (CD4, alcohol status, FIB-4 and concomitant cause of

hepatoxicity) affected by past exposure status [358, 359]. A method which correctly adjusts for confounding in this situation employs weighting schemes such as standardization or inverse probability weighting (IPW). IPW consists in building a logistic regression model to obtain an estimate of the probability of the exposure observed for an individual given an individuals' past history of other covariates and using this probability as a weight in the subsequent analyses. The inverse of this predicted probability is what is used to control for confounding or to account for loss to follow-up (known as selection bias) to estimate causal effects of the exposure on outcome. In this analysis, both informative censoring and time-varying confounders affected by prior exposure (e.g. CD4 which may affect the chance of acquiring HCV infection and, in turn, be modified by HCV infection) were identified and IPW used to appropriately control for both potential source of bias [239, 360] [361]

The outcome of this analysis was stopping due to all reasons except simplification or failure so that stops due to simplification/failure are the censoring events. In this analysis, a logistic censoring model (modelling the probability of events not happening, i.e. to remain uncensored) was fitted to obtain stabilized weights. One key step in this process is to check that the weights have a mean of one and to verify that there is not an issue with positivity (i.e. very large weights) [360, 361]

The goal is to create inverse probability weights for each person at each follow-up time point which are proportional to the person's probability of receiving her own exposure history (e.g. time-fixed covariates, current CD4). The assumption is that all predictors of censoring are known and measured. A pseudo-population is created in which the observation for each participant is repeated by a specific number of replicates equal to the inverse of the weight. In the pseudo-population there is no longer selection bias due to confounding or censoring. These weights are obtained by calculating probabilities (of not experiencing the event, i.e. not discontinuing) from the first logistic model which includes baseline covariates. These probabilities serve as the numerator of the weights while other probabilities obtained from fitting another logistic model which additionally includes time-

dependent covariates serve as the denominator. The final weight is then obtained by the ratio numerator/denominator.

These standardised weights are finally incorporated into the Poisson regression models to produce the weighted estimates. The following steps below detail the process to construct the censoring weights (the process to control for confounding is similar and not shown here) [239].

Step one

A logistic model was fitted to model the probability that participants remain uncensored at each time point independent of baseline covariates included in the primary analysis alone (i.e. variables included in model 3). In this case, the censoring process was determined by stopping a drug for reasons not related to intolerance/toxicity. Thus the binary outcome variable in the logistic model was set so that it took values 1 = discontinued due to failure/simplification, 0 = did not discontinue due to failure/simplification (includes all other reasons) and the probability of 1 is modelled. The probabilities obtained would be the estimates for the numerator of the censoring weights.

Step two

Another logistic model was fitted to model the probability that individuals remain uncensored at each time point independent of both baseline covariates and adjusted for time-dependent covariates (in this case, CD4 and HIV-RNA). The outcome in the logistic model again was set so that it took values 1 = discontinued due to failure/simplification, 0 = did not discontinue due to failure/simplification (included other reasons). The probabilities obtained would be the estimates for the denominator of the censoring weights.

Step three

To obtain the censoring probabilities at the current follow-up visit, the probability of remaining uncensored given the individual was uncensored at the previous time point are multiplied for each time point. This was done for both the numerator (step one) and the denominator (step two) model. Thus the final weight was the inverse

probability that the individual remains under follow-up in the analysis i.e. the ratio numerator/denominator.

Step four

In case of positivity, to exclude extreme values, truncation of weights at the 1st and 99th percentiles and a check of the mean (SD) of the final distribution of weights was performed.

Step five

A weighted Poisson regression model was fitted instead of the standard model adjusting for covariates. Of note, when constructing the weights it was possible to include one or more potential confounders at the time and follow the sequential steps used in the standard unweighted approach. The results obtained from these Poisson models are termed as weighted estimates and are unaffected by potential selection bias induced by non-informative censoring.

5.5 Results

5.5.1 Individuals included in the ARV drug analysis

As of 30th June 2016, the Icona cohort had enrolled 14,532 participants of whom 3,895 were excluded from this analysis because they either: i) had not started one of the drugs under evaluation, or ii) were still cART-naïve at the time of the analysis or iii) were coinfected with HBV. Therefore, this analysis included the remaining 10,637 HIV-positive individuals who initiated cART including at least one of the drugs under evaluation. Table 5.3 Number of HIV-positive individuals starting each drug and distribution of regimens

Table 5.4 Baseline characteristics when starting cART by HCVAb statusTable 5.3 shows the frequency of availability of data in each of the analysis involving specific drugs. Most individuals had initiated either TDF or FTC based regimens (n=7717 and n=7302 respectively) and among the PI/rs, DRV/r and ATV/r (n=2120 and

n=2206 respectively) were the most common third drugs in the regimen initiated. Among the INIs; RAL (n=1134) was the most common.

Table 5.7 Number of HIV-positive individuals starting each drug and distribution of regimens

	N (*%)
NRTIs	
Abacavir (ABC)	2363
ABC/3TC/DLG	204 (8.6)
ABC/3TC/EFV	277(11.7)
ABC/3TC/LOP/r	152 (6.4)
ABC/3TC/NVP	151 (6.4)
ABC/3TC/DRV/r	268 (11.3)
ABC/3TC/ATZ/r	304(12.8)
ABC/3TC/ZDV	434 (18.4)
Lamivudine (3TC)	5207
3TC/ABC/EFV	337 (6.5)
3TC/ABC/IND	610 (11.7)
3TC/ZDV/LOP/r	350 (6.7)
3TC/ZDV/NVP	418(8.0)
Tenofovir (TDF)	7717
TDF/FTC/EFA	1648 (21)
TDF/FTC/ELV	484 (6.3)
TDF/FTC/LOP/r	568 (7.4)
TDF/FTC/RIL	955 (12.4)
TDF/FTC/DRV/r	981 (12.7)
TDF/FTC/ATV/r	1069 (13.9)
Emtricitabine (FTC)	7302
FTC/TDF/EFA	1793 (24.6)
FTC/TDF/ELV	486 (6.7)
FTC/TDF/LOP/r	623 (8.5)
FTC/TDF/RIL	965 (13.2)
FTC/TDF/DRV/r	991 (13.6)
FTC/TDF/ATV/r	1203 (16.5)

	N (*%)
NNRTIs	
Efavirenz (EFA)	3142
TDF/FTC/EFA	1693 (53.8)
3TC/ZDV/EFA	542 (17.3)
Rilpivirine (RIL)	1803
3TC/ABC/RIL	104 (5.8)
TDF/FTC/RIL	1647 (91.4)
PIs	
Lopinavir/r (LPV/r)	1612
ABC/3TC/LOP/r	99 (6.1)
3TC/TDF/LOP/r	94 (5.8)
TDF/FTC/LOP/r	567 (35.2)
3TC/ZDV/LOP/r	466 (28.9)
Darunavir/r (DRV/r)	2120
ABC/3TC/DRV/r	269 (12.7)
DRV/r/RAL	143 (6.8)
TDF/FTC/DRV/r	1255 (59.2)
TDF/FTC/DRV/r/RAL	128 (6.0)
Atazanavir/r (ATV/r)	2206
3TC/ABC/ATV/r	269 (12.1)
3TC/TDF/ATV/r	129 (5.8)
TDF/FTC/ATV/r	1373 (62.2)
INIs	
Raltegravir (RAL)	1134
3TC/ABC/RAL	100 (8.8)
DRV/r/RAL	165 (14.5)
TDF/FTC/RAL	433 (38.8)
TDF/FTC/DRV/r/RAL	139 (12.3)
Dolutegravir (DLG)	652
3TC/ABC/DOL	263 (40.0)
TDF/FTC/DOL	284 (43.6)
Elvitegravir (ELV)	774
TDF/FTC/ELV	748 (96.6)

*The percentages relate to the proportion of individuals initiating the regimen.

5.5.2 Baseline characteristics

Table 5.4 Baseline characteristics when starting cART by HCVAb status

Table 5.5 Calendar year of starting any cART by HCVAb statusTable 5.4 shows baseline characteristics of the 10,637 participants included in this analysis stratified by HCV infection status at time of starting cART. The majority of individuals were male 76% (n=8,044), median age [IQR] 38 [32-46]. In most cases, mode of HIV transmission was through heterosexual contact 40% (n=4,263) or MSM 36% (n=3,773). However, as expected, among HCVAb positive individuals, as shown in chapter 3, PWID was the most common mode of HIV transmission 69.3% (714/1030) compared to HCVAb negative 2.4% (112/4633). HIV/HCV coinfected individuals started cART in earlier calendar periods compared to HIV mono-infected: median [IQR] year of starting 2001 [1998 – 2011] and 2012 [2007 – 2014]. Table 5.5 Calendar year of starting any cART by HCVAb status

Table 5.6 Number of individuals on cART for each drug and frequency of drug discontinuations by main reason for discontinuationTable 5.5 shows the distribution of calendar year of initiating cART stratified by HCV infection status. Forty-eight percent of HCVAb positive individuals started prior to the year 2000 compared to 14% and 20% of HCVAb negative and unknown, respectively, confirming that calendar year of starting is a major potential confounder in the association between HCV status and discontinuation. As discussed in chapter 3, this calendar time distribution of HCV is likely explained by the reduction over time in people acquiring HIV through PWID in Italy. In order to try to control for this imbalance, calendar year was adjusted for in the multivariable analyses as a continuous variable. Also the analysis involving 3TC, was restricted to individuals starting cART after 2002 and results were similar – See Table 5.15 Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification (ONLY FOR LAMIVUDINE)

Table 5.16 Association of HCV status on discontinuation of PIs and INIs: Poisson regression models stratified by HCVAb status (time-dependent) – Stopping for all reasons except stopping for failure/simplificationTable 5.15.

Overall, median [IQR] CD4 at cART initiation was 315 [170-459] cells/mm^3 and median [IQR] log_{10} HIV-RNA was 4.6 [3.9 - 5.2] copies/ml. Baseline median values of HIV-related factors (i.e. CD4 and HIV-RNA) were similar by HCV infection status. There were some differences in terms of socio-demographics, HCVAb positive individuals were more likely to have lower levels of education and more likely to be unemployed Table 5.4 Baseline characteristics when starting cART by HCVAb status

Table 5.5 Calendar year of starting any cART by HCVAb statusTable 5.4.

Table 5.10 Baseline characteristics when starting cART by HCVAb status

	HCVAb negative N=4633	**HCVAb positive N=1030**	**HCVAb unknown N=4974**	**Total N=10637**
Gender, n(%)				
Female	1091 (23.5)	258 (25.0)	1244 (25.0)	2593 (24.4)
Male	3542 (76.5)	772(75.0)	3730(75.0)	8044(75.6)
Age (years)				
Median (IQR)	38 (31, 47)	38 (34, 44)	39 (33, 45)	38 (32, 46)
Region, n(%)				
North	2343 (50.6)	543 (52.7)	2841 (57.1)	5727 (53.8)
South	557 (12.0)	191 (18.5)	613 (12.3)	1361 (12.8)
Center	1733 (37.4)	296 (28.7)	1520 (30.6)	3549 (33.4)
Nationality, n(%)				
Italian	3659 (79.0)	944 (91.7)	4352 (87.5)	8955 (84.2)
Non-Italian	974 (21.0)	86 (8.3)	622 (12.5)	1682 (15.8)
Mode of HIV transmission, n(%)				
Heterosexual	2211 (47.7)	173 (16.8)	1879 (37.8)	4263 (40.1)
MSM	1933 (41.7)	107 (10.4)	1733 (34.8)	3773 (35.5)
PWID	112 (2.4)	714 (69.3)	1011 (20.3)	1837 (17.3)
Other/unknown	377 (8.1)	36 (3.5)	351 (7.1)	764 (7.2)

	HCVAb negative N=4633	HCVAb positive N=1030	HCVAb unknown N=4974	Total N=10637
Year starting cART				
Median (IQR)	2012 (2007, 2014)	2001 (1998, 2011)	2010 (2002, 2013)	2011 (2002, 2013)
Education, n(%)				
Primary school (<11)	1350 (29.1)	345 (33.5)	1601 (32.2)	3296 (31.0)
Middle school (11-16)	366 (7.9)	104 (10.1)	318 (6.4)	788 (7.4)
Secondary school (16-18)	978 (21.1)	373 (36.2)	1215 (24.4)	2566 (24.1)
University (18+)	1459 (31.5)	181 (17.6)	1384 (27.8)	3024 (28.4)
Other/unknown	480 (10.4)	27 (2.6)	456 (9.2)	963 (9.1)
Employment, n(%)				
Employed	2826 (61.0)	563 (54.7)	3143 (63.2)	6532 (61.4)
Unemployed	562 (12.1)	313 (30.4)	728 (14.6)	1603 (15.1)
Other	482 (10.4)	72 (7.0)	436 (8.8)	990 (9.3)
Unknown	763 (16.5)	82 (8.0)	667 (13.4)	1512 (14.2)
Previous AIDS diagnosis, n(%)				
Yes	891 (19.2)	244 (23.7)	617 (12.4)	1752 (16.5)
CD4 cell count, cells/mm^3				
Median (IQR)	269 (110, 423)	243 (110, 382)	361 (250, 504)	315 (170, 459)
≤200	1657 (35.8)	427 (41.5)	782 (15.7)	2866 (26.9)
201-350	1063 (22.9)	265 (25.7)	1367 (27.5)	2695 (25.3)
>350	1466 (31.6)	295 (28.6)	2403 (48.3)	4164 (39.1)
Unknown	447 (9.6)	43 (4.2)	422 (8.5)	912 (8.6)
HIV-RNA (log10 copies/ml)				
Median (IQR)	4.84 (4.15, 5.40)	4.79 (4.08, 5.30)	4.42 (3.58, 5.00)	4.64 (3.87, 5.19)
≤1000	346 (7.5)	94 (9.1)	740 (14.9)	1180 (11.1)
1001-10000	528 (11.4)	128 (12.4)	840 (16.9)	1496 (14.1)
>10000	3227 (69.7)	748 (72.6)	2927 (58.8)	6902 (64.9)
Unknown	532 (11.5)	60 (5.8)	467 (9.4)	1059 (10.0)
Alcohol consumption, n(%)				
Abstain	1682 (36.3)	257 (25.0)	1465 (29.5)	3404 (32.0)
Moderate	1027 (22.2)	114 (11.1)	1129 (22.7)	2270 (21.3)
Hazardous	273 (5.9)	79 (7.7)	344 (6.9)	696 (6.5)

	HCVAb negative N=4633	HCVAb positive N=1030	HCVAb unknown N=4974	Total N=10637
Unknown	1651 (35.6)	580 (56.3)	2036 (40.9)	4267 (40.1)

Table 5.13 Calendar year of starting any cART by HCVAb status

Year	HCVAb negative		HCVAb positive		HCVAb unknown	
	N=4633	%	N=1030	%	N=4974	%
1997	172	3.7	195	18.9	98	2.0
1998	223	4.8	169	16.4	297	6.0
1999	65	1.4	58	5.6	276	5.6
2000	200	4.3	74	7.2	281	5.7
2001	61	1.3	40	3.9	178	3.6
2002	121	2.6	43	4.2	178	3.6
2003	99	2.1	28	2.7	192	3.9
2004	81	1.8	27	2.6	185	3.7
2005	60	1.3	21	2.0	143	2.9
2006	69	1.5	24	2.3	134	2.7
2007	80	1.7	8	0.8	132	2.6
2008	121	2.6	19	1.8	156	3.1
2009	189	4.1	22	2.1	192	3.9
2010	235	5.1	28	2.7	270	5.4
2011	483	10.4	54	5.2	325	6.5
2012	493	10.6	57	5.5	410	8.2
2013	435	9.4	58	5.6	444	8.9
2014	541	11.7	41	4.0	451	9.1
2015	626	13.5	49	4.8	462	9.3
2016	279	6.0	15	1.5	170	3.4

5.5.3 Reasons for cART discontinuation

There were 15,464 drug discontinuations among the 10,637 individuals included in this analysis. Table 5.6 Number of individuals on cART for each drug and

frequency of drug discontinuations by main reason for discontinuation

Table 5.7 Toxicity/intolerability reasons for discontinuation by drugTable 5.6 shows the frequency distribution of reasons of ARV drug discontinuation; this was the breakdown: viral/immunological failure (6%), toxicity/intolerability (19%), simplification (22%), non-adherence (11%), other (24%) and unknown (18%). In this analysis, the ‘other’ category was the most common reason, recorded in about a quarter of cases. Consistently with other recent analyses of the database and those of other cohorts, stopping for simplification was the next most common reason for discontinuation, especially in recent years. Looking at specific drugs, for TDF, FTC, EFV, DRV/r, RAL and DTG based regimens, the most common reason for cART discontinuation was still simplification. In contrast, for ABC, 3TC, RPV, LPV/r, ATV/r and EVG-based regimen intolerance/toxicity was the most frequently observed reason for stopping. Further exploratory analyses on the specific reasons for cART discontinuation due to toxicity/intolerability are shown in for each drug**.** In general, discontinuations due to toxicity were mostly due to liver, kidney, gastrointestinal or metabolism toxicity, however the frequencies of total discontinuation is small for some of the drugs. Although very small number, CNS was the most common reason of discontinuation for DTG (Table 5.7 Toxicity/intolerability reasons for discontinuation by drug

Table 5.8a Summary of number of events and incidence rate and incidence rate ratios of cART discontinuation of at least one drug by drug class for potential confoundersTable 5.7).

Table 5.16 Number of individuals on cART for each drug and frequency of drug discontinuations by main reason for discontinuation

Drug	N	Total discontinuations	Viral/ immunological failure	Toxicity/ intolerability	Simplification	Non-adherence	Other	Unknown
		15464	853 (%)	2979(%)	3401(%)	1720(%)	3775(%)	2736(%)
NRTIs								
Abacavir	2363	821	71 (8.6)	170 (20.7)	124 (15.1)	142 (17.3)	162 (19.7)	152 (18.5)
Lamivudine	5207	2930	266 (9.1)	585 (20.0)	390 (13.3)	575 (19.6)	783 (26.7)	331 (11.3)
Tenofovir	7717	3339	127 (3.8)	632 (18.9)	856 (25.6)	277 (8.3)	879 (26.3)	568 (17.0)
Emtricitabine	7302	2966	90 (3.0)	557 (18.8)	810 (27.3)	223 (7.5)	765 (25.8)	521 (17.6)
NNRTIs								
Efavirenz	3142	1928	91 (4.7)	331 (17.2)	446 (23.1)	174 (9.0)	577 (29.9)	309 (16.0)
Rilpivirine	1803	164	2 (1.2)	29 (17.7)	20 (12.2)	8 (4.9)	23 (14.0)	82 (50.0)
PI/rs								
Lopinavir/r	1612	1104	87 (7.9)	208 (18.8)	167 (15.1)	130 (11.8)	208 (18.8)	304 (27.5)
Darunavir/r	2120	665	34 (5.1)	111 (16.7)	229 (34.4)	46 (6.9)	117 (17.6)	128 (19.2)
Atazanavir/r	2206	999	50 (5.0)	263 (26.3)	190 (19.0)	108 (10.8)	168 16.8)	220 (22.0)
INIs								
Raltegravir	1134	436	28 (6.4)	72 (16.5)	141 (32.3)	30 (6.9)	68 (15.6)	97 (22.2)
Dolutegravir	652	70	7 (10.0)	10 (14.3)	25 (35.7)	4 (5.7)	14 (20.0)	10 (14.3)
Elvitegravir	774	42	-	11 (26.2)	3 (7.1)	3 (7.1)	11 (26.2)	14 (33.3)

Table 5.19 Toxicity/intolerability reasons for discontinuation by drug

Abacavir (N = 2363)	**n (%)**	**Lamivudine (N = 5207)**	**n (%)**	**Tenofovir (N = 7717)**	**n (%)**
Total number of discontinuations	821	Total number of discontinuations	2930	Total number of discontinuations	3339
Other	651	Other	2345	Other	2707
Toxicity	170	Toxicity	585	Toxicity	632
Liver	19 (11)	Liver	53 (9)	Liver	54 (8.5)
Kidney	11 (7)	Kidney	25 (4)	Kidney	125 (20)
Gastrointestinal tract	39 (23)	Gastrointestinal tract	132 (23)	Gastrointestinal tract	95 (15)
Cardiovascular	0 (0)	Cardiovascular	0 (0)	Cardiovascular	1 (0.3)
CNS/PNS	15 (9)	CNS/PNS	34 (6)	CNS/PNS	84 (13)
Metabolism	31 (18)	Metabolism	123 (21)	Metabolism	128 (20)
Hematology	6 (3)	Hematology	102 (17)	Hematology	1 (0.2)
Other/Unspecified	49 (29)	Other/Unspecified	114 (20)	Other/Unspecified	143 (23)
Emtricitabine (N = 7302)	**n (%)**	**Efavirenz (N = 3142)**	**n (%)**	**Rilpivirine (N = 1803)**	**n (%)**
Total number of discontinuations	2966	Total number of discontinuations	1928	Total number of discontinuations	164
Other	2409	Other	1597	Other	135
Toxicity	557	Toxicity	331	Toxicity	29
Liver	39 (7)	Liver	21 (6)	Liver	3 (10)
Kidney	111 (20)	Kidney	19 (6)	Kidney	2 (7)
Gastrointestinal tract	89 (16)	Gastrointestinal tract	45 (14)	Gastrointestinal tract	5 (17)
Cardiovascular	2 (0.4)	Cardiovascular	0 (0)	Cardiovascular	0 (0)
CNS/PNS	76 (14)	CNS/PNS	92 (28)	CNS/PNS	3 (10)
Metabolism	115 (20)	Metabolism	68 (20)	Metabolism	7 (24)
Haematology	2 (0.4)	Haematology	10 (3)	Haematology	1 (3)
Other/Unspecified	123 (22.2)	Other/Unspecified	76 (23)	Other/Unspecified	8 (29)
Lopinavir/r (N = 1612)	**n (%)**	**Darunavir/r (N = 2120)**	**n (%)**	**Atazanavir/r (N = 2206)**	**n (%)**
Total number of discontinuations	1104	Total number of discontinuations	665	Total number of discontinuations	999
Other	896	Other	554	Other	736
Toxicity	208	Toxicity	111	Toxicity	263
Liver	23 (11)	Liver	6 (5)	Liver	37 (14)
Kidney	25 (12)	Kidney	8 (7)	Kidney	32 (12)
Gastrointestinal tract	48 (23)	Gastrointestinal tract	39 (35)	Gastrointestinal tract	66 (25)
Cardiovascular	0 (0)	Cardiovascular	2 (2)	Cardiovascular	0 (0)
CNS/PNS	15 (7)	CNS/PNS	5 (4)	CNS/PNS	12 (4)
Metabolism	39 (19)	Metabolism	28 (25)	Metabolism	39 (15)
Haematology	13 (6)	Haematology	4 (3)	Haematology	3 (1)
Other/Unspecified	45 (22)	Other/Unspecified	19 (17)	Other/Unspecified	74 (28)
Raltegravir (N = 1134)	**(%)**	**Dolutegravir (N = 652)**	**(%)**	**Elvitegravir (N = 774)**	**(%)**
Total number of discontinuations	436	Total number of discontinuations	70	Total number of discontinuations	42
Other	364	Other	50	Other	31
Toxicity	72	Toxicity	10	Toxicity	11
Liver	11 (16)	Liver	0 (0)	Liver	1 (9)
Kidney	5 (7)	Kidney	3 (30)	Kidney	2 (18)
Gastrointestinal tract	19 (26)	Gastrointestinal tract	1 (10)	Gastrointestinal tract	5 (45)
Cardiovascular	1 (1)	Cardiovascular	0 (0)	Cardiovascular	0 (0)
CNS/PNS	4 (6)	CNS/PNS	4 (40)	CNS/PNS	2 (18)
Metabolism	15 (21)	Metabolism	1 (10)	Metabolism	0 (0)
Haematology	1 (1)	Haematology	0 (0)	Haematology	0 (0)
Other/Unspecified	16 (22)	Other/Unspecified	1 (10)	Other/Unspecified	1 (9)

5.5.4 Incidence rates of cART discontinuation by HCVAb infection status

Table 5.8a summarizes incidence rate and incidence rate ratios of cART discontinuation of at least one drug and for potential confounders. All individuals included in the analysis, contributed a total of 82,416 PYFU corresponding to an overall incidence rate (95% CI) 18.8 (18.5 – 19.1) per 100 PYFU.

Overall, rates of cART discontinuation were similar across HCV infection antibody tested groups, slightly lower in people for which test results were not available (HCVAb unknown). Among the HCVAb positive individuals there were 1,046 discontinuations in 4,816 PYFU corresponding to an incidence rate of 21.7 (20.4 - 23.1) cases per 100 PYFU. For the HCVAb negative, there were 5,574 discontinuations in 25,982 PYFU corresponding to a similar rate of 21.5 (20.9 - 22.0) and for HCVAb unknown there were 8,844 discontinuations in 51,617 PYFU corresponding to a lower rate of 17.1 (16.8 - 17.5) cases per 100 PYFU (Table 5.8b).

Individuals who were older age vs. younger age, having diabetes vs. not, AIDS diagnosis vs. not, advanced liver disease vs. mild/moderate liver disease and unknown HIV-RNA vs. HIV-RNA <1000 were more likely to discontinue at least one cART drug. In contrast, individuals residing in south vs. north and CD4>350 vs CD4≤200 were less likely to discontinue cART (Table 5.8a).

When restricting to episodes of NNRTIs-based cART, there were 2,092 discontinuations in 11,010 PYFU for an overall rate of 19.0 (18.2 – 19.8) per 100 PYFU. In this subgroup, incidence rates of cART discontinuation were lower for HIV/HCV coinfected people compared with HIV mono-infected (18.1 (16.0 – 23.6) vs. 24.8 (23.2 – 26.5); p=0.02). For all other analyses restricted to a specific drug class (NRTI, PI and INSTI) rates of cART discontinuations across HCV infection groups were similar to those estimated overall. It is worth noting that these are

crude comparisons and they may be affected by confounding bias due to factors such as calendar year of starting (Table 5.8b).

Table 5.22a Summary of number of events and incidence rate and incidence rate ratios of cART discontinuation of at least one drug by drug class for potential confounders

	No.Events (PYFU)	Rate/ 100 PYFU (95% CI)	IRR (95% CI)	p-value
ALL drugs				
Overall	15464 (82415.9)	18.76 (18.47, 19.06)	-	-
HCV infection				
HCVAb positive	5574 (25982.1)	21.45 (20.89, 22.02)	1.00	
HCVAb negative	1046 (4816.44)	21.71 (20.42, 23.07)	1.14 (0.99, 1.32)	0.079
HCVAb unknown	8844 (51617.4)	17.13 (16.77, 17.49)	0.99 (0.82, 1.20)	0.918
Age				
18-35	383 (6968.3)	5.50 (4.97, 6.07)	1.00	
>35	770 (11216.6)	6.86 (6.40, 7.37)	1.25 (1.10, 1.41)	<0.001
Gender				
Male	876 (13730.2)	6.38 (5.97, 6.82	1.00	
Female	277 (4455.1)	6.27 (5.53, 6.99)	0.98 (0.85, 1.12)	0.761
Region				
North	568 (8470.7)	6.71 (6.18, 7.28)	1.00	
Center	352 (5327)	6.61 (5.95, 7.33)	1.01 (0.89, 1.15)	0.888
South	87 (1978.1	4.40 (3.56, 5.43)	0.67 (0.54, 0.85)	0.001
Nationality				
Non-Italian	145 (2424.2)	5.98 (5.08, 7.03)	1.00	
Italian	1008 (15761)	6.40 (6.01, 6.80)	1.07 (0.90, 1.28)	0.444
Mode of HIV transmission				
Heterosexual	476 (7687.2)	6.19 (5.66, 6.77)	1.00	
MSM	405 (6111.6)	6.63 (6.01, 7.30)	1.06 (0.93, 1.22)	0.359
PWID	207 (3108.7)	6.66 (5.81, 7.63)	1.08 (0.91, 1.27)	0.383
Other/unknown	65 (1277.7)	5.09 (3.99, 6.49)	0.82 (0.63, 1.06)	0.128
Diabetes				
No	1108 (17713.1)	6.26 (5.90, 6.63)	1.00	
Yes	45 (472.2)	9.53 (7.11, 12.76)	1.53 (1.14, 2.05)	0.005
Previous AIDS diagnosis				
No	886 (15139.8)	5.85 (5.48, 6.25)	1.00	
Yes	267 (3045.4)	8.76 (7.78, 9.88)	1.51 (1.31, 1.74)	<0.001
FIB-4				

	No.Events (PYFU)	Rate/ 100 PYFU (95% CI)	IRR (95% CI)	p-value
≤3.25	1063 (17179.1)	6.19 (5.83, 6.57)	1.00	
>3.25	70 (587.1)	11.92 (9.43, 15.07)	1.93 (1.51, 2.48)	<0.001
CD4 cell count, cells/mm^3				
≤200	377 (5469.6)	6.89 (6.23, 7.62)	1.00	
201-350	326 (5139.8)	6.34 (5.69, 7.07)	0.92 (0.79, 1.06)	0.255
>350	349 (6385.8)	5.46 (4.92, 6.07)	0.79 (0.68, 0.91)	0.002
Unknown	101 (1190.1)	8.49 (6.98,10.31)	1.23 (0.98, 1.54)	0.076
HIV-RNA (log10 copies/ml)				
≤1000	81 (1363.3)	5.94 (4.78, 7.39)	1.00	
1001-10000	135 (2358.9)	5.72 (4.83, 6.77)	0.97 (0.73, 1.27)	0.803
>10000	802 (13018.8)	6.16 (5.75, 6.60)	1.04 (0.83, 1.30)	0.740
Unknown	135 (1444.2)	9.35 (7.90, 11.06)	1.58 (1.19, 2.08)	0.001
Alcohol consumption				
Abstain	479 (7340.9)	6.53 (5.97, 7.14)	1.00	
Moderate	123 (1979.3)	6.21 (5.21, 7.42)	0.95 (0.78, 1.16)	0.645
Hazardous	15 (259.4)	5.78 (3.49, 9.59)	0.89 (0.53, 1.49)	0.661
Unknown	536 (8605.7)	6.23 (5.72, 6.78)	0.95 (0.84, 1.07)	0.429

Table 5.8b Summary of number of events and incidence rate of cART discontinuation of at least one drug by drug class stratified by HCV status

	No.Events (PYFU)	Rate/ 100PYFU (95% CI)
NRTIs		
Overall	10056 (52145)	19.28 (18.90, 19.66)
HCVAb negative	3642 (16839)	21.62 (20.93, 22.34)
HCVAb positive	775 (3480)	22.27 (20.73, 23.89)
HCVAb unknown	5639 (31827)	17.71 (17.26, 18.18)
NNRTIs		
Overall	2092 (11009.6)	19.00 (18.20, 19.83)
HCVAb negative	673 (2710.4)	24.83 (23.21, 26.50)
HCVAb positive	108 (553.2)	18.11 (16.02, 23.58)
HCVAb unknown	1311 (7746)	21.45 (16.02, 17.87)
PIs		
Overall	2768 (15930.5)	17.37 (16.73, 18.03)
HCVAb negative	1024 (5420)	19.54 (18.36, 20.77)
HCVAb positive	139 (684.5)	20.30 (17.07, 23.98)

	No.Events (PYFU)	Rate/ 100PYFU (95% CI)
HCVAb unknown	1605 (10006)	16.04 (15.26, 16.84)
INIs		
Overall	548 (3149.9)	17.40 (15.97, 18.91)
HCVAb negative	235 (1012.7)	23.20 (20.33, 26.37)
HCVAb positive	24 (98.74)	24.30 (15.57, 36.16)
HCVAb unknown	289 (2038.4)	14.18 (12.59, 15.91)

5.5.5 Role of HCVAb for the risk of discontinuation of specific ARV drugs

NRTIs

As shown in Table 5.9 Association of HCV status on discontinuation of NRTI and NNRTIs: Incidence rates and Poisson regression models stratified by HCVAb status

Table 5.10 Association of HCV status on discontinuation of PIs and INIs: Incidence rates and Poisson regression models stratified by HCVAb statusTable 5.9, crude incidence rates (95% CI) of discontinuation of Abacavir and Lamivudine were significantly higher in HCVAb positive individuals compared to HCVAb negative 18.8 (13.5 - 25.6) vs. 11.9 (10.2 - 13.9) (p=0.007) and 24.0 (21.8 – 26.4) vs. 20.7 (19.4 - 22.0) (p=0.006) cases per 100 PYFU respectively. In contrast, there were no differences in incidence rates of cART discontinuations of Tenofovir and Emtricitabine by HCV status: HCVAb positive compared to HCVAb negative individuals 20.8 (17.8 - 24.2) vs. 23.4 (22.2 - 24.7) (p=0.077) and 20.7 (17.6 - 24.4) vs. 23.0 (21.8 - 24.4) (p=0.112) cases per 100 PYFU, respectively.

In the multivariable models, unadjusted analysis showed a 53% increased risk (incidence rate ratio IRR = 1.53 (1.07 - 2.18; p=0.018) in stopping Abacavir for HCVAb positive compared to HCVAb negative. For HCVAb unknown the risk was not significantly elevated (IRR = 1.13 (0.94 - 1.35; p=0.194)) compared to HCVAb negative. However, adjustment for demographics and metabolic factors greatly impacted the results: there was no longer a difference by HCV status in stopping

Abacavir: aIRR = 0.95 (0.66 – 1.37); p=0.774 and aIRR = 0.91 (0.75 – 1.10); p=0.313 for HCVAb positive and HCVAb unknown compared to HCVAb negative respectively. The attenuation was mostly explained by calendar year of starting cART. The aIRR remained similar after further controlling for HIV-related factors, liver-related factors and concomitant cART use.

The unadjusted analysis showed a 17% increased risk (IRR = 1.17 (1.04 – 1.31); p=0.009) in discontinuation of Lamivudine comparing HCVAb positive individuals with HCVAb negative individuals. For HCVAb unknown, the estimate was IRR = 1.03 (0.95 – 1.11; p=0.521)) compared to HCVAb negative individuals. After adjustment for potential confounders (model 1), the risk was attenuated (aIRR=0.93 (0.81 – 1.07; p=0.292) and aIRR=0.99 (0.90 – 1.08; p=0.822)) for HCVAb positive and HCVAb unknown respectively and further attenuated after controlling for mode of HIV transmission and diabetes status. HIV/HCV coinfection was not associated with the risk of discontinuing Tenofovir or Emtricitabine. In contrast HCVAb unknown status was associated with reduced risk of discontinuation of Tenofovir or Emtricitabine compared to HCVAb negative (aIRR=0.86 (0.79 – 0.93; p<0.001) and aIRR=0.86 (0.78 – 0.93; p<0.001)) respectively.

Table 5.25 Association of HCV status on discontinuation of NRTI and NNRTIs: Incidence rates and Poisson regression models stratified by HCVAb status

	No. events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Abacavir							
Overall	821	6302	13.03 (10.79, 12.28)				
HCVAb negative	161	1351	11.91 (10.21, 13.90)	1.00	1.00	1.00	1.00
HCVAB positive	40	213.3	18.75 (13.75, 25.56)	1.53 (1.07, 2.18)	0.95 (0.66, 1.37)	1.01 (0.69, 1.48)	0.98 (0.67, 1.43)
				0.018	0.774	0.948	0.905
HCVAB unknown	620	4738	13.09 (12.10, 14.16)	1.13 (0.94, 1.35)	0.91 (0.75, 1.10)	0.97 (0.78, 1.20)	0.95 (0.77, 1.18)
				0.194	0.313	0.765	0.652
Lamivudine							
Overall	2930	13770	21.28 (16.97, 18.13)				
HCVAb negative	930	4504	20.65 (19.36, 22.02)	1.00	1.00	1.00	1.00
HCVAB positive	424	1769	23.97 (21.79, 26.36)	1.17 (1.04, 1.31)	0.93 (0.81, 1.07)	0.94 (0.82, 1.09)	0.94 (0.82, 1.08)
				0.009	0.292	0.410	0.396
HCVAB unknown	1576	7497	21.02 (20.01, 22.09)	1.03 (0.95, 1.11)	0.99 (0.90, 1.08)	1.03 (0.93, 1.13)	1.03 (0.93, 1.13)
				0.521	0.822	0.551	0.566
Tenofovir							
Overall	3339	16259	20.54 (16.51, 17.57)				
HCVAb negative	1308	5591	23.40 (22.16, 24.70)	1.00	1.00	1.00	1.00
HCVAB positive	164	788.3	20.80 (17.85, 24.24)	0.91 (0.77, 1.07)	0.93 (0.78, 1.11)	0.92 (0.77, 1.10)	0.92 (0.77, 1.09)
				0.242	0.416	0.388	0.330
HCVAB unknown	1867	9880	18.90 (18.06, 19.77)	0.82 (0.77, 0.88)	0.79 (0.73, 0.85)	0.85 (0.78, 0.92)	0.86 (0.79, 0.93)
				<.001	<.001	<.001	<.001
Emtricitabine							
Overall	2966	15814	18.76 (15.28, 16.32)				
HCVAb negative	1243	5393	23.05 (21.80, 24.37)	1.00	1.00	1.00	1.00

	No. events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
HCVAB positive	147	709.3	20.72 (17.63, 24.36)	0.91 (0.76, 1.08) 0.264	1.00 (0.84, 1.20) 0.968	1.00 (0.83, 1.20) 0.988	0.99 (0.82, 1.19) 0.911
HCVAB unknown	1576	9712	16.23 (15.45, 17.05)	0.72 (0.67, 0.78) <.001	0.74 (0.68, 0.80) <.001	0.84 (0.77, 0.92) <.001	0.86 (0.78, 0.93) <.001
Efavirenz							
Overall	1928	8707	22.14 (17.40, 18.87)				
HCVAb negative	623	2107	29.57 (27.33, 31.98)	1.00	1.00	1.00	1.00
HCVAB positive	104	506.5	20.53 (16.94, 24.88)	0.78 (0.63, 0.97) 0.026	0.87 (0.68, 1.10) 0.243	0.86 (0.68, 1.10) 0.233	0.86 (0.68, 1.10) 0.237
HCVAB unknown	1201	6094	19.71 (18.63, 20.86)	0.70 (0.64, 0.78) <.001	0.73 (0.65, 0.81) <.001	0.78 (0.70, 0.88) <.001	0.81 (0.72, 0.90) <.001
Rilpivirine							
Overall	164	2302	7.12 (5.70, 7.67)				
HCVAb negative	50	603.4	8.29 (6.28, 10.93)	1.00	1.00	1.00	1.00
HCVAB positive	4	46.67	8.57 (3.22, 22.84)	1.03 (0.36, 3.00) 0.953	0.77 (0.26, 2.30) 0.644	0.80 (0.26, 2.47) 0.692	0.80 (0.26, 2.51) 0.708
HCVAB unknown	110	1652	6.66 (5.52, 8.03)	0.80 (0.58, 1.12) 0.198	0.67 (0.48, 0.95) 0.022	0.59 (0.42, 0.84) 0.003	0.57 (0.40, 0.81) 0.002

Model 1: age, gender, ethnicity, geographical region, mode of HIV transmission, diabetes, BMI, calendar year of starting cART;
Model 2 : Model 1 + previous AIDS diagnosis, CD4 cell count, HIV-RNA, Previous ART use, concomitant ART use (ABC - 3TC, DRV/r, ATV/r, EFV; 3TC - ZDV, NVP, EFA; TDF- FTC, DRV/r, ATV/r, EFV; FTC - TDF, EFA, LPV/r, DRV/r, ATV/r ; EFA – TDF/FTC, ZDV/3TC ; RIL- TDF/FTC);
Model 3: Model 2 + liver fibrosis, (FIB-4 and alcohol use)

NNRTIs

As shown in Table 5.9 Association of HCV status on discontinuation of NRTI and NNRTIs: Incidence rates and Poisson regression models stratified by HCVAb status

Table 5.10 Association of HCV status on discontinuation of PIs and INIs: Incidence rates and Poisson regression models stratified by HCVAb statusTable 5.9, incidence rates (95% CI) of discontinuation of Efavirenz were significantly lower in HCVAb positive and HCVAb unknown individuals compared to HCVAb negative: 20.5 (16.9 - 24.9) and 19.7 (18.6 - 20.9) respectively vs. 29.6 (27.3 - 32.0) (p=0.002) cases per 100 PYFU.

The unadjusted analysis showed a reduction in risk of 22% (IRR = 0.78 (0.63 - 0.97; p=0.026) in stopping Efavirenz in HCVAb positive individuals compared to HCVAb negative individuals. A reduction in risk was also observed in HCVAb unknown (IRR = 0.70 (0.64 – 0.78; p<0.001) compared to HCVAb negative. However, after adjusting for demographics and metabolic factors this difference in risk was attenuated (aIRR = 0.87 (0.68 – 1.10); p=0.243) and the aIRR remained similar after further controlling for HIV-related factors liver-related factors and concomitant ART use. For the HCVAb unknown group, even after adjustment for all potential confounders, a reduction in risk was still observed (aIRR = 0.81 (0.72 – 0.90); p<0.001) compared to HCVAb negative individuals. There was no difference in incidence rates of cART discontinuation of Rilpivirine among HCVAb positive and HCVAb unknown individuals compared to HCVAb negative individuals 8.6 (3.2 - 22.8) and 6.7 (5.5 - 8.0) respectively vs. 8.3 (6.2 - 10.9) (p = 0.453).
Both in the unadjusted and adjusted analyses there was no evidence that HIV/HCV coinfection was associated with the risk of stopping Rilpivirine. However, HCVAb unknown was associated with a reduction in risk of discontinuation, even after adjusting for all potential confounders (aIRR = 0.57 (0.40 – 0.81); p=0.002) compared to HCVAb negative individuals.

PIs

As shown in Table 5.10**,** there were no differences in crude incidence rates for discontinuation of Lopinavir/r (24.6 (19.3 – 31.4 vs. 27.3 (24.8, 30.0); p = 0.225) or Atazanavir (15.2 (11.1 – 20.7) vs. 17.1 (15.3 – 19.1); p = 0.242) by HCV status. In contrast, the incidence rate for discontinuation of Darunavir/r was significantly higher in HCVAb positive individuals compared to HCVAb negative individuals (21.8 (15.6 – 30.3) vs. 15.8 (14.1 – 17.7); p = 0.043). In the unadjusted analysis, HIV/HCV coinfected individuals showed an increased risk of 39% of stopping Darunavir/r which was marginally significant (IRR = 1.39 (0.95 – 2.03); p=0.089). After adjusting for demographics and HIV related factors HIV/HCV coinfection remained significantly associated with higher risk of discontinuation (IRR = 1.50 (1.01 – 2.22); p = 0.045 (model 2). In contrast, HCVAb unknown was associated with reduced risk of discontinuation of Darunavir/r (IRR = 0.81 (0.69 – 0.94); p=0.008) compared to HCVAb negative. However, after adjustment of potential confounders, this association was attenuated. Both the unadjusted and adjusted analysis showed that HIV/HCV coinfected was not associated with the risk of discontinuing Lopinavir/r or Atazanavir/r. HCVAb unknown was associated with reduced risk of discontinuation of Atazanavir/r (IRR = 0.77 (0.67 – 0.89); p<0.001) compared to HCVAb negative. However, after adjustment for potential confounders, this association was also attenuated.

INIs

As shown in Table 5.10 Association of HCV status on discontinuation of PIs and INIs: Incidence rates and Poisson regression models stratified by HCVAb status

Table 5.11 Summary of number of events and incidence rate of discontinuation by HCVAb/HCV-RNA statusTable 5.10 there were no differences in crude incidence rates of discontinuation of Raltegravir (26.3 (17.0 – 40.7) vs. 29.5 (25.6 – 34.0); p = 0.323) or Dolutegravir (32.9 (8.2 – 131.5) vs. 16.4 (11.1 – 24.3); p = 0.177) or Elvitegravir (12.1 (3.0 – 48.2) vs. 7.9 (4.8 – 12.9); p=0.263) between HCVAb positive and HCVAb negative respectively. Of note, the unadjusted incidence was two-fold higher in HCVAb positive compared to HCVAb negative individuals for Dolutegravir and Elvitegravir although it is based on <30 discontinuations so the

analysis is likely to be underpowered for these comparisons and wide CIs were observed. After adjusting for all potential confounders considered, HCV infection was not associated with the risk of discontinuing any of the drugs in the INI class. In a similar pattern to that seen for other drugs, HCVAb unknown was associated with reduced risk of discontinuation of RAL compared to HCVAb negative [aIRR = 0.74 (0.60- 0.93); p=0.010)].

Table 5.28 Association of HCV status on discontinuation of PIs and INIs: Incidence rates and Poisson regression models stratified by HCVAb status

	No. events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Lopinavir							
Overall	1104	4217	26.18 (19.67, 21.85)				
HCVAb negative	408	1496	27.27 (24.75, 30.05)	1.00	1.00	1.00	1.00
HCVAB positive	64	260.1	24.61 (19.26, 31.44)	0.89 (0.68, 1.16)	1.10 (0.83, 1.45)	1.06 (0.81, 1.40)	1.07 (0.81, 1.42)
				0.400	0.512	0.666	0.620
HCVAB unknown	632	2460	25.69 (23.76, 27.77)	0.96 (0.85, 1.09)	1.06 (0.93, 1.20)	1.07 (0.93, 1.23)	1.10 (0.95, 1.26)
				0.518	0.404	0.333	0.204
Darunavir/r							
Overall	665	4666	14.25 (11.60, 13.37)				
HCVAb negative	297	1876	15.83 (14.13, 17.74)	1.00	1.00	1.00	1.00
HCVAB positive	35	160.7	21.78 (15.64, 30.34)	1.39 (0.95, 2.03)	1.47 (1.00, 2.17)	1.50 (1.01, 2.22)	1.42 (0.96, 2.12)
				0.089	0.053	0.045	0.083
HCVAB unknown	333	2629	12.67 (11.38, 14.10)	0.81 (0.69, 0.94)	0.89 (0.76, 1.03)	0.93 (0.78, 1.11)	0.94 (0.79, 1.12)
				0.008	0.125	0.438	0.481
Atazanavir/r							
Overall	999	7049	14.17 (11.70, 13.14)				
HCVAb negative	319	1868	17.07 (15.30, 19.06)	1.00	1.00	1.00	
HCVAB positive	40	263.7	15.17 (11.13, 20.68)	0.89 (0.63, 1.25)	0.84 (0.58, 1.20)	0.86 (0.60, 1.23)	0.85 (0.59, 1.21)
				0.506	0.330	0.395	0.362
HCVAB unknown	640	4917	13.02 (12.05, 14.06)	0.77 (0.67, 0.89)	0.83 (0.72, 0.97)	0.93 (0.79, 1.10)	0.92 (0.78, 1.08)
				<.001	0.017	0.386	0.325
Raltegravir							
Overall	436	2192	19.90 (15.20, 18.04)				
HCVAb negative	194	657.8	29.49 (25.62, 33.95)	1.00	1.00	1.00	1.00

	No. events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
HCVAB positive	20	76.08	26.29 (16.96, 40.75)	0.90 (0.55, 1.47)	0.94 (0.57, 1.55)	0.94 (0.57, 1.55)	0.93 (0.56, 1.54)
				0.670	0.816	0.808	0.785
HCVAB unknown	222	1458	15.23 (13.35, 17.37)	0.53 (0.43, 0.64)	0.65 (0.53, 0.80)	0.75 (0.60, 0.94)	0.74 (0.60, 0.93)
				<.001	<.001	0.013	0.010
***Dolutegravir**							
Overall	70	411.3	17.02 (11.54, 17.82)				
HCVAb negative	25	152.1	16.44 (11.11, 24.33)	1.00	1.00	1.00	1.00
HCVAB positive	2	6.08	32.88 (8.22, 131.5)	2.00 (0.46, 8.67)	2.10 (0.50, 8.82)	2.30 (0.57, 9.20)	2.58 (0.75, 8.83)
				0.355	0.311	0.240	0.132
HCVAB unknown	43	253.2	16.98 (12.60, 22.90)	1.03 (0.63, 1.69)	0.93 (0.54, 1.61)	0.99 (0.52, 1.89)	1.10 (0.57, 2.12)
				0.897	0.798	0.985	0.786
***Elvitegravir**							
Overall	42	546.6	7.68 (5.20, 9.35)				
HCVAb negative	16	202.8	7.89 (4.83, 12.88)	1.00	1.00	1.00	1.00
HCVAB positive	2	16.58	12.06 (3.02, 48.22)	1.52 (0.34, 6.86)	1.76 (0.35, 8.77)	1.45 (0.29, 7.34)	1.16 (0.27, 4.97)
				0.586	0.490	0.654	0.844
HCVAB unknown	24	327.2	7.34 (4.92, 10.94)	0.93 (0.50, 1.74)	1.01 (0.52, 1.96)	1.20 (0.60, 2.42)	1.00 (0.46, 2.17)
				0.825	0.985	0.610	0.997

*Events <100, interpret with caution

Model 1: age, gender, ethnicity, geographical region, mode of HIV transmission, diabetes, BMI, calendar year of starting cART; Model 2 : Model 1 + previous AIDS diagnosis, CD4 cell count, HIV-RNA, Previous ART use, concomitant ART use (LOP - TDF/FTC, ZDV/3TC; DRV/r - TDF/FTC, ABC/3TC; ATV - TDF/FTC, ABC/3TC; RAL - TDF/FTC, ABC/3TC TDF/FTC/DRV/r; DOL - TDF/FTC, ABC/3TC; ELV- TDF/FTC); Model 3: Model 2 + liver fibrosis, (FIB-4 and alcohol use)

5.5.6 Incidence rates of cART discontinuation by HCVAb/HCV-RNA infection status in the subset of HCVAb+ participants

Some of the previously published analyses reported discrepancies when rate of discontinuations were compared stratifying participants according to the results of serology tests alone as compared to using also HCV-RNA test results. This section of the results illustrate the associations found after further classifying HCVAb positive people according to their HCV-RNA results. Table 5.11 Summary of number of events and incidence rate of discontinuation by HCVAb/HCV-RNA status

Table 5.12 Association of HCV status on discontinuation of NRTIs and NNRTIs: Incidence rates and Poisson regression models stratified by HCV-RNA statusTable 5.11 summarizes incidence rates of cART discontinuations by HCV-RNA status. HCVAb positive individuals included in this analysis contributed 30,639 PYFU corresponding to an overall incidence rate [95% CI] of discontinuation of 21.6 (21.4 – 22.5). There was no difference in the incidence rates for HCVAb+/HCV-RNA positive compared with HCVAb negative (23.0 (20.4 – 25.8) vs. 21.6 (21.0 – 22.1); p = 0.152). In contrast the incidence rates for HCVAb+/HCV-RNA negative were much lower (17.5 (13.9 – 21.7)) compared to HCVAb negative. An indication that aviremic individuals are at reduced risk of cART discontinuation in this overall analysis.

When the analysis was stratified by drug class, similar rates of cART discontinuations were observed regardless of participants' HCV-RNA status for the NRTI and NNRTI class but not for the PI class. Incidence rate of PI drugs discontinuation was significantly higher in HCVAb+/HCV-RNA positive compared to HCVAb negative (27.1 (20.7 – 34.7) vs. 19.6 (18.4 – 20.8); p = 0.008). For INIs, the numbers are too small to make any meaningful conclusion.

Table 5.31 Summary of number of events and incidence rate of discontinuation by HCVAb/HCV-RNA status

	No. Events (PYFU)	Rate/ 100PYFU (95% CI)
ALL drugs		
Overall	6620 (30639)	21.6 (21.42, 22.48)
HCVAb-	5574 (25802.1)	21.6 (21.04, 22.18)
HCVAb+/HCV-RNA+	297 (1292.9)	22.9 (20.43, 25.74)
HCVAb+/HCV-RNA-	84 (479.3)	17.5 (13.98, 21.69)
HCVAb+/HCV-RNA unk	665 (3064.7)	21.7 (20.08, 23.41)
NRTIs		
Overall	4417 (20319)	21.7 (21.10, 22.39)
HCVAb-	3642 (16839)	21.6 (20.93, 22.34)
HCVAb+/HCV-RNA+	188 (859.07)	21.9 (18.87, 25.24)
HCVAb+/HCV-RNA-	55 (292.75)	18.8 (14.15, 24.45)
HCVAb+/HCV-RNA unk	532 (2328.7)	22.8 (20.94, 24.87)
NNRTIs		
Overall	781 (3264.4)	23.9 (22.27, 25.67)
HCVAb-	673 (2710.4)	24.8 (22.98, 26.78)
HCVAb+/HCV-RNA+	36 (152.8)	23.6 (16.50, 32.62)
HCVAb+/HCV-RNA-	6 (38.28)	15.7 (5.75, 34.11)
HCVAb+/HCV-RNA unk	66 (362.2)	18.2 (14.09, 23.18)
PIs		
Overall	1163 (5924.4)	19.63 (18.51, 20.79)
HCVAb-	1024 (5240)	19.6 (18.36, 20.77)
HCVAb+/HCV-RNA+	62 (229.2)	27.1 (20.75, 34.71)
HCVAb+/HCV-RNA-	19 (120.8)	16.1 (9.46, 24.55)
HCVAb+/HCV-RNA unk	58 (334.4)	19.7 (13.16, 24.42)
[+]INIs		
Overall	259 (1111.4)	23.30 (20.55, 26.32)
HCVAb-	235 (1012.7)	17.4 (20.33, 26.37)
HCVAb+/HCV-RNA+	11 (51.8)	15.3 (10.60, 37.98)
HCVAb+/HCV-RNA-	4 (27.5)	27.1 (3.96, 37.21)
HCVAb+/HCV-RNA unk	9 (39.4)	22.5 (10.43, 43.31)

5.5.7 Role of HCV-RNA for the risk of discontinuation of specific ARV drugs

NRTIs

In Table 5.12 Association of HCV status on discontinuation of NRTIs and NNRTIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

Table 5.13 PIs and INIs: Incidence rates and Poisson regression models stratified by HCV-RNA statusTable 5.12**,** crude incidence rates (95% CI) of discontinuation of Abacavir for HCVAb+/HCV-RNA positive and HCVAb+/HCV-RNA unknown was significantly higher compared to HCVAb negative IR= 23.3 [14.3 – 38.0] vs. 11.9 (10.2 – 13.9) (p=0.019) and 19.0 (12.5 – 28.9) vs. 11.9 (10.2 – 13.9) (p=0.047) respectively. For the HCVAb+/HCV-RNA negative, incidence rates (95 CI%) of discontinuation was lower compared to HCVAb negative: 6.9 (1.7 – 27.4) vs. 11.9 (10.2 – 13.9). It is worth noting that because of the restriction to HCVAb positive participants with HCV-RNA data available, the numbers of specific drug discontinuations here are also very small. For Lamivudine, incidence rates were higher in HCVAb+/HCV-RNA unknown compared to HCVAb negative IR = 24.0 (21.7 – 26.5) vs. 20.6 (19.4 – 22.0) (p=0.016). There were no differences in incidence rates of discontinuation of Tenofovir or Emtricitabine across HCV-RNA groups compared to HCVAb negative individuals.

The unadjusted analysis showed an 81% increased risk of discontinuation of Abacavir (IRR = 1.81 (1.14–2.88; p=0.013) in HCVAb+/HCV-RNA positive individuals compared to HCVAb negative. However, after adjusting for demographics and metabolic factors this risk was greatly attenuated (IRR = 1.14 (0.57 – 2.29); p=0.712) and aIRR remained similar after further controlling for HIV-related factors liver-related factors and concomitant ART use. For Lamivudine, unadjusted analysis showed a 16% increased risk of cART discontinuation for HCVAb+/HCV-RNA unknown compared to HCVAb negative (IRR = 1.16 (1.03 – 1.31); p = 0.013). After adjusting for potential confounders, there was no association of cART discontinuation by HCV-RNA status (aIRR=1.00 (0.82 – 1.22); p=0.978). Similarly, no association was observed for the risk of stopping Tenofovir or Emtricitabine by HCV-RNA in both the unadjusted and adjusted analyses.

NNRTIs

In Table 5.12 Association of HCV status on discontinuation of NRTIs and NNRTIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

Table 5.13 PIs and INIs: Incidence rates and Poisson regression models stratified by HCV-RNA statusTable 5.12 , crude incidence rates (95% CI) of discontinuation of Efavirenz for people who were HCVAb+/HCV-RNA unknown was lower compared to HCVAb negative IR= 21.6 (16.8 – 27.7) vs. 23.0 (21.8 – 24.4) ($p<0.001$). In the unadjusted analysis, the estimate of the rate ratio for Efavirenz was IRR = 0.65 (0.50 – 0.85); $p = 0.002$). However, after adjustment of potential confounders, HCVAb+/HCV-RNA unknown was not associated with cART discontinuation. Moreover, no difference in incidence rate was observed for HCVAb+/HCV-RNA positive compared with HCVAb negative 25.3 (18.1 – 35.4) vs. 23.0 (21.8 – 24.4) ($p=0.389$). A small number of discontinuations of Rilpivirine were observed but unadjusted incidence rates appeared to be similar for this drug.

Table 5.34 Association of HCV status on discontinuation of NRTIs and NNRTIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

	No.events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Abacavir							
Overall	201	1565	12.85 (9.94, 12.91)				
HCVAB-	161	1351	11.91 (10.21, 13.90)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	16	68.67	23.30 (14.27, 38.03)	1.81 (1.14, 2.88)	1.14 (0.57, 2.29)	1.31 (0.66, 2.61)	1.32 (0.65, 2.65)
				0.013	0.712	0.442	0.442
HCVAb+/HCV-RNA-	2	29.17	6.86 (1.71, 27.42)	0.47 (0.17, 1.26)	0.38 (0.18, 0.78)	0.39 (0.17, 0.87)	0.39 (0.17, 0.91)
				0.133	0.008	0.022	0.029
HCVAb+/HCV-RNA unk	22	115.5	19.05 (12.54, 28.93)	1.65 (1.00, 2.72)	1.04 (0.58, 1.86)	1.06 (0.58, 1.93)	1.03 (0.56, 1.88)
				0.050	0.904	0.850	0.925
Lamivudine							
Overall	1354	6273	21.59 (16.90, 18.62)				
HCVAB-	930	4504	20.65 (19.36, 22.02)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	36	150.9	23.85 (17.21, 33.07)	1.20 (0.84, 1.70)	1.18 (0.80, 1.75)	1.21 (0.82, 1.79)	1.20 (0.80, 1.78)
				0.319	0.396	0.336	0.377
HCVAb+/HCV-RNA-	12	49.58	24.20 (13.74, 42.62)	1.17 (0.64, 2.12)	1.24 (0.70, 2.17)	1.24 (0.71, 2.17)	1.17 (0.66, 2.07)
				0.611	0.462	0.455	0.581
HCVAb+/HCV-RNA unk	376	1569	23.97 (21.67, 26.52)	1.16 (1.03, 1.31)	1.02 (0.83, 1.24)	1.02 (0.83, 1.24)	1.00 (0.82, 1.22)
				0.013	0.874	0.868	0.978
Tenofovir							
Overall	1472	6379	23.08 (17.89, 19.62)				
HCVAB-	1308	5591	23.40 (22.16, 24.70)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	71	317.4	22.37 (17.73, 28.23)	0.97 (0.75, 1.24)	0.95 (0.72, 1.25)	0.96 (0.73, 1.26)	0.95 (0.72, 1.26)
				0.782	0.715	0.762	0.728
HCVAb+/HCV-RNA-	20	109.7	18.24 (11.77, 28.27)	0.80 (0.51, 1.24)	0.77 (0.49, 1.22)	0.81 (0.52, 1.25)	0.80 (0.52, 1.24)

	No.events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
				0.318	0.268	0.338	0.316
HCVAb+/HCV-RNA unk	73	361.3	20.21 (16.07, 25.42)	0.89 (0.70, 1.13)	0.87 (0.65, 1.16)	0.92 (0.69, 1.23)	0.91 (0.68, 1.23)
				0.343	0.341	0.562	0.549
Emtricitabine							
Overall	1390	6102	22.78 (17.68, 19.44)				
HCVAB-	1243	5393	23.05 (21.80, 24.37)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	65	322.1	20.18 (15.83, 25.73)	0.89 (0.68, 1.14)	0.92 (0.69, 1.23)	0.97 (0.73, 1.29)	0.96 (0.72, 1.28)
				0.352	0.562	0.831	0.761
HCVAb+/HCV-RNA-	21	104.3	20.13 (13.12, 30.87)	0.86 (0.57, 1.30)	0.90 (0.59, 1.35)	0.97 (0.64, 1.45)	0.96 (0.64, 1.44)
				0.472	0.602	0.876	0.832
HCVAb+/HCV-RNA unk	61	282.9	21.56 (16.78, 27.71)	0.95 (0.73, 1.23)	0.97 (0.71, 1.31)	1.05 (0.78, 1.43)	1.05 (0.77, 1.42)
				0.704	0.833	0.740	0.768
Efavirenz							
Overall	727	2614	27.82 (20.38, 23.18)				
HCVAB-	623	2107	29.57 (27.33, 31.98)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	34	134.3	25.33 (18.10, 35.44)	1.12 (0.80, 1.55)	1.03 (0.66, 1.60)	1.06 (0.67, 1.66)	1.05 (0.66, 1.66)
				0.517	0.902	0.811	0.847
HCVAb+/HCV-RNA-	6	29.08	20.63 (9.27, 45.92)	0.67 (0.30, 1.52)	0.62 (0.28, 1.42)	0.62 (0.28, 1.37)	0.61 (0.27, 1.36)
				0.336	0.260	0.234	0.225
HCVAb+/HCV-RNA unk	64	343.2	18.65 (14.60, 23.83)	0.65 (0.50, 0.85)	0.95 (0.66, 1.35)	1.00 (0.70, 1.43)	0.99 (0.69, 1.42)
				0.002	0.768	0.994	0.960
Rilpivirine							
Overall	54	650.1	8.31 (5.82, 9.74)				
HCVAB-	50	603.4	8.28 (6.28, 10.93)				
HCVAb+/HCV-RNA+	2	18.5	10.81 (2.71, 43.22)				
					Not estimable		
HCVAb+/HCV-RNA-	0	9.2	Not estimable				
HCVAb+/HCV-RNA unk	2	19.0	10.53 (2.63, 42.09)				

PIs

As shown in Table 5.13 PIs and INIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

Table 5.14 Association of HCV status on discontinuation of NRTIs and NNRTIs: Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplificationTable 5.13 , there were no differences in crude incidence rates [95% CI] of discontinuation for Lopinavir/r and Atazanavir/r by HCV-RNA status. Incidence rates for discontinuation for Lopinavir/r and Atazanavir/r tended to be lower in HCV-RNA negative group compared to the HCVAb negative group. However, the numbers of discontinuations were few and confidence intervals are wide so results need to be interpreted with caution. The unadjusted analysis showed no association between HCV-RNA status and risk of discontinuation of Lopinavir/r, however after additional adjustment for factors such as liver fibrosis and alcohol use, there was evidence for a 67% increased risk of discontinuation of Lopinavir/r (IRR = 1.67 (1.01 - 2.76; p=0.045) in HCV-RNA positive patients compared to HCVAb negative. No difference was observed for the risk of stopping Atazanavir/r by HCV-RNA status in both the unadjusted and adjusted analysis.

In contrast, similar to findings when comparing people according to their HCVAb status (Table 5.10), incidence rates for discontinuation of Darunavir/r, were significantly higher in HCV-RNA positive compared to HCVAb negative IR = 29.6 (18.4 – 47.6) vs. 15.8 [14.1 – 17.7] (p=0.019). Of interest, the incidence was higher in HCV chronic infected participants than in the HCVAb+ group as a whole [21.8 (15.6 – 30.3). There was a 86% increased risk of discontinuation of Darunavir/r for HCV-RNA positive patients compared to HCVAb negative in the unadjusted analysis (IRR = 1.86 (1.12 – 3.09); p = 0.016). After additional adjusting for liver fibrosis and alcohol use, the risk of DRV/r discontinuation remained 2-fold higher (IRR = 2.0 (1.09 – 3.79); p = 0.025) for HCV-RNA positive compared to HCVAb negative, despite the small number of stops and PYFU contributing to this analysis.

INIs

As shown in Table 5.13 PIs and INIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

Table 5.14 Association of HCV status on discontinuation of NRTIs and NNRTIs: Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplificationTable 5.13 , there were no differences in incidence rates by HCV-RNA status compared to HCVAb negative for the risk of Raltegravir discontinuation similarly to findings when comparing participants stratified by HCV antibody groups. Number of events were too few to obtain accurate incidence rate ratios for Dolutegravir and Elvitegravir therefore multivariable Poisson models were not fitted. In the multivariable analysis for Raltegravir, there was no association of HCV-RNA status with discontinuation. Again, although not significant, the difference in unadjusted incidence was large but the number of events was less than 20 so there is great uncertainty around the estimates.

It is worth noting that the marginal incidence rate ratios for HCV-RNA negative are lower than HCV-RNA+ group in all results presented in and . This is generally apparent for the multivariable as well as univariable analysis. This could be an indication of the role of viremic HCV status on cART discontinuation. Specifically, these finding could indicate that participants in whom HCV was spontaneously cleared or eradicated were better at tolerating HIV treatment than individuals with viremic HCV infection.

Table 5.37 PIs and INIs: Incidence rates and Poisson regression models stratified by HCV-RNA status

	No.events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Lopinavir							
Overall	472	1756	26.87 (19.51, 22.90)				
HCVAB-	408	1496	27.27 (24.75, 30.05)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	23	65.08	35.34 (23.48, 53.18)	1.22 (0.80, 1.86)	1.54 (0.96, 2.46)	1.53 (0.95, 2.45)	1.67 (1.01, 2.76)
				0.345	0.074	0.077	0.045
HCVAb+/HCV-RNA-	7	41.67	16.80 (8.01, 35.24)	0.67 (0.29, 1.53)	0.87 (0.38, 1.96)	0.89 (0.39, 2.01)	1.01 (0.44, 2.31)
				0.340	0.733	0.771	0.990
HCVAb+/HCV-RNA unk	34	153.3	22.17 (15.84, 31.03)	0.79 (0.56, 1.11)	1.22 (0.81, 1.83)	1.11 (0.72, 1.72)	1.19 (0.77, 1.84)
				0.174	0.337	0.638	0.425
Darunavir/r							
Overall	332	2037	16.30 (12.65, 15.44)				
HCVAB-	297	1876	15.83 (14.13, 17.74)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	17	57.50	29.57 (18.38, 47.56)	1.86 (1.12, 3.09)	2.20 (1.20, 4.04)	2.11 (1.12, 3.95)	2.04 (1.09, 3.79)
				0.016	0.011	0.020	0.025
HCVAb+/HCV-RNA-	7	32.33	21.65 (10.32, 45.41)	1.38 (0.59, 3.19)	1.46 (0.65, 3.27)	1.53 (0.67, 3.48)	1.54 (0.67, 3.52)
				0.455	0.360	0.313	0.311
HCVAb+/HCV-RNA unk	11	70.83	15.53 (8.60, 28.04)	1.00 (0.52, 1.92)	1.15 (0.59, 2.24)	1.03 (0.51, 2.06)	0.99 (0.48, 2.04)
				0.988	0.679	0.942	0.973
Atazanavir/r							

	No.events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Overall	359	2132	16.84 (13.06, 15.82)				
HCVAB-	319	1868	17.07 (15.30, 19.06)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	22	106.6	20.64 (13.59, 31.35)	1.21 (0.77, 1.89)	1.32 (0.78, 2.26)	1.39 (0.81, 2.38)	1.41 (0.82, 2.40)
				0.413	0.303	0.237	0.211
HCVAb+/HCV-RNA-	5	46.83	10.68 (4.44, 25.65)	0.61 (0.26, 1.40)	0.65 (0.28, 1.49)	0.70 (0.30, 1.62)	0.69 (0.31, 1.53)
				0.241	0.306	0.404	0.362
HCVAb+/HCV-RNA unk	13	110.3	11.79 (6.85, 20.31)	0.70 (0.39, 1.25)	0.81 (0.39, 1.65)	0.81 (0.40, 1.65)	0.81 (0.40, 1.65)
				0.225	0.556	0.570	0.570
Raltegravir							
Overall	214	733.8	29.16 (19.97, 25.29)				
HCVAB-	194	657.8	29.49 (25.62, 33.95)	1.00	1.00	1.00	1.00
HCVAb+/HCV-RNA+	10	20.92	47.81 (25.72, 88.85)	1.52 (0.85, 2.72)	1.47 (0.76, 2.85)	1.42 (0.73, 2.77)	1.45 (0.73, 2.89)
				0.154	0.256	0.302	0.286
HCVAb+/HCV-RNA-	2	21.42	9.34 (2.34, 37.34)	0.32 (0.08, 1.33)	0.33 (0.08, 1.32)	0.37 (0.10, 1.35)	0.36 (0.10, 1.24)
				0.117	0.117	0.132	0.104
HCVAb+/HCV-RNA unk	8	33.75	23.70 (11.85, 47.40)	0.84 (0.39, 1.80)	1.12 (0.46, 2.70)	1.12 (0.45, 2.77)	1.11 (0.43, 2.88)
				0.645	0.809	0.806	0.823
Dolutegravir							
Overall	27	158.17	17.07 (9.88, 20.00)				
HCVAB-	25	152.1	16.43 (11.1, 24.3)				
HCVAb+/HCV-RNA+	0	2.4	Not estimable				
					Not estimable		
HCVAb+/HCV-RNA-	1	2.6	38.71 (5.45, 274.80)				

	No.events	PYFU	Rate/ 100 PYFU (95% CI)	Unadjusted Rate ratio (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
HCVAb+/HCV-RNA unk	1	1.1	92.31 (13.0, 655.30)				
Elvitegravir							
Overall	18	219.4	8.21 (4.57 , 11.26)				
HCVAB-	16	202.8	7.89 (4.83, 12.88)				
HCVAb+/HCV-RNA+	1	28.5	11.76 (1.66, 83.51)				
					Not estimable		
HCVAb+/HCV-RNA-	1	3.5	28.57 (4.02, 202.83)				
HCVAb+/HCV-RNA unk	0	4.6	0.00 (0.00, -)				

*Events <100 should be interpreted with caution

5.5.8 Sensitivity analysis: Discontinuation for all reasons except stopping for simplification of viral/immunological failure (inverse probability of censoring weights method)

As detailed in the methods section, when reasons for stopping drugs are those reported by the treating physician in the observational setting, misclassifications are possible. Furthermore, the primary reason recorded, used in these analyses, may not always fully capture the main reasons for stopping if two or more reasons were important. In order to make sure that all discontinuations for which the underlying cause was attributable to intolerance/toxicity to antiretroviral were included, an alternative endpoint was evaluated, defined as stopping for all reasons except stopping for simplification or viral/immunological failure. In addition, the inverse probability method was used to control for both time-fixed and time-dependent confounding as well as for potential informative censoring. shows the incidence rates for this alternative analysis and the results from the corresponding weighted Poisson regression models in which the same list of confounders used in the primary analysis were used to construct the weights.

NRTIs

In Table 5.14 Association of HCV status on discontinuation of NRTIs and NNRTIs: Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification

Table 5.15 Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification (ONLY FOR LAMIVUDINE)Table 5.14, when considering this alternative endpoint, in the unadjusted analysis, HCVAb positive individuals showed an increased risk of discontinuation of Abacavir (IRR = 1.28 (1.07 – 1.54); p = 0.008). However, after further adjustments for potential confounders, no association was observed [aIRR=1.05 (0.78 – 1.39); p=0.763]. Similarly, both in the unadjusted and adjusted analysis, no association between HCVAb infection status

and discontinuation of Tenofovir or Emtricitabine was observed. When using this endpoint, the association with the risk of stopping by HCVAb status for Lamivudine (unadjusted IRR = 1.26 (1.15 – 1.38); p<0.001) was stronger than that observed in the main analysis. Even after adjusting for demographics and metabolic factors, HCVAb infection was still associated with the risk of discontinuation due to toxicity (adjusted IRR = 1.19 (1.02 – 1.38); p=0.025) and after further controlling for liver fibrosis and alcohol use, HCVAb infection still showed an association, although marginally non-significant (IRR = 1.15 (0.99 – 1.35); p = 0.061).

Finally, because of the observed large imbalance of calendar year of starting Lamivudine-based cART by HCVAb status, I restricted the analysis to individuals initiating Lamivudine after 2002 and this analysis provided similar results (Table 5.15 Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification (ONLY FOR LAMIVUDINE)

Table 5.16 Association of HCV status on discontinuation of PIs and INIs: Poisson regression models stratified by HCVAb status (time-dependent) – Stopping for all reasons except stopping for failure/simplificationTable 5.15) . One possible explanation for the increased risk of discontinuation observed with lamivudine is the fact that 3TC is often used in combination with zidovudine or abacavir the latter also shown here to be associated with higher risk of stopping.

NNRTIs, PI/rs and INIs

Neither the risk of stopping Efavirenz or Rilpivirine was associated with HCVAb status in this alternative analysis (Table 5.16 Association of HCV status on discontinuation of PIs and INIs: Poisson regression models stratified by HCVAb status (time-dependent) – Stopping for all reasons except stopping for failure/simplification

Table 5.16). Similar results were found for Lopinavir/r, Darunavir/r, Atazanavir/r or Raltegravir but not reaching statistical significance. Even when using this

expanded definition for the outcome, a small number of discontinuations were observed for Dolutegravir and Elvitegravir so Poisson regression models have not been fitted.

Table 5.40 Association of HCV status on discontinuation of NRTIs and NNRTIs: Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification

	All reasons			All reasons except failure/simplification			Failure/simplification			Estimates from weighted Poisson models, i.e. stopping for failure/simplification treated as a competing risk			
	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	Unadjusted IRR (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Abacavir													
Overall	821	6302	13.03 (10.79, 12.28)	626	6302	9.93 (8.37, 9.72)	195	6302	3.09 (2.60, 3.43)				
HCVAb negative	547	4588	11.92 (10.96, 12.96)	399	4588	8.70 (7.88, 9.59)	148	4588	3.22 (2.74, 3.78)	1.00	1.00	1.00	1.00
HCVAb positive	225	1346	16.72 (14.67, 19.05)	191	1346	14.19 (12.32, 16.35)	34	1346	2.52 (1.81, 3.54)	1.28 (1.07, 1.54)	1.07 (0.81, 1.42)	1.08 (0.81, 1.44)	1.05 (0.78, 1.39)
										0.008	0.638	0.605	0.763
HCVAb unknown	49	368.5	13.30 (10.05, 17.59)	36	368.5	9.77 (7.05, 13.54)	13	368.5	3.52 (2.05, 6.07)	1.16 (0.81, 1.67)	1.04 (0.72, 1.49)	0.97 (0.67, 1.41)	0.97 (0.67, 1.41)
										0.419	0.851	0.869	0.881
Lamivudine													
Overall	2930	13770	21.28 (16.97, 18.13)	2274	13770	16.51 (13.64, 14.72)	656	13770	4.76 (4.21, 4.89)				
HCVAb negative	1765	8809	20.04 (19.12, 20.99)	1312	8809	14.89 (14.11, 15.72)	453	8809	5.14 (4.69, 5.63)	1.00	1.00	1.00	1.00
HCVAb positive	954	3981	23.96 (22.49, 25.53)	795	3981	19.97 (18.63, 21.41)	159	3981	3.99 (3.41, 4.66)	1.26 (1.15, 1.38)	1.19 (1.02, 1.38)	1.16 (0.99, 1.34)	1.15 (0.99, 1.35)
										<.001	0.025	0.060	0.061
HCVAb unknown	211	979.5	21.54 (18.82, 24.65)	167	979.5	17.05 (14.65, 19.84)	44	979.5	4.49 (3.34, 6.04)	1.07 (0.91, 1.26)	1.07 (0.90, 1.28)	1.03 (0.86, 1.22)	1.03 (0.87, 1.23)
										0.412	0.460	0.753	0.717
Tenofovir													
Overall	3339	16259	20.54 (16.51, 17.57)	2356	16259	14.49 (12.18, 13.14)	983	16259	6.04 (5.36, 6.05)				
HCVAb negative	2410	11547	20.87 (20.05, 21.72)	1671	11547	14.47 (13.79, 15.18)	739	11547	6.40 (5.95, 6.87)	1.00	1.00	1.00	1.00
HCVAb positive	600	3058	19.62 (18.11, 21.26)	473	3058	15.47 (14.14, 16.93)	127	3058	4.15 (3.49, 4.94)	1.01 (0.90, 1.12)	1.10 (0.96, 1.28)	1.13 (0.98, 1.30)	1.11 (0.96, 1.28)
										0.924	0.176	0.097	0.148
HCVAb unknown	329	1654	19.89 (17.86, 22.16)	212	1654	12.82 (11.20, 14.67)	117	1654	7.07 (5.90, 8.48)	0.89 (0.77, 1.03)	0.87 (0.75, 1.01)	0.84 (0.73, 0.98)	0.85 (0.73, 0.98)
										0.108	0.063	0.022	0.030
Emtricitabine													
Overall	2966	15814	18.76 (15.28, 16.32)	2066	15814	13.06 (11.09, 12.03)	900	15814	5.69 (5.04, 5.73)				
HCVAb negative	2194	11253	19.50 (18.70, 20.33)	1510	11253	13.42 (12.76, 14.11)	684	11253	6.07 (5.64, 6.55)	1.00	1.00	1.00	1.00

	All reasons			All reasons except failure/simplification			Failure/simplification			Estimates from weighted Poisson models, i.e. stopping for failure/simplification treated as a competing risk			
HCVAb positive	461	2933	15.72 (14.35, 17.22)	358	2933	12.21 (11.01, 13.54)	103	2933	3.51 (2.89, 4.26)	0.89 (0.79, 1.01)	1.02 (0.87, 1.20)	1.09 (0.93, 1.27)	1.06 (0.91, 1.25)
										0.067	0.828	0.312	0.452
HCVAb unknown	311	1628	19.10 (17.09, 21.35)	198	1628	12.16 (10.58, 13.98)	113	1628	6.94 (5.77, 8.34)	0.89 (0.77, 1.04)	0.86 (0.74, 1.00)	0.83 (0.72, 0.97)	0.84 (0.72, 0.98)
										0.133	0.053	0.019	0.029
Efavirenz													
Overall	1928	8707	22.14 (17.40, 18.87)	1391	8707	15.98 (13.11, 14.45)	537	8707	6.17 (5.34, 6.29)				
HCVAb negative	1362	6058	22.48 (21.32, 23.71)	973	6058	16.06 (15.08, 17.10)	389	6058	6.42 (5.81, 7.09)	1.00	1.00	1.00	1.00
HCVAb positive	387	2052	18.86 (17.07, 20.84)	302	2052	14.72 (13.15, 16.47)	85	2052	4.14 (3.34, 5.12)	0.97 (0.85, 1.11)	1.00 (0.82, 1.21)	0.99 (0.82, 1.21)	0.97 (0.80, 1.18)
										0.685	0.972	0.944	0.770
HCVAb unknown	179	597.1	29.98 (25.89, 34.71)	116	597.1	19.43 (16.20, 23.31)	63	597.1	10.55 (8.24, 13.50)	0.90 (0.74, 1.10)	0.88 (0.71, 1.08)	0.85 (0.69, 1.05)	0.88 (0.71, 1.08)
										0.300	0.213	0.122	0.216
Rilpivirine													
Overall	164	2302	7.12 (5.70, 7.67)	142	2302	6.17 (4.92, 6.77)	22	2302	0.96 (0.59, 1.38)				
HCVAb negative	128	1793	7.14 (6.00, 8.49)	112	1793	6.25 (5.19, 7.52)	16	1793	0.89 (0.55, 1.46)	1.00	1.00	1.00	1.00
HCVAb positive	23	210.9	10.90 (7.25, 16.41)	20	210.9	9.48 (6.12, 14.70)	3	210.9	1.42 (0.46, 4.41)	1.54 (0.95, 2.50)	1.09 (0.61, 1.95)	1.02 (0.56, 1.86)	0.99 (0.54, 1.83)
										0.078	0.774	0.955	0.980
HCVAb unknown	13	298.0	4.36 (2.53, 7.51)	10	298.0	3.36 (1.81, 6.24)	3	298.0	1.01 (0.32, 3.12)	0.54 (0.28, 1.03)	0.54 (0.28, 1.05)	0.56 (0.27, 1.17)	0.55 (0.27, 1.13)
										0.061	0.070	0.121	0.104

Model 1: age, gender, ethnicity, geographical region, mode of HIV transmission, diabetes, BMI, calendar year of starting cART; Model 2 : Model 1 + previous AIDS diagnosis, time dependent.CD4 cell count, HIV-RNA, Previous ART use, concomitant ART use (ABC - 3TC, DRV/r, ATV/r, EFV; 3TC - ZDV, NVP, EFA; TDF- FTC, DRV/r, ATV/r, EFV; FTC - TDF, EFA, LPV/r, DRV/r, ATV/r ; EFA – TDF/FTC, ZDV/3TC ; RIL- TDF/FTC); Model 3: Model 2 + liver fibrosis, (FIB-4 and alcohol use)

Table 5.43 Poisson regression models using Inverse probability weighting stratified by HCVAb status (time-dependent) - Stopping for all reasons except stopping for failure/simplification (ONLY FOR LAMIVUDINE)

	All reasons			All reasons except failure/simplification			Failure/simplification			Estimates from weighted Poisson models, i.e. stopping for failure/simplification treated as a competing risk			
	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	Unadjusted IRR (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Lamivudine													
Overall	2412	12103.75	19.93 (16.01, 17.23)	1853	12104	15.31 (12.72, 13.84)	559	12104	4.62 (4.06, 4.78)				
HCVAb negative	1562	8082.5	19.32 (18.39, 20.31)	1158	8083	14.33 (13.53, 15.18)	404	8083	5.00 (4.53, 5.51)	1.00	1.00	1.00	1.00
HCVAb positive	721	3336.17	21.61 (20.09, 23.25)	599	3336	17.95 (16.57, 19.45)	122	3336	3.66 (3.06, 4.37)	1.20 (1.08, 1.33)	1.20 (1.01, 1.41)	1.10 (0.93, 1.31)	1.10 (0.93, 1.31)
										<.001	0.035	0.272	0.269

HCVAb unknown	129	685.08	18.83 (15.85, 22.38)	96	685.1	14.01 (11.47, 17.12)	33	685.1	4.82 (3.42, 6.78)	1.01 (0.82, 1.25) 0.918	1.04 (0.81, 1.32) 0.771	0.96 (0.75, 1.21) 0.712	0.97 (0.77, 1.24) 0.823

Table 5.46 Association of HCV status on discontinuation of PIs and INIs: Poisson regression models stratified by HCVAb status (time-dependent) – Stopping for all reasons except stopping for failure/simplification

	All reasons			All reasons except stopping for failure/simplification			Stopping for failure/simplification			Estimates from weighted Poisson models, i.e. stopping for failure/simplification treated as a competing risk			
	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	Unadjusted IRR (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
Lopinavir/r													
Overall	1104	4217	26.18 (19.67, 21.85)	850	4217	20.16 (15.76, 17.82)	254	4217	6.03 (5.02, 6.38)				
HCVAb negative	775	2762	28.06 (26.16, 30.11)	590	2762	21.37 (19.71, 23.16)	185	2762	6.70 (5.80, 7.73)	1.00	1.00	1.00	1.00
HCVAb positive	252	1213	20.77 (18.36, 23.50)	199	1213	16.40 (14.27, 18.85)	53	1213	4.37 (3.34, 5.72)	0.86 (0.73, 1.02) 0.079	0.94 (0.74, 1.20) 0.634	0.96 (0.76, 1.23) 0.760	0.97 (0.76, 1.23) 0.795
HCVAb unknown	77	241.8	31.85 (25.48, 39.82)	61	241.8	25.23 (19.63, 32.43)	16	241.8	6.61 (4.05, 10.81)	0.97 (0.75, 1.25) 0.816	1.03 (0.79, 1.34) 0.838	1.01 (0.78, 1.32) 0.935	1.01 (0.77, 1.31) 0.966
Darunavir/r													
Overall	665	4666	14.25 (11.60, 13.37)	402	4666	8.62 (7.20, 8.69)	263	4666	5.64 (4.73, 5.98)				
HCVAb negative	487	3487	13.97 (12.78, 15.26)	294	3487	8.43 (7.52, 9.45)	193	3487	5.53 (4.81, 6.37)	1.00	1.00	1.00	1.00
HCVAb positive	84	642.7	13.07 (10.55, 16.19)	57	642.7	8.87 (6.84, 11.50)	27	642.7	4.20 (2.88, 6.13)	1.07 (0.80, 1.43) 0.660	1.27 (0.90, 1.79) 0.172	1.28 (0.90, 1.82) 0.162	1.26 (0.89, 1.79) 0.189
HCVAb unknown	94	536.1	17.53 (14.33, 21.46)	51	536.1	9.51 (7.23, 12.52)	43	536.1	8.02 (5.94, 10.81)	1.13 (0.83, 1.52) 0.437	0.90 (0.67, 1.23) 0.521	0.84 (0.62, 1.15) 0.283	0.83 (0.61, 1.14) 0.259
Atazanavir/r													
Overall	999	7049	14.17 (11.70, 13.14)	759	7049	10.77 (9.07, 10.39)	240	7049	3.40 (2.89, 3.71)				
HCVAb negative	714	4933	14.47 (13.45, 15.57)	530	4933	10.74 (9.87, 11.70)	184	4933	3.73 (3.22, 4.31)	1.00	1.00	1.00	1.00
HCVAb positive	218	1667	13.08 (11.45, 14.94)	177	1667	10.62 (9.17, 12.31)	41	1667	2.46(1.81, 3.34)	1.03 (0.86, 1.23) 0.763	0.93 (0.70, 1.22) 0.597	0.92 (0.70, 1.21) 0.549	0.91 (0.69, 1.20) 0.484
HCVAb unknown	67	449.2	14.92 (11.74, 18.95)	52	449.2	11.58 (8.82, 15.19)	15	449.2	3.34 (2.01, 5.54)	1.05 (0.79, 1.39) 0.745	0.98 (0.73, 1.30) 0.863	0.89 (0.67, 1.18) 0.407	0.93 (0.70, 1.24) 0.612
Raltegravir													
Overall	436	2192	19.90 (15.20, 18.04)	267	2192	12.18 (9.66, 12.12)	169	2192	7.71 (6.15, 8.23)				
HCVAb negative	321	1609	19.95 (17.88, 22.26)	196	1609	12.18 (10.59, 14.01)	125	1609	12.96 (8.46, 19.90)	1.00	1.00	1.00	1.00

	All reasons			All reasons except stopping for failure/simplification			Stopping for failure/simplification			Estimates from weighted Poisson models, i.e. stopping for failure/simplification treated as a competing risk			
	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	No. events	PYFU	Rate/ 100PYFU (95% CI)	Unadjusted IRR (95% CI) p-value	Model 1 IRR (95% CI) p-value	Model 2 IRR (95% CI) p-value	Model 3 IRR (95% CI) p-value
HCVAb positive	68	420.8	16.16 (12.74, 20.49)	45	420.8	10.69 (7.98, 14.32)	23	420.8	5.47 (3.63, 8.22)	0.89 (0.64, 1.23)	0.96 (0.61, 1.52)	0.97 (0.61, 1.54)	0.97 (0.61, 1.54)
										0.478	0.870	0.904	0.895
HCVAb unknown	47	161.8	29.04 (21.82, 38.65)	26	161.8	16.07 (10.94, 23.60)	21	161.8	7.77 (6.52, 9.26)	1.26 (0.83, 1.91)	1.04 (0.67, 1.60)	1.03 (0.66, 1.60)	0.98 (0.62, 1.55)
										0.271	0.865	0.909	0.945
***Dolutegravir**													
Overall	70	411.3	17.02 (11.54, 17.82)	38	411.3	9.24 (6.07, 11.20)	32	411.3	7.78 (5.00, 9.80)				
HCVAb negative	46	308.8	14.89 (11.16, 19.89)	25	308.8	8.09 (5.47, 11.98)	21	308.8	6.80 (4.43, 10.43)	1.00			
HCVAb positive	8	31.33	25.53 (12.77, 51.05)	6	31.33	19.15 (8.60, 42.62)	2	31.33	6.38 (1.60, 25.52)	2.37 (0.96, 5.81)			
										0.060			
HCVAb unknown	16	71.17	22.48 (13.77, 36.70)	7	71.17	9.84 (4.69, 20.63)	9	71.17	12.64 (6.58, 24.30)	1.11 (0.48, 2.58)			
										0.803			
***Elvitegravir**													
Overall	42	546.6	7.68 (5.20, 9.35)	39	546.6	7.14 (4.79, 8.81)	3	546.6	0.54 (0.11, 1.38)				
HCVAb negative	29	409.3	7.08 (4.92, 10.19)	26	409.3	6.35 (4.32, 9.33)	3	409.3	0.73 (0.24, 2.27)	1.00			
HCVAb positive	6	39.67	15.13 (6.80, 33.67)	6	39.67	15.13 (6.80, 33.67)	0	39.67	0.00 (0.00, .)	2.37 (0.96, 5.81)			
										0.060			
HCVAb unknown	7	97.58	7.17 (3.42, 15.05)	7	97.58	7.17 (3.42, 15.05)	0	97.58	0.00 (0.00, .)	1.11 (0.48, 2.58)			
										0.803			

*Events <100

Model 1: age, gender, ethnicity, geographical region, mode of HIV transmission, diabetes, BMI, calendar year of starting cART; Model 2 : Model 1 + previous AIDS diagnosis, time dependent.CD4 cell count, HIV-RNA, Previous ART use, concomitant ART use (LOP - TDF/FTC,ZDV/3TC; DRV/r - TDF/FTC, ABC/3TC; ATV - TDF/FTC, ABC/3TC; RAL - TDF/FTC, ABC/3TC TDF/FTC/DRV/r; DOL - TDF/FTC, ABC/3TC; ELV- TDF/FTC }; Model 3: Model 2 + liver fibrosis, (FIB-4 and alcohol use)

5.6 Discussion

The aim of this chapter was to assess the impact of HCV on the risk of specific cART discontinuation in HIV-positive individuals specifically focusing on recommended regimens in Italy as per 2016 guidelines [362]. In this analysis 10,637 individuals were included and 15,464 discontinuations were observed, just under half of which were for unknown or 'other' reason. For the remainder, the most frequent reason was simplification, followed by with the following distribution: toxicity/intolerability non-adherence (11%), and then virological/immunological failure (6%). The proportion of ARV drug discontinuations due to toxicity/intolerance in this study was generally lower (19% or 23% when the unknow group was excluded) than that shown in previous similar studies in which proportions of discontinuations of up to 41% were reported [230, 324, 346, 349, 354, 363]. A possible explanation for this difference is the fact that these other cohorts included individuals receiving drug regimens which were no longer recommended in 2016, while in this analysis I intentionally focussed on modern drugs only. In another recent study in two large HIV cohorts in South Africa, the rate of cART discontinuation was more similar to that described here, with a risk of 12% in 15,396 HIV-positive individuals [364]. The exact definition of discontinuation of at least one cART drug varies across different studies (for example in the length of interruption required to classify as a discontinuation) and this may further explain differences in the observed incidence. Furthermore, some studies investigated, the complete interruption of a cART rather than just one drug [346-348] [355].

Discontinuation because of simplification emerged as the most common reason for discontinuation in recent years and this is consistent with a recent Icona analysis evaluating the rate of discontinuation of cART in people initiating their first-line cART after January 2008 [231]. This analysis also observed relatively low rates of discontinuation due to failure or non-adherence confirming the fact that modern ARV drug regimens are much improved in terms of both efficacy and safety resulting in better adherence to treatment [343, 362]. Previously, *Cicconi et al* had showed evidence from the Icona cohort that the pattern of reasons for cART

discontinuation was changing over time with the incidence of stops due to simplification surpassing that shown for intolerance/toxicity [341]. This was also confirmed in a study conducted in the USA and another one by the SWISS cohort. [363, 365].

Overall, in the analysis investigating the effect of drug classes as a whole, I found little differences in incidence rates of cART discontinuation according to HCVAb infection status. However, for NNRTIs, incidence rates of cART discontinuation was lower for HIV/HCV coinfected people compared with HIV mono-infected. In this sample data analysis the majority of individuals in this analysis had initiated TDF/FTC backbone; both drugs are less affected by liver disease as they are mostly secreted in the kidneys. Interestingly this same result was also observed in one of the EuroSIDA publications and explained with the fact that NNRTI are often used with TDF/FTC [349]. However it's worth noting that, risk for hepatoxicity may still be higher for individuals on regimens including Nevirapine [326]

Incidence rates for all other drug classes were similar across HCV status categories. When the risk of discontinuation was evaluated generically for cART as a whole, some previous studies found evidence for an association with HCV infection [230, 347, 349] and others did not [231, 324, 350, 351]. Again, a possible explanation for the discrepancy is the fact that the former studies which showed an association evaluated ARV drugs more likely to cause an interaction between HCV and hepatic impairment. Case-mix of the study population is another potential explanation as the studies reporting an association included a large number of HCV-viremic individuals. In addition, some of the cohorts in these analyses included both cART-naïve and treatment experienced patients while, by definition, people are cART-naive at entry in Icona [348, 349]. Another explanation could be residual confounding as the factors included in the models may not have fully accounted for all bias in the relationship between HCV infection and cART discontinuation.
Similarly, in the analysis assessing cART discontinuation by drug classes according to HCV-RNA status, there was no evidence for an association. However, because HCV-RNA was available only in a subset of all HCVAb positive

participants, this might be partly explained by the lack of power to detect differences.

When considering incidence rates of discontinuations for specific ARVs, Abacavir and Lamivudine were shown to have higher incidence of cART discontinuation in HIV/HCV coinfected individuals compared to HIV mono-infected. The unadjusted analysis for Abacavir showed a 53% increased risk of cART discontinuation; however after adjustment for potential confounders, the risk was attenuated and no longer significant. Similarly, Lamivudine showed a 17% increases risk of cART discontinuation in the unadjusted analysis but after adjustment for potential confounders this risk was attenuated. In contrast, *Mocroft et al*, in the EuroSIDA cohort found these same drugs to be associated with an increased risk of cART discontinuation in HCV-positive even after adjustment of potential confounders (gender, calendar year of starting cART, time on cART, cART naïve status, CD4, type of cART and region) [348].

In the case of NNRTI, Efavirenz was found to have a reduction in risk in discontinuation of 22% for HIV/HCV coinfected individuals compared to mono-infected. However, after adjustment, there was no association, mostly driven by IDU or diabetes. In contrast some studies have found an association between HIV/HCV coinfection and discontinuation of Efavirenz [348]. This could possibly be explained by factors adjusted for in the models as well as the type of population included in the analysis. Another explanation could be type of backbone (i.e zidovudine/lamivudine, didanosine/stavudine, stavudine/lamivudine) used in the cART regimen which are associated with drug discontinuation. Interestingly *Law et al* found neuropsychiatric disorders to be associated with discontinuation of Efavirenz in HIV-positive individuals[366]. Discontinuation of Rilpivirine also showed no association with HCV status.

When assessing the PI/r class, HIV/HCV coinfection was marginally (i.e. borderline statistical significance) associated with a 50% increased risk of discontinuation of Darunavir/r independently of demographics, HIV related factors, previous cART

and concomitant use of cART. After additionally adjusting for liver fibrosis and alcohol use, the effect was further attenuated but remained significant. This is the only ARV for which there was convincing evidence that HCV status impacted on risk of discontinuation. In a previous Icona analysis, evaluating the association between the incidence of liver enzyme elevation and HIV/HCV coinfection, Darunavir/r was found to be well tolerated in both coinfected and mono-infected patients [(367)] . The authors noted that such a finding could possibly explained by close monitoring of liver enzyme elevations using ALT and AST as surrogate markers triggering pro-active discontinuations [(367)].

Integrase inhibitors are now widely used as part of cART regimens in cART- naïve HIV-positive individuals [(343)]. In particular, because of its profile of high potency and good safety, Dolutegravir has been approved for used in first-line regimens even in resource limited settings and it is therefore destined to become one of the most frequently used drug in HIV worldwide [(35)]. This analysis did separately evaluate the association between HCV infection and the risk of discontinuing drugs belonging to the INI class. The data showed no evidence for an association, although differences in unadjusted rates of discontinuation by HCV status were detected which were not significant possibly because of low statistical power. However, there seems to be general consensus that the safety profile of these drugs in HIV/HCV coinfected individuals is more favourable that that seen for PIs or NNRTI based regimens [(36)]. A systematic review of RCTs involving 6,407 HIV-positive individuals evaluating the effectiveness of Dolutegravir showed that the rate of discontinuation of this drug in the wider population of PLWH was lower compared to non-Dolutegravir containing regimens [(368)].

The analysis assessing the effect of HCV viremia in HCVAb positive individuals, found incidence rates of PI/rs discontinuation to be higher in HCV-viremic individuals compared to HCVAb negative individuals. However, when assessing specific drugs, HCV viremia was marginally associated with the risk of stopping of Lopinavir/r even after adjusting for important confounders such as liver fibrosis and alcohol use. Indeed, the presence of liver damage and high alcohol intake both

have detrimental effect on the liver which in turn can play a role in cART discontinuation [346]. At the same time, these same factors may affect the probability of prescription of PI/r. Therefore, importantly, this analysis shows the effect of Lopinavir/r could not be explained by this confounding mechanism. This result was also consistent with what found by *Grint et al* when exploring the association between the use of Lopinavir/r and the risk of discontinuation due to toxicity or patient/physician choice [349]. This EuroSIDA study also found that HCV viremia patients had a 2-fold increased risk of Darunavir/r discontinuation for any reason. As Darunavir/r is metabolised in the liver, this result is expected in patients with hepatic impairment caused by HCV. Although, the analysis in this chapter restricted to people with available HCV-RNA test results was likely to be underpowered, the association between the risk of stopping Darunavir/r and HCV status was confirmed in my main analysis stratified by HCV antibody status.

In a secondary analysis in which CD4 and HIV-RNA were both fitted as time-dependent variables appropriately as well as controlling for potential informative censoring using IPW, the overall findings did not differ from those of the primary analysis when the expanded definition of discontinuation due to toxicity/intolerability was used as the endpoint. Because after relaxing the assumption of independence between the reasons for stopping, results were similar, it is conceivable to conclude that little bias had been introduced from the presence of informative censoring. However, the two analyses answer slightly unrelated questions as the latter evaluated the effect associated with current HCV status which might differ from the status recorded prior to starting cART. Furthermore, the sensitivity analysis had a different endpoint, in that discontinuations with specific reasons (except for failure/simplification) were not counted as positive for the outcome.

HCV-RNA positive status also showed a weak association in the Icona data analysis with discontinuation of Lopinavir/r. Lopinavir is a drug which is mainly metabolized in the liver and therefore the association of HCV viremia with the risk

of stopping due to intolerance/toxicity is a possible validation of my hypothesis. This was consistent with the results of other studies that considered discontinuation due to toxicity in sensitivity analyses and reinforce the idea that there might be a causal link between HCV and the risk of interruption of this drug via the P450 enzymes drug concentration pathway [347, 349].
It is important to note that both Darunavir/r and Lopinavir/r are most commonly used as Ritonavir-boosted regimens and the booster has been previously implicated in a higher frequency of discontinuations among HIV/HCV coinfected people.

Some studies have found a higher risk of discontinuation of Lamivudine in the HCV infected which was independent of confounding factors only in one analysis and not confirmed in the Icona data. Lamivudine, Lopinavir/r and Darunavir/r are important in that they were used as rtv-boosted as this is likely to be the issue were the three ARVs which were identified as implicated in a higher frequency of discontinuations among HIV/HCV coinfected people. However, Lamivudine is a generally well tolerated drug with little biological plausibility in the mechanism leading to drug discontinuations because of liver impairment. A possible explanation for the observed higher risk of discontinuation of Lamivudine in HIV/HCV coinfected people is the fact that it is typically used in combination with other toxic drugs such as ZDV and Abacavir which is known to be partly metabolized by the cytochrome P450 enzyme system (CYP 3A) [326]. Another possible explanation for the risk associated with use of 3TC was residual confounding by calendar year that was not fully removed by adjustment, as Lamivudine was more widely used in early calendar years when the risk of stopping due to toxicity was greater. However, in a sensitivity analysis restricting to people who started Lamivudine-based cART after 2002, there was still some evidence for an association between HIV/HCV coinfection and discontinuation of Lamivudine.

5.7 Limitations

In addition, it is important to highlight that evaluating the incidence of drug discontinuation is not equivalent to estimate the rate of adverse events on a drug. Indeed it is possible that for a given degree of severity, not all adverse events necessarily lead to the discontinuation of a drug, as switching decisions are at the discretion of the treating physicians. On the other hand, it is possible that specific drugs are more likely to be stopped in the HCV-infected populations for ingrained beliefs in clinicians that a certain antiretroviral drug is associated with hepatotoxicity. However, I attempted to address this concern by counting all discontinuations as events, regardless of the reason. In addition, a secondary analyses was carried out restricting to discontinuations due to toxicity/intolerability and appropriately controlling for possible censoring bias by means of IPW adjustment. The results of this sensitivity analysis were broadly similar to those of the main analysis.

The analysis evaluated modern drugs which are currently recommended by European guidelines and frequently used in the clinics but for some of the drugs which very newly introduced statistical power was limited. Nevertheless, at the time of this analysis little was known about the hepatotoxicity of the INSTIs so it was important to confirm that the safe toxicity profile of this drug class also in people with HCV. This is particularly important for drugs such as Dolutegravir which is now used in first line treatment in people infected with HIV worldwide.

The primary analysis considered HCVAb infection status at entry in the cohort as well as potential confounders measured at baseline. It is possible that the HCVAb infection status may change over time as HCVAb-negative individuals may seroconvert for HCV which could have diluted the association. In turn, although HCVAb serology status cannot revert from positive to negative, HCV-RNA positive individuals can spontaneously clear the infection or be cured by therapy. However, for the main analysis with HCV exposure status based on serology test results, I performed a secondary analysis after fitting HCVAb status, CD4 and HIV-RNA all as time-dependent factors and results did not differ from those of the main

analysis. In addition, much consideration was given to the construction of the multivariable models and investigation of confounding by sequentially adding single variables or groups of variables in the models.

One identified major source of potential confounding was calendar year of cART initiation as the prevalence of HIV/HCV coinfected individuals was much higher in earlier calendar years when the rate of discontinuations of ART was also higher. There was clearly considerable confounding for some of the drugs, as the estimated rate ratios changed considerably from the unadjusted result to those in the adjusted models.

However, it is also important to state that although the multivariable analysis was controlled for a large number of measured potential confounders, because of the observational nature of the study it is not possible to rule out the presence of unmeasured confounders that were not accounted for. Additionally potential drug drug interactions between DAAs and cART are not captured as reasons if discontinuation. In the DAA era, this could potentially be an important reason for cART discontinuation.

5.8 Conclusion

In this chapter, the incidence rates of stopping ARV drugs have been estimated and compared according to HCV infection status taking into account both according to HCVAb status and HCV-RNA status. The key finding is that there seems to be no substantial difference in the incidence rates of ARV discontinuations according to HCV-infection status for most of the drugs evaluated. The only exceptions were possibly Lamivudine, Lopinavir/r and especially for Darunavir/r for which there was some evidence that HIV/HCV coinfection may have played a role in increasing the risk of stopping these drugs. The most compelling case was possibly for Darunavir/r in which evidence of an association of coinfection with stopping was very strong in magnitude. At the time of performing

this analysis the risk of ARV discontinuation in people coinfected with HCV was an important issue for the management of PLWH.
Following these results, drugs such as Abacavir or Darunavir/r have been used more parsimoniously in people with HCV because they were shown to be associated with greater rates of discontinuation. In the current era of universal access to highly effective HCV treatment, in which >90% of HIV/HCV coinfected individuals are cured for HCV, these results have fewer practical implications for daily clinical practice. However, a key implication of this finding is that even today perhaps Darunavir/r should not be a priority drug for PLWH who did not achieve cure on DAA or at least delay its use until cure is obtained.

The data also confirm that for modern ARV drugs, discontinuation due to simplification is by large the most frequent reason for discontinuation This supports the notion that newer drugs are better tolerated and that pro-active switches aiming to reduce the number of drugs, pill burden or ARV costs are increasingly common in clinical practice, at least in the Italian setting. This analysis also provides an updated detailed description of the management of HIV/HCV coinfected individuals in people seen for care in Italian infectious disease units as well the pattern of ARV drugs currently used in routine clinical care.

Finally, the issue of drug-to-drug interaction for people about to initiate DAA (which was not covered at all in this thesis) still constitutes a problem although typically consists in a temporary stop of the ARV followed by resumption after achievement of HCV eradication.

CHAPTER 6

6 WHAT IS THE ROLE OF LATE HCV PRESENTATION ON ALL CAUSE MORTALITY AND HCV TREATMENT INITIATION AMONG NEWLY DIAGNOSED COINFECTED HIV INDIVIDUALS SEEN FOR ROUTINE CLINICAL CARE IN ITALY?

6.1 Aim and objectives

The aim of this chapter is to identify factors associated with late HCV presentation, and investigate the association of late HCV presentation with risk of all-cause mortality as well as the probability of starting HCV therapy, among individuals newly diagnosed with HIV.

The specific objectives are, among individuals newly diagnosed with HIV at entry to the Icona cohort:

1. To estimate the proportion of individuals tested for HCV
2. To compare characteristics between those HCV tested and those not HCV tested
3. To assess the prevalence of late HCV presentation at entry in the cohort among HIV/HCV coinfected individuals, and compare individuals' characteristics between late HCV presenters and non-late HCV presenters
4. To evaluate changes over calendar time in the prevalence of late HCV presentation among HIV/HCV coinfected individuals
5. Among HIV/HCV coinfected individuals, to investigate the association between late HCV presentation and;
    - Subsequent risk of all-cause mortality
    - Subsequent probability of starting HCV therapy

6.2 Introduction

As part of the HCV continuum of care (CoC), diagnosing HCV early means individuals can be treated and cured. Screening for HCV (based on detection of HCVAb or HCV-RNA) is the first step to identify HCV-positive individuals; however there is still a large proportion of people with chronic HCV infection who are not aware of being infected [5, 142]. Interestingly, Italy has the highest burden of HCV in Europe, primarily transmitted through people who inject drugs. However, as highlighted in chapter 1 (section 1.3.5) and discussed more extensively in chapter 7 section 7.3.3, there has been great improvements in treatment, with policies now in place indicating universal access to DAA as of March 2017 for all HCV-positive individuals in Italy regardless of stage of liver disease [160]. In 2015, around 146,000 individuals with chronic HCV in the EU were treated and those in UK, Italy, Spain and France accounted for more than 80% of all individuals treated [160].

According to the EACS 2019 version 10 HIV treatment guidelines, all HIV/HCV coinfected individuals should be treated with DAAs, regardless of stage of liver disease [38]. More so, the current EACS 2019 version 10 guidelines recommend HCV testing at the time of HIV diagnosis and yearly subsequently. Once somebody is found to be infected with HCV, extent of liver damage also needs to be assessed [38]. If individuals enter care who already have an indication of advanced liver disease, this implies that there have been some missed opportunities to detect HCV infection earlier. It is well known that HCV is asymptomatic, and if regular tests or screening are not done promptly after HIV diagnosis, the opportunities to detect HCV infection early will be missed. Limited access to care is another issue that results in failure to test HIV-positive individuals for HCV [158]. It is crucial to test everybody with HIV for the presence of HCVAb/HCV-RNA, not only those perceived to be at high risk of HCV infection.

Following a consensus definition of late HCV presentation, endorsed by EASL in 2015, it is possible to classify individuals based on whether they present late or not into care [369]. In this chapter, I applied these definitions to a cohort of individuals

newly diagnosed with HIV and coinfected with HCV in the Icona cohort in order to better estimate the current burden of individuals presenting late into care in Italy [38]. One of the objectives in this chapter was to identify the determinants of HCV testing which is considered as the very first step in the HCV CoC (more of this in chapter 7). Ultimately, the results of this analysis should help identify people at greater risk of being tested for HCV and guide target interventions. The other aims were to provide a robust estimate of the prevalence of HCV late presentation in the cohort, to assess the correlates of late presentation, and to evaluate the association between late presentation and both treatment and all-cause mortality.

6.3 Literature review

In this chapter, the literature review focuses on globally published research relating to late HCV presentation in HCV mono-infected and HIV/HCV coinfected individuals in pre and post DAA era. The literature search was first done up to April 2019 and subsequently updated to include additional evidence published up to February 2021.

6.3.1 Prevalence of late HCV presentation among people with HIV

Although we are in the era of new and improved HCV therapy, people are still presenting into care with advanced liver disease [1, 3, 370-376]. In a recent study including individuals enrolled between 2014 and 2016 assessing the HCV CoC in cohorts of HIV-positive individuals in Europe, stage of liver of disease was evaluated in individuals when first entering HIV care. In people identified with chronic HCV infection it was found that approximately 16% entered HIV care with advanced fibrosis or cirrhosis [377].

Another study in Denmark involving 570 individuals enrolled between 2007 and 2016 with chronic HCV infection aimed to estimate the prevalence of late presentation for care and late stage liver disease in individuals attending their first consultation in clinic [378]. In this analysis, late presentation for care was defined as

individuals presenting with liver stiffness measurement kPa>9.5. Late stage of liver disease was defined as presence of hepatocellular carcinoma or decompensation within 6 months of first consultation. This study found 32% (169/570) of individuals presented late with HCV and among these, 5% (28/169) also had late stage liver disease [378]. Older age and heavy alcohol consumption were found to be risk factors for presenting late with HCV. In contrast HIV infection was not associated with late HCV presentation, possibly explained by very few individuals included in the study[378]. *Moorman et al* evaluated data from the Chronic Hepatitis Cohort Study in the US between 2006 and 2011 [370]. In this analysis, late diagnosis of HCV infection was defined as having cirrhosis or FIB-4 score >5.88 during the period of 3 months prior to 12 months after the initial diagnosis of HCV. The study included 6,166 HCV-positive individuals of whom 17% (n=1,056) were defined as having late HCV diagnosis and a higher prevalence of late HCV diagnosis was found in the older age groups (born before 1945) and in individuals relying on the country's health insurance systems [370]. A recent analysis from the same authors, this time looking at newly diagnosed chronic HCV infection during the period of 2014 to 2016, included 2,694 HIV-positive individuals of whom 21% (n=576) had late HCV diagnosis. This analysis therefore showed a 5% increase in prevalence of late HCV diagnosis compared to the previous study [379]. In this same study, out of the 42 individuals who had HIV/HCV coinfection with newly diagnosed HCV and were not included in the main analysis, 24% (n=10) were found to have severe liver disease [379].

In another cohort study (British Columbia Hepatitis Tester's Cohort) in Canada, late HCV diagnosis was defined as the detection of decompensated cirrhosis or hepatocellular carcinoma within two years of HCV diagnosis [373]. The study included 4,827 with HCV infection of whom 32% (n=1,566) were found to have decompensated cirrhosis and 6% (283/4827) were found to have hepatocellular carcinoma within two years of HCV diagnosis. In this cohort, however, only 3% (142/4827) were HIV/HCV coinfected. Among those diagnosed with decompensated cirrhosis a similar proportion of 2.6% (40/1566) were HIV/HCV coinfected individuals.

In these studies, the variability in the estimates of the prevalence of late diagnosis of HCV among HIV-positive individuals (ranging between 10% to 30%) can be partly explained by the method of assessing stage of liver disease. For example, in some studies, this assessment was reliant on laboratory data to calculate FIB-4 score while clinical diagnosis using liver biopsy has been used to assess stage of liver disease in some other cases. It is therefore challenging to directly compare these estimates of the prevalence of HIV-positive individuals presenting late with HCV coming from the various studies. Also, the prevalence of late HCV diagnosis may depend on the health care setting, as they are still differences by countries in terms of universal access to care. For example, *Gupta et al*, carried out a retrospective analysis of individuals (with or without HIV infection) with confirmed HCV infection in sub-Saharan Africa. Two hundred and fifty-three individuals were included of whom 30% (n=67) were diagnosed with HCV within a year of study enrolment and 21% (n=37) had advanced liver disease, although only a small percentage 4% (n=10) were HIV/HCV coinfected [380]. Such low prevalence of HCV infected individuals engaged in healthcare could be explained by the fact HCVAb testing only became available in sub-Saharan Africa in 2016 [380]. This is a general issue in low-resource limiting countries which are affected by lack of resources needed to screen individuals for HCV in the first place. Certainly, taken together, these results indicate that the prevalence of late diagnosis of HCV among HIV-positive individual is at least 10%, which seems concerning.

Marcellin et al conducted a retrospective review of medical records of individuals with chronic HCV infection (with or without HIV), looking at the methods that physicians used to assess severity of liver disease in five European countries (France, UK, Italy, Spain and Germany). There were 4,594 individuals included in the analysis. Liver biopsy was found to be less frequently used in Italy, France, Germany or Spain compared to the UK. The study also showed that, once the individuals had been assessed using Fibroscan, this reduced the probability of undergoing liver biopsy [381]. The use of different methods to assess the stage of liver disease is likely to lead to differences in prevalence rates of stage liver disease in different countries or settings [377, 381]. Certainly, reported prevalence

rates also vary depending on the characteristics of the target population being studied (i.e. age, mode of HIV transmission, etc.) [375]. For an example, *Chirikov et al*, assessed the presence of advanced liver disease (with or without HIV infection) in three age groups defined as those born prior to 1945, between 1945 and 1965, and those born after 1965 [375]. They found higher prevalence rates of advanced liver disease in the elderly group 28%, followed by 23% and 15% in each of the other age groups, respectively [375]. In the same study, HIV/HCV coinfection was associated with reduced risk of advanced liver diagnosis PR = 0.63 (0.50-0.80, P<0.001).

6.3.2 Association between late HCV presentation with risk of all-cause mortality in HIV-positive individuals

In this section, I collated relevant studies that investigated the impact of late HCV diagnosis on the risk of advanced liver disease and all cause-mortality, including studies regardless of whether all participants were coinfected with HIV.

It is well known that untreated chronic HCV can result in cirrhosis in 20-30% of individuals by 20 years from the time of infection and this contributes to increased mortality [382]. Indeed, mortality rates amongst HIV/HCV coinfected are high compared to HIV mono-infected in the cART era [221, 383-386]. This is reported in a meta-analysis by *Chen T-Y et al* who assessed changes in the mortality rate between the pre-cART and post-cART eras in HIV/HCV coinfected individuals. The meta-analysis included ten studies conducted in the pre-CART era which included 4,413 HIV/HCV coinfected individuals and 10,213 HIV mono-infected individuals. Perharps surprisingly, the authors first reported a pooled mortality adjusted RR of 0.69 (95% CI: 0.54 – 0.88) in HIV/HCV coinfected compared to HIV mono-infected who were cART-naive. The likely explanation for this finding is that, in a period in which there was no effective treatment for HIV, HIV mono-infection was more lethal than HIV/HCV coinfection. However, the meta-analysis also included 27 studies conducted in the cART era including 25,319 HIV/HCV coinfected and 61,697 HIV mono-infected individuals. In contrast here, the pooled adjusted RR indicated an

increased mortality rate in the HIV/HCV coinfected population compared to HIV mono-infected: aRR=1.35 (95% CI: 1.11 – 1.63) [221]. In the cART era, liver-related death is the leading cause of mortality rather than AIDS events among HIV/HCV coinfected individuals, likely because HIV treatment has improved over time and HIV/HCV coinfected individuals are now living longer and therefore morbidity related to HCV dominates.

The association between late HCV presentation and risk of all-cause mortality was assessed by *Moorman et al* who studied health outcomes of individuals with HCV infection seen for routine clinical care between 2006 and 2011 in the USA. They found a mortality rate of 6.7/100 person-years in people with late HCV diagnosis compared to 1.8/100 person years in those classified as non-late HCV diagnosis. The estimated mortality incidence risk ratio was therefore 3.8 (95% CI: 3.2 - 4.2). Seventeen percent (1056/6166) of the included individuals appeared to be in the late stage of HCV disease, with a mean (SD) of 3.4 (2.2) and 5.8 (5.5) years from HCV diagnosis in the late and non-late HCV diagnosis group, respectively [370].

In the EuroSIDA cohort, *Grint et al* assessed factors associated with all-cause and liver-related death in 3,941 HIV/HCV coinfected individuals who contributed 16,091 PYFU. A total of 670 deaths were observed with an overall all-cause mortality rate of 41.6 (95% CI: 38.6 – 44.7) per 100 PYFU, and the most common cause of death was liver-related in 22% of the events. Unsurprisingly, they also found a strong association between advanced liver disease (F4 fibrosis) measured at baseline and the risk of liver related death: adjusted HR = 6.25 (95% CI: 4.08 – 9.58; p<0.0001) compared to those with mild liver disease [197] adjusted for age, sex, mode of HIV transmission, region of EuroSIDA, cardiovascular events, diabetes diagnosis, HCV genotype, calendar year, CD4 cell count, CD4 cell count nadir, HIV-RNA, HBsAg status, minimum duration of HCV infection and staging of liver fibrosis [197]. Interestingly, having a history of HCV infection longer than 10 years was also associated with a higher risk of liver related death HR = 1.95 (95% CI: 1.03 – 3.71; p=0.041) compared to those who were HCV infected for less than two

years [197]. These results highlight the key importance of the timing diagnosis of HCV and liver disease staging to reduce risk of mortality.

A study in France enrolling individuals from two cohorts with or without HIV between 2005 and 2016, compared mortality rates between HIV/HCV coinfected and HCV mono-infected in the DAA era [387]. The study included 1,253 individuals with cirrhosis i.e. advanced liver disease of whom 14% (n=175) were HIV/HCV coinfected individuals. In this study, similar death rates were observed in the HIV/HCV coinfected and HCV mono-infected group with a reported overall 5-year crude mortality rate of 12.9% vs. 10.8% respectively HR=1.1 (95% CI: 0.72 – 1.72). The lack of evidence for a difference might be explained by the fact that the majority of individuals included in this analysis had undetectable HIV-RNA and therefore were protected against HIV disease progression [387]. However, upon stratification by age, the authors found that in older HIV/HCV coinfected individuals there was an increased risk of mortality vs. mono-infected HR=1.88 (95% CI: 1.15 – 3.06; p=0.001). It is possible that older age is an indicator of late HCV presentation and late HCV presentation in HIV/HCV coinfected individuals was an effect-modifier for the risk of death in this analysis. The authors did not perform a formal test for interaction with age.

Studies of HCC can also give insight into late diagnosis of HCV. *Merchante et al*, recently examined the risk of death after HCC diagnosis in HIV/HCV coinfected individuals [388]. The cohort Group for the Study of Hepatitis virus (GEHEP-002) enrols all HIV/HCV coinfected individuals diagnosed with HCC in Spain and the analysis included data collected between 1999 and 2017. The total population included N=457 participants, of whom 74% (n=339) were HIV/HCV coinfected and 26% (n=118) HCV mono-infected. In participants who were screened for HCC within a year prior to HCC diagnosis, hepatocellular carcinoma was present in 57% (192/339) HIV/HCV coinfected and in 52% (73/118) HCV mono-infected individuals. Using the Barcelona Clinic Liver Cancer stage at diagnosis, 17% (57/339) HIV/HCV coinfected people had severe liver damage compared to 6% (17/118) in the HCV mono-infected group (p<0.001). Out of the total 457 people

studied, 73% (n=334) died and in 91% (303/334) of these deaths was due to HCC. Estimates from a Kaplan Meier plot showed a 2-year probability of death of 65% in HIV/HCV coinfected vs. 57% in HCV mono-infected. However, after adjusting for potential confounders (age, gender, alcohol consumption, HIV infection, previous achievement of SVR, BCLC stage at presentation), the authors concluded that the higher mortality rates observed in HIV/HCV coinfected individuals was due to late diagnosis of HCC [388].

A further issue relevant to the impact of late diagnosis of HCV is that, there is ongoing debate as to whether there is a clinical benefit in treating individuals with very advanced liver disease, such as decompensated cirrhosis, with DAAs. The main uncertainty surrounds whether full recovery is possible following treatment with DAAs and over the duration of this recovery. More studies are needed with longer follow-up to monitor the regression of liver disease post HCV cure [389, 390]. Additionally, it is also likely that individuals found to be in the very late stages of liver disease, may have additional health complications and require more complicated therapy strategies including liver transplantation besides DAA treatment [389, 390]. Therefore, in individuals with very advanced liver disease, although DAAs may improve the prognosis of the disease it may not necessarily fully eliminate the risk of progression [389, 390].

Carrat et al have looked at clinical outcomes in individuals with advanced liver disease following treatment with DAAs and found a reduction in risk for mortality after end of treatment [391]. The authors compared incidence of mortality, HCC and decompensated cirrhosis in individuals treated with DAAs vs. those not treated in the French ANRS CO22 Hepather cohort [391]. In the adjusted analysis, individuals treated with DAA were at a reduced risk of mortality compared to individuals not treated HR = 0.48 (95% CI: 0.33 – 0.70); $p<0.001$[391]. The advantage of initiating DAA in terms of survival was also found in a study involving the Women's Interagency HIV study (WIHS). The authors compared 10-year all-cause mortality risk among people with HIV/HCV coinfection receiving DAA at study entry compared with the risk in those not receiving DAA at study entry. The 10-year all-

cause mortality risk was 14.9 (95% CI: 9.2 – 24.4) in HIV/HCV coinfected treated vs. 18.7 (95% CI: 10.8 - 30.5) in the untreated or a risk difference of -3.8 (95% CI: -9.22 - 0.89) [392].

In summary, several studies have reported an association between advanced liver disease and all-cause mortality, more so in older age groups, which suggests the impact of late diagnosis on HCV in terms of risk of mortality. In addition, there is an impact of DAA initiation on reduced risk on mortality in HIV/HCV coinfected population, suggesting the importance of timely HCV therapy following HCV infection. However, there is still ongoing debate with regards to risk of progression of HCV following HCV therapy in individuals with very advanced or severe liver disease. The issues are that individuals with very advanced liver disease may not fully benefit from treatment with DAAs because the disease is too far advanced and the liver damage is beyond repair.

6.3.3 Association between late HCV presentation and treatment initiation

With the exception of individuals with a very short life expectancy due to very advanced liver disease who cannot be treated, according to current European HCV guidelines in 2021 (AASLD and EASL), treatment of all individuals with chronic HCV infection is recommended regardless of stage of liver disease, [142, 393]. This also applies to HIV/HCV coinfected populations. However, in the first few years of the introduction of DAA treatment, advanced stage of liver disease were predictors of early initiation of treatment [380, 394]. More so, only 2.5% of the 71 million people globally infected with HCV initiated HCV therapy in 2016. However, about 86% of individuals initiating HCV therapy were treated with DAA (WHO–progress report) [160, 395]. Also, in Italy free access to DAAs [391] offered to all HIV/HCV coinfected people accessing care started from March 2017 [160].
The situation is similar in other European countries. Universal access to DAA in France was in 2017, with Spain and the UK was in 2018 [396]. Figure 6.0 shows proportion of HCV infected people in the general population initiating DAA treatment[11]. Iceland, Egypt and Georgia stand has countries with >70%

individuals with HCV infected were treated. Indicating the different policies and strategies in place in different countries[11].

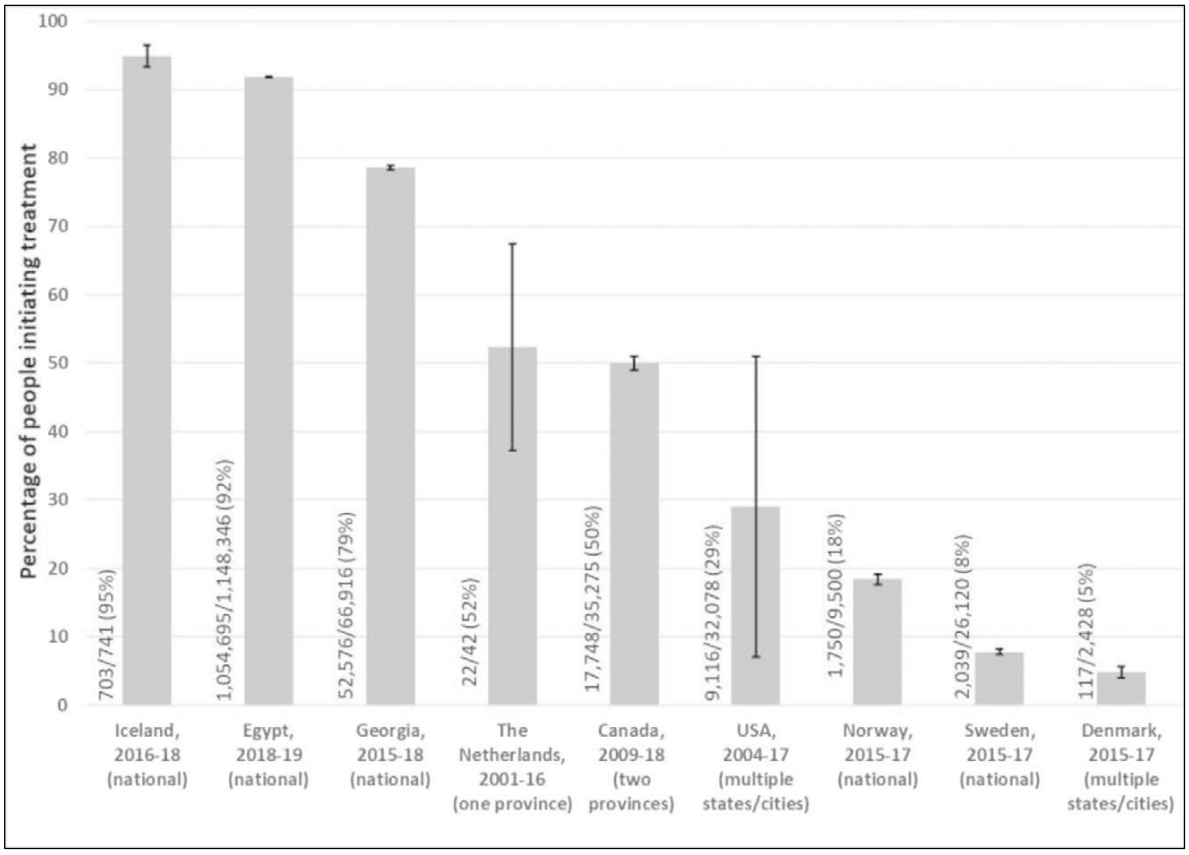
Juarez-Rivero et al carried out a study, six months post introduction of universal access to DAA in Spain. Of the 3,474 HIV/HCV coinfected individuals included, 22% (n=764) had advanced stage of liver and among these 7% (53/764) developed hepatic decompensation, an indication that, even in the DAA era, people are still presenting late for treatment. In terms of rates of treatment initiation, 24% (n=834) of individuals were treated within the first six months of the implementation of universal access to DAA [397]. In an analysis of an HCV mono-infected cohort in France followed-up between 2012 and 2015, comparing characteristics of patients treated with DAA with those of the not treated, severity of liver disease, longer duration of HCV disease and comorbidities were all associated with DAA use [391]. Since the analysis was conducted shortly after the first introduction of DAA, it is not surprising that those with more advanced HCV disease were treated first. However, universal access to treatment is still proving to be challenging in some countries. For example, in the Chronic Hepatitis C cohort in the USA, there is still a proportion of individuals who are not yet tested for HCV and because the HCV population is aging, the prevalence of advanced liver disease is likely to increase due to lack of access to HCV therapy [379].

The emergence of the COVID-19 pandemic has had a huge impact on HCV treatment initiation as countries have diverted public health resources to tackling the pandemic [398, 399]. This is likely to impact on HCV elimination targets as set by WHO [400].

In summary, studies highlight that varying policies of access to DAA in different countries will impact on access to HCV treatment in individuals with late HCV

presentation.

Figure 6.0 Proportion of individuals diagnosed with who received DAA among the general population [11]



M. T. Yousafza et al, 2021. Global cascade of care for chronic hepatitis C virus infection: A systematic review and meta-analysis. J Viral Hepat 2021 Vol. 28 Issue 10 Pages 1340-1354

6.3.4 Summary of literature review

The literature review highlights that because treatment with DAA has been shown to be effective in eliminating HCV-infection from the HIV/HCV coinfected population, now more than ever before, late HCV diagnosis should be prevented to reduce the risk of unfavourable outcomes. Nevertheless, there is a relatively limited amount of research specifically looking at late diagnosis of HCV in the HIV/HCV coinfected populations, particularly in settings with universal healthcare.

Although there is now an agreed definition of late HCV presentation which was formalised in 2015, some of these studies were carried out before this consensus was achieved, or have sufficient information collected in order to implement the agreed definitions in the analyses so there is variability in how late presentation of HCV was defined. Consequently, it is difficult to standardise these estimates in order to grasp the real scale of the problem and the variation in prevalence of late HCV presentation across the settings.

The studies included in the literature review also have some methodological limitations, worth highlighting. Some of the studies used exploratory analyses such as multivariable analyses aiming at the identification of factors independently associated with late HCV diagnoses. As suggested by *Greenland et al* [401], the interpretation of the results of such exploratory analyses are often problematic. It occurs frequently in the literature that estimates for the primary exposure of interest as well as estimates for what can be termed as secondary risk factors are all included in the same model and presented together in a single result table. Causal diagrams are a useful tool that allows to transparently describe these relationships and identify and distinguish between confounders mediators and colliders (as mentioned in chapter 2 section 2.8.5) [234].

After a clear identification and definition of the exposure (late HCV diagnosis) and outcome (all-cause mortality and initiation of treatment) the strategy to construct multivariable models in this chapter was to include in the final multivariable model all potential confounding factors for this association. Most importantly, I made sure that none of the models included colliders or mediators as mentioned in chapter section 2.8.5. I have used DAG graphs to describe the hypothesised relationships between exposure outcome and other variables included.

In terms of other limitations of previous analyses in the literature, some studies have used retrospective routine clinic data which are prone to bias such as under reporting of HCV infection status, recall bias in an individual reporting when the HCV test was done or lack of standardised methods to establish HCV infection.

One of the strengths of using the Icona cohort is that it is a prospective ongoing cohort study with data collected in the real-world setting in an unselected population and data collection standardised across all the sites involved.

In this chapter, only participants in Icona recently diagnosed with HIV are included in the analysis. I focused on people newly diagnosed with HIV in order to include only true incident cases, as these people should also be tested for HCV at the point of HIV diagnosis. This was a limitation in other studies of late HCV presentation in coinfected people, as individuals included were not limited to newly diagnosed HIV individuals. Also, considering that, after 2014, universal access to DAA was recommended in Italy, results are likely to reflect the magnitude of late HCV diagnosis in the DAA era.
An additional strength of my analysis is that it uses the standard definition of late HCV presentation currently proposed by a panel of European experts.

Very few studies have looked at outcomes related to late HCV diagnosis, as well as its possible impact on the probability of treatment initiation. In particular, in this chapter, as well as providing an estimate of the prevalence of late HCV diagnosis in Italy, I will also look at the probability of HCV treatment initiation and whether this is affected by late presentation and stage of liver disease. The rate of HCV-treatment uptake is an important outcome to look at because although universal treatment is now recommended it remains key to monitor if these guidelines are implemented in clinical practice and how quickly individuals are accessing treatment.

All these data should improve our understanding of some of the challenges around HCV testing that remain for achieving elimination of HCV.

6.4 Methods

6.4.1 Inclusion criteria

Figure 6.2 Participant Flow diagram

Figure 6.2 shows patient flow diagram of the selection of individuals for inclusion into the analyses. This analysis included all individuals enrolled in Icona Foundation Study cohort up to January 2018 who were diagnosed with HIV within six months of the date of enrolment and subsequently had at least one month of follow-up in the cohort. The rationale for restricting only to persons newly diagnosed with HIV was to select a population of people who were eligible for testing for HCV at that time and had likely not been tested previously.

The analyses in this chapter were developed in two stages. The first stage focuses on the comparison of the characteristics of the newly HIV-diagnosed individuals for whom there was an available HCV serology test result at entry in the cohort vs. those who had not been tested for HCV.

The second stage only includes a subset of the study population included in stage 1: those for whom the HCV test result was available, and this result was positive (HCVAb positive). To be included in stage 2 analysis, individuals needed to have had an HCVAb/HCV-RNA test result at the time of enrolment. HCV infection status was then established either from the serology test (a positive HCVAb test result) or, if serology was not available, from an HCV-RNA positive (quantitative or qualitative) test result or from the availability of HCV genotype. Specifically, the date of first diagnosis of HCV was defined as the earliest of: first positive HCVAb; first positive HCV-RNA, qualitative test or date of HCV genotype test result. Individuals with positive serology but who were HCV-RNA-negative were excluded from this stage 2 prospective analysis because they had spontaneously cleared

their HCV infection. Finally, the availability of a measure of age, ALT, AST and PLT at entry in the cohort was also required to be included in the stage 2 analysis to determine participants' stage of liver disease. In particular, the value of these markers was used to calculate the FIB-4 score at enrolment. This second stage aimed to estimate the prevalence of late HCV presentation among all those with HCV, assess the factors associated with late HCV presentation and evaluate the association of late presentation with time to treatment initiation and all-cause mortality.

A time window of six months to capture data on liver disease was used because clinical visits in the Icona Foundation Study cohort are scheduled on average every six months so to ensure that the most recent status of liver disease was captured.

6.4.2 Definitions of late HCV presentation

The analysis uses a consensus definition of late presentation of viral hepatitis which was developed in 2015, by a group of experts in viral hepatitis within the EASL and HIV in Europe Initiative. According to this panel of experts, late presentation of viral hepatitis B or C comprises of two definitions based either on presentation with 'advanced liver disease' or presentation with 'late stage liver disease' [369]. To distinguish between the two definitions, I abbreviate the definitions as ALD (advanced liver disease) and LSLD (late stage liver disease) throughout this chapter to avoid confusion and collectively defined them as late HCV presentation. Essentially, the definition of ALD is based on values of biomarkers only, mostly measured using non-invasive procedures, while the diagnosis of LSLD is based on clinical evaluation. A composite endpoint of ALD or LSLD is also often used. Untreated patients who are diagnosed late with HCV should be immediately entered into care to prevent liver disease progression[369].

1. **Advanced liver disease in untreated patients with chronic hepatitis B or C**

According to this definition *"A patient with chronic hepatitis B or C and significant fibrosis assessed by one of the following: serologic fibrosis score≥F3 (assessed by APRI score > 1.5, FIB-4 > 3.25, Fibrotest >0.59 or alternatively a transient elastography (FibroScan) > 9.5 kPa) or liver biopsy (≥ METAVIR stage F3) in patients with no previous antiviral treatment"* is defined as a patient presenting with advanced liver disease (ALD) [369]

2. **Late stage liver disease in untreated patients with chronic hepatitis B or C**

According to this definition *"Presence of at least one symptom of decompensated cirrhosis (jaundice, hepatic encephalopathy, clinically detectable ascites, variceal bleeding) and/or hepatocellular carcinoma in patients with no previous antiviral treatment"* is sufficient to define a patient as presenting with late stage liver disease (LSLD) [369].

For the analysis in this chapter, I followed the exact two definitions described above additionally including untreated individuals at study enrolment and I presented results separately for ALD alone and ALD or LSLD combined. In the analysis with ALD as the endpoint, individuals classified as having ALD did not have LSLD.

6.4.3 Target population, design of analysis and main exposures and outcomes

The sections below detail the target population, design of analysis, main exposures and main outcomes considered for each of the analysis stages.

This first stage of the analyses in this chapter considered HCV testing (stage 1a) and the evaluation of participants' stage of liver disease to identify late presenters (stage 1b) in individuals newly HIV diagnosed within six months of study entry in Icona. It is worth noting that analysis stage 1a (assessing HCV testing) and stage 1b (assessing late HCV presentation), are exploratory analyses aiming to identify

potential risk factors from a range of demographic, HIV-related factors, lifestyle, socioeconomic, HBV infection and HCV genotype measured at baseline for these outcomes. Detecting HCV infection was done by first testing for anti-HCV antibodies and, in people who are HCVAb positive, to subsequently test for HCV-RNA to confirm chronic infection. Following HCV diagnosis, an assessment of fibrosis and cirrhosis was done to ascertain extent of liver damage.

Stage 1a- HCV testing among people with newly diagnosed HIV

Target population

- All Icona participants with an HIV diagnosis within 6 months of enrolment in the cohort. This was done to exclude prevalent HIV infections, which are also likely to be prevalent cases of HCV.

Design of analysis: cross-sectional at enrolment

Main exposure factors

- The factors shown in Table 6.1, which were measured at baseline, were considered for this analysis).

Main outcome

- HCV serology testing at enrolment in Icona (Yes/No)

Stage 1b- Late HCV presentation among those with HCV

Target Population

- All Icona participants with an HIV diagnosis within 6 months of entry in the cohort, ever diagnosed with HCV either HCVAb or HCV-RNA and with available data on FIB-4 or Fibroscan or clinical liver disease stage diagnosis (a subset of the target population of stage 1a).

Design of analysis: cross-sectional at enrolment

Main exposure factors

- Same at stage 1a (Table 6.1)

Main outcomes

- Advanced liver disease ((ALD) and the combined endpoint (ALD or LSLD)) (Yes/No)

Stage 2 – All-cause mortality and treatment initiation among HCV-positive individuals

As described above, stage 1b of the analysis focused on comparing the main characteristics of individuals classified as late HCV presenters (ALD or LSLD) vs. those of participants who could not be classified as late HCV presenters at entry in Icona. These same groups were also compared in terms of their risk of experiencing specific outcomes over follow-up: all-cause mortality, and the secondary outcome of HCV therapy initiation (stage 2 analysis). In this second aetiological analysis, because the interest was on the causal effect, a more formal assessment and minimisation of potential biases was performed. Baseline in this analysis was the date of HCV diagnosis.

Target Population

- Same as stage 1b (Table 6.1)

Design of analysis: prospective cohort

Main exposure(s)

- Late presentation of HCV (defined two possible ways):
    - Presentation with ALD - (Yes/No)
    - Presentation with (ALD or LSLD) - (Yes/No)

Main outcomes

- All-cause mortality (assessed from CoDE)
- Time to starting any HCV treatment (includes IFN/RBV or DAA) post HCV diagnosis from HCV diagnosis

Therefore the main outcome in the cross-sectional analysis in stage 1b (late HCV presentation defined as ALD or LSLD) becomes the key exposure of interest for the prospective analysis in stage 2.

6.5 Data

The potential risk factors considered in the exploratory analysis for stages 1a and 1b are shown in Table 6.1 Potential risk factors considered measured at baseline

Table 6.1. Indeed, there is evidence in the literature that some of these are both predictors of late HCV presentation and clinical outcome. For example, age has been found to be associated with late presentation, i.e. older people are more likely to show advanced liver disease and because HCV infection occurred a while ago, symptoms only begin to present themselves at late stage of their life. Also PWIDs, who are more likely to have high alcohol consumption and not to seek care are more likely to present with late HCV. HIV-related factors are also potentially associated with the risk of late presentation with HCV, as people who are newly diagnosed with HIV are typically also tested for HCV at the same time. Socio-economic factors such as level of education, could also be associated with the risk of late HCV presentation as lack of education of the disease was also shown to be a barrier for testing [383, 402]. As mentioned in the literature review in section 6.3.2, demographics, HIV-related factors, calendar year of HCV diagnosis are often considered as confounders for the association between late HCV presentation and all-cause mortality. Finally, geographical region of the participating site is also likely to play a role because health care policies vary across Italian regions (this is the main exposure of interest in the analysis included in chapter 7 relating to HCV CoC).

Table 6.2 Potential risk factors considered measured at baseline

	Variables	Classification
Demographics	Age (years)	Continuous (age per 10 years older)
	Gender	Male, Female
	Nationality	Italian, Non-Italian
	Recruitment site	North, South, Center
	Mode of HIV transmission	PWID, MSM, Heterosexual, Other/unknown
	Calendar year enrolled	1997-2002, 2003-2008, 2008-2012, 2013 - January 2018
HIV related factors	AIDS diagnosis	No, Yes
	CD4 cell count (cells/mm^3)	≤200, >200, unknown
	HIV-RNA (copies/ml)	≤10k, 10001 – 100k, >100k, Unknown
Lifestyle	Alcohol consumption[1]	Abstain, Moderate Hazardous, unknown
Social Economic factors	Education	Primary, Secondary, College, University, Other/unknown
	Employment	Unemployed, Employed, Other, unknown
Hepatitis	Hepatitis B	Negative, Positive, Not tested
	HCV Genotype status	(1/4), (2/3), Other

[1]As classified in Chapter 4

6.6 Statistical analysis

In stage 1a, the analysis included all individuals with HIV diagnosis ≤6 months prior to enrolment. Participants were stratified according to whether they have been tested for HCVAb or not at study entry in the cohort and their baseline characteristics were compared using chi-squared test for categorical and Mann-Whitney test for continuous variables. Cross sectional date for the analysis is defined as the date of enrolment in Icona.

Stage 1b analysis includes only individuals found to be HCVAb positive and in whom stage of their liver disease was assessed. The proportion of individuals with late HCV presentation (ALD or LSLD) per period of enrolment was calculated (calendar time was categorized into 5-year periods [1997-2001; 2002-2007; 2008-2012; 2013 to January 2018]. The uncertainty surrounding this estimate was presented with 95% CIs around these proportions which were calculated using the binomial exact method. The binomial exact method was used in this analysis because there is an assumption that for large samples of data, the binomial distribution is similar to the normal distribution. Therefore, confidence intervals of point estimates such as proportions from sample data can be estimated using the normal approximation [403]. Trends in proportion over time were presented graphically and tested using the chi-square test for trend.

Baseline characteristics of participants stratified by late (ALD or LSLD)) HCV presentation or not were presented and factors compared between the two groups using chi-squared test for categorical and Mann-Whitney test for continuous variables.

As mentioned above, potential determinants of late HCV presentation (ALD or LSLD) were identified among a number of potential factors (Table 6.1). To identify factors associated with the probability of late presentation of HCV, first unadjusted logistic regression models were fitted. Then bivariate logistic regression models were fitted for each of the potential factors, all adjusted for age (as age is likely to be a confounding factors for all of the others) to assess which factors remained independently associated with late HCV presentation. Due to small numbers of people with available measurements of HCV genotype, and the low prevalence of people with AIDS and comorbidities, these three factors were not included in the logistic regression models.

The association of late HCV presentation with the primary endpoint, the risk of death in the prospective stage 2 analysis was evaluated using standard survival

analysis by means of Kaplan-Meier (KM) plots and Cox regression model. Baseline date was defined at date of HCV diagnosis. Individuals were followed up until the date of experiencing death or their follow-up time was censored at the date of their last clinical visit. KM plots were presented comparing participants who presented late with HCV with non-late presenters, separately for the late presenting definition ALD alone and ALD or LSLD combined. For this survival analysis I focussed on late HCV presentation as the main exposure and used a more rigorous approach than previously to assess potential confounders. Only time-fixed factors at baseline were evaluated in the model. Potential confounders considered in this analysis were selected on the basis of the literature results and axiomatic knowledge. A causal model was hypothesized and the assumptions of this model were depicted using a direct acyclic graph (DAG) (Figure 6.1 DAG model for impact of late HCV presentation on all-cause mortality

Figure 6.2 Participant Flow diagramFigure 6.1). I used the DAGitty [238] R software to visualize such a graph. The software was used also to identify, under the assumed DAG, the minimally sufficient set of variables to include in the multivariable model to minimise all sources of measured confounding.

In detail, the DAG shows the hypothesised causal relationship between late HCV presentation (the key exposure, represented by the ‘play’ sign in green) and all-cause mortality (the outcome in blue, represented by the letter ‘I’, which stands for dependent variable). All possible other variables considered in the model were depicted in the DAG as distinct nodes. The blue nodes represent variables that are not considered confounders because they are predictors of outcome but not factors causing the exposure. For example, region is a predictor of death but not considered to be an ancestor of late HCV presentation. In contrast, all variables in pink represent potential confounders, either directly or through a chain. For example, there is backdoor path between late HCV presentation and all-cause mortality by year of HCV diagnosis (which is a common cause of both). Indeed, it is possible that year of HCV testing/diagnosis changes the risk of observing late

stage of liver disease at entry in the cohort and also likely to impact on all-cause mortality risk (because of changes related to management and treatment of HCV over time). This backdoor path is (late HCV presentation ← year of HCV diagnosis → all-cause mortality) and adjusting for year of HCV diagnosis closes this back door path resulting in the association of interest no longer being confounded by year of HCV diagnosis. There is also an example in the graph, in which more than one variable lies along a back door path so that adjusting for only one of these is sufficient to remove confounding. One of these back door paths (late HCV presentation ← nationality → employment → geographical region → all-cause mortality) in which adjusting for either nationality, employment or region is sufficient to block this confounding pathway. In contrast, the variable AIDS (in blue) could be seen as a factor causing an M-bias relationship (late HCV presentation ← CD4 →AIDS ←HIV-RNA → all-cause mortality) and should not be controlled for as it would open this backdoor path and introduce bias, more details as mentioned in chapter 2 section 2.8.5.

The factors identified by the DAGitty software as confounders were included in the multivariable Cox regression model to minimise all sources of measured confounding bias.

Thus, if the assumptions in the DAG are correct, a number of distinct models were suggested as minimally sufficient for removing all confounding pathways when estimating the causal link between late HCV presentation and the risk of death.

a) Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + HCV genotype[1] + CD4 + HIV-RNA + Alcohol consumption + HBV status + Region

b) Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + HCV genotype[1] + CD4 + HIV-RNA + Alcohol consumption + HBV status + Employment status

c) Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + HCV genotype[1] + CD4 + HIV-RNA + Alcohol consumption + HBV status + Nationality

[1]HCV genotype not included in the fitted model, due to missing values.

Although the model relies on untestable assumptions, under these, any of these adjustments are equally good at controlling for measured confounding.

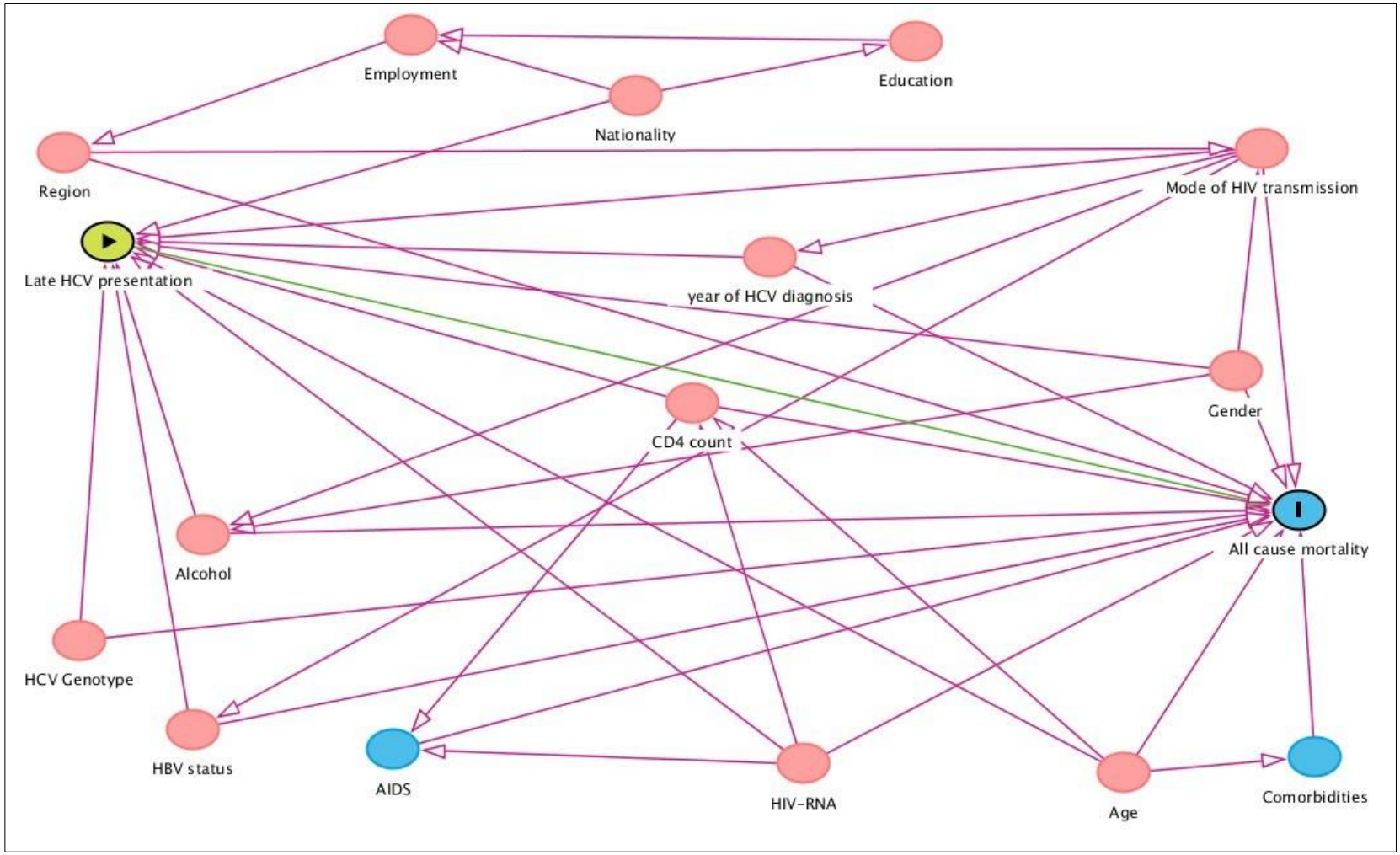
To assess the association of late presentation of HCV with the probability of the secondary outcome of starting HCV therapy, I also used the KM method and Cox regression model for the analysis. The date of HCV therapy initiation was defined at the time a participant started any HCV therapy after the date of HCV diagnosis. If no treatment initiation event was observed, participants' follow-up was truncated at the date at which they were last seen still off treatment and alive (this was defined on the basis of the earliest the date of death and date of last clinical visit). The same approach for the identification and adjustment for confounding factors used for the outcome all-cause mortality (Figure 6.1 DAG model for impact of late HCV presentation on all-cause mortality

Figure 6.2 Participant Flow diagramFigure 6.1) was also applied to the time to HCV therapy initiation outcome.

Sensitivity analysis

A sensitivity analysis for the outcome of starting HCV therapy was restricted to individuals who were enrolled in Icona after 2014 DAA teatment was in use. This additional analysis was performed to assess whether, in the DAA era, everybody in the study had the same chance of being treated regardless of their stage of liver disease. The analysis was carried out similarly to the main analysis (although due to the small sample size, adjustment was made only for age and year of HCV diagnosis).

Figure 6.2 DAG model for impact of late HCV presentation on all-cause mortality



Legend: exposure outcome ancestor of outcome ancestor of exposure *and* outcome causal path biasing path

6.7 Results

6.7.1 Patient Flow diagram

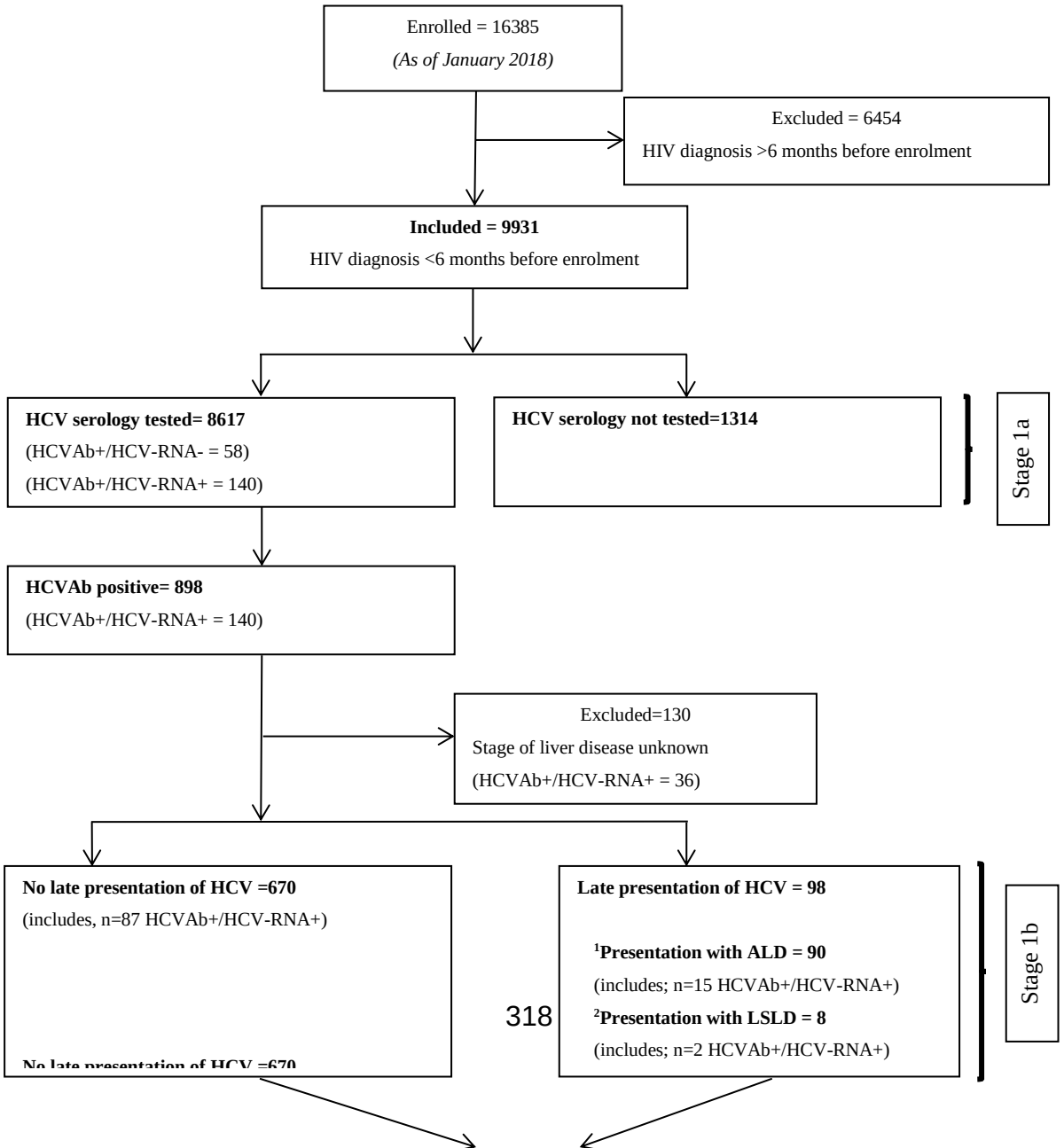
As of January 2018, 16,385 participants were enrolled into the Icona cohort and 39% (n=6,454) of these were excluded from all analyses in this chapter as their HIV diagnosis was recorded more than 6 months prior to enrolment in the cohort. This was done to exclude prevalent cases of HCV infection. The resulting 60% (n=9,931) of the Icona individuals were classified as newly diagnosed with HIV. Of these, 87% (n=8,617) were tested for HCV antibodies around the time of enrolment and 9% (898/9931) were found to be HIV/HCV coinfected [16% (140/898) were also HCV-RNA positive at this time Figure 6.2 Participant Flow diagram

Figure 6.2].

Of the total 898 HIV/HCV coinfected participants, 86% (n=768) also had data on parameters needed to assess stage of liver disease. According to combined definition of late HCV presentation, 13% (98/768) were defined as presenting late with HCV (ALD or LSLD). Presentation with advanced liver disease (ALD - based on FIB-4 or Fibroscan only) was found in 12% (90/768) and 1% (8/768) presented with late stage liver disease (LSLD - based on clinical diagnosis only). Of the total 768 HIV/HCV coinfected individuals included in stage 2 of the analysis, 8% (n=63), died and 17% (n=132) started HCV therapy. Figure 6.3 Cascade from newly HIV diagnosis to late presentation of HCV of individuals enrolled in Icona up to January 2018

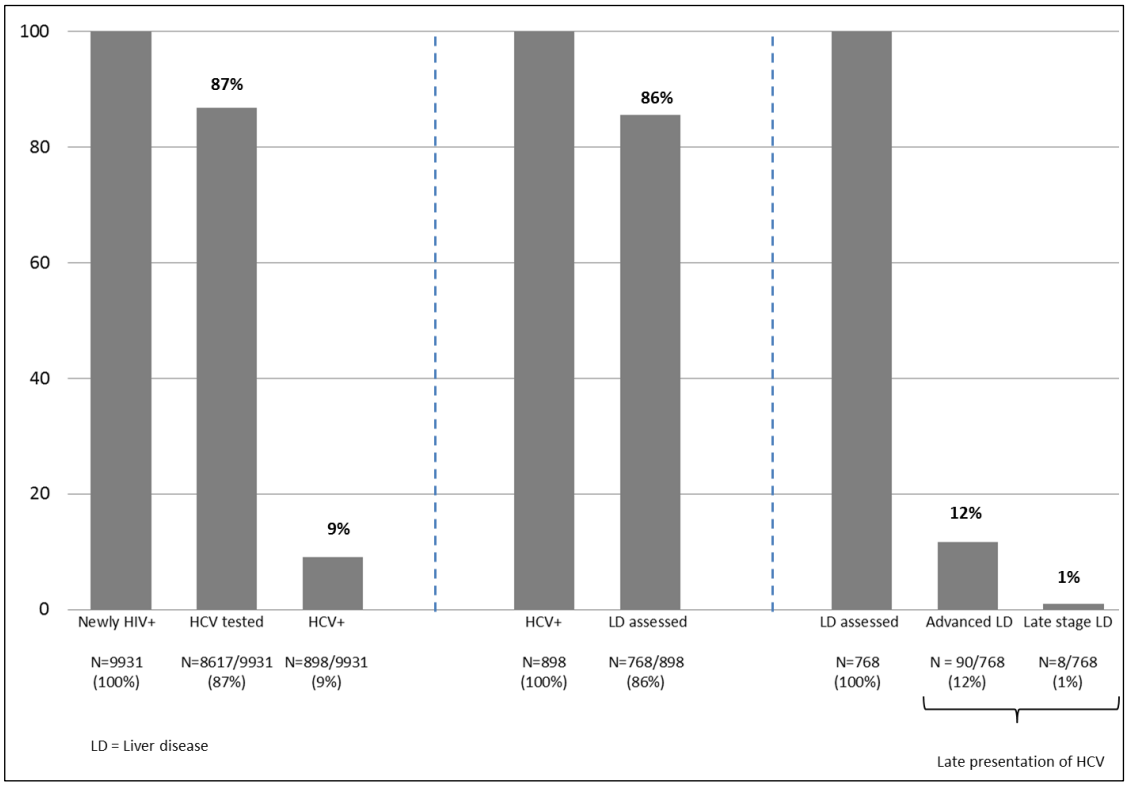
Table 6.2 Participant characteristics at study enrolment stratified by HCV tested vs HCV not tested (stage 1a)Figure 6.3 shows a summary of the cascade from the initial population of all newly HIV diagnoses to the identification of the proportion of participants presenting late with HCV at entry in the cohort.

Figure 6.5 Participant Flow diagram



[1]ALD= advanced liver disease based on FIB-4 or Fibroscan

Figure 6.8 Cascade from newly HIV diagnosis to late presentation of HCV of individuals enrolled in Icona up to January 2018



6.7.2 Participant characteristics stratified by HCV tested vs HCV not tested (stage 1a)

Participant characteristics of individuals newly diagnosed with HIV at enrolment in Icona is shown in Table 6.2 Participant characteristics at study enrolment stratified

by HCV tested vs HCV not tested (stage 1a)

Figure 6.4 Trend of proportion of individuals with late presentation of HCV from 1997 to 2018Table 6.2 stratified by HCV tested vs. not HCV tested (stage 1a). The overall median (IQR) calendar year of enrolment of Icona in this population was 2012 (2007 – 2015). The majority of individuals were males (79%), median (IQR) age was 37 (30-46) years, half of the participants were receiving care in the northern regions of Italy and the most common route of HIV transmission was heterosexual contacts (43%) and MSM (42%). In terms of HIV-related factors, the majority had a CD4 >200 cells/mm^3 (58%) and high HIV-RNA (>10,000 copies/mL) (68%) at entry in the cohort. The majority also achieved a level of education at or above primary school (90%) and declared to be employed (56%). Alcohol consumption was common with almost a quarter of the individuals classified as moderate consumer and HBV coinfection was present in only 3% of individuals.

The two groups differed for most of the baseline characteristics studied with the exception of gender. Briefly, individuals tested for HCV were younger (median age 37 vs. 39 years old, p<0.001), more likely to be receiving care in the north or central region of Italy, p<0.001), more likely to have acquired HIV through heterosexual or MSM contacts (p<0.001), more likely to have a CD4>200 cells/mm^3 (60% vs. 46%, p<0.001), high HIV-RNA (p<0.001), more likely to consume alcohol (26% vs. 17%, p<0.001), were enrolled in the cohort in earlier calendar periods (median calendar year enrolled 2012 vs. 2014, p<0.001) and more likely to be infected with HBV (3.4% vs. 1%, p<0.001, Table 6.2 Participant characteristics at study enrolment stratified by HCV tested vs HCV not tested (stage 1a)

Figure 6.4 Trend of proportion of individuals with late presentation of HCV from 1997 to 2018Table 6.2). Regional differences are important as they may reflect access to, or quality of care, and region is specifically considered as the key exposure of interest in the following chapter 7 which examines the impact of region on different stages (outcomes) of the HCV CoC.

Interestingly using the alcohol classification established in chapter 4, there was a higher prevalence of hazardous alcohol consumption in people who were tested for HCV at entry in the cohort as compared to those that were not tested (5% vs 2.5%, p<0.001). Additionally, individuals not tested for HCV were also likely to have missing data on alcohol consumption, as also shown in chapter 3.

Table 6.5 Participant characteristics at study enrolment stratified by HCV tested vs HCV not tested (stage 1a)

	HCV tested N= 8617	**HCV not tested** N= 1314	**Total** N= 9931	**p-value**
Gender, n(%)				0.698
Female	1844 (21.4)	275 (20.9)	2119 (21.3)	
Male	6773 (78.6)	1039 (79.1)	7812 (78.7)	
Age (years), n(%)				<.001
Median (IQR)	37 (30, 46)	39 (31, 49)	37 (30, 46)	
Region, n(%)				<.001
North	4289 (49.8)	757 (57.6)	5046 (50.8)	
South	1194 (13.9)	214 (16.3)	1408 (14.2)	
Center	3127 (36.3)	341 (26.0)	3468 (34.9)	
Nationality, n(%)				<.001
Italian	6539 (75.9)	896 (68.2)	7435 (74.9)	
Mode of HIV transmission, n(%)				<.001
Heterosexual	3739 (43.4)	523 (39.8)	4262 (42.9)	
MSM	3617 (42.0)	529 (40.3)	4146 (41.7)	
PWID	578 (6.7)	112 (8.5)	690 (6.9)	
Other	683 (7.9)	150 (11.4)	833 (8.4)	
CD4 cells/mm^3, n(%)				<.001
CD4≤200	2498 (29.0)	402 (30.6)	2900 (29.2)	
CD4>200	5135 (59.6)	598 (45.5)	5733 (57.7)	
CD4 unknown	984 (11.4)	314 (23.9)	1298 (13.1)	
HIV-RNA copies/ml, n(%)				<.001
HIV-RNA≤10k	1478 (17.2)	210 (16.0)	1688 (17.0)	
HIV-RNA 10k-100k	3041 (35.3)	339 (25.8)	3380 (34.0)	
HIV-RNA>100k	2978 (34.6)	422 (32.1)	3400 (34.2)	
HIV-RNA unknown	1120 (13.0)	343 (26.1)	1463 (14.7)	
Education, n(%)				<.001
Primary school	2826 (32.8)	717 (54.6)	3543 (35.7)	
Secondary school	584 (6.8)	50 (3.8)	634 (6.4)	
College	1717 (19.9)	150 (11.4)	1867 (18.8)	
University	2539 (29.5)	269 (20.5)	2808 (28.3)	

	HCV tested N= 8617	HCV not tested N= 1314	Total N= 9931	p-value
Other/Unknown	951 (11.0)	128 (9.7)	1079 (10.9)	
Employment, n(%)				0.003
Employed	4974 (57.7)	617 (47.0)	5591 (56.3)	
Unemployed	1182 (13.7)	191 (14.5)	1373 (13.8)	
Other	869 (10.1)	113 (8.6)	982 (9.9)	
Unknown	1592 (18.5)	393 (29.9)	1985 (20.0)	
Alcohol use, n(%)				<.001
Abstainer	2530 (29.4)	161 (12.3)	2691 (27.1)	
Moderate	1615 (18.7)	151 (11.5)	1766 (17.8)	
Hazardous	428 (5.0)	33 (2.5)	461 (4.6)	
Unknown	4044 (46.9)	969 (73.7)	5013 (50.5)	
Calendar year enrolled, n(%)				<.001
1997-2001	1692 (19.6)	156 (11.9)	1848 (18.6)	
2002-2007	838 (9.7)	57 (4.3)	895 (9.0)	
2008-2012	3593 (41.7)	379 (28.8)	3972 (40.0)	
2013-2018	2493 (28.9)	583 (44.4)	3076 (31.0)	
Calendar year enrolled				<.001
Median (IQR)	2012 (2007, 2015)	2014 (2011, 2016)	2012 (2007, 2015)	
HBV infection, n(%)				<.001
HBV negative	6087 (70.6)	102 (7.8)	6189 (62.3)	
HBV positive	295 (3.4)	13 (1.0)	308 (3.1)	
HBV not tested	2235 (25.9)	1199 (91.2)	3434 (34.6)	

6.7.3 Proportion of individuals with late HCV presentation from 1997 to 2018 (stage 1b)

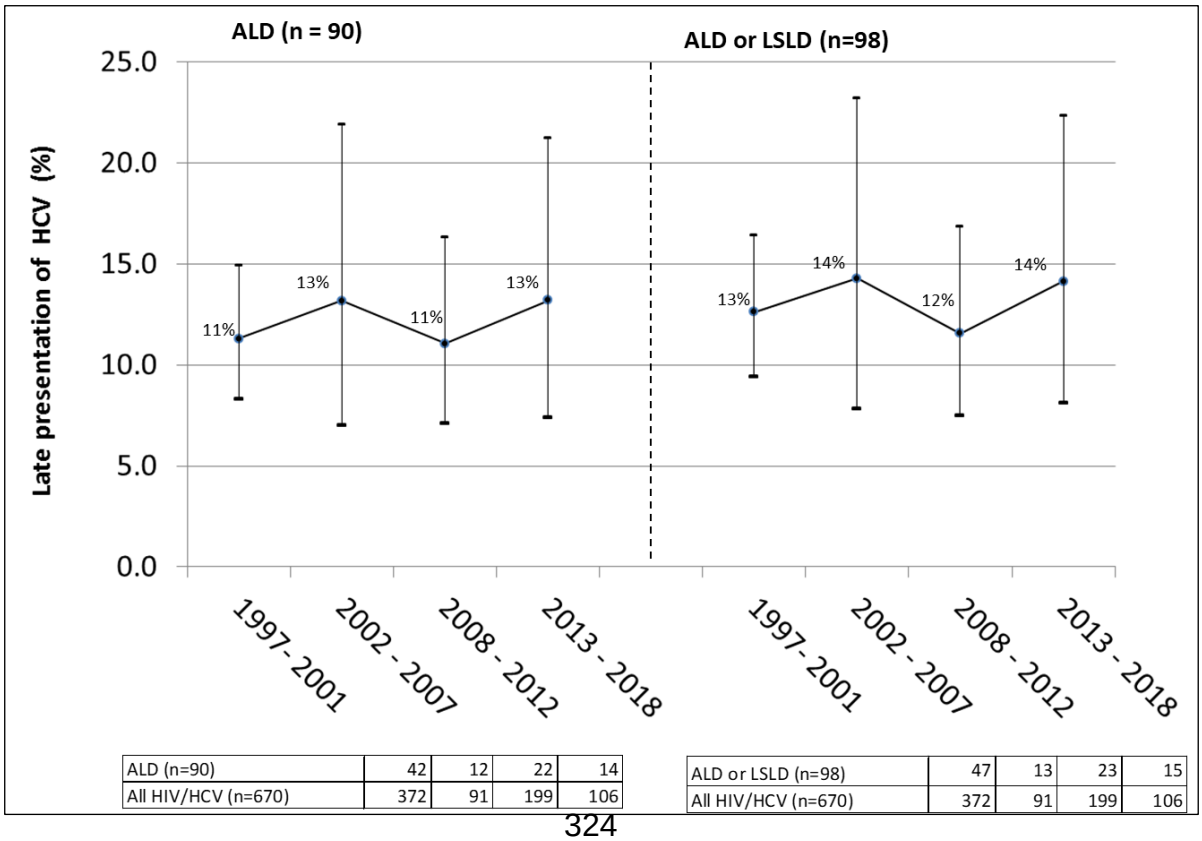
Figure 6.4 Trend of proportion of individuals with late presentation of HCV from 1997 to 2018

Figure 6.4 shows the proportion of individuals presenting late for HCV from 1997 to January 2018; (the left-hand panel shows the proportion of individuals with ALD only while the right panel the proportion of ALD or LSLD combined) among the 768 HIV/HCV coinfected individuals with evaluable stage of liver disease and included in this stage 1b analysis. The general trend suggests a stable pattern of late HCV

presentation over the 20 years of observation. The proportion of late HCV presentation ranges from 11% to 14% over time. Chi-squared test for trend for ALD (p=0.764) and ALD or LSLD (p=0.932) respectively are consistent with the null hypothesis of no change in proportion of individuals presenting with late HCV over time. The effect of calendar time adjusted for age is presented in Table 6.5.

Figure 6.11 Trend of proportion of individuals with late presentation of HCV from 1997 to 2018

Denominator in year x analysis = person who remains undiagnosed for HCV at the beginning of year x and with >=1 clinical visit in year x. Numerator in year x analysis = person who satisfies the definition of late presenter of liver disease in year x



ALD (n=90)	42	12	22	14
All HIV/HCV (n=670)	372	91	199	106

ALD or LSLD (n=98)	47	13	23	15
All HIV/HCV (n=670)	372	91	199	106

6.7.4 Participant characteristics stratified by late HCV presentation among HIV/HCV coinfected individuals with stage of liver disease assessed (stage 1b)

Table 6.3: Participant characteristics stratified by late HCV diagnosis among HIV/HCV coinfected and stage of liver disease assessed (Stage 1b)

Table 6.4 Stage of liver disease stratified by late presentation of HCVTable 6.3 shows participant characteristics of HCV-positive participants included in stage 1b analysis stratified by the combined variable late presentation of HCV (yes/no) and, within the group of late HCV presenters, further stratified by ALD alone or LSLD. There were some differences in terms of age, CD4, HIV-RNA and HCV genotype between individuals presenting late and not presenting late for HCV. Individuals presenting late for HCV (ALD or LSLD) were more likely to be male (p=0.016), of older age (p<0.001), have a CD4 ≤200 cells/mm^3 (p<0.001) and high HIV-RNA (p=0.073) and more likely to have HCV genotype 1 or 4 (p=0.017). Although they were only eight individuals identified as presenting with LSLD, the data indicates that these individuals were also more likely to be older, also with CD4≤200 cells/mm^3 and high HIV-RNA.

Table 6.8: Participant characteristics stratified by late HCV diagnosis among HIV/HCV coinfected and stage of liver disease assessed (Stage 1b)

	Not Late presentation of HCV (N=670)	Late presentation of HCV		Total (N=768)	p-value (comparing not late vs ALD or LSLD)
		Advanced liver disease (N=90)	Late stage liver disease (N=8)		
Gender, n(%)					0.016
Male	516 (77.0)	79 (87.8)	7 (87.5)	602 (75.4)	
Female	154 (23.0)	11 (12.2)	1 (12.5)	166 (21.6)	
Age (years), n(%)					<.001
median (IQR)	36 (31, 42)	43 (36, 49)	41 (36, 43)	37 (32, 44)	
Region, n(%)					0.494
North	320 (47.8)	48 (53.3)	3 (37.5)	371 (48.3)	
South	125 (18.7)	19 (21.1)	1 (12.5)	145 (18.9)	
Center	225 (33.6)	23 (25.6)	4 (50.0)	252 (32.8)	
Nationality, n(%)					0.418
Italian	567 (84.6)	81 (90.0)	5 (62.5)	653 (85.0)	
Mode of HIV transmission, n(%)					0.111
Heterosexual	184 (27.5)	20 (22.2)	1 (12.5)	205 (26.7)	
MSM	157 (23.4)	16 (17.8)	0 (0.0)	173 (22.5)	
PWID	289 (43.1)	48 (53.3)	6 (75.0)	343 (44.7)	
Other	40 (6.0)	6 (6.7)	1 (12.5)	47 (6.1)	
CD4 cells/mm^3, n(%)					<.001
CD4≤200	201 (30.0)	45 (50.0)	6 (75.0)	252 (32.8)	
CD4>200	412 (61.5)	31 (34.4)	1 (12.5)	444 (57.8)	
CD4 unknown	57 (8.5)	14 (15.6)	1 (12.5)	72 (9.4)	
HIV-RNA copies/ml, n(%)					0.073
HIV-RNA≤10k	157 (23.4)	16 (17.8)	2 (25.0)	175 (22.8)	
HIV-RNA 10k-100k	220 (32.8)	25 (27.8)	2 (25.0)	247 (32.2)	
HIV-RNA>100k	225 (33.6)	33 (36.7)	2 (25.0)	260 (33.9)	
HIV-RNA unknown	68 (10.1)	16 (17.8)	2 (25.0)	86 (11.2)	
Education, n(%)					0.844
Primary school (<11)	59 (8.8)	6 (6.7)	1 (12.5)	66 (8.6)	
Secondary school (11-16)	189 (28.2)	31 (34.4)	0 (0.0)	220 (28.6)	
College (16 - 18)	155 (23.1)	17 (18.9)	3 (37.5)	175 (22.8)	
University (18+)	37 (5.5)	2 (2.2)	0 (0.0)	39 (5.1)	
Other/Unknown	230 (34.3)	34 (37.8)	4 (50.0)	268 (34.9)	
Employment, n(%)					0.419

	Not Late presentation of HCV (N=670)	Late presentation of HCV		Total (N=768)	p-value (comparing not late vs ALD or LSLD)
		Advanced liver disease (N=90)	Late stage liver disease (N=8)		
Employed	385 (57.5)	55 (61.1)	3 (37.5)	443 (57.7)	
Unemployed	158 (23.6)	20 (22.2)	5 (62.5)	183 (23.8)	
Other	54 (8.1)	6 (6.7)	0 (0.0)	60 (7.8)	
Unknown	73 (10.9)	9 (10.0)	0 (0.0)	82 (10.7)	
Alcohol use, n(%)					0.148
Abstainer	152 (22.7)	16 (17.8)	2 (25.0)	170 (22.1)	
Moderate	93 (13.9)	8 (8.9)	0 (0.0)	101 (13.2)	
Hazardous	37 (5.5)	9 (10.0)	0 (0.0)	46 (6.0)	
Unknown	388 (57.9)	57 (63.3)	6 (75.0)	451 (58.7)	
Year enrolled, n(%)					0.888
1997-2001	325 (48.5)	42 (46.7)	5 (62.5)	372 (48.4)	
2002-2007	78 (11.6)	12 (13.3)	1 (12.5)	91 (11.8)	
2008-2012	176 (26.3)	22 (24.4)	1 (12.5)	199 (25.9)	
2013-2018	91 (13.6)	14 (15.6)	1 (12.5)	106 (13.8)	
HCV genotype (in HCV-RNA positive, n=104), n(%)					0.017
Genotype(1/4)	46 (6.9)	14 (15.6)	1 (12.5)	61 (7.9)	
Genotype(2/3)	18 (2.7)	1 (1.1)	1 (12.5)	22 (2.9)	
Genotype unknown	23 (3.0)	0 (0)	0 (0)	23 (2.6)	
Presence of AIDS, n(%)	100 (14.9)	17 (18.9)	3 (37.5)	120 (15.6)	0.163
Comorbidities, n(%)	66 (9.9)	11 (12.2)	2 (25.0)	79 (10.3)	0.299
HBV infection, n(%)					0.893
HBV negative	508 (75.8)	67 (74.4)	6 (75.0)	581 (75.7)	
HBV positive	24 (3.6)	3 (3.3)	0 (0.0)	27 (3.5)	
HBV not tested	138 (20.6)	20 (22.2)	2 (25.0)	160 (20.8)	

6.7.5 Stage of liver disease stratified by late HCV presentation (stage 1b)

Table 6.4 Stage of liver disease stratified by late presentation of HCV

Table 6.4 shows the distribution of liver disease parameters used to determine late presentation of HCV. By definition, there were differences in terms of fibrosis

score, liver stiffness and clinical diagnosis of liver disease among late and not-late presenters. Individuals presenting with ALD had FIB-4 >3.25 and median FIB-4 (IQR) of 5.25 (3.27 – 47.59) and this parameter was even higher in individuals presenting with LSLD 5.34 (0.45 - 15.09). Among the individuals with LSLD, four individuals were found to have HCC or compensated cirrhosis and another four with clinical diagnosis of late stage based on hospitalization records. Numbers were too small to carry out any formal statistical comparisons between the groups.

Table 6.11 Stage of liver disease stratified by late presentation of HCV

	Not Late presentation of HCV (N=670)	Late presentation of HCV		Total (N=768)
		Advanced liver disease (N=90)	Late stage liver disease (N=8)	
Fibrosis score, n	669	90	8	767
Median (IQR)	1.13 (0.22, 3.23)	5.25 (3.27, 47.59)	5.34 (0.45, 15.09)	1.27 (0.22, 47.59)
Liver stiffness, n	11	5	1	17
Median (IQR)	1.28 (0.32, 7.15)	5.56 (3.66, 14.50)	8.04 (8.04, 8.04)	1.62 (0.32, 14.50)
Clinical diagnosis, n(%)				
HCC, compensated cirrhosis	0 (0%)	0 (0%)	4 (50.0%)	4 (0.5%)
*Clinical diagnosis from ICD-9 codes	0 (0%)	0 (0%)	4 (50.0%)	4 (0.5%)

*ICD9 codes included all reasons for hospitalization reported as ‘571’

6.7.6 Factors associated with late HCV presentation (ALD or LSLD) (stage 1b)

For this stage 1b, analysis exploring factors independently associated with the risk of late HCV presentation (ALD or LSLD) in a total of 768 new HCV diagnoses, unadjusted ORs from fitting a logistic regression model are shown in Table 6.5 Logistic regression models for the odds of late presentation of HCV (ALD or LSLD) vs not presenting with (ALD and LSLD) (N=768)

Table 6.5 . The odds of presenting with ALD or LSLD was associated with older age OR = 1.72 (95% CI: 1.40 – 2.10) per 10 years older; p<0.001), being male (vs. female OR = 2.14 (95% CI: 1.14 – 4.02); p=0.018), having CD4>200 cells/mm^3 (vs. CD4≤ 200 OR = 0.30 (95% CI: 0.19 – 0.49; p≤0.001) with some evidence for an association with being PWID (vs heterosexuals OR = 1.64 (95% CI: 0.96 – 2.80; p=0.072. There was no evidence of association with nationality, HIV-RNA, alcohol consumption, education, employment, calendar year and HBV infection. In the bivariate analysis, after adjusting for age; male gender (global p = 0.013), CD4 (global p <0.001) and PWID mode of HIV transmission (global p = 0.005) remained independently associated with the risk of late HCV presentation Table 6.5 Logistic regression models for the odds of late presentation of HCV (ALD or LSLD) vs not presenting with (ALD and LSLD) (N=768)

Table 6.5.

Table 6.14 Logistic regression models for the odds of late presentation of HCV (ALD or LSLD) vs not presenting with (ALD and LSLD) (N=768)

	Unadjusted OR (95% CI)	**p-value**	**g-pv**	**[1]Adjusted OR (95% CI)**	**p-value**	**g-pv**
Age, years						
per 10 years older	1.72 (1.40, 2.10)	<.001	<.001	-	-	-
Gender						
Female	1.00			1.00		

	Unadjusted OR (95% CI)	p-value	g-pv	[1]Adjusted OR (95% CI)	p-value	g-pv
Male	2.14 (1.14, 4.02)	0.018	0.018	2.27 (1.19, 4.33)	0.013	0.013
Nationality						
Italian	1.00			1.00		
Non-Italian	0.77 (0.41, 1.46)	0.419	0.419	0.80 (0.42, 1.55)	0.515	0.515
***CD4* cells/mm^3**						
≤200	1.00		<.001	1.00		<.001
>200	0.30 (0.19, 0.49)	<.001		0.37 (0.23, 0.60)	<.001	
Unknown	1.04 (0.54, 1.98)	0.912		1.10 (0.57, 2.14)	0.763	
HIV-RNA copies/ml						
HIV-RNA≤10k	1.00		0.072	1.00		0.229
HIV-RNA 10k-100k	1.07 (0.57, 2.01)	0.832		1.08 (0.57, 2.04)	0.816	
HIV-RNA>100k	1.36 (0.74, 2.48)	0.322		1.17 (0.63, 2.17)	0.611	
HIV-RNA unknown	2.31 (1.13, 4.71)	0.021		1.97 (0.95, 4.10)	0.066	
Mode of HIV transmission						
Heterosexual	1.00		0.11	1.00		0.005
MSM	0.89 (0.45, 1.77)	0.746		1.04 (0.51, 2.11)	0.920	
PWID	1.64 (0.96, 2.80)	0.072		2.69 (1.48, 4.89)	0.001	
Other	1.53 (0.61, 3.85)	0.363		1.21 (0.46, 3.19)	0.701	
Alcohol use						
Abstainer	1.00		0.147	1.00		0.147
Moderate	0.73 (0.30, 1.74)	0.472		0.79 (0.33, 1.92)	0.604	
Hazardous	2.05 (0.85, 4.94)	0.108		1.93 (0.79, 4.75)	0.152	
Unknown	1.37 (0.79, 2.39)	0.266		1.63 (0.92, 2.89)	0.094	
Region						
North	1.00		0.494	1.00		0.224
South	1.00 (0.58, 1.75)	0.989		1.18 (0.66, 2.10)	0.570	
Center	0.75 (0.46, 1.24)	0.263		0.70 (0.42, 1.16)	0.165	
Education						
Primary (<11)	1.00		0.488	1.00		0.299
Secondary (11-16)	1.38 (0.58, 3.30)	0.466		1.88 (0.75, 4.72)	0.179	
College (16-18)	1.09 (0.44, 2.71)	0.857		1.26 (0.49, 3.26)	0.635	
University (18+)	0.46 (0.09, 2.31)	0.343		0.55 (0.11, 2.92)	0.491	
Other/unknown	1.39 (0.59, 3.28)	0.448		1.69 (0.69, 4.16)	0.250	
Employment status						
Unemployed	1.00		0.844	1.00		0.203
Employed	1.05 (0.63, 1.74)	0.849		1.22 (0.73, 2.05)	0.849	
Other	0.74 (0.30, 1.79)	0.501		0.39 (0.15, 1.03)	0.058	
Unknown	0.82 (0.39, 1.72)	0.598		0.65 (0.30, 1.41)	0.275	
Calendar year enrolled						
1997-2001	1.00		0.888	1.00		0.456
2002-2007	1.15 (0.59, 2.23)	0.674		0.96 (0.48, 1.91)	0.909	
2008-2012	0.90 (0.53, 1.54)	0.709		0.67 (0.38, 1.16)	0.154	

	Unadjusted OR (95% CI)	p-value	g-pv	[1]Adjusted OR (95% CI)	p-value	g-pv
2013-2018	1.14 (0.61, 2.13)	0.682		0.78 (0.41, 1.51)	0.468	
HBV						
HBV negative	1.00		0.893	1.00		0.937
HBV positive	0.87 (0.26, 2.96)	0.823		0.86 (0.25, 3.00)	0.808	
HBV not tested	1.11 (0.66, 1.85)	0.691		1.07 (0.64, 1.82)	0.781	

[1]Age adjusted for each variable

6.7.7 Association between late HCV presentation with risk of all-cause mortality (stage 2)

Individuals were followed-up after HCV diagnosis in the cohort for a median (IQR) of 3.6 years (0.9 - 7.9). Table 6.6 Cause of death stratified by late presentation of HCV (ALD, LSLD or both)

Figure 6.5 Cumulative risk of all-cause mortality stratified by late presentation of HCV (ALD vs. not ALD)Table 6.6 shows the breakdown of the causes of death stratified by late HCV presentation among those who died. A total of 8% (63/768) died and of these 22% (14/63) presented late for HCV (ALD or LSLD). The most common cause of death among those presenting with ALD or LSLD was liver related. Although this is a small sample, and the analysis is restricted to those who died, this is not surprising as liver-related mortality is the leading cause of death among HIV/HCV coinfected individuals.

Table 6.17 Cause of death stratified by late presentation of HCV (ALD, LSLD or both)

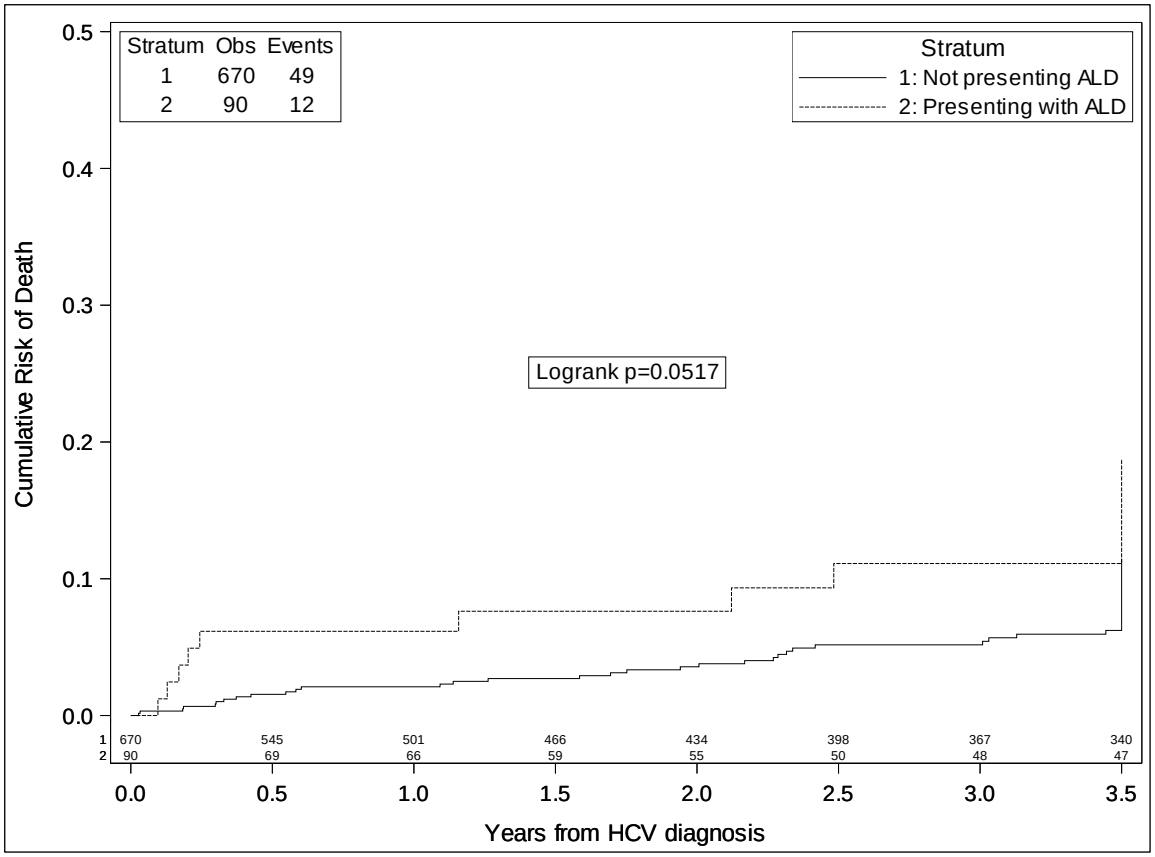
Cause of death	Not presenting with ALD and LSLD (N=49)	Presenting with ALD (N=12)	Presenting LSLD (N=2)	Total N=63
Liver related	5 (10%)	4 (33%)	2 (100%)	11 (17%)
AIDS	7 (15%)	3 (25%)	0 (0%)	10 (16%)
Non-AIDS	10 (20%)	2 (17%)	0 (0%)	12 (19%)

Other	22 (45%)	3 (25%)	0 (0%)	25 (40%)
Unknown	5 (10%)	0 (0%)	0 (0%)	5 (8%)

The KM plots of cumulative risk of all-cause mortality are shown in Figure 6.5 (comparing ALD vs. not ALD) and in Figure 6.6. (comparing ALD or LSLD vs. not). In this unadjusted analysis, there was weak evidence for an increased risk of all-cause mortality (log rank p=0.052) in individuals classified as presenting with ALD vs. not ALD. However, the association was stronger when I evaluated the composite exposure defintion of late HCV presentation (ALD or LSLD) (log rank p=0.020), which was mostly driven by the additional LSLD cases. The KM estimates of the cumulative risk of all-cause mortality by one and three years from HCV diagnosis are shown in Table 6.7 Cumulative probability of death from the KM plot from year one to year three stratified by late presentation of HCV

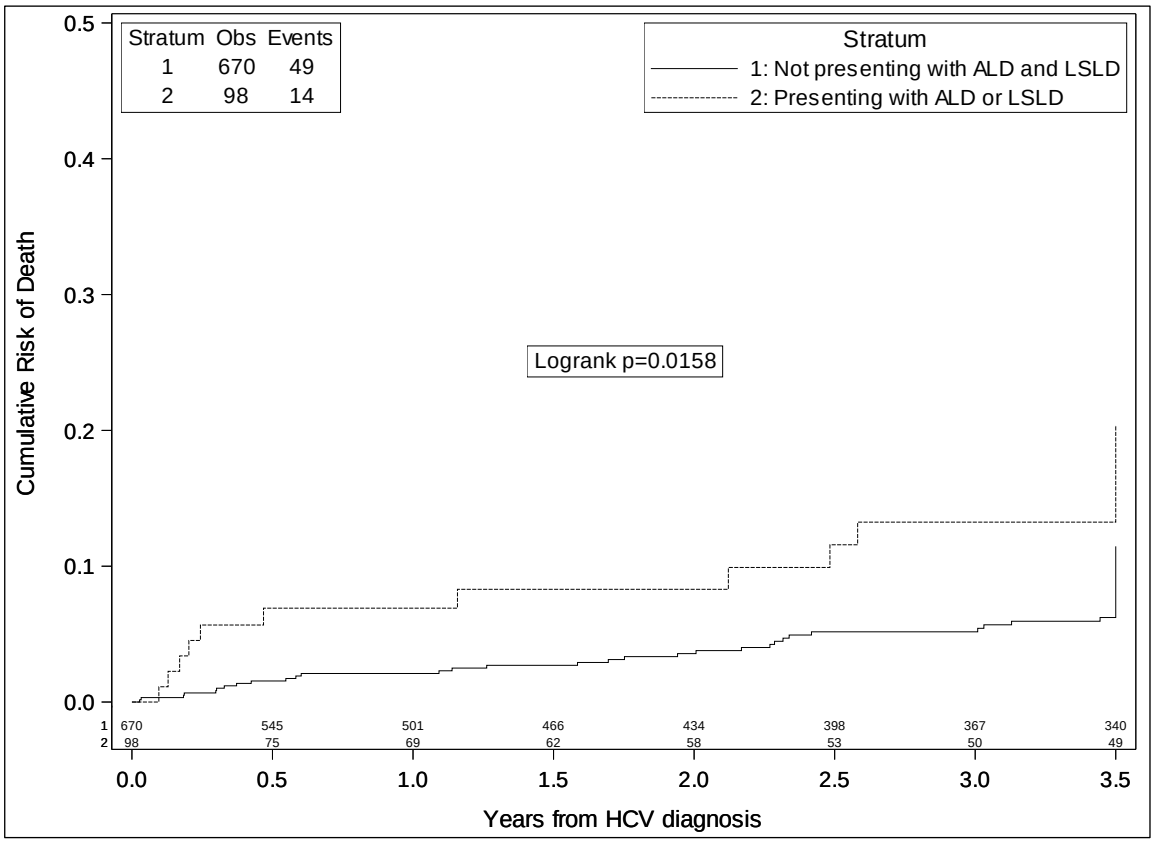
Table 6.8 Univariable and Multivariable Cox regression model for risk of death (any cause of death)Table 6.7 . By 3 years, the estimated cumulative risk of death (95% CI) in non-late presenters and those presenting with ALD were 6.2% (4.4 – 8.8) and 11.1% (5.7 – 21.2) respectively. In addition, by 3 years the estimated cumulative risk of death (95% CI) from baseline in those presenting with ALD or LSLD was13.3% (7.3 – 23.5). Thus, there was an additional 2% difference in risk by 3 years comparing HCV-infected people with advanced disease with those without advanced disease when including in the exposed also the clinically diagnosed LSLD.

Figure 6.14 Cumulative risk of all-cause mortality stratified by late presentation of HCV (ALD vs. not ALD)



*Individuals with ALD did not have LSLD

Figure 6.17 Cumulative risk of all-cause death stratified by late presentation of HCV (ALD or LSLD vs. not)



*Individuals with ALD did not have LSLD

Table 6.20 Cumulative probability of death from the KM plot from year one to year three stratified by late presentation of HCV

	1-year (%) (95% CI)	**3-year (%) (95% CI)**
Late presentation of HCV (n=760)		
Not presenting with ALD	2.30 (1.30, 3.90)	6.20 (4.40, 8.80)
Presenting with ALD (n=90)	7.60 (3.50, 16.30)	11.10 (5.70, 21.20)
Late presentation of HCV (n=768)		
Not presenting with ALD or LSLD	2.30 (1.30, 3.90)	6.20 (4.40, 8.80)

	1-year (%) (95% CI)	3-year (%) (95% CI)
Presenting with ALD or LSLD (n=98)	8.30 (4.00, 16.70)	13.30 (7.30, 23.50)

In the unadjusted Cox regresssion model (shown inTable 6.8 Univariable and Multivariable Cox regression model for risk of death (any cause of death)

Table 6.9 Univariable and Multivariable Cox regression model for probability of starting HCV therapyTable 6.8), there was weak evidence for a higher risk of death in people with ALD compared to those not presenting with ALD (RH = 1.84 (0.98 – 3.47); p=0.057). According to model assumptions as suggested by the DAG, a number of different approaches can block all back-door paths from exposure to outcome. All these multivariable models showed similar findings i.e increased risk of death in people with ALD compared to those not presenting with ALD but not reaching statistical significance. Arbitrarily picking one of the models suggested by the DAG (Figure 6.1 DAG model for impact of late HCV presentation on all-cause mortality

Figure 6.2 Participant Flow diagramFigure 6.1) the risk of all-cause mortality was attenuated, as compared to the unadjusted estimate - model#1 - aRH = 1.41 (0.73 – 2.75); p=0.311.

Results (not shown in table) from a fitted model adjusting for age only yielded an adjusted aRH = 1.60 (0.84 – 3.06); p=0.152 and additionally adjustting for and year of HCV diagnosis yielded an adjusted aRH = 1.51 (0.79 – 2.88); p=0.216, showing that these factors accounted for much of the attenuation. Of note, under our assumptions, controlling for only these two variables is not even sufficient to remove all of the potential confounding.

In the analysis adding individuals presenting with LSLD in the group of the exposed, there was an estimated 2-fold increased risk of death in people with advanced disease compared to those not presenting with neither ALD or LSLD in the unadjusted analysis RH = 2.05 (1.13 – 3.71); p=0.018). However, also for this comparison after adjustment for the same set of chosen potential confounders (as suggested by the DAG) the risk of all-cause mortality was largely attenuated, model#1 - aRH = 1.56 (0.83 – 2.93); p=0.166 . Similarly, this attenuation was mostly driven by key confounding factors such as age diagnosis. In all models, low statistical power of the analysis could also explain the lack of statistical significance as few events have been observed.

Table 6.23 Univariable and Multivariable Cox regression model for risk of death (any cause of death)

	Unadj RH (95% CI)	pv	Model1 RH (95% CI)	pv	Model2 RH (95% CI)	pv	Model3 RH (95% CI)	pv
Late presentation of HCV (n=760)								
Not presenting with ALD	1.00		1.00		1.00		1.00	
Presenting with ALD	1.84 (0.98, 3.47)	0.057	1.41 (0.73, 2.75)	0.311	1.55 (0.79, 3.03)	0.197	1.50 (0.77, 2.92)	0.232
Late presentation of HCV (n=768)								
Not presenting with ALD and LSLD	1.00		1.00		1.00		1.00	
Presenting with ALD or LSLD	2.05 (1.13, 3.71)	0.018	1.56 (0.83, 2.93)	0.166	1.71 (0.91, 3.21)	0.096	1.66 (0.88, 3.10)	0.116

Table 6.26 Univariable and Multivariable Cox regression model for probability of starting HCV therapy

	Unadj RH (95% CI)	pv	Model1 RH(95% CI)	pv	Model2 RH(95% CI)	pv	Model3 RH(95% CI)	pv
Late presentation of HCV (n=760)								
Not presenting with ALD	1.00		1.00		1.00		1.00	
Presenting with ALD	1.79 (1.15, 2.79)	0.010	2.02 (1.24, 3.29)	0.004	2.03 (1.25, 3.27)	0.004	2.06 (1.27, 3.32)	0.003
Late presentation of HCV (n=768)								
Not presenting with ALD and LSLD	1.00		1.00		1.00		1.00	
Presenting with ALD or LSLD	1.85 (1.21, 2.85)	0.005	2.03 (1.27, 3.26)	0.003	2.05 (1.29, 3.27)	0.003	2.12 (1.33, 3.38)	0.002

Model 1: Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + CD4 + HIV-RNA + Alcohol + HBV status + Region
Model 2: Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + CD4 + HIV-RNA + Alcohol + HBV status + Employment status
Model 3: Age + Gender + Mode of HIV transmission + Year of HCV diagnosis + CD4 + HIV-RNA + Alcohol + HBV status + Nationality

6.7.8 Association between late HCV presentation and probability of starting HCV therapy

There were 17% (132/768) of individuals who started HCV therapy for the first time after HCV diagnosis. A breakdown of the first HCV therapy started, stratified by exposure groups in the 132 who initiated therapy is shown in Table 6.10 HCV therapy started stratified by late presentation of HCV

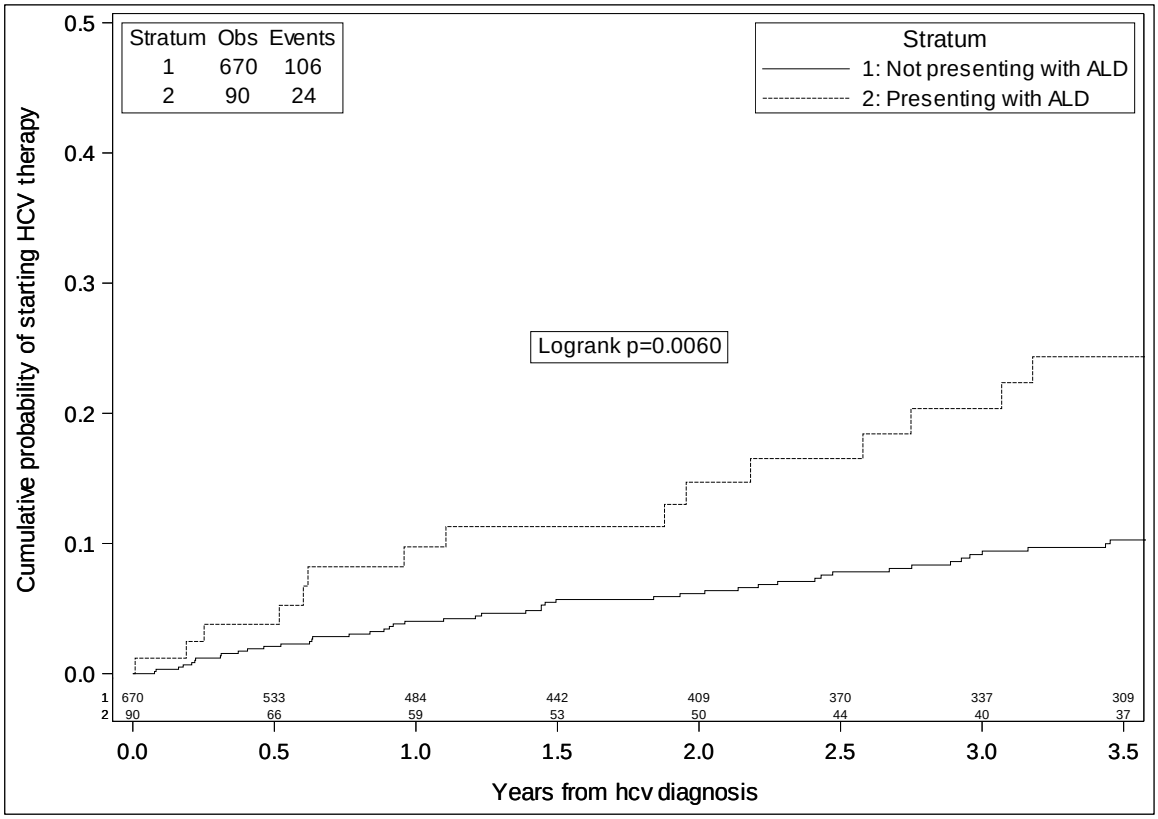
Figure 6.7 Cumulative probability of starting HCV therapy stratified by late presentation of HCV (ALD vs. not ALD)Table 6.10. Participants were enrolled on average in 2012 and diagnosed with HCV prior to the introduction of DAA in 2014. Nevertheless, almost half of people initiating HCV therapy started a treatment with IFN/RBV (69/132, 52%). However, it is reassuring that the remaining 41% (54/132) were started on second generation DAAs. At the time of the analysis, the data also showed that of the 69 people who failed IFN/RBV, no participant subsequently started DAA, however this could be delay in reporting of the DAA and not a true reflection of current practice.

Table 6.29 HCV therapy started stratified by late presentation of HCV

HCV therapy started	Not presenting with advanced and late stage LD N=106 (%)	Presenting with advanced LD N=24 (%)	Presenting with late stage LD N=2 (%)	Total N=132 (%)
IFN/RBV	64 (60)	5 (21)	0 (0)	69 (52)
Telaprevir	2 (2)	2 (8)	0 (0)	4 (3)
Boceprevir	1 (1)	0 (0)	0 (0)	1 (1)
Sofosbusvir	4 (4)	1 (4)	0 (0)	5 (4)
Daclatasvir	12 (11)	5 (21)	1 (50)	18 (13)
Harvoni	9 (9)	4 (17)	0 (0)	13 (10)
Viekirax	8 (7)	2 (8)	1 (50)	11 (8)
Epclusa	3 (3)	2 (8)	0 (0)	5 (4)
Zepatier	2 (2)	0 (0)	0 (0)	2 (2)
Other	1 (1)	3 (13)	0 (0)	4 (3)

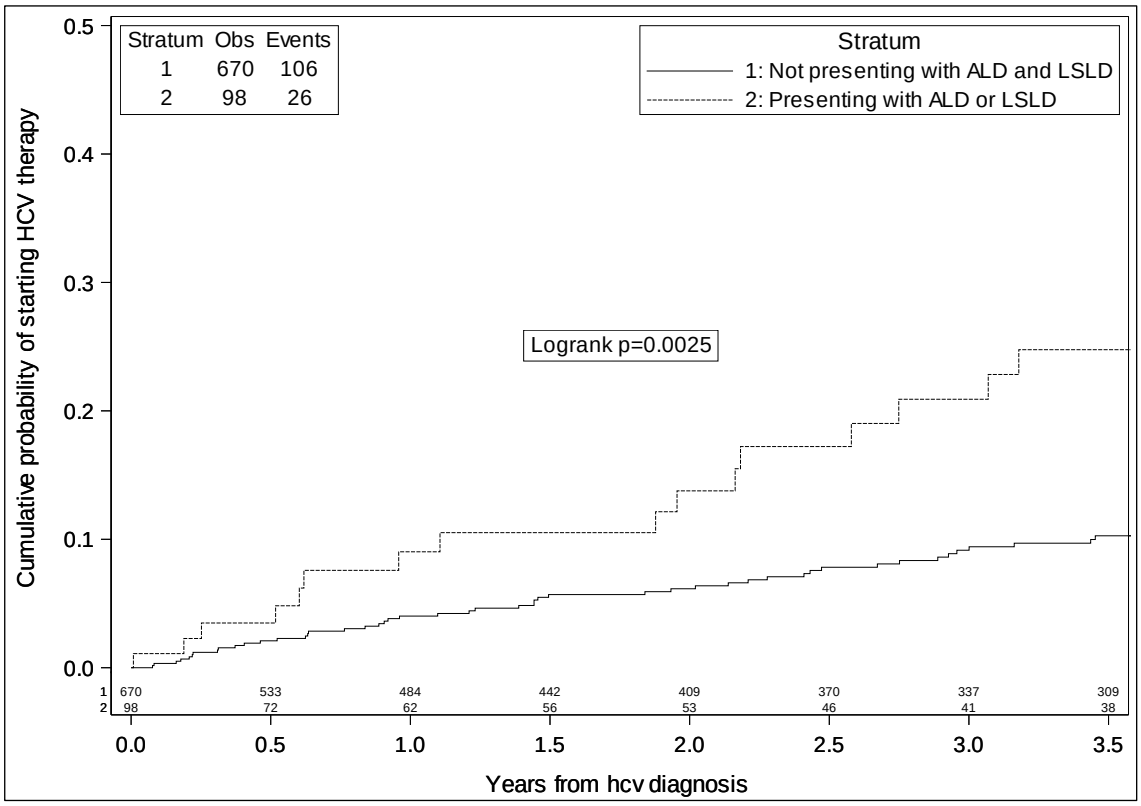
Ledispasvir/Sofobusvir – Harvoni ; Ombitasvir/Paritaprevir/Ritonavir – Viekrax ; Sofosbuvir/Velpatasvir – Epclusal
Grazoprevir/Elbasvir – Zepatier

Figure 6.20 Cumulative probability of starting HCV therapy stratified by late presentation of HCV (ALD vs. not ALD)



*Individuals with ALD did not have LSLD

Figure 6.23 Cumulative probability of starting HCV therapy stratified by late presentation of HCV (ALD or LSLD vs. not)



*Individuals with ALD did not have LSLD

The KM plots of the cumulative probability of starting HCV therapy are shown in Figure 6.7 Cumulative probability of starting HCV therapy stratified by late presentation of HCV (ALD vs. not ALD)

Figure 6.7 (comparing individuals not presenting with ALD vs. those presenting with ALD) and in Figure 6.8 Cumulative probability of starting HCV therapy stratified by late presentation of HCV (ALD or LSLD vs. not)

Figure 6.8 (comparing individuals not presenting with ALD or LSLD vs. those presenting with either of those). HCV-infected people with ALD initiated HCV

treament significantly sooner that people without ALD (log rank p = 0.006). Results were similar when classifying as exposed individuals presenting with ALD or LSLD (log rank p=0.003).

The estimated cumulative probabilities of starting HCV therapy at one and three years are shown inTable 6.11 Cumulative probability of starting HCV therapy from the KM plot from year one to year five stratified by late presentation of HCV

Figure 6.9 Cumulative probability of starting DAA stratified by late presentation of HCV (ALD or LSLD vs. not) in individuals enrolled after 2014Table 6.11. Thus, by three years from enrolment the estimated cumulative probability (95% CI) of starting HCV treatment in individuals not presenting with ALD was 9.4% (7.1 – 12.4) vs. 22.3% (13.7 – 25.1) in those presenting with ALD. Slightly higher cumulative probability (95% CI) was observed by three years in those presenting with ALD or LSLD 22.8% (14.3 – 35.4).

Table 6.32 Cumulative probability of starting HCV therapy from the KM plot from year one to year five stratified by late presentation of HCV

	1-year (%) (95% CI)	**3-year(%) (95% CI)**
Late presentation of HCV (n=760)		
Not presenting with ALD	4.40 (2.80, 6.30)	9.40 (7.10, 12.40)
Presenting with ALD	11.30 (5.80, 21.40)	22.30 (13.70, 35.10)
Late presentation of HCV (n=768)		
Not presenting with ALD and LSLD	4.40 (2.80, 6.30)	9.40 (7.10, 12.40)
Presenting with ALD or LSLD	10.50 (5.40, 20.00)	22.80 (14.30, 35.40)

In the unadjusted Cox regresssion model (Table 6.9 Univariable and Multivariable Cox regression model for probability of starting HCV therapy

Table 6.9), presenting with ALD was associated with higher probability of starting

HCV therapy compared to not presenting with ALD unadjusted RH = 1.79 (95% CI: 1.15 – 2.79); p=0.010). After model adjustment, probability of starting HCV therapy as by model assumptions described in the DAG (Figure 6.1 DAG model for impact of late HCV presentation on all-cause mortality

Figure 6.2 Participant Flow diagramFigure 6.1) presenting with ALD was even more strongly associated with the probability of starting HCV therapy, model #1 - adjusted RH = 2.02 (95% CI: 1.24 – 3.29); p=0.004. These findings were similar when additionally including individuals presenting with LSLD. Unadjusted RH = 1.85 (95% CI: 1.21 – 2.85); p=0.005) and model #1 - adjusted RH = 2.03 (95% CI: 1.27 – 3.26); p=0.003 Table 6.9 Univariable and Multivariable Cox regression model for probability of starting HCV therapy

Table 6.9.

6.7.9 Sensitivity analysis

The additional analysis restricted to individuals who were enrolled in Icona after 2014, indicated no difference (log rank p = 0.117) in the rate of DAA therapy initiation between those presenting with ALD or LSLD and individuals not presenting with ALD and LSLD (Figure 6.9 Cumulative probability of starting DAA stratified by late presentation of HCV (ALD or LSLD vs. not) in individuals enrolled after 2014

Table 6.12 Cox regression model for probability of starting HCV therapy in individuals enrolled after 2014Figure 6.9). Although the sample size in this sensitivity analysis was small, the results seem to be more consistent with the hypothesis that the probability of starting treatment for HCV was less correlated with the stage of liver disease in this period. This might be partly due to changes of treatment guidelines over time progressing towards universal treatment access. In

the adjusted analysis (model including age and year of HCV diaganosis at baseline) the estimated difference in risk was RH = 1.44 (95% CI: 0.79 – 2.62); p=0.238 . Therefore, the analysis still carried some evidence to suggest a higher probability of starting DAA for individuals with ALD or LSLD vs. not, but was not a statistically significant finding. The magnitude of the association is moderate and the confidence interval shows that a substantial larger effect cannot be ruled out.

Figure 6.26 Cumulative probability of starting DAA stratified by late presentation of HCV (ALD or LSLD vs. not) in individuals enrolled after 2014

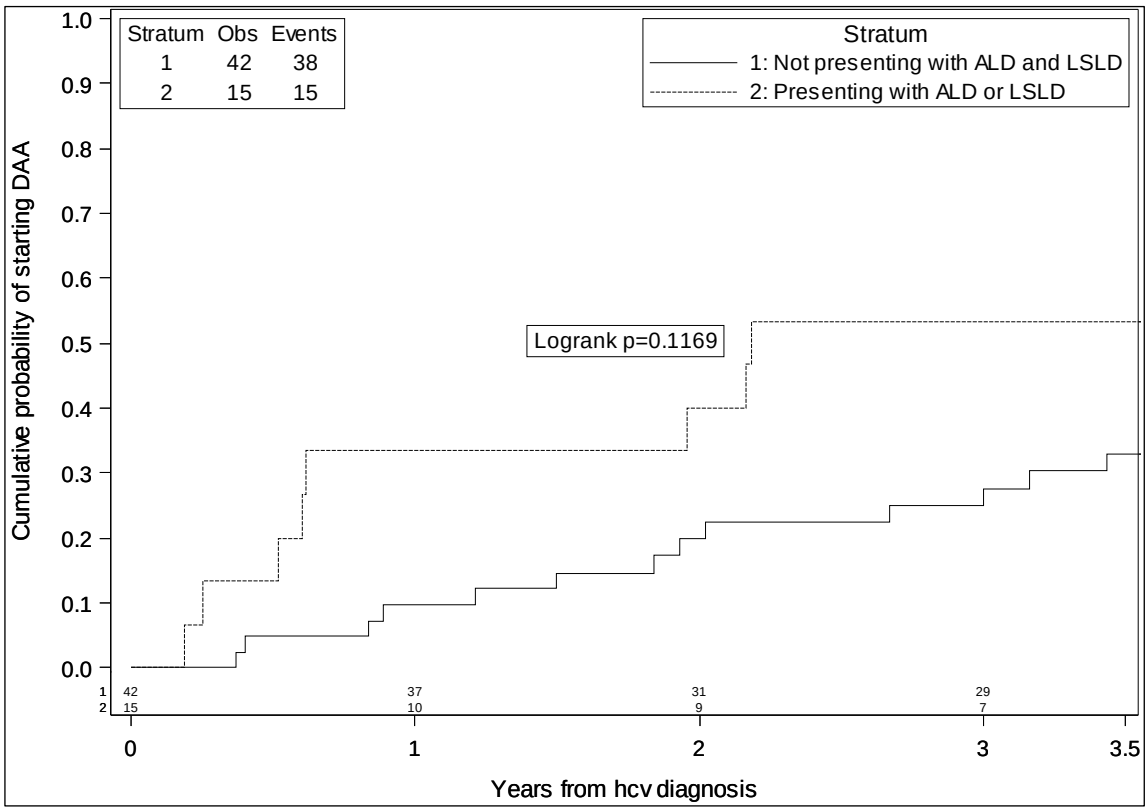


Table 6.35 Cox regression model for probability of starting HCV therapy in individuals enrolled after 2014

	Unadjusted RH (95% CI)	pv	[1]Adjusted RH (95% CI)	pv
Late presentation of HCV (n=768)				

Not presenting with ALD and LSLD	1.00		1.00	
Presenting with ALD or LSLD	1.36 (0.75, 2.47)	0.315	1.44 (0.79, 2.62)	0.238

[1]Adjusted for Age and Year of HCV diagnosis

6.8 Discussion

In this chapter I set out to investigate the prevalence of late HCV presentation and factors associated with this, and to assess the impact of late HCV presentation on risk of all-cause mortality as well as probability of starting HCV therapy in newly diagnosed HIV-positive individuals seen for routine clinical care in infectious disease units across Italy. To classify participants as late presenters I used the consensus definition for late HCV presentation as of 2015 [369]. This identified those presenting with advanced liver disease (based on FIB-4 score or Fibroscan) and those presenting with late stage of liver disease (based on clinical diagnosis) at entry in the Icona cohort.

Firstly, following the introduction of DAA the guidelines for HCV testing have changed so that everybody with HIV should be universally tested for HCV at point of entry in care, regardless of mode of HIV acquisition or other factors. This modification in recommendations was needed to maximise the chance of quickly identifying all individuals infected with HCV[38]. Following that, WHO recommends that patients' stage of liver disease needs to be determined to establish the right course of treatment. Not testing everybody for HCV when they first have access to care for HIV is likely to result in a missed opportunity to treat and cure [158].

The first stage of this analysis identified a non-negligible proportion of individuals enrolled in the Icona cohort who were not tested for HCV at enrolment. Overall, 87% (8617/9931) were tested for HCV antibodies at entry in the cohort and the remaining 13% were not. This proportion is close to the WHO recommended target of testing at least 90% of individuals in order to reach the 2030 goal of HCV elimination[5]. Therefore, it is re-assuring that, in the context of the HIV/HCV coinfected population seen for care in Italy, screening for HCV approximates the WHO target. This is under the assumption that Icona is a representative sample of the target population of interest.

When looking at factors associated with HCV testing at study enrolment, there were differences in terms of demographics (with the exception of gender), HIV-related factors, socio-economic status, HBV coinfection which suggests that historically, in Italy HCV testing was more likely to occur for individuals perhaps considered at risk for HCV infection. Thus, although testing is available to all, individuals' characteristics were associated with the probability of accessing HCV testing. There were also regional differences in the uptake of HCV testing, possibly due to differences in health care setting and policies. These results taken altogether indicate that a number of potential barriers to testing still exist.

The target population in this analyses includes a proportion of individuals who were enrolled prior to the introduction of DAA when there was still prioritisation in terms of who was getting treated. As a side note, clinical sites participating in the Icona Network are also involved in a separate protocol extended to people who are not cART-naïve at entry aiming at evaluating trends in HCV testing at first contact with medical care (data produced after I left UCL which are not included in this thesis). Encouraging results were presented at the EACS 2019 Conference showing an increase of HCV testing outside of the historical groups at higher risk for HCV acquisition [229].

The first definition of late HCV presentation that I used in this chapter relates to individuals presenting with FIB-4>3.25 or kPa>9.5 within six months of HCV diagnosis. Out of the 898 newly diagnosed HIV/HCV coinfected participants, 15% (n=130) had to be excluded from this analysis as they did not have data available for staging their liver disease. Of the remaining, there were 11% (n=98) who were found to be presenting with advanced or late stage liver disease. This percentage appeared to remain stable over the study period ranging between 11% and 14%, with some uncertainty around these estimates, given the small sample size. These data also have implications; a lack of reduction of the fraction of people presenting late over time indicates that current frequency of HCV testing is still insufficient to detect infections early and represents a missed opportunity to early achievement of

HCV eradication. In addition, it is known that there is also a large margin of improvement for the rate of HIV testing and many people are still not diagnosed with HIV until they develop symptoms[158]. Therefore, increased rate of testing for both HIV and HCV are desirable targets for WHO and from a public health prospective in general.

My estimate of the prevalence of late HCV presentation is slightly lower than that observed in similar observational studies in other parts of Europe with a prevalence ranging over [15 - 32%] [370, 373, 375, 378]. This discrepancy is likely to be due to the different definitions used to classify late HCV presentation. For example in studies conducted in the USA, a person with a FIB-4 cut-off >5.88 was considered to have advanced stage of liver disease, while the European consensus definition used here has the much lower cut-off of FIB-4 >3.25. Also the time period from HCV diagnosis used to define late HCV presentation varies across different studies. In my analysis, I used a time window of six months to capture data on liver disease because clinical visits are on average scheduled every six months in Icona. In most of the other studies a much larger window period from HCV diagnosis was used, in some studies even a two-year period was considered a reasonable time window for late presentation and this could have contributed to the difference in prevalence estimates seen [379]. Another factor likely to explain some of the variability between prevalence estimates is the case-mix of the study population in recent years most of the participants in Icona acquired HIV/HCV through heterosexual or MSM contacts, but the prevalence of MSM is typically higher in other study populations (e.g. UK, North Europe [174]).

Despite these differences in prevalence rates of late HCV presentations, this analysis identified risk factors associated with increased risk of presenting late which are consistent with those found also by other studies [371, 373, 378]. Older age, male gender and in the case of HIV/HCV coinfected people, having a low CD4 were independently associated with increased risk of presenting late for HCV. The associations with age and CD4 could be explained by the fact that HIV and HCV

infection was acquired many years prior to enrolment and remained asymptomatic until the date of seeking medical care and enrolment in the cohort. Indeed, in this analysis, individuals with advanced or late stage liver disease were of median age >40 years and average CD4 of <200 cells/mm^3.

In HIV/HCV coinfected individuals, having a CD4 <200 cells/mm^3 is indicative of progressed HIV disease which is consistent with also having advanced or late stage liver disease. It is conceivable that, at least for the PWIDs included here, HIV and HCV infections occurred approximately at the same time. Of note, the magnitude of these associations are likely to be underestimated because the study population only included those who were newly diagnosed with HIV (within 6 months of enrolment) and therefore a proportion of prevalent HCV infections were excluded.

The impact of late HCV presentation on risk of all-cause mortality was also assessed. In the Cox regression analysis, assessing the association between late HCV presentation and risk of death, the unadjusted analysis showed a 2-fold increased risk of all-cause mortality in individual with ALD or LSLD compared to those with no disease at presentation. However, after adjustment for potential confounding factors, this effect was attenuated, the modification of the hazard ratio was mostly driven by age and year of HCV diagnosis. These results are partially in contrast with those of other studies that found an independent effect of late HCV disease on the risk of death [197, 387] [370]. Nevertheless, most of the deaths among our cohort of HIV/HCV coinfected individuals was liver-related [197, 387].

There were 17% (132/768) individuals who started HCV therapy post HCV diagnosis (including IFN/RBV-based and DAA therapy). Less than a half of the study population (41%) were started on DAA treatment. This is likely to be because second generation DAA were not yet available for these individuals when treatment initiation was recommended for them. In the multivariable analyses, after adjusting for (age, gender, mode of HIV transmission, calendar year enrolled, region, CD4,

HIV-RNA, alcohol use, HBV infection and region), late HCV presentation was independently associated with an increased probability of starting HCV therapy adjusted RH = 2.02 (95% CI: 1.24 – 3.29); p=0.004). Again, this is likely to be the result that most therapy initiation occurred in a period preceding the change of guidelines to universal treatment when therapy prioritisation was guided by factors such as liver stiffness and stage of liver disease. These results are not unexpected as prior to the universalisation of DAA treatment, ALD (which is based on FIB-4 and liver stiffness and more generally the stage of HCV disease) was used as a factor to prioritise treatment initiation.

Of interest, a sensitivity analysis restricted to the individuals enrolled after 2014, when DAAs were introduced, the date were more compatible with the null hypothesis of no differences in the rate of DAA therapy uptake comparing late and non-late HCV presenters. This is consistent with universal access to DAA treatment in Italy as per current guidelines. However, the direction of the association showed an increased probability of starting DAA in individuals presenting with late HCV (also after adjusting for age and year of HCV diagnosis) and this might indicate that clinicians are still selecting individuals for treatment. Also, we cannot rule out that the lack of statistical association was purely due to low statistical power.

6.9 Strengths and limitations

One of the strengths of this analysis was in the fact that the estimate of the prevalence of late HCV presentation was based on two consensus definitions which are standardised and approved by a panel of experts [369]. This enables standardised comparisons between studies and over time [369]. However, with less than 100 people classifiable as advanced liver disease, there remains uncertainty around the prevalence estimates relating to late and the trend over time. Nevertheless a dramatic reduction in this proportion in recent years can be excluded by the Icona data.

Another strength which applies to stage 2 analysis in this chapter, was the careful construction of an underlying model for the identification of potential confounders affecting the association between late HCV presentation and clinical and treatment outcomes. The main aim was to establish whether there is a causal link between late HCV presentation and the risk of these specific outcomes after controlling for all possible pathways of measured confounding. The model assumptions have been depicted using DAGs. This is a novel and transparent tool to construct multivariable models, to summarise the assumptions made regarding the relationship between the exposure and outcome variables.

There are also some limitations. First, as these analyses use routinely collected data, we cannot rule out the existence of a delay in reporting HCV diagnosis and indeed treatment initiation. The number of individuals identified as presenting late with HCV was low especially when using the definition involving late stage liver disease. Another possible limitation is that the differences between characteristics of individuals with ALD could not be compared with those of participants with LSLD as the number were too small. Also, the generalisation of estimate of the prevalence to the wider HIV/HCV population in Italy relies on the assumption that the HIV/HCV coinfected population sampled in Icona was representative of the target population of coinfected residents in Italy and cART-naïve. It is indeed conceivable that inclusion in the cohort is conditioned on individual's severity of HCV symptoms and therefore collider bias cannot be excluded.

The relatively low prevalence of late HCV presentation also resulted in a lack of statistical power in some analyses in which the condition was the exposure variable of interest. Additionally, HCV infection was mainly based on the results of the antibody test because HCV-RNA testing has not been routinely introduced in Icona clinical sites until 2002. It is also possible that some of the participants with positive serology had spontaneously cleared the infection and there was no HCV-RNA data to document this. However, of note, this is the first analysis of the Icona

cohort, that has assessed the frequency of late HCV presentation using consensus standardised definitions. These are more robust measures of stage of liver disease as they use a number of surrogate biomarkers values (i.e. FIB-4, Fibroscan) as well as clinical diagnosis compared to other studies that relied only on medical notes, or retrospectively collected data.

Since this is an observational study, causal links are impossible to prove. Indeed, despite the rigorous method used for the identification and removal of all possible confounding, the results still rely on the assumptions of a correctly specified model and no unmeasured confounding being present. This latter is a very strong assumption as, for example, perceived adherence is likely to be an unmeasured confounder of the association between advanced liver disease and rate of treatment uptake. In general, it is important to bear in mind that all these methods aiming to reduce confounding or collider bias rely on untestable assumptions.

6.10 Conclusion

In conclusion, this analysis of data of newly HIV-positive individuals, as of January 2018, found that >80% of individuals were tested for HCV at the time of their first contact with medical care. Additionally, among those HCV tested, 13% were found to have advanced or late stage liver disease. Male gender, PWID as mode of HIV transmission and lower CD4 were found to be associated with the risk of late HCV presentation (independently of age), suggesting specific patients characteristics to look out for in routine HCV screening. When considering outcomes relating to late HCV presentation, not enough evidence was found for an association between late HCV presentation and risk of all-cause mortality. The association found in unadjusted analysis was mainly explained by differences in age, CD4 and year of HCV diagnosis.

In this cohort, most HCV therapy was initiated prior to the universalisation of DAA treatment. Therefore, although results indicated a 2-fold increased probability of initiating HCV therapy in people with advanced HCV disease, when restricting to

the period after 2014, data were more compatible with the null hypothesis of no difference suggesting consistency with universal access to DAA treatment in Italy as per current guidelines.

6.11 Further work

Overall, there was evidence that some of the analyses were underpowered and although these initial data are interesting, further research is needed to better correlate late HCV presentation with clinical and treatment outcome, especially in the DAA era. Specifically, in the analysis presented, there was no attempt to quantify how much of the risk of death attributable to late HCV presentation was adverted by initiation of therapy. It will be important to relate the HCV testing data to those more recently produced by the Icona Network as well in other settings regarding the proportion of HIV-positive people who are screened for HCV and its impact on HCV eradication. Certainly, a further area of interest would also be to look at re-infection rates of HCV and rate of eradication in people who have failed their first DAA regimen, especially in individuals not presenting late for HCV.

CHAPTER 7

7 ARE THERE ANY REGIONAL DIFFERENCES IN TERMS THE CONTINUUM OF CARE FOR HCV AMONG HIV/HCV COINFECTED INDIVIDUALS SEEN FOR ROUTINE CLINICAL CARE IN ITALY SINCE JANUARY 2015?

7.1 Aim and objectives

The overall aim of this chapter is to develop and evaluate the hepatitis C continuum of care (CoC) in HIV/HCV coinfected individuals seen for routine clinical care in Italy and enrolled in the Icona Network cohorts since January 2015. I specifically focus on describing regional differences (North vs. Centre vs. South of Italy) as region is considered a factor potentially associated with differential access to health care. Region was defined on the basis of the location of the clinical site attended by the participants.

The specific objectives are:

1. To estimate the prevalence of individuals tested for HCV-RNA among HCVAb positive individuals and investigate whether the probability of HCV-RNA testing varies by geographical region
2. To estimate the frequency of direct acting antiviral (DAA) treatment uptake amongst HCV-RNA positive (chronically infected) individuals and investigate whether the probability of initiating DAA treatment varies by geographical region
3. To estimate the proportion of individuals achieving sustained virological response (SVR) amongst those starting DAA treatment and investigate whether the probability of achieving SVR varies by geographical region

7.2 Introduction

In 2015, the estimated global prevalence of chronic HCV infection was 71 (range: 62-79) million of which 20% were diagnosed with HCV (14 million). Of those diagnosed, only 7.4% (1.1 million) initiated treatment, with very few individuals initiating newer DAA drugs [158]. In 2016, 1.76 million people were additionally treated bringing the global coverage of hepatitis C treatment to an estimated 13% of all those infected with HCV [158]. Of those individuals started on treatment, about 50% were started on DAA [158]. In Europe, the picture was similar, the 2015 estimates showed that among an estimated 15 million people living with HCV infection in the European region, 37% were diagnosed with HCV [404]. According to the WHO Global progress for HCV, the updated estimates as of end of 2017 show that 7% (5 million) people of the 71 million people globally infected with HCV have been treated with DAA[158].

The WHO has set out prevention and treatment targets towards the elimination of HCV by 2030 [158]. Prevention targets include; 100% of blood donations to be screened in a quality-assured manner, 90% of injections are given using safe devices. Treatment targets include; ensuring all individuals considered at risk of HCV to be tested for HCV and among these individuals who are diagnosed with HCV, at least 80% should be treated for HCV. The impact of meeting these goals is estimated to be a 90% reduction in incidence of chronic HCV infections and 65% reduction mortality from chronic HCV between 2016 and 2030 [5]. Even more recently, the WHO 2021 Global progress report for HCV include guidelines for a 'treat all' approach for all those with active viremia should be treated with DAA[405].

In line with the plan for HCV eradication, the Italian Medicines Agency (AIFA) has redefined the treatment criteria for individuals with chronic HCV infection recommending universal access to DAAs as of March 2017. Universal access implies that all individuals for whom therapy is required should be treated

immediately; prior to March 2017 only people with advanced fibrosis were receiving treatment [406]. This was similar also in other European countries [11]. For the HIV/HCV coinfected population, according to the treatment criteria from AIFA, coinfected individuals with any stage of liver disease should also be treated as of March 2017 [406]. Despite the introduction of DAA treatment for chronic HCV with universal access, barriers to treatment access might still be present and further research is needed to identify factors associated with failing to transition through stages in the HCV CoC pathway in HIV/HCV coinfected populations. For example, stigmatisation [407], PWID or not started on HIV treatment[408]. This chapter specifically evaluates the hypothesis that in Italy there are regional barriers to HCV testing and treatment. As mentioned in chapter 1 (section 1.3.8), regional differences potentially exist in terms of health care access. In chapter 6, when I looked at the newly diagnosed HIV/HCV coinfected participants in an exploratory analysis, there was some evidence that the rate of HCVAb testing varies by region.

In Italy, the National Health Service (NHS) covers all medical costs in hospitals and consultations with doctors [252]. However, according to a health profile report of Italy by the European Commission in 2019, Italy's NHS service is de-centralised and varies by region in terms of health care access [252, 409]. For example, unmet needs defined as longer waiting times for treatment and longer distances to health care facilities are more common in the south than in the northern region[252]. There has been a change in the regulations so that individual regions have the responsibilities of ensuring that all citizens and foreign residents have access to health care, replacing a centralised control at National level [252]. In addition, regions have a choice to offer health care services beyond the standard of care. Therefore, individuals have a choice to receive care in other regions besides their region of residence [252]. However, there are policy concerns over regional differences in terms of delivery, accessibility and capacity of health care as well as the quality of health services offered [409]. This is mainly due to variations in resources available in each region. In general, the northern and central regions are known to have higher capacity of resources, more advanced technology and better quality of care

than the southern region [409]. The southern region is highly populated with people on low-income meaning affordability of any additional health care required is likely to be limited to lower health care quality services [409]. Additionally lower hospital capacity has been reported in the southern region with more individuals turning to private medical insurance. This will in turn have an impact on associated access to health care and individuals residing in the south are more likely to want to receive care in the north or central regions [252]. The new system also proved to be inefficient toward fighting new emerging pandemics such as COVID-19 disease which has also disrupted essential access to health care [410].

The main aim of this chapter is therefore to investigate the HCV CoC for HIV/HCV coinfected individuals in Italy and assess whether regional disparities in HCV CoC continue to exist in infectious disease units in Italy in the DAA era. The results of this analysis are important as they add to the literature in terms of further understanding of health care barriers and may help policy makers in developing targeted interventions.

7.3 Literature review

The literature review in this section focuses on studies that have evaluated the HCV CoC generally and also in HIV/HCV coinfected individuals. In particular, I reviewed studies across all middle to high-income countries which analysed the same three cascade outcomes evaluated in this chapter (HCV-RNA testing, starting HCV treatment (specifically studies in the DAA era i.e. after 2014) and achieving sustained virological response (SVR)). The literature search was done up to January 2019 and the updated in January 2021.

7.3.1 World Health organization framework of HCV continuum of care

Following the launch of the WHO setting targets towards prevention and elimination of HCV, there has been an increased focus on the evaluation of the

HCV CoC [5]. The HCV CoC is a framework used to identify individuals with chronic HCV infection with the aim of linking them to care and providing treatment to achieve cure [411, 412]. According to WHO, the CoC for HCV spans a range of possible interventions that are required in order to achieve the 2030 target [5].
Figure 7.1 Proposed continuum of care of viral hepatitis according to WHO [5]

Figure 7.1 shows the proposed CoC for viral hepatitis. It has been proposed that HCV CoC starts with identifying all people at risk of infection (this includes HIV-positive individuals); interventions related to this stage may identify possible ways of preventing HCV infection and knowing which populations to target to identify undiagnosed infections. The next step involves testing the target population and linking into appropriate medical care those who are found to be viremic for HCV (chronically infected). To access medical care means that a chronically infected individual is initiated on treatment and a complete course of treatment is ensured. The aim of initiating and completing treatment is to cure the chronic HCV infection by achieving SVR [5]. As shown in the Figure 7.1 Proposed continuum of care of viral hepatitis according to WHO [5]

Figure 7.1 awareness of HCV infection status can also be regarded as one of the first stages in the HCV CoC. This step may result in educating people about how to prevent HCV transmission or directing individuals towards appropriate medical care and has not been evaluated in this analysis [413].

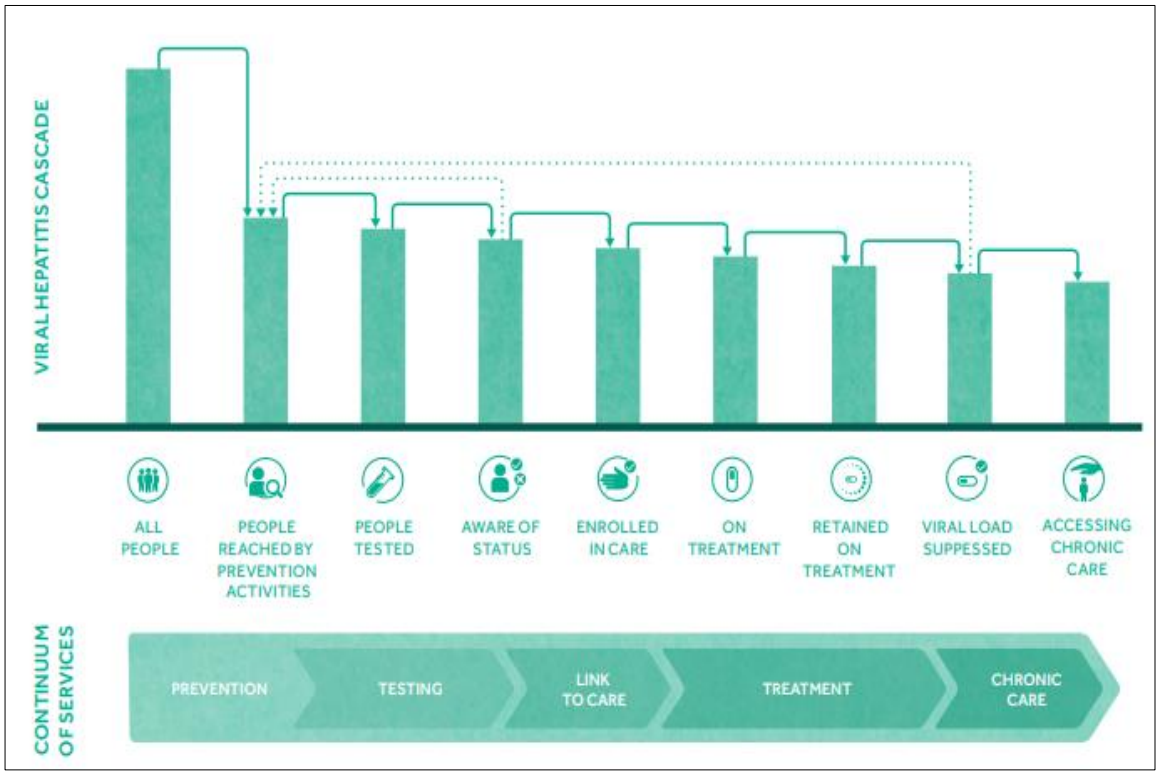
Studies assessing HCV CoC may either include all or some of the stages of the continuum. It is at each of the stages that individuals can be lost in the HCV care pathway for various reasons including potentially removable barriers. The number of stages evaluated in research studies typically depends on what data are available and which population is being studied. As a result, the design or the development of the HCV CoC may be different according to region of the world and specific setting. For example, among individuals identified with chronic HCV infection, some studies have gone a step further by evaluating the HCV genotype

and assessing stage of liver disease [414-418]. In their HCV CoC assessment, most studies have at least included the following stages: [377, 419-426].

- HCVAb testing among those identified to be at risk
- Proportion tested for HCV-RNA among those found to be HCVAb positive
    - Proportion found to be HCV-RNA positive among those tested for viremia
- Proportion starting treatment among those found to be HCV-RNA positive
- Proportion cured (defined as having a negative HCV-RNA test at the end of HCV treatment) among those who started/completed treatment.

In 2019, a group of health experts from Europe, Australia and North America developed a consensus definition of HCV CoC [427]. The agreed consensus relating to stages of continuum of care for HCV included four stages; infected, diagnosed treated and cured [427]. However, the experts noted that the proposed stages of HCV CoC could be used simply as an alternative to the already existing WHO framework [427].

Figure 7.2 Proposed continuum of care of viral hepatitis according to WHO [5]



WHO. Global Health Sector Strategy on Viral Hepatitis 2016-2021 towards ending viral hepatitis. 2016

7.3.2 Testing for HCV-RNA in HCV mono-infected and HIV/HCV coinfected populations

In the pre-DAA era, studies have typically reported a prevalence of >80% of individuals with positive HCV-RNA among those who were HCVAb positive [377, 418, 422, 423, 428-431]. These estimates have remained high even in the DAA era [402, 415, 424, 432, 433]. In specific risk groups and among HCVAb positive individuals, proportion of individuals tested for chronic HCV infections remains low [417, 420, 426, 430, 434]. For example, a study in Argentina, conducted on electronic medical records in 2018 found that only 49% (803/1650) of HIV-positive PWIDs were positive for HCV serology and 21% of these were tested for HCV-RNA [417]. Similarly, *Iversen et al*

studied 2,222 PWIDs considered to have a history of HCV testing and found that over half of them 57% (n=1173) were HCVAb positive; of these individuals 54% (637/1173) were tested for HCV-RNA [426].

One of the main reasons for the low rate of HCV-RNA testing observed in these studies is the fact that the source of data to establish HCV testing is often heavily reliant on the accuracy of medical records [426]. In the Icona cohort, for example, data on HCV-RNA have not been routinely collected in the database until 2002. In the EuroSIDA cohort of HIV positive individuals, in order to fill the gap in routine data collection, HCV-RNA was measured retrospectively on plasma stored samples to define their population of chronically HCV-infected participants [201].

In another study, *Viner et al* used the data obtained from hepatitis surveillance programs in the USA, between 2010 and 2013, which included 14,000 individuals in active care defined as having more than two HCV-RNA tests done over the previous six months. Of the 14,000 individuals included in the study 47% (n=6383) were shown to be chronically infected with HCV [420]. The authors noted that individuals in their study population did not recognize themselves as being at high risk of HCV infection and so the need to be tested was not seen as a priority. Also there could have been some people who were excluded from the analysis because they did not satisfy the definition of being under active care; it is possible that if the defining window period was extended beyond six months the study could have captured more individuals [420].

Screening for HCV-RNA in the initial target population of HIV/HCV coinfected gives an indication of the proportion of individuals who may need access to HCV treatment. A recent study by *Sacks-Davis et al* looking at linkage and retention in HCV care for HIV/HCV coinfected individuals in the DAA era, reported cascade of care data from seven HIV/HCV coinfected cohort studies spanning six countries (Canada, Australia, Netherlands, France, Switzerland and Georgia) [402]. In these six countries, the overall estimated number of PLWH ranged from 9,600 (in

Georgia) to 149,900 (in France) and of these, the estimated proportion also found to be HCVAb positive ranged from 12% (Netherlands) to 40% (in Georgia) [402]. In the Icona cohort, estimates of the number of participants coinfected with HIV/HCV fall in this range, being approximately 35% of all those enrolled. In the seven countries study, the proportion of individuals found to have chronic HCV infection ranged between 6% (estimated in the CEASE cohort study in Australia) to 20% (estimated in the CCC cohort study in Canada) [402].

Testing individuals for HCV-RNA among those found to be HCVAb positive to determine if a person is chronically HCV infected is also considered one of the first stages in the HCV CoC process. This is an important step aiming to identify potential candidates for treatment and provides an estimate of the rate of natural clearance of hepatitis C infection [5]. The proportion of individuals identified to be chronically infected will largely depend on the testing methods used, as well as populations targeted. For example, PWIDs have a higher prevalence of HCV infection globally compared to other risk groups, although HCV-RNA testing in these populations may have its own challenges [3]. Additionally over time, screening methods have improved, and geographical disparities are possible due to differences in technological advances [3]. Prevalence of HCV infection in individuals tested for HCV-RNA can also be impacted by spontaneous clearance of HCV. In HIV-positive populations spontaneous clearance of HCV infection was reported in one study to be 15.0% (95% CI: 11.5 – 19.3) in MSMs to 16.5% (95% CI: 12.5 – 19.6) in PWIDs [107]. In a meta-analysis of 675 HIV-negative populations, spontaneous clearance of HCV infection was reported to be 26% (95% CI: 22 – 29) [435].

Interestingly in the DAA era, diagnosis of chronic HCV infection is still low in specific populations [413], and especially in deprived populations [424]. *Mah et al* identified that social economic factors play a role in awareness of HCV [413]. Awareness comprised of both knowledge and willingness, both assessed by a series of questions asked on a Likert scale relating to HCV transmission,

knowledge regarding natural history of HCV disease and willingness to be treated. High scores were indicative of higher levels of knowledge and willingness to be treated [413]. They also found that lack of awareness of HCV (i.e. scoring low levels on questions relating to knowledge) resulted in lack of engagement to HCV care [413]. Interestingly, regions known to be associated with areas of poverty were associated with lack of HCV knowledge [413]. In chapter 6, I showed how a non-negligible proportion of participants of the Icona cohort were not tested for HCV antibodies until they already had developed liver disease. Thus, lack of testing delays the diagnosis which in turn delays treatment initiation, the consequences of which remain to be fully investigated.

In a study by *Ireland et al* assessing HCV CoC from diagnosis to treatment in PWIDs included in the analysis between 2008 and 2013 (pre DAA era) and in 2014 (early DAA era) [436]. Among individuals using drug services in Sentinel Surveillance of Blood Borne Virus Testing (SSBBV), 16,707 were tested for HCV of whom (n=3123) 19% were HCVAb positive and in the early DAA era (n=2340/3123) 75% were HCV-RNA tested. Of the 2,340 HCV-RNA tested, (n=1666) 53% were HCV-RNA positive, of whom (n=233) 8% were treated [436]. This shows that in the early years of the DAA era, low proportion of people received treatment, especially more so in high-risk groups. [436]

7.3.3 Starting treatment for HCV and cure rates or achieving SVR in chronically infected HIV/HCV coinfected populations

A meta-analysis by *Yehia et al*, found that large gaps appeared to exist between diagnosis and treatment of HCV [416]. The review included 3.5 million individuals identified to have chronic HCV infection and only 50% were found to be aware of their infection which represents an important initial barrier to receiving care [416]. Among people who are HCV-RNA positive, the number of people initiating HCV treatment in the pre-DAA era has been shown to be low mainly because historically treatment was targeted at specific groups, e.g. individuals with advanced stage of

liver disease. However, challenges in accessing treatment from health care providers also played a part [377, 417-419, 421, 425].

In a recent analysis of the EuroSIDA cohort, *Amele S et al* established a methodology to evaluate HCV CoC in HIV/HCV coinfected individuals in Europe prior to widespread use of DAA in January 2015 [377]. The study identified 3,876 HIV/HCV coinfected individuals with chronic HCV infection of which 44% (n=1673) reported ever having received HCV treatment [377]. There were 31% (n=1195) who had data for SVR assessment and of whom 53% (633/1195) achieved SVR. In this analysis one of the factors investigated was region of Europe (central, northern and southern Europe (which includes Italy)). Regional differences were observed at each stage of the HCV CoC with >80% of HIV/HCV coinfected individuals who were tested for HCV-RNA, however only 50% were HCV-RNA tested in eastern Europe (Russia was included) [377]. When addressing treatment initiation, the highest proportion of individuals receiving DAA was reported in northern Europe with 15% of individuals [377]. In this analysis, the cure rates (mainly based on non-DAA treatment) among those who had chronic HCV infection had completed treatment, were reported to be <20% across regions i.e. ranging from 11% in central-eastern Europe to 19% northern and southern Europe [377].

Fursa et al extended this analysis by including HIV/HCV coinfected individual's follow-up in 2019. The study included 4,773 HIV/HCV coinfected individuals of whom 93% (95%CI: 92-94) were HCV-RNA tested and (n=4300) 90% (95%CI: 89-91) were chronically infected [437]. In terms of treatment initiation, (3116/4300) 73% (95%CI: 71-74) had started treatment [437]. There was an improvement in cure rates, that is, (2985/4300) 56% (95%CI: 54-58) of chronically infected individuals were cured overall [437].

Roberson et al found an improvement in rate of treatment initiation between the pre-DAA era and the DAA era [421]. The study was carried out in the USA and involved 408 HIV/HCV coinfected individuals enrolled between 2008 and 2013

(pre-DAA era group) and 300 HIV/HCV coinfected individuals enrolled between 2014 and 2015 (DAA era group) [421]. The study found no difference in the proportion of individuals diagnosed for HCV and engaged in care by time period. However, when it came to comparing the rate of treatment initiation, 5% (pre-DAA era group) vs. 35% (DAA era group) had started treatment [421]. These findings are consistent with *Sacks-Davis et al* assessment of HCV cascade of care in seven HIV/HCV coinfected cohort studies over a period in which DAAs became broadly available [402]. The study found large variability by country in the proportion of HIV/HCV coinfected individuals initiating DAA treatment, ranging from 36% (in Georgia- DAA became available in 2015) to 74% (in the Netherlands- DAA became available in 2015) [402]. Also shown in chapter 6, Figure 6.0 Iceland and Egypt have a high proportion of >80% of HCV positive individuals receiving DAA among the general population [11].

In another more recent study conducted in the USA between October 2015 and September 2016 and involving 187 HIV-positive individuals, 40% of these were HIV/HCV coinfected and treatment uptake increased over time [433]. The study reported that 60% of the individuals included (n=113) initiated DAA with 57% (n=107) completing treatment and overall 53% (n=100) were cured corresponding to a SVR of 95% (100/107) [433]. In another study assessing trends in HCV DAA treatment initiation between 2013 and 2015, an increase in the rate of DAA initiation was observed from 8 per 100 person-year (95% CI: 6-11) to 28 per 100 person years (95% CI: 23-33) [438]. In the Swiss HIV Cohort Study 12,401 HIV/HCV coinfected individuals were enrolled between January 2001 and December 2013, of whom 17% (n=2,107) were HCV-RNA positive [439]. Of these 30% (n=636) had started HCV treatment, resulting in an incidence rate of treatment uptake of 5.8/100 PY (95% CI: 5.3 – 6.2) [439]. Of note, the incidence of treatment uptake did not appear to change significantly by calendar period [439, 440]. In a more recent analysis of the Swiss HIV Cohort Study between April 2014 and December 2015 when second generation DAAs were introduced in Switzerland, 876 were found to be HCV-RNA positive. Of these 876, 20% (n=180) had started HCV treatment with

second generation DAAs [441]. The estimated incidence rate for treatment uptake was higher than previously estimated at 22.4/100 PY [441].

A recent analysis using the data of the Icona and Hepaicona cohorts evaluated the rates of access to DAA according to AIFA eligibility. The study population included 2,607 viremic HIV/HCV coinfected individuals of whom 35% (n=920) initiated DAA, and found that overall 21% (n=545) were cured [143]. Although in this analysis only approximately 40% of the included individuals were eligible for DAA treatment at the time, data from EASL-HEPAHEALTH reports on treatment uptake in Italy show an increase from 2013 to 2016 (Figure 7.2a) [4]. Additionally, recent data from AIFA show an increase in the rate of DAA initiation as of January 2021, especially for specific populations with advanced stage of liver disease or presence of HIV/HCV coinfection [442]. In general, shows large variability in the proportion of treatment uptake according to specific criteria/groups.

In contrast, as part of the HCV CoC, a more recent study conducted in a cohort of HIV/HCV coinfected individuals in Argentina in 2016, found that less individuals initiated DAA treatment compared to non-DAA treatment [417]. The study included 320 HIV/HCV coinfected chronically infected individuals, of whom 37% (n=118) started treatment with Interferon based therapies and only 11% (n=35) were started on DAA [417]. Similarly, low rates of DAA initiation have been observed in the Canadian HIV/HCV coinfection cohort [438]. This was found despite the fact that approval of second generation DAAs came into effect in 2013 [438]. The study consisted of 911 HIV/HCV coinfected individuals chronically infected with HCV and considered eligible for HCV treatment. They found that 22% (n=199) initiated DAA with an SVR rate (among those initiating and completing DAA) of 88% (176/199) [438]. In the SWISS HIV/HCV coinfected cohort, *Beguellin et al* observed HIV/HCV coinfected individuals initiating DAA to be 20% (180/876) between 2014 and 2015 with overall cure rates reported at 20% (n=173). In terms of the rate of SVR (among those initiating and completing DAA treatment), this was 96% (173/180) [441].

It is reassuring to see that the cure rates among HIV/HCV coinfected individuals who start DAAs are comparable regardless of the sub-populations studied and similar to those seen in the HCV mono-infected populations [402, 421, 441]. However, it is clear from the literature that there is variability in DAA treatment initiation across countries, suggesting that DAA uptake serves as an additional barrier in the care pathway for HCV elimination [11].

Despite a general improvement in levels of HCV treatment initiation among HCV/HIV coinfected individuals after the introduction of DAAs, some individuals considered eligible for treatment continue to face challenges in completing the HCV CoC pathway [413, 417, 421, 424]. For example, patient-level barriers such as being PWID [436], or alcohol consumer and even living in a region considered to be economically deprived may reduce the chance of accessing health care [402, 438]. In terms of regional barriers, not all regions may be able to afford the cost of DAA, there might be issues related to limited staff in small clinical sites in poorer areas or because of lack of resources access to treatment may still be dependent on stage of liver disease [402, 438]. Additionally other factors may include, personal beliefs about consequences of treatment, fear of diagnosis or treatment, negative experiences with health care services or even lack of knowledge for both patients and clinicians [408] [407].

In terms assessing HCV uptake in specific high-risk groups such as PWIDs, the Enhancing Treatment of Hepatitis C in Opioid Substitution (ETHOS) cohort study in Australia evaluated factors associated with receiving HCV treatment in participants enrolled in the study between 2018 and 2019 [443]. *Valerio et al* included 1,443 PWIDs of whom (n=331) 24% were HCV positive. When assessing predictors of HCV infection; homelessness aOR = 1.47 (95%CI: 1.00-2.16), history of incarceration vs. never aOR = 1.79 (95%CI: 1.38-3.01) and daily injecting drug use aOR = 2.29 (95%CI: 1.45-3.62) [443]. *Valerio et al* also assessed self-report of ever initiating HCV treatment and among individuals who had previous chronic and

current HCV infection, which included 55% (788/1443) of individuals [443]. Among the 788 individuals, 66% (n=520) self-reported ever initiating treatment between 2016 and 2018 [443]. Interestingly, factors associated with reduced likelihood of treatment initiation was being female, homeless, daily injecting drug use vs no injecting in the last year. In contrast older age and individuals receiving opioid agonist therapy were more likely to receive HCV treatment [443].

Figure 7.2a Number DAA treatment courses by country [4]

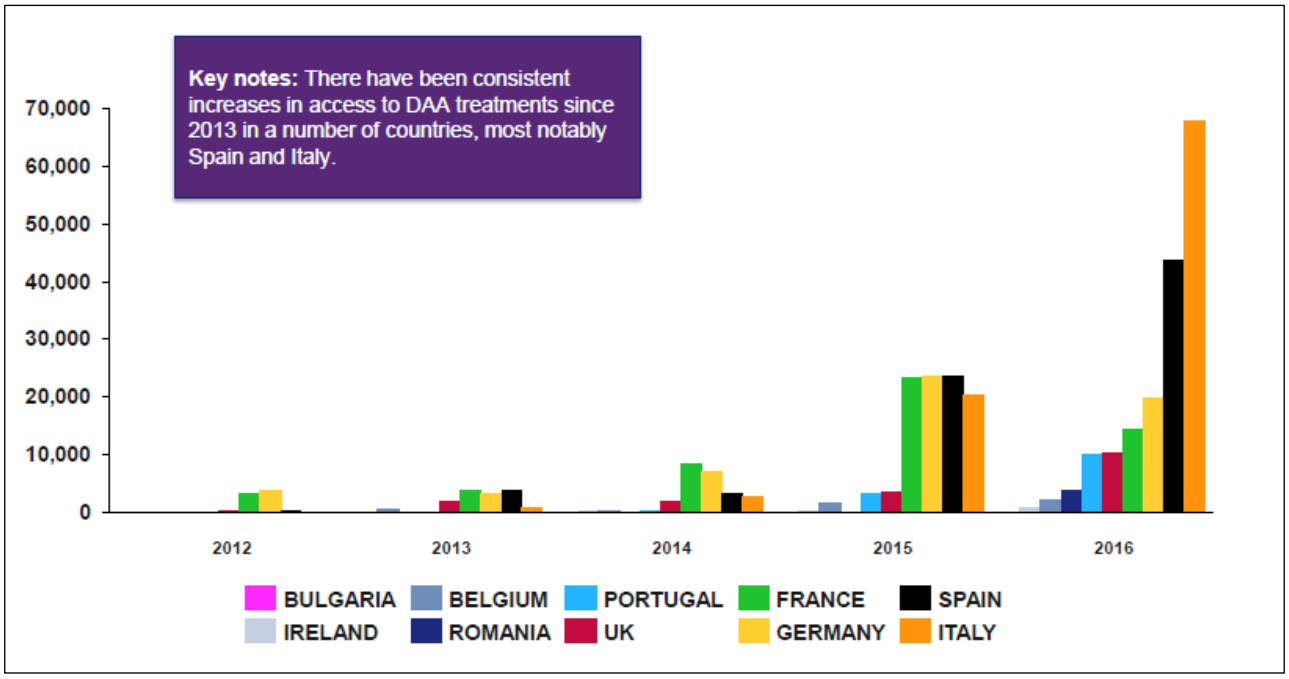
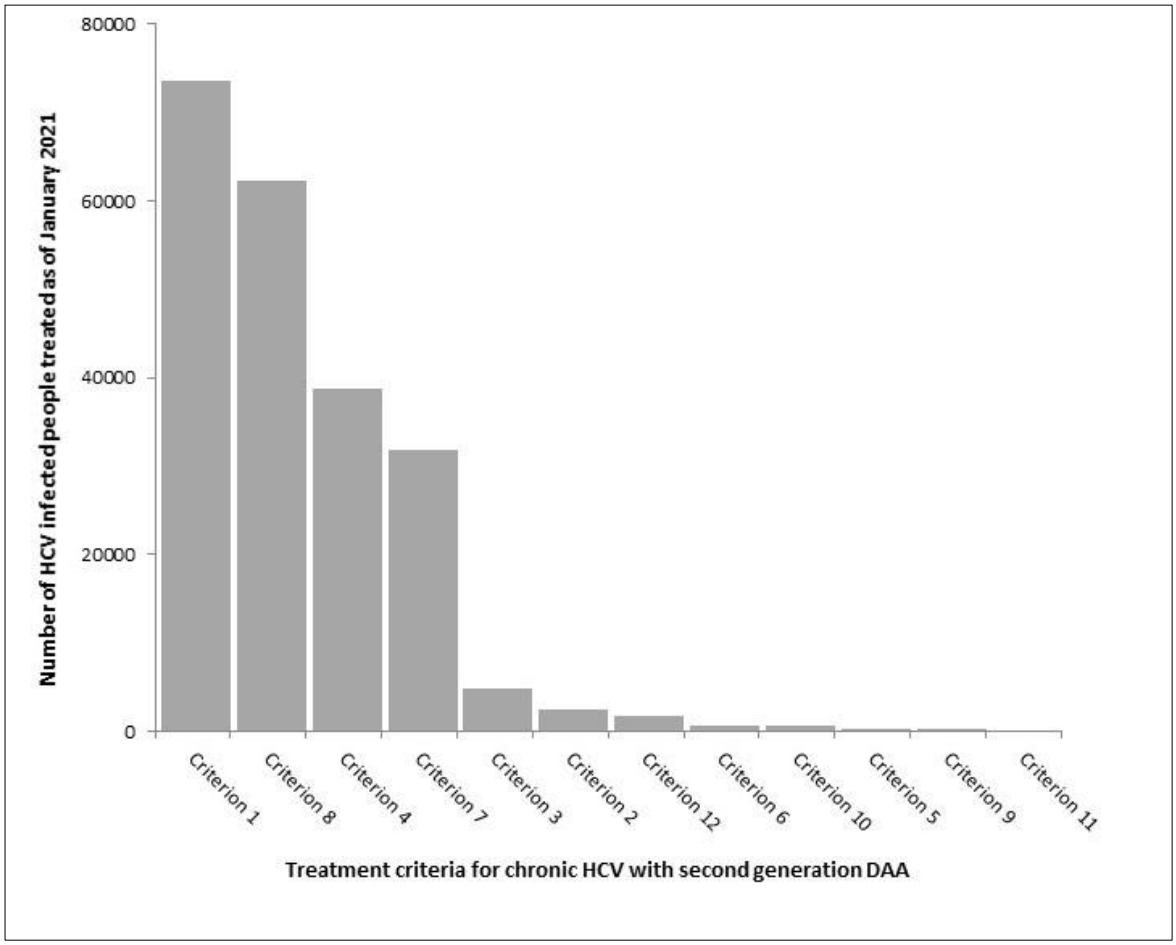

Key notes: There have been consistent increases in access to DAA treatments since 2013 in a number of countries, most notably Spain and Italy.
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
2012
2013
2014
2015
2016
BULGARIA
BELGIUM
PORTUGAL
FRANCE
SPAIN
IRELAND
ROMANIA
UK
GERMANY
ITALY

Figure 7.5 Number of people initiating DAA in Italy reported by AIFA according to treatment criterion as of January 2021 [6]



AIFA Aggiornamento dati Registri AIFA DAAs - Epatite C cronica[6]

The 12 treatment criteria for chronic HCV with second generation DAA defined by AIFA as of January 2021 are listed in Table 7.1 Criterion for treatment with second generation DAA defined by AIFA as of January 2021 [6]

Table 7.2 Potential baseline (prior to January 2015) factors consideredTable 7.1 [6]. With Figure 7.2 showing the majority of HCV positive individuals treated satisfied Criterion 1 (patients with cirrhosis or HCC considered not suitable for

transplantation) and Criterion 8 (patients with chronic hepatitis with mild fibrosis or with comorbidities such as HIV or HBV resulting in increased risk of liver damage.)

Table 7.2 Criterion for treatment with second generation DAA defined by AIFA as of January 2021 [6]

Criterion	**Definition**
Criterion 1	Patients with cirrhosis in Child A or B class and / or with HCC with complete response to surgical or loco-regional resective therapies not suitable for liver transplantation in which liver disease is decisive for the prognosis.
Criterion 2	HCV-RNA positive recurrent hepatitis of the transplanted liver in a clinically stable patient with optimal levels of immunosuppression.
Criterion 3	Chronic hepatitis with severe HCV-related extra-hepatic manifestations (cryoglobulinemic syndrome with organ damage, B-cell lymphoproliferative syndromes, renal failure).
Criterion 4	Chronic hepatitis with fibrosis METAVIR F3 (or corresponding Ishak).
Criterion 5	Listed for liver transplant with cirrhosis MELD <25 and / or with HCC within the Milan criteria with the possibility of waiting on the list of at least 2 months.
Criterion 6	Chronic hepatitis after solid organ (non-liver) or bone marrow transplantation in a clinically stable patient with optimal levels of immunosuppression.
Criterion 7	*Chronic hepatitis with fibrosis METAVIR F2 (or corresponding Ishak) and / or comorbidities at risk of liver damage progression [HBV coinfection, HIV coinfection, chronic non-viral liver disease, diabetes mellitus under drug treatment, obesity (body mass index ≥30 kg / m^2), congenital hemoglobinopathies and coagulopathies].*
Criterion 8	*Chronic hepatitis with fibrosis METAVIR F0-F1 (or corresponding Ishak) and / or comorbidities at risk of liver damage progression [HBV coinfection, HIV coinfection, chronic non-viral liver disease, diabetes mellitus under drug treatment, obesity (body mass index ≥30 kg / m^2), hemoglobinopathies and congenital coagulopathies].*
Criterion 9	Infected health workers.
Criterion 10	Chronic hepatitis or liver cirrhosis in a patient with chronic renal failure undergoing dialysis treatment.
Criterion 11	Chronic hepatitis in the patient on the waiting list for solid organ (not liver) or bone marrow transplantation.

Criterion	Definition
Criterion 12	Chronic hepatitis or liver cirrhosis in patients who cannot access a liver biopsy and / or fibroscan for social welfare reasons

7.3.4 The role of region of care on stages of HCV continuum of care

Although HCV elimination targets have been set by the WHO, not all regions or countries have implemented policies to ensure that WHO targets could be met and this in itself is a potential barrier [402]. At various stages of the HCV CoC, geographical region within countries has been shown to play a role or act as an additional barrier partly because of variability in terms of health care resources available at regional levels [402, 409]. Also at country level, there are variations in intervention strategies (mainly involving testing, access to treatment, health care professional, media campaigns and risk reduction) that have been implemented for HCV elimination [402].

A recent study in England involving HCV mono-infected individuals found region to be associated with differential rate of HCV testing [419]. The study included 40,856 people who were HCVAb positive and found 72% (n=29,557) to be HCV-RNA positive, with a 21% increased probability of being HCV-RNA tested in primary care if the individual resided outside of the London region vs. those resident in London OR =1.21 (95% CI: 1.13 - 1.30; p-value not reported) [419]. Year of the HCVAb test has also been shown to play a role in HCV-RNA testing, the more recent the HCVAb test the higher was the probability of being tested for HCV-RNA [419]. In a study involving 705 female sex workers, 43% (n=302) self-reported to be HCV-positive[425]. The study showed that region of residence had an impact on self-reporting of HCV testing [425]. Specifically, residing in an area which was defined as being near a drug use epicenter, was associated with an increased risk of HCV testing in comparison to residing outside the area OR = 3.19 (95% CI: 1.78 – 5.73; p <0.001) [425]. It is not unusual in epidemiological studies that the prevalence of testing is higher due to better connectedness or because the

residing area is near an outbreak so it is subject to greater medical vigilance. The authors also found sexual/gender minority OR= 1.89 (95% CI: 1.10 – 3.24; p= 0.020) and PWID OR = 2.00 (95% CI: 1.19 – 3.34; p=0.008) to be associated with higher probability of HCV testing [425]. In contrast being a migrant was associated with reduced chance of being tested OR = 0.24 (95% CI: 0.12 – 0.48; p<0.001) [425].

Interestingly in Switzerland, health access is universal and this was assessed by *Brezzi et al* who investigated the association between geographic origin of individuals living Switzerland and probability of access to therapy using participants enrolled in the SWISS Hepatitis C Cohort Study up to 2017 [394]. This study included 5,356 participants of whom 7% (n=375) were HIV/HCV coinfected. In terms of the probability of initiating HCV therapy (including DAA and non-DAA treatment), unadjusted and adjusted OR was 1.66 (95% CI: (1.36 - 2.02) and 1.28 (95% CI: 0.99 - 1.66) respectively for Italian born nationals compared to Swiss born nationals. This was independent of other factors (sex, age, region of residence, education income, IDU, alcohol consumption status, calendar year of enrolment, HCV genotype). However, this finding is possibly explained by Italians being more likely to have advanced liver disease at enrolment and showing incident cirrhosis, therefore they were more likely to be treated [394].
Concerning other nationalities (Germany, Portugal, Eastern Europe, Southern Europe, Western Europe, Asia, Africa or America), no evidence for a difference was found in terms of probability of treatment initiation [394]. This is consistent with the fact that access to health care is universal in Switzerland [394].

In addition, treatment initiation was found to be associated with socioeconomic deprivation, as individuals living in areas that were defined as being deprived were also less likely to be treated [413]. This is consistent with the results of a study by *Noska et al* who observed differences in treatment initiation among homeless and non-homeless veterans in the USA. Among 32,449 homeless veterans infected with HCV, 23% (n=7421) received DAA treatment [424]. This was lower than non-

homeless veterans of whom 31% (58,321/188,156) received DAA treatment [424]. This is an indication of treatment disparities within a country in socially deprived areas which is another barrier to overcome towards HCV elimination [424].

In the Canadian Coinfection Cohort study, 911 HCV-positive individuals were included of whom 22% (n=199) had initiated DAA. The authors found that only one person 1% (1/199) of those who initiated DAA reported to be residing in Saskatchewan. In this region, not all health benefits are free, some need to be paid for [444]. This finding was only partially explained by treatment health inequities as the region had HIV/HCV coinfected individuals who tended to be younger PWIDs, and the region had lower prevalence of advanced liver disease [438]. In this same study, when looking at predictors of DAA initiation, living in Saskatchewan was found to be associated with the reduced chance of starting DAA aHR = 0.04 (95% CI: 0.01 - 0.11)) vs. living in British Columbia, the latter known for good social economic status [438]. The Saskatchewan region was associated with reduced likelihood of starting DAA compared to individual residing in British Columbia independently of other factors (such as age, gender, PWID, income alcohol use, undetectable HIV-RNA, fibrosis, HCV genotype).

Some of these results suggest that financial resources available to health care institutions is an additional barrier to HCV medical care, likely to impact on the probability of providing treatment despite current policies of universal access to DAA. Although second generation DAAs were introduced in 2013 in Canada, not all geographical regions had immediate universal access to DAA. This is because of barriers such as the cost of DAA which led to a delay in the introduction of the drugs as individual countries and regions had to negotiate for affordable rates [402]. *Douglass et al* described the pricing variation of DAAs in different countries [445]. This is dependent on how well each government was able to negotiate reasonable discounts or prices that pharmaceuticals have set. High-income countries including Italy, France, Finland, Iceland, Norway, Portugal, Scotland, Spain and Sweden have all managed to negotiate good prices for DAA[445]. The implication of this is

that, potentially, all HCV-positive individuals in these countries can be treated and cured regardless of their stage of liver disease. In 2017, Italy was able to negotiate prices with Gilead to about 4,000 Euros per course of treatment which was very competitive at the time [446]. Of note, up until 2018, in other European countries such as Denmark and Poland treatment was still prioritised to individuals with advanced liver fibrosis or PWID due to high DAA cost [445] [447].

7.3.5 Summary of literature review and what this chapter adds

Although we are now in the era of new and improved drugs to treat and cure HCV, the literature has shown that there are still ongoing barriers that limit the full impact of DAAs. Certainly, there has been a drastic improvement in terms of cure rates when comparing individuals who initiated treatment in the pre-DAA vs. post DAA eras. However, this has not occurred in all regions of the world (e.g. low to middle income countries) and even in high income settings, not all HCV viremic individuals are receiving treatment. In terms of the continuum of care, the literature shows that even at the first stage of testing for HCV-RNA to establish chronic infection status, some individuals are not being retained, and some characteristics such as female gender, PWIDs, poorer socio-economic status have been linked to reduced likelihood of HCV-RNA testing. In addition, some studies have shown that individuals' knowledge of HCV infection status and willingness to be treated could influence the probability of being tested. However, perhaps more importantly, regional differences continue to exist, possibly due to differential levels of social deprivation or availability of financial resources. Additionally, testing for HCVAb may not be part of routine care in some risk groups. For example, testing is typically more commonly done in PWID but not so much in MSM's or heterosexuals. This is true also in the HIV/HCV coinfected populations.

The studies included in this literature review have some limitations that are worth highlighting. Some studies have only included specific populations, so that generalizability of the results is problematic. In addition, some studies have

reported diagnoses of HCVAb infection via self-report which are subject to recall bias or have used medical records that are subject to inaccuracies or omission of information. This may result in an under estimation or inaccuracy of the prevalence of HCV chronic infection. The sample sizes reported in these studies are also worth mentioning, some studies being small which might have limited the statistical power to investigate factors associated with stages of HCV CoC.

Most of the studies that have provided estimates of the prevalence of individuals reaching each of the stages of the CoC were carried out in the pre-DAA era. There is a lack of studies that have investigated factors associated the probability of retention in care at the various stages in the DAA era, particularly regarding the possible impact of region of care. Because second generations DAAs have been introduced relatively recently the identification of barriers to treatment uptake in this modern era remains an unmet need. This work should help in targeting specific interventions to minimise/remove barriers at specific points in the CoC and facilitate the achievement of HCV elimination towards the 2030 goal set by WHO.

As mentioned previously, in Italy there is particular concern regarding regional disparities as the north and central regions have more capacity, advanced technology and better quality of life than the southern regions. Additionally lower hospital capacity has been reported in the southern regions with more individuals turning to private medical insurance. This will in turn have an impact on the associated rate of access to health care.

This chapter evaluates the HCV CoC in Italy, giving insights into the impact of region of care on the various stages of HCV CoC using the data of the Icona and Hepaicona cohorts. The main strengths of both cohorts is that they are heterogeneous in nature, including PWIDs, MSMs and heterosexuals. Hepaicona specifically enrols chronically infected individuals and collects detailed data on DAA treatment. Data have been collected in a timely manner so that, by combining the two cohorts (each providing data for a number of stages of the CoC), we could

include larger number of people treated with DAA compared to those included in other similar national cohorts. A huge effort has been made in Italy in treating HCV in the HIV-positive population as compared to most other European countries considering the very large number of individuals who needed treatment and the resources available. AIFA was key in negotiating a very competitive price of DAAs with pharmaceutical companies.

Of note, most of the studies evaluating the rate of retention at various stages of the HCV CoC have used standard epidemiological research methods for the analysis when assessing the impact of factors on success at each stage. It is not uncommon that bias is introduced in the analysis of observational data because there is residual confounding or redundant adjustment for mediators or colliders. The analysis carried out in this chapter assessing the impact of region follows recently published guidance on how to raise the rigor of the work when it comes to the construction of multivariable models. Specifically, I have used directed acyclic graphs (DAGs) as described in chapter 2 to illustrate the assumed underlying causal structure of the data and the R software daggity.net to identify the minimal set of covariates sufficient to block all backdoor confounding pathways [(1)] when investigating the association of region with HCV-RNA testing, initiating DAA and achieving SVR. The adoption of this framework enhances the validity of the science in the field of real-world epidemiology studies and improves the communication of research findings.

7.4 Methods

7.4.1 Inclusion criteria of individuals included in this analysis

This analysis includes HIV/HCV coinfected individuals from both Icona and Hepaicona cohorts who were alive and under active follow-up in 2014. Specifically, participants were defined to be alive and under active follow-up if, their last clinical visit was registered after 01st January 2014 (maximum 1 year prior to the baseline

date of 01st January 2015). I set the baseline as January 2015 because it is the official date in which IFN-free DAAs were licensed for universal use in Italy. Therefore, all individuals included have at baseline the same non-zero probability of starting DAA if tested HCV-RNA positive. The date of HCVAb positive test could be at any time prior to baseline. Participants were defined as HCV-RNA positive if they had ≥1 HCV-RNA positive test result (qualitative or quantitative) prior to baseline.

7.4.2 Data

Key participants' characteristics were extracted at baseline date (Table 7.2 Potential baseline (prior to January 2015) factors considered

Table 7.2 shows the list of factors used in the analyses). Stage of liver disease was determined from baseline FIB-4 score (classified as: ≤ 3.25 and >3.25), liver stiffness measured using Fibroscan (≤9.5kPa and >9.5kPa) (although Fibroscan data is only available in a subset in individuals), and clinical diagnosis of liver disease obtained from hospitalization records or assessed at clinical visits.

Table 7.5 Potential baseline (prior to January 2015) factors considered

	Variables	**Classification**
Demographics	Age (years)	Continuous (age per 10 years older)
	Gender	Male, Female
	Nationality	Italian, Non-Italian
	Region of care	North, South, Centre
	Mode of HIV Transmission	PWID, MSM, Heterosexual, Other/unknown
	Calendar year of HCV test	Continuous
HIV related factors	AIDS diagnosis	No, Yes
	CD4 cell count (cells/mm^3)	Continuous

	Variables	Classification
	HIV-RNA (copies/ml)	Continuous (log transformed)
	cART status	cART naïve, cART experienced
Lifestyle	Alcohol consumption	Abstain, Moderate Hazardous, Unknown
Social Economic factors	Education*	Primary, Secondary, College, University, Other/Unknown
	Employment	Unemployed, Employed, Other, Unknown
Hepatitis	Hepatitis B	Negative, Positive, Not tested
Liver disease	FIB-4, Fibroscan, clinical diagnosis of liver disease	≤F3 (FIB-4≤3.25 or kPa≤9.5 >F3 (FIB-4>3.25, kPa >9.5, clinical diagnosis of liver disease

*Data not collected in Hepaicona

7.4.3 Defining stages of the HCV Continuum of Care

HCV CoC is conceived as separate stages along the pathway from HCVAb positive diagnosis to testing for HCV-RNA, initiation of treatment, and finally achieving SVR; the exact stages included and definitions used in this analysis are presented in Table 7.3 Stages of HCV CoC based on Icona and Hepaicona cohorts

Table 7.3. Due to a limitation of the data available in both cohorts, some of the WHO stages described in the literature review of this chapter are not evaluated here. These include the first stage of HCVAb positive testing (CoC starts with all people with positive serology), the number of people reached by prevention activities and the number of those aware of status and accessing chronic care (all these information is unmeasured in the Icona Network datasets).

Table 7.8 Stages of HCV CoC based on Icona and Hepaicona cohorts

HCV CoC Stage	Description	Inclusion	Main CoC outcome
Icona cohort only			
0. HCVAb positive	To identify HCVAb+ prevalence among HIV diagnosed individuals. People HCVAb positive were used as the denominator to estimate the proportion of HCV-RNA tested	HCVAb positive (prior to January 2015)	N/A
1.HCV-RNA testing	To identify proportion of individuals who were HCV-RNA tested among HCVAb positive individuals identified in Stage 0	All individuals HCVAb positive	HCV-RNA tested (Yes/No) Denominator = All HCVAb positive
[1]1a. HCV-RNA positive	To identify proportion of individuals who ever were HCV-RNA positive amongst those HCV-RNA tested identified in Stage 1	HCV-RNA+ tested	N/A
Icona and Hepaicona cohorts			
1.HCV-RNA positive	To identify individuals ever HCV-RNA positive	HCV-RNA positive (prior to January 2015)	N/A
2.Starting DAA	To identify proportion of individuals diagnosed with chronic HCV (HCVAb+ and HCV-RNA positive) starting IFN-free DAA	All individuals diagnosed with chronic HCV infection (HCVAb+ and HCV-RNA positive)	Starting DAA (Yes/No) Denominator = All HCVAb+ and HCV-RNA positive
3. Cured Achieved SVR 12/24	Cure relates to the proportion of individuals diagnosed with chronic HCV who received IFN-DAA treatment and had a negative HCV-RNA test at more than 12/24 post treatment among individuals ever HCV-RNA positive		N/A SVR (Yes/No)

HCV CoC Stage	Description	Inclusion	Main CoC outcome
	Achieved SVR 12/24 relates to individuals with chronic HCV (HCVAb+ and HCV-RNA positive) who received IFN-free DAA treatment and had an EOT[2] HCV-RNA for SVR assessment	All individuals starting IFN-free DAA treatment and those with availability of EOT[2] HCV-RNA value	Denominator = All HCVAb+ and HCV-RNA positive who started DAA and had an EOT HCV-RNA value available

[1]This was done to identify who is included of those participating in Icona in the denominator for Stage 2.
[2]HCV-RNA value determined 12/24 post end of treatment

7.4.4 Exposures and outcomes

Main exposure

The main exposure of interest for this analysis was region of care defined as the geographical region of the Icona Network clinical site in which the participant was enrolled, and it was categorized as north, centre and southern Italy.

Main outcome(s):

The main outcomes (all coded as binary variables) based on the stages of the HCV CoC in this analysis were:

- HCV-RNA testing (Yes/No) among all those HCVAb positive
    - Calculation of the proportion HCV-RNA positive
- Initiating DAA (Yes/No) among those HCV-RNA positive
- Achieving SVR (Yes/No) among those who started DAA and also had availability of an HCV-RNA end of treatment (EOT) value

7.5 Statistical analysis

The analysis of the first stage was conducted using only the data of Icona as having a positive HCV-RNA at entry was a criterion for Hepaicona. Thus, the denominator for the first stage included all individuals identified as HCVAb positive in the Icona cohort alone. Following the scheme of other similar analyses, the proportion of individuals transitioning to various stages was depicted using bar graphs. The proportion of individuals tested for HCV-RNA among those HCVAb positive was compared between region of care using a chi-squared test. For the remaining stages of the CoC pathway the combined data of Icona and Hepaicona were used. For these steps, a similar analysis was conducted by calculating first the proportion of individuals starting DAA among those HCV-RNA positive, and eventually the proportion of individuals achieving SVR among those starting DAA. Again the rates of retention by region were calculated and compared.

Baseline characteristics for individuals identified as HCVAb positive in the Icona cohort and individuals identified as HCV-RNA positive in both cohorts were also compared after stratification by region of care using non-parametric tests for continuous variables and chi-squared tests for proportions (categorical variables).

7.5.1 Model selection using www.DAGitty.net

For this analysis I used the DAGitty software to construct a DAG to visualize the main hypothesized causal pathway between exposure (region) and outcome (retention stage) and the assumed causal links between these and all the other factors considered in the analysis. This DAG was also used to identify minimal sets of covariates that were sufficient to block all backdoor pathways generating confounding. The variables and the relationship between variables included in the DAG were decided on the basis of research hypotheses, associations published in the literature and other axiomatic knowledge.

Stage 1: DAG for effect of region of care on the probability of HCV-RNA testing

Figure 7.3 shows the DAG for the impact of region of care on HCV-RNA testing i.e. the hypothesised causal relationship between region of care and the probability of being tested for HCV-RNA (stage 1 of the CoC). The variable in green (with the 'play' sign) is the exposure of interest 'region of care'. All the other variables (red, blue) except the outcome (with an 'I' sign) represent all other measured or unmeasured (grey) variables which we considered in the model. All green lines are defined as causal paths and all red lines are defined as biasing paths. From a DAG, all variables in 'red' represent potential confounders, common causes of exposure and outcome, either directly or through a chain. For an example, this DAG shows that there is a back door path from exposure to outcome through mode of HIV transmission (i.e. it is possible to walk from the region of care node to the HCV-RNA test node walking through the arrow which goes into region of care from mode of HIV transmission).

Indeed, prevalence of mode of HIV transmission might be different by region of Italy and we have also shown how mode of HIV transmission is linked to the probability of being HCV-RNA tested. In Figure 7.8, this back-door pathway is [region of care ← mode of HIV transmission → HCV-RNA test] and adjusting for mode of HIV transmission will close this back door path resulting in the association of interest no longer being confounded by mode of HIV transmission.

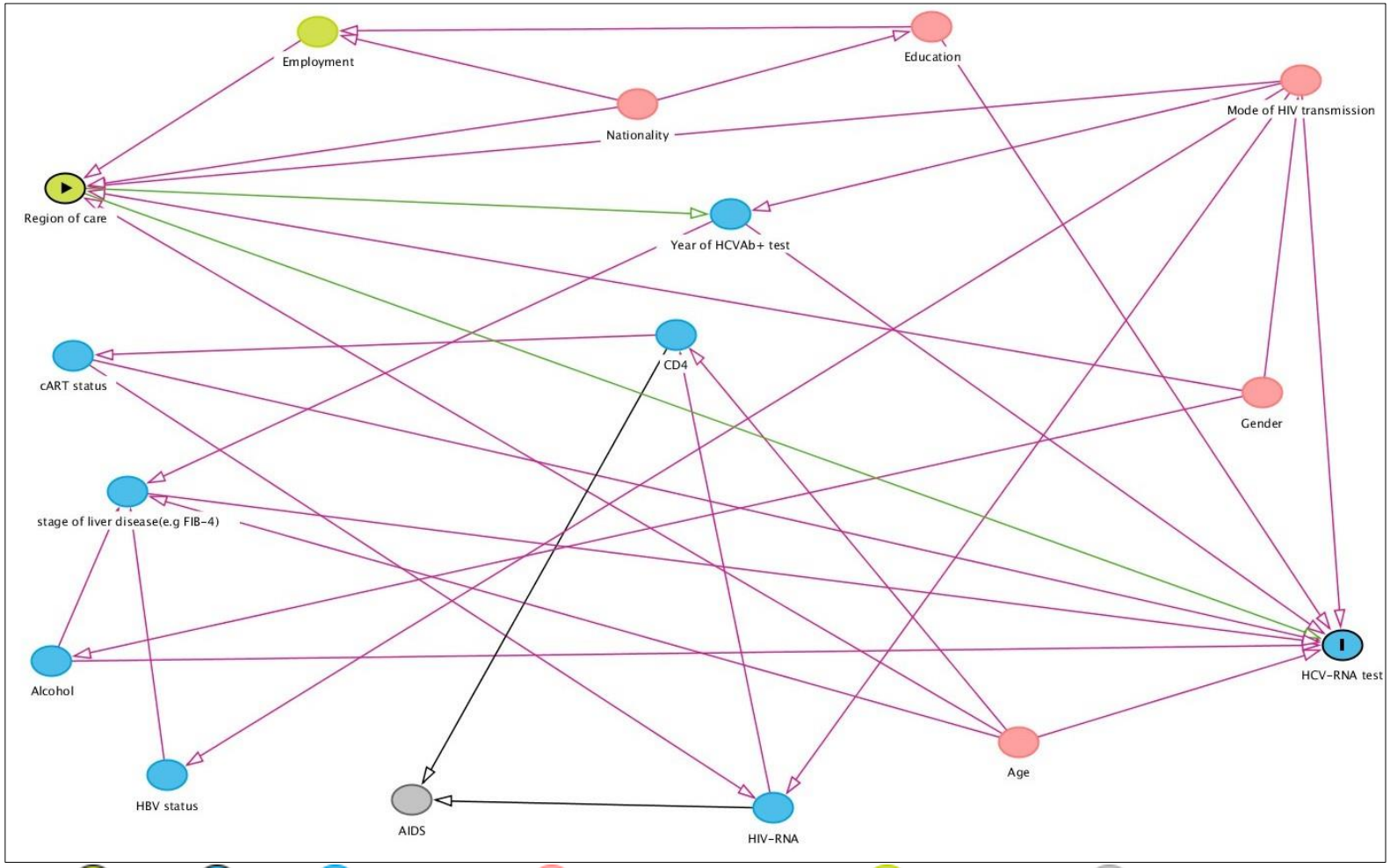
The graph also shows the existence of mediators i.e. year of HCVAb positive test (as serology testing could have been implemented in different time periods in different regions) which is an open indirect causal path between region of care and HCV-RNA testing (region of care → year of HCVAb positive test → HCV-RNA test). Because modality of HIV transmission is a common cause of year of HCVAb testing and of the outcome, year of serology testing is also a collider and adjusting for this variable would open a back door path thus introducing confounding. As a result, the estimate of the association between region and HCV-RNA test will be biased. In the case where more than one variable lies along the same back door path, adjusting for one single confounder is sufficient. For an example in the path (region of care ← gender → alcohol consumption → HCV-RNA test), adjusting for either gender or alcohol consumption will be sufficient to remove confounding. Also in this other scenario of (region of care ← employment ← education → HCV-RNA test), adjusting for either education or employment will be sufficient.

HIV-RNA could also be seen as a variable causing an M-bias (i.e. another case of a collider variable but in this case neither region of care or HCV-RNA test is a direct cause of HIV-RNA) relationship (region of care ← mode of HIV transmission → HIV-RNA ← cART status → HCV-RNA test). Again, adjusting for HIV-RNA (which is a collider and not a confounder) should be avoided as it opens this back door path thus introducing confounding. Therefore, if the assumptions in the DAG are correct (this includes the strong assumptions that it is a linear model without interactions and that all possible measured and unmeasured variables are included in the DAG), four equivalent sets of adjusting factors (see below) can be identified.

Any of these four multivariable models (shown below) can be used to estimate the total un-confounded effect of region of care on the probability of HCV-RNA testing. All four models have been fitted to verify that all provided similar estimates for the association between exposure and outcome. However, under the DAG assumptions, it does not really matter which model is the main model used, all conclusion should be similar.

a) Region of care + age + education + mode of HIV transmission + alcohol consumption
b) Region of care + age + nationality + employment + mode of HIV transmission + alcohol consumption
c) Region of care + age + gender + education + mode of HIV transmission
d) Region of care + age + gender + nationality + employment + mode of HIV transmission

Figure 7.8 DAG for the effect of region of care on HCV-RNA testing



Legend: exposure outcome ancestor of outcome ancestor of exposure *and* outcome ancestor of exposure other variable causal path biasing path

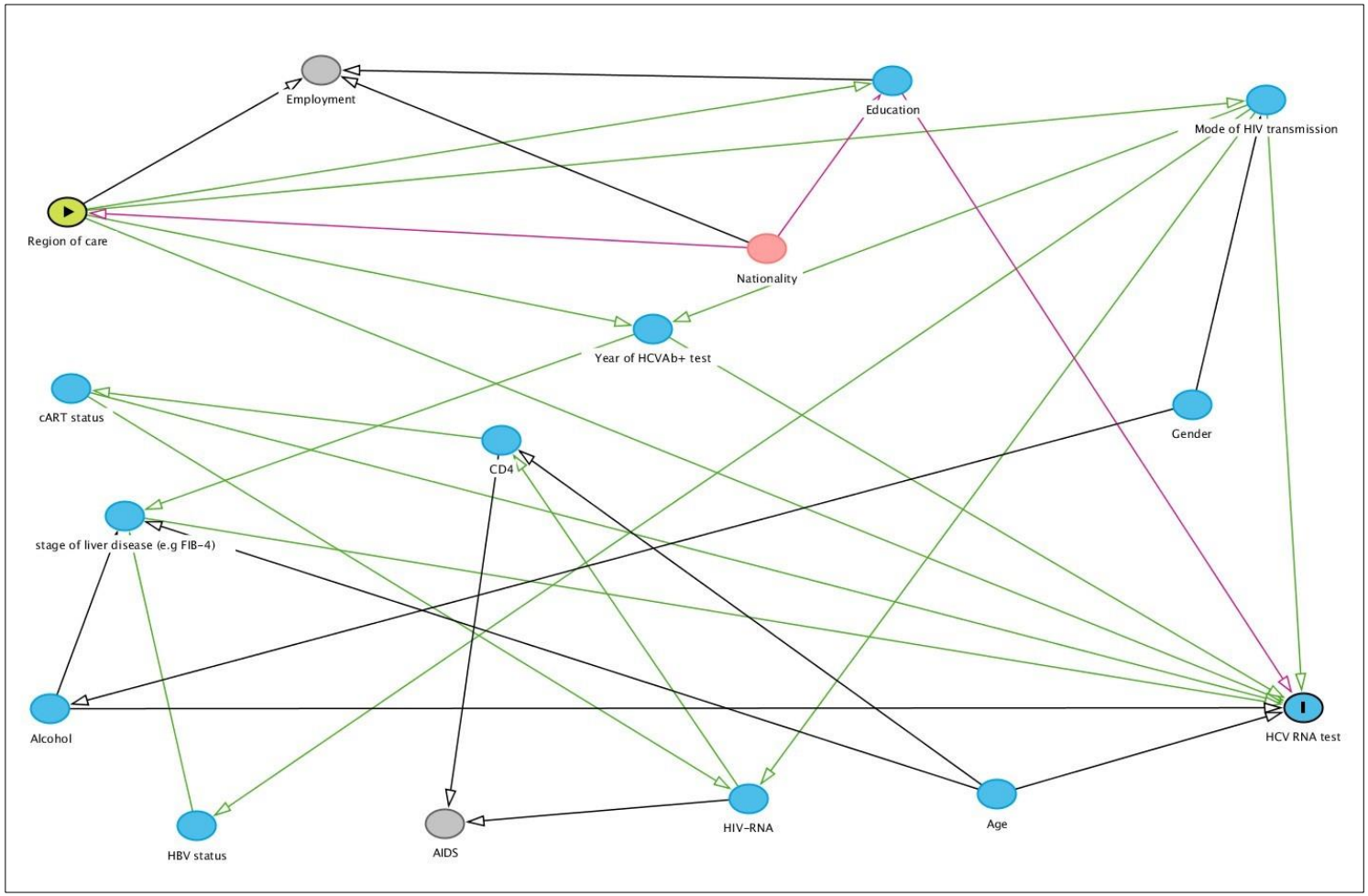
Stage 1: DAG for effect of region of care on the probability of HCV-RNA testing (changing directional assumptions)

The arrows in DAGs are usually unidirectional to represent assumed causal effects between variables, this is why the graphs are called ‘acyclic’. Occasionally, especially in cross-sectional analysis where temporality is more difficult to establish, there might be ambiguous choices for the direction of the arrow. For example I originally assumed in Figure 7.4 DAG for the effect of region on HCV-RNA with changed direction of causal pathways

Figure 7.4 that employment status might influence where you receive care but in fact could be the other way around (that geographical region determines the chance to find a job) as shown in Figure 7.4 DAG for the effect of region on HCV-RNA with changed direction of causal pathways

Figure 7.4. Therefore, under this alternative assumption the DAGitty software indicates that adjusting only for nationality is sufficient to estimate the total un-confounded effect of region of care on HCV-RNA testing. This will be further addressed in the discussion section, but briefly, this demonstrates that DAGs are heavily reliant on directionalities of effects, which are not always known, and it is important to perform sensitivity analyses after considering alternative model assumptions. However, one of the strengths of using DAGs is the direct communication of the model assumptions through a very transparent graphical language and the flexibility to changes of these assumptions.

Figure 7.9 DAG for the effect of region on HCV-RNA with changed direction of causal pathways



Legend: exposure outcome ancestor of outcome ancestor of exposure *and* outcome other variable causal path biasing path

Stage 2: DAG for effect of region of care on the probability of starting DAA

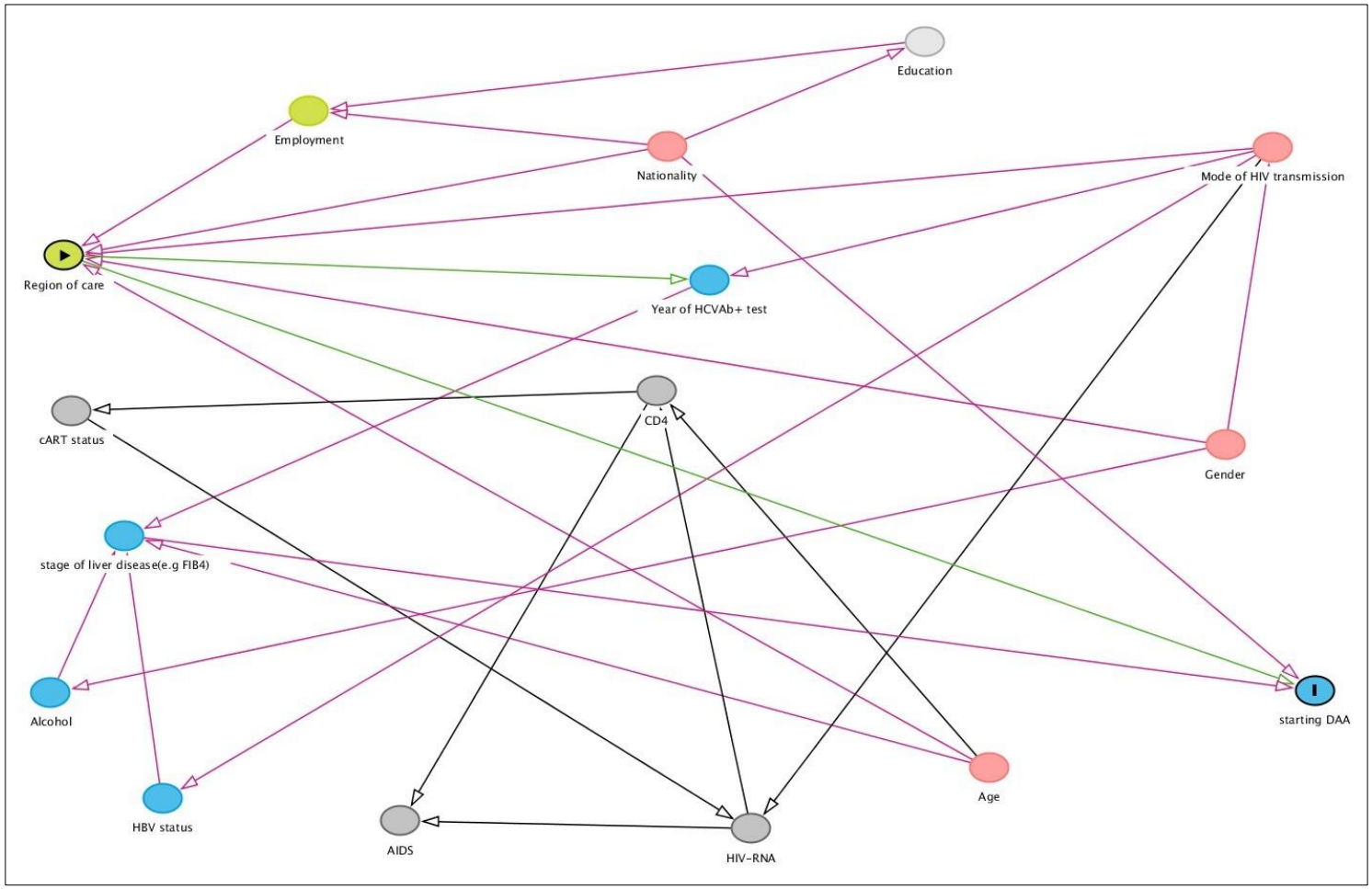
The same approach was used for identifying potential confounders for the outcome starting DAA and Figure 7.5 DAG for the effect of region of care on starting DAA

Figure 7.6 DAG for the effect of region on achieving SVRFigure 7.5 depicts the assumed causal relationships between variables. This time one of the variables included ('education') is shown in light grey (because it is not measured in Hepaicona). Of note, it is possible and recommended to include both measured and unmeasured factors in a DAG. The presence of unmeasured variables increases the chance that not all backdoor pathways can be blocked resulting in unmeasured confounding being present (luckily not the case here). Interestingly, there is a back door path between region of care and starting DAA via three variables (mode of HIV transmission, year of HCVAb positive test and stage of liver disease). Again, adjustment for mode of HIV transmission is sufficient to block the back-door pathway [region of care ← mode of HIV transmission → year of HCVAb positive test → stage of liver disease → starting DAA]. Another identified back door path is [region of care ← gender → alcohol consumption → stage of liver disease → starting DAA]. Adjusting for a single variable on this pathway will be sufficient to close this other back door path.

In summary, the diagram identifies two equivalent sets of adjusting factors (see below) for estimating the total un-confounded effect of region of care on the probability of starting DAA. These are shown below and again both models have been fitted and results compared.

a) Region of care + age + nationality + mode of HIV transmission + alcohol consumption
b) Region of care + age + gender + nationality + mode of HIV transmission

Figure 7.12 DAG for the effect of region of care on starting DAA



Legend: exposure outcome ancestor of outcome ancestor of exposure *and* outcome ancestor of exposure other variable causal path biasing path unobserved (latent)

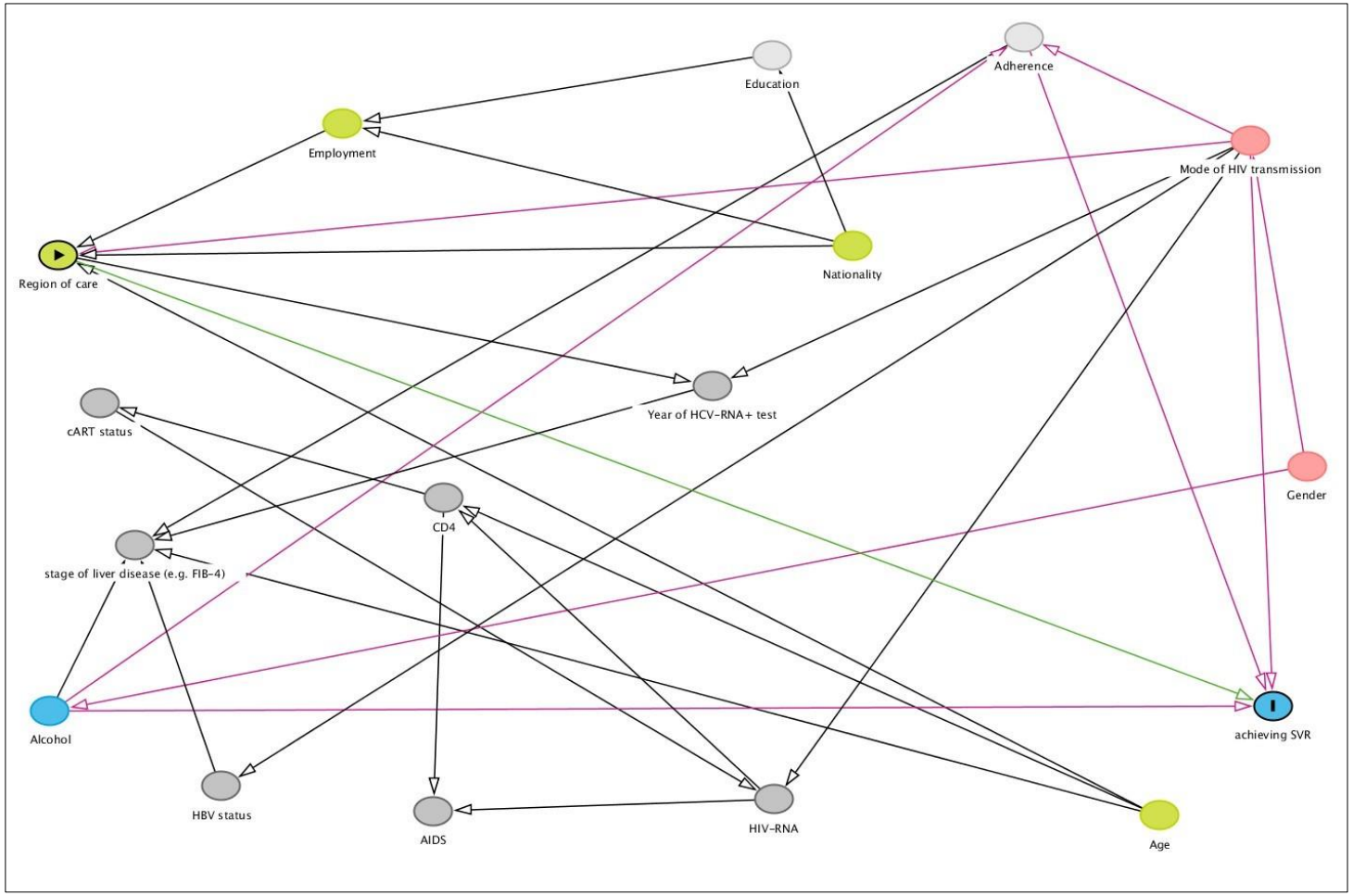
Stage 3: DAG for effect of region of care on the probability of achieving SVR

As before, Figure 7.6 DAG for the effect of region on achieving SVR

Figure 7.7 Stages of the HCV Continuum of Care– overall proportions (Icona and Hepaicona participants)Figure 7.6 depicts the assumed causal relationships for the outcome of achieving SVR. Using the same approach as previously, the assumed connection between alcohol consumption or mode of HIV transmission with the probability of achieving SVR is based on the hypothesis that adherence to DAA might be different in these sub-populations. Of note, adherence is not collected in either of the two cohorts so is another unmeasured factor besides education (grey nodes). In this DAG, mode of HIV transmission is a confounder and needs to be adjusted for to block the (region of care ← mode of HIV transmission →achieving SVR) back door path. If the assumptions of this DAG hold, it identifies a single set of confounders that need to be controlled for to estimate the total effect of region on achieving SVR including only two variables:

a) Region of care + mode of HIV transmission

Figure 7.15 DAG for the effect of region on achieving SVR



Legend: exposure outcome ancestor of outcome ancestor of exposure *and* outcome ancestor of exposure other variable causal path biasing path unobserved (latent)

7.6 Results

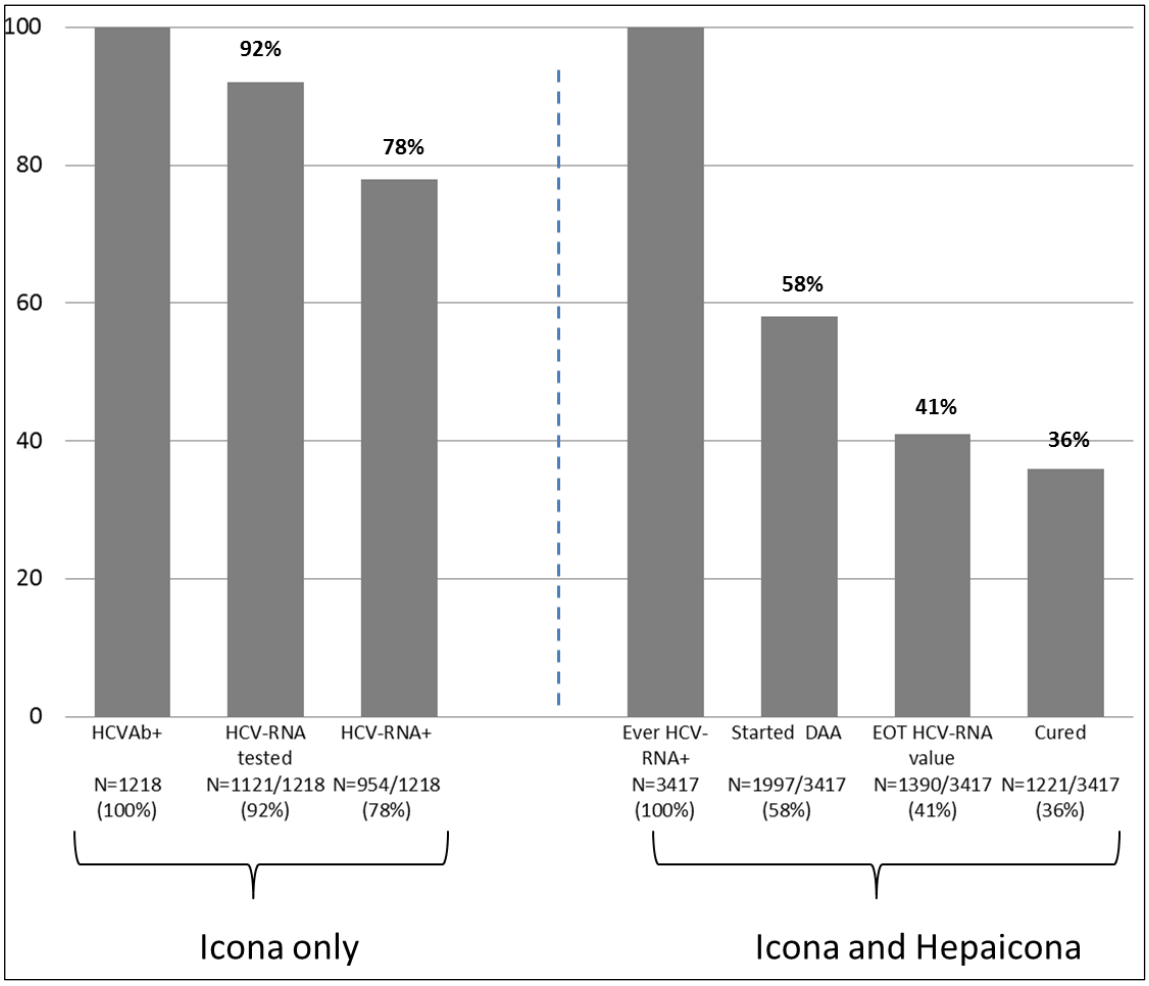
7.6.1 Stages of HCV CoC: descriptive summaries

The denominator for stage 1 of the HCV CoC included 1,218 HIV/HCV coinfected individuals with positive HCV serology and in active follow-up in the Icona cohort only at or after January 2014. HCV-RNA was tested in 92% (n=1,121) and of these, 85% (954/1121) were HCV-RNA positive (Figure 7.7 Stages of the HCV Continuum of Care– overall proportions (Icona and Hepaicona participants)

Figure 7.7). In the subsequent HCV CoC stages, participants in both Icona and Hepaicona cohorts were included. Overall, 3,417 individuals identified to be chronically infected were included (n=954 from Icona and n=2,463 Hepaicona which only includes HCV-RNA positive at entry by definition). Of these 3,417 HCV-RNA positive participants, 58% (n=1,997) started DAA, 41% (n=1,390) had an HCV-RNA value at end of treatment (EOT), and 36% (n=1,221) were cured Figure 7.7 Stages of the HCV Continuum of Care– overall proportions (Icona and Hepaicona participants)

Figure 7.7; SVR at 12/24 weeks among those with an EOT HCV-RNA value was 88% (1221/1390) (not shown in the CoC chart). The proportion of individuals starting DAA treatment may be underestimated because of a delay in data capture of the actual number of HIV/HCV coinfected individuals who started DAA in Italy. Although this is not directly comparable, in a previous Icona analysis by *Monforte et al*, 35.3% of HIV/HCV coinfected enrolled on or after January 2013 individuals were estimated to have initiated DAA among those considered eligible for treatment according to AIFA [(143)].

Figure 7.18 Stages of the HCV Continuum of Care– overall proportions (Icona and Hepaicona participants)



Stage 1

When looking at the regional differences (Figure 7.8 Stages of the HCV Continuum of Care stratified by region (Icona and Hepaicona participants)

Table 7.4 Baseline characteristics of HCVAb positive individuals identified in Icona as of January 2015 stratified by region of careFigure 7.8) in terms of HCV-RNA testing, in the Icona cohort, small but significant differences between region of care

in terms of proportions of individuals tested for HCV-RNA were observed (north 669/736 - 91% vs centre 331/347- 95% vs south 121/135 - 90%; $\chi 2$ test p=0.021). In contrast, there were no differences in terms of proportions of individuals found to be HCV-RNA positive in those who were tested (north 568/736 - 77% vs centre 280/347 - 81% vs south 106/135 - 79%; $\chi 2$ test p=0.423).

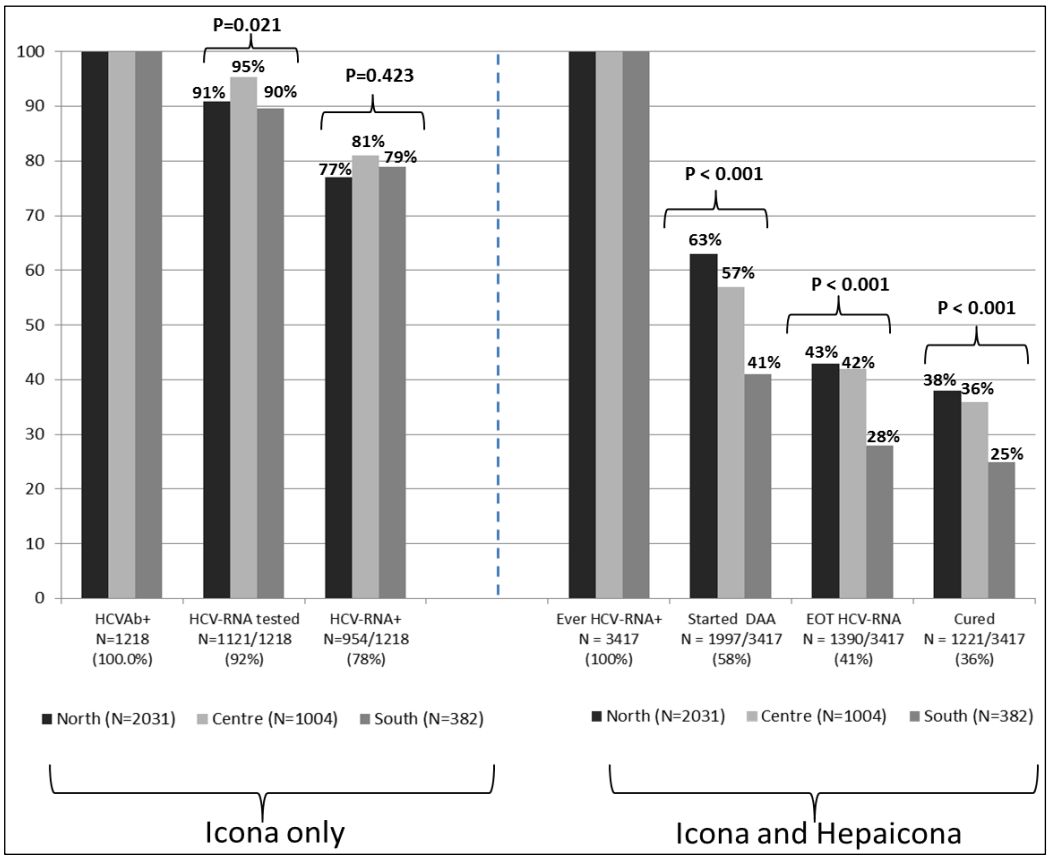
Stages 2-3

Figure 7.8 Stages of the HCV Continuum of Care stratified by region (Icona and Hepaicona participants)

Table 7.4 Baseline characteristics of HCVAb positive individuals identified in Icona as of January 2015 stratified by region of careFigure 7.8 shows the subsequent HCV CoC stages including data from both the Icona and Hepaicona cohorts, when stratified by region of care; there were significant differences between regions of care at each of these later stages of the HCV CoC.

In particular, the proportion of individuals starting DAA was higher in the north (1272/2031 - 63%) and centre (568/1004 - 57%) than in the south 157/382 - 41%); $\chi 2$ test p<0.001). There were regional differences also in terms of cure rates (north 769/2031 - 38%, centre 358/1004 - 36% and south 94/382-25%; $\chi 2$ test p<0.001). Eventual differences in cure rates as well as those in the proportions of individuals lost at the previous stage would have been indicative of a significant variation in the health care received in different regions of Italy. Reassuringly, when looking at SVR 12/24 rates (denominator here is out of those with EOT HCV-RNA value n=1,390), similar rates were observed across all regions: north (769/863- 89%) and centre (358/421- 85%) than the south 94/106 - 89%); $\chi 2$ test p=0.107) (not shown in the Figure). This was somewhat anticipated as many other studies have shown that the probability of SVR does not seem to vary by patients' characteristics, including region of care, i.e. once treated for HCV with DAA, most individuals are cured from HCV. These crude univariable analyses have been taken a step further starting from section 7.6.3, by formally fitting a logistic regression model.

Figure 7.21 Stages of the HCV Continuum of Care stratified by region (Icona and Hepaicona participants)



7.6.2 HCV CoC: HCV-RNA testing among those HCVAb positive in Icona cohort only (N=1,218)

Baseline characteristics stratified by region care

Baseline characteristics of individuals included in the denominator for stage 1 i.e. HCVAb positive stratified by region of care are shown in Table 7.4 Baseline characteristics of HCVAb positive individuals identified in Icona as of January 2015 stratified by region of care

Table 7.4. Of the 1,218 HCVAb positive individuals identified from the Icona cohort, 60% (n=736), 28% (n=347) and 11% (n=135) were seen for care in the north, centre and south, respectively. Overall, the majority of individuals included in this analysis were males (74%), median (IQR) age was 37 (33 – 43) years old, and the most common mode of HIV transmission was PWID in 64%. Overall, median (IQR) calendar year of first HCVAb positive test prior to January 2015 was 2006 (2001 – 2011), interestingly HCVAb positive testing in the southern region appeared to be less frequently performed as part of screening compared the north or centre. This might perhaps indicate the limitations of resources or the limited capacity of trained staff to screen for HCV. However, delay or lack of notification cannot be completely ruled out. Also, this is mostly apparent in the Icona data, a cohort which started much earlier in calendar time than the Hepaicona cohort and focussed on HIV infection rather that specifically in the HIV/HCV coinfected population. HCVAb testing has now been promoted across the Icona Network clinical sites and rates of improvement will be tested as part of a separate research protocol called NoCo (not included in this thesis) [448].

In terms of socio-economic variables, overall the majority of participants reported college or university as their highest degree of education (64%) and overall approximately 50% reported alcohol consumption (moderate or hazardous drinking) which was more frequent in the south (53%) than the north (48%) and central (42%) regions. Focusing on the southern region, individuals were more likely to be female ($p=0.009$), to have acquired HIV through heterosexual contact ($p<0.001$), not recently diagnosed with HCV infection ($p<0.001$), with low CD4 ($p=0.043$), less likely to have university or college education ($p<0.001$), most likely to consume alcohol ($p=0.004$), and more likely to be infected with HCV genotype 2 ($p<0.001$). Interestingly, some of these factors were identified as potential confounders in the DAG model for stage 2 of the HCV CoC (i.e. gender, age, mode of HIV transmission, nationality, employment status, level of education and alcohol consumption) so that they need to be taken into account when evaluating the association between geographical region and probability of HCV-RNA testing.

Table 7.11 Baseline characteristics of HCVAb positive individuals identified in Icona as of January 2015 stratified by region of care

	North N= 736	**Centre** N= 347	**South** N= 135	**Total** N= 1218	**P-value**
Gender, n(%)					0.009
Male	558 (75.8)	247 (71.2)	86 (63.7)	891 (73.2)	
Female	178 (24.2)	100 (28.8)	49 (36.3)	327 (26.8)	
Age(years)					0.081
Median (IQR)	37 (33, 43)	37 (33, 43)	36 (32, 40)	37 (33, 43)	
Nationality, n(%)					0.005
Non-Italian	37 (5.0)	36 (10.4)	10 (7.4)	83 (6.8)	
Italian	699 (95.0)	311 (89.6)	125 (92.6)	1135 (93.2)	
Mode of HIV transmission, n(%)					<.001
Heterosexual	107 (14.5)	73 (21.0)	32 (23.7)	212 (17.4)	
MSM	126 (17.1)	47 (13.5)	6 (4.4)	179 (14.7)	
PWID	476 (64.7)	213 (61.4)	87 (64.4)	776 (63.7)	
Other	27 (3.7)	14 (4.0)	10 (7.4)	51 (4.2)	
Calendar year of HCV positive test					<.001
Median (IQR)	2007 (2001, 2012)	2006 (2002, 2012)	2003 (1999, 2010)	2006 (2001, 2011)	
CD4 cell count cells/mm^3					0.043
Median (IQR)	438 (257, 621)	424 (230, 564)	346 (180, 591)	425 (235, 597)	
HIV-RNA (log10)					0.093
Median (IQR)	4 (3, 5)	4 (3, 5)	4 (4, 5)	4 (3, 5)	
AIDS Diagnosis, n(%)	114 (15.5)	57 (16.4)	25 (18.5)	196 (16.1)	0.665
cART Naive, n(%)	69 (9.4)	19 (5.5)	13 (9.6)	101 (8.3)	0.079
Education, n(%)					0.001
Primary school (<11)	139 (18.9)	55 (15.9)	37 (27.4)	231 (19.0)	

	North N= 736	Centre N= 347	South N= 135	Total N= 1218	P-value
Secondary school (11-16)	62 (8.4)	39 (11.2)	19 (14.1)	120 (9.9)	
College(16 - 18)	319 (43.3)	143 (41.2)	50 (37.0)	512 (42.0)	
University (18+)	182 (24.7)	91 (26.2)	28 (20.7)	301 (24.7)	
Other/Unknown	34 (4.6)	19 (5.5)	1 (0.7)	54 (4.4)	
Employment, n(%)					0.083
Employed	507 (73.2)	225 (67.0)	77 (58.8)	809 (69.7)	
Unemployed	149 (21.5)	86 (25.6)	35 (26.7)	270 (23.3)	
Other	28 (4.0)	17 (5.1)	16 (12.2)	61 (5.3)	
Unknown	9 (1.3)	8 (2.4)	3 (2.3)	20 (1.7)	
Alcohol consumption, n(%)					0.004
Abstainer	291 (39.5)	169 (48.7)	56 (41.5)	516 (42.4)	
Moderate	313 (42.5)	124 (35.7)	68 (50.4)	505 (41.5)	
Hazardous	40 (5.4)	22 (6.3)	4 (3.0)	66 (5.4)	
Unknown	92 (12.5)	32 (9.2)	7 (5.2)	131 (10.8)	
HBV Infection, n(%)					0.822
Negative	645 (87.6)	302 (87.0)	122 (90.4)	1069 (87.8)	
Positive	32 (4.3)	16 (4.6)	6 (4.4)	54 (4.4)	
Not tested	59 (8.0)	29 (8.4)	7 (5.2)	95 (7.8)	
Stage of liver disease, n(%)					0.547
≤F3	523 (71.1)	256 (73.8)	95 (70.4)	874 (71.8)	
>F3	101 (13.7)	47 (13.5)	13 (9.6)	161 (13.2)	
***Genotype, n(%)**					<.001
1	247 (33.6)	91 (26.2)	45 (33.3)	383 (31.4)	
2	11 (1.5)	2 (0.6)	9 (6.7)	22 (1.8)	
3	151 (20.5)	77 (22.2)	30 (22.2)	258 (21.2)	
4	47 (6.4)	26 (7.5)	7 (5.2)	80 (6.6)	

*only in HCV-RNA+

7.6.3 Effect of region of care on the probability of receiving HCV-RNA testing in Icona cohort only (N=1,218)

The unadjusted analysis (Table 7.5 Odds ratios from fitting logistic regression models of HCV testing vs. not HCV testing (in Icona only N=1,218)

Table 7.5) showed regional differences for the probability of HCV-RNA testing (unadjusted global p = 0.015). Specifically, compared to individuals attending a site in the north, those who were seen for care in the central region were twice as likely be tested for HCV-RNA following HCVAb positive test (unadjusted OR = 2.07 (95% CI: 1.18 – 3.65)) while there was no difference in the odds of HCV-RNA testing in the southern region (unadjusted OR = 0.87 (95% CI: 0.47 – 1.59)). According to model assumptions, a number of different approaches (#1 region of care + age + mode of HIV transmission + education + alcohol consumption; #1 + nationality + employment ; #3 region of care + age + gender + mode of HIV transmission + education; #4 region of care + age + mode of HIV transmission + gender + nationality + employment) can block all back-door paths from exposure to outcome. All these multivariable models indeed showed similar findings i.e. the odds of being tested for HCV-RNA in the central region was twice greater than that of being tested in the northern region and also higher compared to the south, while there was no difference in terms of odds of testing for HCV-RNA between south and north regions. Using one of the approaches described above, after adjusting for age, gender, nationality, employment and mode of HIV transmission, region of care remained independently associated with HCV-RNA testing, centre OR = 2.29 (95% CI: 1.29 – 4.06); p=0.005, and south OR = 1.01 (95% CI: 0.54 – 1.91); p = 0.966 compared to north , global p = 0.008 (Table 7.5 Odds ratios from fitting logistic regression models of HCV testing vs. not HCV testing (in Icona only N=1,218)

Table 7.5). It is worth mentioning that, in all models, the type 3 global p-values are driven by the comparisons of the centre vs. north and south, the latter two regions being similar.

Applying the assumptions depicted in the alternative DAG in Figure 7.4 DAG for the effect of region on HCV-RNA with changed direction of causal pathways

Figure 7.4 , in which the direction of causation for employment status was inverted, nationality was the only variable identified as potential confounder. Results of this

alternative model (#1 region of care + nationality) were similar to those found with the main analysis, south OR of HCV-RNA testing = 0.90 (95% CI: 0.48 – 1.63); p = 0.704 and centre OR = 2.21 (95% CI: 1.26 – 3.90) p = 0.006, compared to north, global p = 0.008. This finding could be interpreted in two ways. First, that both models are equally good representation of the reality and therefore able to provide the unbiased causal effect of region on the chance of being tested for HCV-RNA. Alternatively, both set of assumptions are not correct and results were similar by chance. Unfortunately, neither of these hypotheses are testable using the data.

Table 7.14 Odds ratios from fitting logistic regression models of HCV testing vs. not HCV testing (in Icona only N=1,218)

	Unadjusted1 OR (95% CI)	**g pv**	**Model1 OR (95% CI)**	**g pv**	**Model 2 OR (95% CI)**	**g pv**	**Model 3 OR (95% CI)**	**g pv**	**Model 4 OR (95% CI)**	**g pv**
Region		0.015		0.018		0.009		0.016		0.008
North	1.00		1.00		1.00		1.00		1.00	
South	0.87 (0.47, 1.59) p=0.641		0.918 (0.48, 1.74) p=0.792		0.907 (0.48, 1.72) p=0.766		1.047 (0.56, 1.97) p=0.886		1.014 (0.54, 1.91) p=0.966	
Centre	2.07 (1.18, 3.63) p=0.011		2.098 (1.18, 3.72) p=0.011		2.221 (1.25, 3.96) p=0.007		2.143 (1.21, 3.78) P=0.009		2.290 (1.29, 4.06) p=0.005	

Unadjusted (n=1218): Region of care
Model 1 (n=1218): Region of care + Age + Education + mode of HIV transmission + Alcohol consumption
Model 2 (n=1218): Region of care + Age + Nationality + Employment + mode of HIV transmission + Alcohol consumption
Model 3 (n=1218): Region of care + Age + Gender + Education + mode of HIV transmission
Model 4 (n=1218): Region of care + Age + Gender + Nationality + Employment + mode of HIV transmission

7.6.4 HCV CoC: Starting DAA treatment among those chronically infected with HCV in Icona and Hepaicona (N=3,417)

Baseline characteristics stratified by region of care

A total of 3,417 individuals from the Icona (n=954) and Hepaicona cohort (n=2,463) were identified as HCV-RNA positive in need of treatment after January 2014. Baseline characteristics stratified by region of care among those who were ever HCV-RNA positive are shown in Table 7.6 Baseline characteristics of HCV chronically infected individuals identified in Icona and Hepaicona by region of care

Table 7.7 Logistic regression models of starting DAA vs not starting DAA based on DAGs (N=3,417)Table 7.6. Median (IQR) age was 49 (41 – 54) years old, the majority of people included were PWID (72%). Alcohol consumption was common and reported by almost 25% of the individuals included in the analysis and approximately 20% had advanced stage of liver disease. Overall, the most common HCV genotype reported was genotype 1 in almost half of the patients, with HCV genotype 3 as the next common genotype in a quarter of individuals.

There were regional differences in terms of baseline characteristics. Some of the key differences include: individuals in the central region were of slightly older age; approximately 79% were PWIDs in the southern region compared to 70% in the north and 73% in the centre; more recent HCV-RNA positive tests were observed in the north and south with a median calendar year of HCV-RNA test of 2014 compared to 2010 in the central region. This could be partly explained by the fact that the majority of sites participating in Hepaicona are from the north and Hepaicona started to enrol in more recent years. Regarding the more recent calendar year of testing registered in the south, this could be explained by the fact that screening for HCV-RNA has been recently improved in the south to catch up with other regions or due to the high prevalence of PWIDs in this region. A lower proportion of individuals with AIDS diagnosis was also observed in the southern region (9%) compared to north and centre (15% and 16% - respectively). Lower

unemployment rates were observed in the north (14%) compared to centre (20%) and south (30%), reflecting the data seen in the general population. This suggests some obvious disparities in terms of social economic status. There was a higher proportion of participants reporting alcohol consumption in the south (26%) compared to north (19%) and centre (19%). As identified in chapter in 3, there was a lot of missing data for with all regions having >50% of missing data on alcohol consumption. Again, some of these variables were identified as potential confounders in the DAG (i.e. gender, age, mode of HIV transmission, nationality and alcohol consumption). Indeed, these were identified as common causes of exposure and outcome and they need to be taken into account when evaluating the association between geographical region and probability of DAA initiation.

Table 7.17 Baseline characteristics of HCV chronically infected individuals identified in Icona and Hepaicona by region of care

	North N=2031	**Centre N=1004**	**South N=382**	**Total N=3417**	**p-value**
Gender, n(%)					0.003
Male	1525 (75.1)	731 (72.8)	255 (66.8)	2511 (73.5)	
Female	506 (24.9)	273 (27.2)	127 (33.2)	906 (26.5)	
Age(years)					0.003
Median (IQR)	49 (42, 53)	50 (40, 54)	49 (39, 53)	49 (41, 54)	
Nationality, n(%)					<.001
Non-Italian	305 (15.0)	194 (19.3)	37 (9.7)	536 (15.7)	
Italian	1726 (85.0)	810 (80.7)	345 (90.3)	2881 (84.3)	
Mode of HIV transmission, n(%)					<.001
Heterosexual	237 (11.7)	125 (12.5)	54 (14.1)	416 (12.2)	
MSM	186 (9.2)	84 (8.4)	10 (2.6)	280 (8.2)	
PWID	1430 (70.4)	737 (73.4)	302 (79.1)	2469 (72.3)	
Other	178 (8.8)	58 (5.8)	16 (4.2)	252 (7.4)	
Year of HCV-RNA positive test					<.001
Median (IQR)	2014 (2010, 2016)	2010 (2005, 2015)	2014 (2011, 2015)	2013 (2008, 2015)	
CD4 cell count cells/mm^3					0.200

	North N=2031	Centre N=1004	South N=382	Total N=3417	p-value
Median (IQR)	477 (305, 709)	472 (293, 658)	474 (285, 765)	476 (298, 696)	
HIV-RNA (log10)					0.062
Median (IQR)	3 (1, 4)	3 (2, 5)	3 (1, 5)	3 (1, 5)	
AIDS diagnosis, n(%)	302 (14.9)	157 (15.6)	33 (8.6)	492 (14.4)	0.003
cART naive, n(%)	351 (17.3)	158 (15.7)	79 (20.7)	588 (17.2)	0.092
Employment, n(%)					0.008
Employed	936 (46.9)	483 (48.7)	213 (55.9)	1632 (48.5)	
Unemployed	279 (14.0)	214 (21.6)	115 (30.2)	608 (18.1)	
Other	73 (3.7)	87 (8.8)	37 (9.7)	197 (5.9)	
Unknown	707 (35.4)	207 (20.9)	16 (4.2)	930 (27.6)	
Alcohol consumption, n(%)					<.001
Abstainer	343 (16.9)	210 (20.9)	78 (20.4)	631 (18.5)	
Moderate	315 (15.5)	149 (14.8)	86 (22.5)	550 (16.1)	
Hazardous	73 (3.6)	45 (4.5)	13 (3.4)	131 (3.8)	
Unknown	1300 (64.0)	600 (59.8)	205 (53.7)	2105 (61.6)	
HBV Infection, n(%)					0.346
Negative	699 (34.4)	312 (31.1)	131 (34.3)	1142 (33.4)	
Positive	27 (1.3)	10 (1.0)	4 (1.0)	41 (1.2)	
Not tested	1305 (64.3)	682 (67.9)	247 (64.7)	2234 (65.4)	
Stage of liver disease					0.068
≤F3	963 (47.4)	461 (45.9)	202 (52.9)	1626 (47.6)	
>F3	395 (19.4)	192 (19.1)	58 (15.2)	645 (18.9)	
Genotype, n(%)					0.020
1	1002 (49.3)	459 (45.7)	180 (47.1)	1641 (48.0)	
2	43 (2.1)	16 (1.6)	19 (5.0)	78 (2.3)	
3	481 (23.7)	245 (24.4)	95 (24.9)	821 (24.0)	
4	281 (13.8)	131 (13.0)	59 (15.4)	471 (13.8)	

7.6.5 Effect of region of care on the probability of starting DAA among those chronically infected with HCV in Icona and Hepaicona (N=3,417)

There were differences in region of care in terms of the probability to initiate DAA therapy (global p <0.001); specifically this probability was lower in the south and centre compared to the north: unadjusted OR = 0.42 (95% CI: 0.33 – 0.52); p<0.001 and OR = 0.78 (95% CI: 0.67 – 0.91) respectively Table 7.7 Logistic

regression models of starting DAA vs not starting DAA based on DAGs (N=3,417)

Table 7.7 . From the adjusted analysis, using the model described by the DAG in Figure 7.5 DAG for the effect of region of care on starting DAA

Figure 7.6 DAG for the effect of region on achieving SVRFigure 7.5, after controlling for age, nationality, mode of HIV transmission and alcohol consumption, region of care remained independently associated with the probability of starting DAAs (global p<0.001), south OR = 0.44 (95% CI: 0.34 – 0.55); p<0.001, and centre OR = 0.80 (95% CI: 0.69 – 0.94) compared to north; p=0.006. Interestingly, the other model adjustment, including gender instead of alcohol consumption to block the (region of care ← gender → alcohol consumption → stage of liver disease → starting DAA) backdoor path, showed similar results. Again, one possible interpretation is that the model has been correctly specified but it cannot be interpreted as a proof. In reality this additional analysis is useful only if the results of the different models are dramatically different as this can be interpreted as evidence that one of the models was not correctly specified.

Table 7.20 Logistic regression models of starting DAA vs not starting DAA based on DAGs (N=3,417)

	Unadjusted1 OR (95% CI)	**g pv**	**Model1 OR (95% CI)**	**g pv**	**Model2 OR (95% CI)**	**g pv**
Region		<0.001		<0.001		<0.001
North	1.00		1.00		1.00	
South	0.416 (0.33, 0.52) p<0.001		0.437 (0.34, 0.55) p<0.001		0.440 (0.35, 0.55) p<0.001	
Centre	0.777 (0.67, 0.91)		0.802 (0.69, 0.94) p=0.006		0.802 (0.69, 0.94)	

Unadjusted (n=3,417): Region of care
Model 1 (n=3,417): Region of care + Age + Nationality + mode of transmission + Alcohol consumption
Model 2 (n=3,417): Region of care + Age + Gender + Nationality + mode of transmission

	Unadjusted1 OR (95% CI)	g pv	Model1 OR (95% CI)	g pv	Model2 OR (95% CI)	g pv
	p=0.001				p=0.006	

7.6.6 HCV CoC: SVR among those individuals who initiated DAA and had HCV-RNA end of treatment value (N=1,390)

Of the 3,417 individuals identified to be HCV-RNA positive 36% (n=1221) individuals were cured. Although an accurate estimate of the proportion of the overall cured is important for public health, most studies tend to report the SVR rate based on those initiating treatment and having an end of treatment HCV-RNA value as the denominator for assessment. This approach of analysis may introduce bias as discussed in the final paragraph. In this Icona Network sample, as previously reported, there were n=1,997 who initiated DAA but only 70% (n=1,390) of these had an end of treatment HCV-RNA value for assessment at 12/24 weeks post treatment. After restricting the analysis to those with EOT HCV-RNA available, 88% (1221/1390) achieved sustained virological response. The logistic regression analysis reported in the next section is restricted to this subset of 1,390 individuals.

7.6.7 Effect of region of care on achieving SVR among those individuals who initiated DAA and had HCV-RNA end of treatment value (N=1,390)

Interestingly, and in contrast with the results shown for the other CoC stages outcomes, there were no regional differences when comparing the chance of achieving SVR (global p=0.145), and even after adjusting for mode of HIV transmission (the key potential confounder identified in the DAG) region of care was still not associated with SVR, south OR = 1.00 (95% CI: 0.53 – 1.90); p = 0.998, compared to north and only limited evidence for a difference between centre and north OR = 0.711 (95% CI: 0.50 – 1.00) p=0.052. It is worth mentioning that in the unadjusted analyses, centre was associated with reduced odds of SVR, but after adjustment for mode of HIV transmission, which is a potential confounder, this

relationship was attenuated and more compatible with the null hypothesis of no difference.

Although this result was somewhat expected, it is reassuring that the data carry no evidence that the rate of SVR varies by geographical region or any other patients' characteristics (adjusted model1 in Table 7.8 Odd ratios from fitting logistic regression models of achieving SVR vs not achieving SVR based on DAGs (N=1,390)

Table 7.8). Importantly in conclusion, this analysis confirms that DAAs are effective in curing HCV regardless of other factors, including the point of access of care in Italy used. It is also worth noting that we cannot rule out residual confounding due to adherence, which is unmeasured in the cohorts.

Table 7.23 Odd ratios from fitting logistic regression models of achieving SVR vs not achieving SVR based on DAGs (N=1,390)

	Unadjusted1 OR (95% CI)	**global pv**	**Model1 OR (95% CI)**	**g pv**
Region		0.115		0.145
North	1.00		1.00	
South	0.956 (0.51, 1.81) P=0.894		1.00 (0.53, 1.90) P=0.998	
Centre	0.695 (0.49, 0.98) P=0.037		0.711 (0.50, 1.00) P=0.052	

Unadjusted (n=1,390): Region of care
Model 1 (n=1,390): Region of care + mode of HIV transmission

7.7 Discussion

In this chapter I set out to evaluate the HCV CoC in HIV/HCV coinfected individuals seen for routine clinical care in Italy since January 2014. This date was defined as the baseline for analyses, as this is the calendar date in which DAA were officially introduced in Italy. However, people could be included if they were under active follow-up after January 2014. Additionally, I also assessed the impact of region of care on a number of stages of the WHO proposed HCV CoC based on the data collected in the Icona and Hepaicona cohorts. By adaptation of the WHO's framework of continuum of care, three main stages were analysed (rate of HCV-RNA testing, rate of DAA uptake and rate of HCV cure).

Overall, the analysis has shown that, more than a third (36%) of the HCV-RNA positive individuals in the Icona cohort sample had started DAA treatment and were cured. Although, this proportion might seem low, the estimated frequency of people who were successful at each stage of the HCV CoC in Italy were in 2016 close to those set out by WHO at the time. Therefore, the data encouragingly suggested that the achievement of HCV elimination in the Italian HIV/HCV population by 2030 seems to be within reach. In these analyses, regional differences were found in terms of proportion of individuals receiving HCV-RNA testing (north - 91%, centre - 95% and south - 90%) and starting DAA (north - 63%, centre - 57% and south - 41%). Nevertheless, the regional differences were not consistent across stages. On one hand, the results indicate that the lower apparent access to HIV/HCV treatment in the central/south part of the country might constitute a potential barrier to test and treatment. However, reassuringly, for those who did complete the HCV CoC, SVR 12/24 rates were broadly similar across all regions (north - 89%, centre - 85% and south - 89%) indicating that once access to testing and treatment is further improved, good treatment outcomes should be ensured for all regions.

Stage 1 of this analysis was conducted using the data of the Icona cohort alone. It aimed to identify individuals who were HCVAb positive, and among these, to further identify people who were tested for HCV-RNA and to eventually estimate the burden of chronic HCV infection. This is a crucial stage as it has helped in the identification of specific regions in which HCV-RNA testing was less frequent so that these areas could be specifically targeted for more frequent testing in the future. Interestingly, the overall coverage was high, as 92% of the study population underwent HCV-RNA testing with some evidence of more frequent testing in the central sites compared to south. Among those tested for HCV-RNA in this initial stage, 85% (954/1218) were found to be chronically infected. This certainly indicates that the level of coverage of HCV diagnosis in Italy is reasonably good and close to the 90% target currently set by WHO [5]. The findings of this analysis also indicate an improvement in terms of testing for chronic HCV infection in the DAA era, possibly explained by the introduction of universal access to all populations, the use of effective and more tolerated drugs in recent years and the potential for everybody to be treated regardless of their stage of liver disease.

Regional differences were observed as HCV-RNA testing was less common in the southern part of Italy compared to central regions. This was also somewhat expected as regional disparities have been previously reported in terms of health care delivery, access, and resource capacity [409]. In addition, this is partly explained by differences in the social economic status between regions of Italy. These regional differences were also apparent when looking at the baseline characteristics as; unemployment rates were higher in the south than the north (27% vs 21%). However, differences by region remained significant after controlling for unemployment rates. These findings are consistent with those of other studies that reported regional differences as potential barriers in the HCV CoC e.g. rate of testing for HCV-RNA seems to be higher in developed cities than in socially deprived areas [413, 419, 424].

After adjusting for potential confounders identified in the DAG, region of care remained independently associated with the probability of undergoing HCV-RNA testing. One of the adjusted models also including age, gender, nationality, employment and mode of HIV transmission, showed an OR = 2.29 (95% CI: 1.29 – 4.06); p=0.005 of being HCV-RNA tested in the central region compared to south OR = 1.01 (95% CI: 0.54 – 1.91); p=0.966. Of course, type of employment is only a proxy for socio-economic factors so we cannot rule out that residual confounding exist. It is also worth mentioning that the analysis was not adequately powered to detect differences between north and south given the small proportion of people accessing care in the south region.

The findings in this analysis are consistent with those of other published studies such as those by *Simmons et al* in the UK primary setting who reported an adjusted OR of 1.21 (95% CI: 1.13 – 1.30) of HCV-RNA testing if person was tested outside London vs. inside London. These results also suggest that regional barriers might be related to availability of resources to regional health care systems. For example in Italy, the northern and central regions are well known to have better quality of health care in terms of having advanced technology thus potentially explaining the higher HCV testing rates compared to the southern region [(409)].

Stage 2 involved identifying individuals who started DAA among those chronically infected. In this analysis, the study population included 3,417 HCV-RNA positive individuals. Also in this analysis, differences in baseline characteristics of participants were identified across regions of care. As correctly assumed in the DAG, there was a higher proportion of PWIDs in the south (79%) compared to north (70%) or centre (73%). Calendar year of HCV test was more recent in the north and south compared to the centre. This is partly explained by a higher prevalence of PWIDs who are more likely to be tested and perhaps recent efforts to increase coverage of HCV testing in more deprived areas to reduce the proportion of un-diagnosed [(160)].

Overall, 58% (1997/3417) were found to have initiated DAA. This estimate falls at the upper end of the range of those observed in other similar studies conducted in a similar period and reporting rates of treatment uptake of 11–60% [417, 421, 424, 433] . This large variation is most likely due to geographical differences and timing of universal access of DAA[11]. It is possible that geographical region is also correlated with socio-economic factors with lower rates of uptake registered in deprived areas [424]. For example *Noska et al* observed 23% of homeless individuals initiating oral DAA in 2015 compared to 31% of non-homelessness individuals [424]. The low DAA treatment uptake observed in the Icona Network sample is certainly due to the fact that universal access to DAA started relatively recently in Italy and at the time of this analysis there was still a small backlog of people with F0-F1 fibrosis stages that had not been treated. In addition, it is conceivable that priority was given to the population of HCV mono-infected and here we are reporting rates in the population of HIV/HCV coinfected individuals. However, recent reports from WHO suggest that Italy is on target to meeting the WHO 2030 HCV elimination goals [449] [160]. However, this estimate cannot be directly compared with that of our sample, as our sample include HIV/HCV coinfected people fitting inclusion criteria specific to study entry.

In the multivariable analysis, after adjusting for age, nationality, mode of HIV transmission and alcohol consumption, region of care remained independently associated with the chance of starting DAAs. The association being particularly strong for the south region which was associated with a 50% reduced probability of initiating DAA compared to what was seen in the north. This finding is consistent with previous reports from the EU which found that worse financial situations and accessibility to treatment in southern regions of Europe were potential causes of poorer health in these areas. More generally in the 2014 EACS report addressing standard of care for HIV/HCV coinfections in Europe, additional potential barriers attributable to treatment initiation associated with patient characteristics were identified, including the possible of lack of knowledge of the disease, or concerns

regarding potential side effects and even economical marginalisation [450]. Some other possible reasons also listed include: lack of awareness of updated treatment guidelines and, of the impact of HIV on liver disease progression, ongoing concerns about drug-drug interactions in HIV/HCV coinfections [450]. However, unfortunately none of these factors are measured in the Icona Network database so these hypotheses could not be tested and some of these factors are potential confounding or colliders for the associations of interests.

The denominator for the final stage 3 analysis of the proposed CoC included individuals who had started DAA treatment and had an end of treatment HCV-RNA value available. The estimated cure rate of 36% among HCV-RNA positive individuals is similar to what has been observed in other samples of European HIV/HCV coinfected individuals in the DAA era [402, 438]. The final outcome in this analysis was SVR 12/24 which was observed in 88% of individuals among those with an end of treatment HCV-RNA value available for assessment. This is also consistent with what is reported in the HIV/HCV coinfected population elsewhere with average rates >90%. Interestingly, according to our set of assumptions the only measured factor that could confound this association was mode of HIV transmission. The assumption is that more PWID attend clinics in the southern regions compared to other regions, and PWID are less likely to have good response to therapy because of low adherence to DAA or even refusing therapy. When looking at the stage 3 outcome ‘achieving SVR’, there was some evidence for regional differences in the unadjusted analysis with the south doing worse. However, in the multivariable analysis, after adjusting for mode of HIV transmission, region of care was no longer associated with the chance of achieving SVR. This is consistent with previous studies reporting comparable SVR rates in HIV-infected populations between different regions (Australia, Canada, France, Georgia, Switzerland and Netherlands, USA) [402, 424, 438].

7.8 Strengths and limitations

One of the main strengths of this analysis is that the population included all major HIV risk groups; PWIDs, MSM and heterosexuals and included individuals with a wide range of stage of liver disease and differing HCV genotypes. Therefore, these findings should be applicable to the HIV/HCV coinfected population as a whole. As this is an observational cohort, there are some limitations that need to be mentioned.

Firstly, differences in reporting of different stages of the HCV CoC may affect results. Indeed, it is possible that the level of accuracy of the various data items varies and this could have affected the retention of care estimates at specific CoC stages evaluated. For example, by the time the database was locked, it is possible that HCV-RNA tests results were fully updated while not all the DAA initiations dates were sent by the clinics due to a delay in data reporting into the cohort database. This despite the extra effort which had been placed across the Icona Network sites towards HCV-RNA testing and collection of data of starting HCV treatment, to obtain more accurate real time estimates. Luckily, the database was locked before the COVID-19 pandemic which has caused extra delays in data collection and reporting.

Second, although the sample population appears to be very heterogeneous it is important to note that only people who were tested for HCVAb were included. As a consequence, the included sample might be a selected population of people with greater health awareness or simply with risk factors for HCV which influenced the chance of being tested and be selected in the analysis. However, it is unlikely that the retention outcomes of the analysis could also have influenced this selection. Nevertheless, only participants with EOT data were included in the analysis of the comparison between SVR rates. Because reasons for not completing treatment are likely to be also associated with the chance of achieving SVR, stage 3 analysis results are likely to suffer from selection/collider bias

In terms of statistical methodology one of the strengths of these analyses involves the careful identification of potential causes of exposure and outcome with the general aim to generate a model able to evaluate the causal link between region of care and the probability of the various retention stage outcomes. All assumptions were described through the use of the transparent language of causal diagrams (DAGs). *Lederer et al* has recently identified DAGs as a way forward in terms of guiding the choice of rigorous statistical methods aiming to estimate the cause effect of an exposure on outcome [(1)]. The objective of this chapter was to try to identify whether region was associated with the rate of retention in care at different stages of the HCV CoC. The use of this rigorous approach has helped in identifying whether region or other unmeasured factors associated with region are important barriers towards the 2030 HCV elimination goal so that efforts could be concentrated in trying to minimise/remove these barriers when possible. However, as described in previous chapters there was a significant amount of missing data for some confounding factors such as alcohol consumption, which could cause bias in the results.

7.9 Conclusion

In conclusion, this analysis of a large sample of HIV/HCV coinfected individuals seen for routine clinical care in Italy found that more than 90% of participants were tested for HCV-RNA and of these more than 80% were found to be chronically infected with HCV. This importantly documents that Italy is currently already meeting the WHO target for coverage of HCV diagnosis. However, among individuals who were HCV-RNA positive there were a very low proportion of individuals remaining in care post HCV diagnosis with 40% of the study population who apparently have not yet started DAA. This seems particularly alarming in the south of the country where individuals had a 50% reduced chance of starting DAA compared to people in other regions, suggesting that factors such as social deprivation or perhaps lack of resources in poorer areas of Italy represents a major

challenge for achieving the WHO target of HCV elimination in the HIV/HCV coinfected population. These data therefore suggests that more work is needed to try to remove regional barriers in terms of health care service in order to ensure that the WHO 2030 goals are met. As a positive note, this analysis showed no evidence for regional differences in terms of achieving SVR so the main efforts should be directed to reduce the geographical gap in terms of rate of testing and treatment uptake.

7.10 Further work

As a separate effort, all clinical infectious disease sites involved in the Icona Foundation Study are now collecting additional basic information on HCVAb positive testing in all HIV-positive individuals seen for care besides those actually included in the cohort. This extra effort should provide data to better evaluate the first step of the WHO CoC aim at reducing the proportion of people undiagnosed for HCV or diagnosed with HCV too late [229]. Indeed, late diagnosis has potential negative consequences in terms of risk of morbidity and mortality as shown in chapter 6 of this thesis.

CHAPTER 8

8 IMPLICATIONS AND FINAL CONCLUDING REMARKS

In this chapter I will first give a recap of how my research questions have evolved and the rationale. I will summarise the hypotheses and main findings and then I will discuss clinical and public health implications and, when relevant, the implication of my findings for future research. Finally I will discuss general limitations and final concluding remarks.

8.1 Recap

At the start of my PhD, my research questions were focused on the association of HCV coinfection with adverse clinical outcomes among PLWH. I first examined whether HCV was a confounder or/and an effect measure modifier for the relationship between alcohol consumption and risk of liver disease (chapter 4). This analysis is important because data on alcohol consumption in HIV cohorts is rarely available. Data on drinking behaviour are collected in both Icona and Hepaicona cohorts using a few simple questions related to quantity and frequency of drinks. By putting together, the information collected through these questions I was able to categorise drinking behaviour into abstainers, moderate or hazardous drinkers and investigate the relationship with severe liver disease in HIV monoinfected and HIV/HCV coinfected participants. As the alcohol variable had a significant proportion of missing values, I used a multiple imputation approach to check the robustness of my findings..

I also investigated the role HCV infection (both based on serology and HCV viral load) on the risk of ARV discontinuation of specific modern HIV drugs (chapter 5). At the time of working on this chapter, research around the risk of ARV discontinuation among HIV/HCV coinfected individuals showed that the most common reason for ARV drug discontinuation was intolerance/toxicity and there

was a general concern that HIV/HCV coinfection could increase the rate of discontinuations (especially for certain antiretrovirals such PIs and NNRTIs as these are metabolised in the liver). In other words, there was concern that liver impairment was likely to impact on HIV therapy. However, this is no longer an issue as modern HIV drugs are now more effective and are better tolerated.

Indeed, in 2015, the introduction of highly effective DAA treatment for HCV revolutionised the management of chronic HCV infection with RCTs showing cure rates of >95% and favourable safety profiles. Prior to effective DAA, the HIV/HCV coinfected population were considered a special population. This was because of the faster progression of HCV disease among HIV infected individuals. However, over time research has shown that DAA is just as effective in HIV/HCV coinfected as it in HCV mono-infected individuals. In 2016, WHO called for a global HCV elimination strategy towards HCV elimination by 2030. The contribution of health sectors in the fight towards HCV elimination has been outstanding with most countries showing excellent progress towards target.

The accumulation of accurate DAA response data in both Icona and Hepaicona cohorts allowed me to evaluate new potential challenges in the management of HIV/HCV coinfected individuals. At this point, my research questions became focused on identifying factors that may hinder progression through the HCV continuum of care pathway following access to health care. These include barriers to HCV testing and diagnosis, initiation of DAA and response to therapy. I particularly first investigated the prevalence of late HCV diagnosis indicating missed screening opportunities in PLHIV (chapter 6). In newly diagnosed HIV-positive individuals likely to be tested for HCV, I also evaluated potential regional differences in access to health care on outcomes such as HCV-RNA testing, initiating DAA and achieving sustained virological response on treatment (chapter 7). These chapters have a clear rationale in addressing questions of key clinical importance in the DAA era that have potential public health implications.

8.2 HIV/HCV coinfection, alcohol consumption and risk of severe liver disease

In chapter 4, I assessed the hypothesis that co-existence of HCV infection and risky alcohol drinking behaviour is associated with an exacerbation of the risk of SLD. I used routinely collected data of physicians' assessment of alcohol consumption mapped to the Italian national drinking guidelines to classify drinking behaviours. This is the first time that the alcohol consumption variable was used as the main exposure in any analyses of the Icona Network group although restricted to approximately 9,500 HIV-positive individuals enrolled in both the Icona and Hepaicona cohorts between 2002 and 30thJune 2016 prior to which the alcohol information was infrequently collected.

The data carried little evidence for an interaction between HCV and alcohol consumption in relation to risk of SLD, once potential confounding factors had been accounted for. In other words, the impact of alcohol on risk of SLD was similar in PLWH with or without HCV infection, once the effect of factors such as mode HIV transmission and smoking status were taken into account.

Similarly there was some evidence overall for an association between hazardous drinking and risk of SLD after controlling for age, gender, nationality, region, calendar year enrolled, HIV related factors and HBV. However, after further adjustment for mode of HIV transmission, HCV infection and smoking, the strength of this association was attenuated. One possible explanation for the lack of an association in the adjusted analysis besides confounding, is low statistical power, as people enrolled in the Icona cohort prior to 2002 had to be excluded because a high proportion had missing data for the alcohol consumption variable. In addition, as in all observational studies, there is always a possibility of unmeasured confounding. However, the overall findings were consistent with those reported by other HIV cohorts [275, 301, 302].

In terms of implications for clinical and public health, the management of HIV/HCV coinfection in relation to drinking behaviours has historically been an important issue. For example, in the era of IFN/RBV treatment for HCV, excessive alcohol consumption was discouraged since treatment duration was up to 48 weeks and alcohol use was known to impact on the effectiveness of HCV treatment [451] [38]. In contrast, in the DAA era, treatment is as short as 8 weeks, and alcohol consumption may thus not interfere as much with the efficacy of therapy [451]. However, it remains key for public health authorities to assess whether alcohol consumption acts as a barrier in the HCV continuum care pathway. Other know barriers to DAA uptake are; personal beliefs about consequences of treatment, fear of diagnosis or treatment, negative experiences with health care services or even lack of knowledge for both patients and clinicians [408] [407].

In terms of implications for future research, the data also suggest that collection of alcohol consumption information in Icona and other HIV cohort studies needs to be improved. Greater standardisation between studies would also be beneficial to allow better comparison to enable uniform comparisons of the prevalence and risk associated with alcohol consumption between studies and settings. The WHO suggests that alcohol consumption should be included in the analyses as a dose-related risk factor, with no safe threshold as risk of liver disease is increased with heavy drinking [250]. In contrast, I used a slightly different categorical variable as this appeared to be the best use of the data collected. This classification incorporated frequency of drinking and type of drink (taking also exact units into account). The variable was useful to gauge prevalence of alcohol use in both cohorts and it was reassuring to see that it was predicting the risk of liver disease, suggesting that despite the presence of missing data mis-classification of the exposure was minimised.

Of note, the results included in this chapter have been already peer reviewed and a manuscript has been published [14] signalling the importance of the data. In addition, the created variable classifying individuals according to their level of

alcohol consumption was applied in all subsequent analyses of the Icona cohort including those described in the following chapters of this thesis (5 to 7). In these later analyses alcohol consumption was mainly included in the regression models as a confounder for other associations of interest.

8.3 HIV/HCV coinfection and risk of specific ARV drug discontinuation

In chapter 5, my analysis assessed the hypothesis that HCV infection might increase the risk of discontinuation of certain ARV drugs in PLWH. The rationale behind this analysis was that, the presence of HCV could increase the level of toxicity of certain drugs and decrease individuals tolerance leading to discontinuations. Confounding by indication was a challenge in this analysis as the use of some of the ARV drugs may be limited in PLWH who are coinfected with HCV.

At the time of the analysis, the literature included conflicting findings regarding the potential role of HCV infection on the risk of ARV drug discontinuation. Replication and failure of replication of results is an important step of scientific progress as scientific consensus is never typically built around the results of a single study. One key tool to establish consistency is that of ‘direct replication’ i.e. using the same methods on new data, for example collected in a different geographical setting, to replicate the results of another study. In this chapter I carried out an analysis very similar to that of another study conducted elsewhere in Europe although restricting to a cohort of PLWH seen for care in Italy with/without HCV infection. The other element of difference as compared to the previous EuroSIDA analysis is that I focused on HIV drugs recommended for use in Italy as of 2016. This analysis included approximately 10,600 HIV-positive people who started cART and I performed separate analyses for a set of twelve individual drugs. These included both NRTIs used as part of the modern backbone pairs: Abacavir, Lamivudine, Tenofovir, Emtricitabine, and anchor drugs in the remaining classes such as Efavirenz, Rilpivirine, Lopinavir/r, Darunavir, Atazanavir, Raltegravir,

Dolutegravir and Elvitegravir. The analysis carried little evidence for a difference in risk of cART discontinuation when considered as a whole for any reason between HCV-positive, HCV negative and the HCV unknown groups. However, individuals with a positive HCV serology were more likely to discontinue specific drugs (i.e. Darunavir/r) than the HCV negative or HCV unknown. When looking at participants' characteristics, older age, having diabetes, AIDS, advanced liver disease and HIV-related factors were also independently associated with risk of cART discontinuation.

The analysis also showed a decrease of the incidence of discontinuation due to toxicity/intolerance in more recent years, confirming that more modern drugs are safer and better tolerated. Interestingly, simplification emerged over the last few years as the most common reason for discontinuation. These simplifications mainly consist in keeping the same regimen but with a reduction in pill burden or even in reduction of the number of drugs used. These are often switches from triple therapy to INSTI-based dual therapy in PLWH with suppressed HIV-RNA..

In terms of implications for clinical practice, these findings indicate that, besides the rare exception of Darunavir/r, most of the other antiretrovirals used in the HIV mono-infected population appeared to be well tolerated also by the very small proportion of HIV/HCV coinfected population who did not achieve eradication with DAA. However, drug-drug interactions may still be present with first generation DAA and side-effects need to be monitored in the faction of population who might be still using these drugs.

Although an in-detail investigation into drug-drug interactions with DAA was beyond the scope of this chapter and of the thesis, it remains an important and somewhat under studied field of research.
The content of this chapter has also been peer reviewed and a manuscript has been published [452] signalling the importance of the data at that time.

8.4 HIV/HCV coinfection and potential barriers in the HCV care pathway in the era of HCV elimination

In chapter 6, I estimated the prevalence of HIV/HCV coinfected individuals presenting into care with advanced or late stage liver disease (the phenomenon of 'late HCV presentation'). Additionally, the study also assessed the hypothesis that late HCV presentation could be associated with risk of all-cause mortality as well as increased probability of initiating any HCV therapy. The analysis showed that 10-14% of the HIV/HCV coinfected population seen for care in Italy gets diagnosed with HCV when they already have advanced stage of liver disease. Although the estimates from Icona are not consistent with an increase in this prevalence over time, the proportion of late presenters appeared to be a stable with no evidence for a decline at least up to 2018.

The analysis also showed some evidence that late HCV presentation was associated with a higher risk of death in the unadjusted analysis. After controlling for other time-fixed confounders measured at time of HCV diagnosis such as age, gender, mode of HIV transmission, year of HCV diagnosis, CD4, HIV-RNA, alcohol, HBV status and region, the association was largely attenuated.

Overall, almost half of the study population was started on DAA regardless of stage of liver disease. The data showed that there was a tendency for individuals presenting with advanced or late stage liver disease to be more likely to start HCV therapy. This is explained by the fact that before DAA introduction, stage of liver disease was used as a criterion to prioritise treatment initiation.

In contrast, a sensitivity analysis including only people enrolled after 2014, when DAA became readily available, showed no differences in initiating DAA between those presenting late and not presenting late with HCV. These results indirectly confirm the hypothesis that stage of liver disease ceased to be a trigger for therapy initiation in the DAA era.

In terms of possible public health implications of this work, the analysis highlights that a low although non-negligible proportion of the HIV/HCV coinfected population (10-14%) presented for care with late HCV presentation and therefore opportunities to test and treat them earlier were missed.

The analysis is also important as it identified specific populations of PLWH (older people, men, those with low CD4) which are at higher risk of discovering their HCV coinfection when disease has already considerably progressed. Although, current recommendation is that all HIV/HCV coinfected individuals should be routinely tested for HCV (and for HCV-RNA if found HCVAb positive) regardless of individual participants' characteristics the data highlight the need to screen more aggressively in these identified sub-populations. This is even more important in reduced resources settings or in other settings in which it proves difficult to test everyone within a fixed time-frame. In general my findings highlight challenges in screening and diagnosis of HCV in the HIV infected population.

In terms of implications for future research, it remains unclear whether the lack of statistical significance for the association between late HCV presentation an risk of clinical outcome in my analysis was due to the small sample size or the univariable association was due to confounding. Further studies are needed to investigate this issue. Looking at the 10-14% estimate of late HCV presentation under a more positive light, the data suggest that HCV screening and assessing individuals' stage of liver disease is currently done in >90% of newly diagnosed HIV individuals and the majority of the tested were free of advanced liver disease.

In chapter 7, I assessed the hypothesis that despite universal access of DAA, regional differences in Italy in terms of HCV testing, DAA uptake and achieving SVR on treatment might still be present. The main analysis included HIV/HCV coinfected viraemic individuals enrolled in Hepaicona cohort after January 2015 when DAA became readily available.

The analysis indicated that some aspects of these measures of HCV care varied by geographical region in Italy. Specifically, HCV-RNA testing was more frequent in the North and Central regions compared to the South. The data analysis also showed that DAA treatment uptake was also lower in the South compared to the Northern regions. Reassuringly, no regional differences were observed in terms of the probability of achieving SVR among those who initiated DAA. Therefore, overall the data suggest that suboptimal health care settings and resources available in the South might have impacted on the rate of screening/treating for HCV. The observed variability in the rate of access to DAA represents a challenge for the achievement of the WHO target of >80% coverage of HCV therapy in Italy as well as other countries. This is also likely to have an impact on the prevalence of late HCV infection at the population level.

In terms of implication for clinical practice and other public health implications, identifying areas of Italy in which testing and treatment uptake is suboptimal is clearly important. Monitoring outcomes on the HCV care continuum pathway provides insight into the detection of potential barriers. This is important for policy makers who are in charge of deciding where to deploy more health care resources to help meet the 2030 elimination goals. Additionally it is worth mentioning that Italy is one of the countries with one of the most effective national plan/strategies in Europe and has reliable national epidemiological data to assess research questions relating to HCV elimination [160].

As a final point, it is worth noting the analyses in chapters 6 and 7 were carried out soon after 2015, the year of the introduction of modern DAA regimens in Italy so some of the findings are not fully updated using current data or might be less relevant now as the field is fast evolving. Indeed, more recent analyses led by the Icona Network group show higher rates of both HCV serology and HCV-RNA testing and DAA treatment uptake across all regions [229]. In terms of implications for future research, updated analyses using the same methods would highlight any improvement in the HCV continuum of care pathway in the country. Some data at

European levels have been recently reported including the impact of delay in data reporting [437].

8.5 Limitations

In each of the results chapters I discussed limitations of the specific analyses carried out. This section discusses overall limitations to be considered with specific examples.

8.5.1 Generalizability

Both the Icona and Hepaicona are large multicentre observational cohort studies including PLWH enrolled from clinical settings in different regions across the whole of Italy. The period of recruitment has spanned more than twenty years considering both cohorts. Individuals in the cohorts include adult men and women and all HIV modalities of transmissions are well represented. The Icona cohort became enriched in recent years with MSM and persons who acquired HIV thorough heterosexual contacts. However, as expected, when only looking at HIV/HCV coinfected individuals, the most frequent modality of HIV transmission is still PWID. Of note, as described in an early chapter of this thesis, the Hepaicona cohort is an older population of persons with longer history of HIV and treatment compared to patients participating in the Icona cohort. In addition, at study entry approximately 20% of HIV infected individuals in Icona had CD4 <200 cells/mm^3 compared to only 6% in Hepaicona. This is likely to be a direct consequence of the inclusion criteria as Icona enrols ART- naïve individuals and Hepaicona enrols ART-experienced individuals who only need to be DAA-naïve. Therefore individuals enrolled in Hepaicona are more likely to be individuals with extensive HIV treatment history, controlled HIV infection on cART and good immune recovery.

Both cohorts are assumed to be a fairly representative sample of the HIV population with/without HCV enrolled in care in the country. However, selection

bias cannot be ruled out as individuals consenting to enrol in either of the cohorts might be different from non-consenting individuals. There is no routine data for documenting basic demographics for all PLWH in Italy, therefore there is no method of assessing representativeness in both cohorts. Sample selection bias (i.e. collider bias) is more likely when individuals are actively recruited into a research study than when an opt out consent is used. Icona and Hepaicona are non-nested studies (in the sense that the whole population of HIV/HCV infected in Italy is unknown) and therefore it is not possible to directly test the presence of sample selection bias or collider bias.

Finally, some results were inconclusive with point estimates suggesting an association but with confidence intervals which were compatible with the null hypothesis of no difference. Occasionally the sample size was greatly reduced (e.g chapter 6) after including only individuals who strictly satisfied the inclusion criteria of the specific chapter and some of the analyses were likely to be underpowered.

8.5.2 Missing data

A proportion of the participants had missing data for some of the variables collected. Chapter 3 gives an overview of the pattern of missing data for a number of key variables used in the analyses for this thesis, describing also the proportion of participants with missing information over time. Missing data was an issue particularly for alcohol consumption, especially before a certain calendar time, but also for laboratory markers (e.g. HCV-RNA) and other socio-demographic factors such as level of employment and education. This is a pity because socio-demographic variables are key confounders in observational studies of PLWH and seldom collected in HIV cohorts.

Researchers are commonly faced with the problem of missing data in observational studies, which may introduce biased results as well as a loss of statistical power and precision. The STROBE [453, 454] and the ROBINS-I guidelines

proposed by *Sterne et al* [455] recommend that cohort studies report on the amount of missing data, reasons for non-participation or non-response and the methods used to handle missing data in the analyses. I used different approaches to handle missing data. A first approach was the *Missing Indicator Method* which is typically applied to categorical exposures. This amounts to including an extra category of the exposure variable for those participants with missing data. Then indicator variables are created for inclusion in the regression models, including an indicator for the missing data category [312]. This method is simple to implement, and one of the advantages is that no participants are excluded so that the denominator is the same in all analyses enabling comparisons of nested models using a standard likelihood test. However, the method can produce biased results in many settings, even when the data are 'Missing Completely At Random' (MCAR).

In chapter 4, I took an additional step and carried out multiple imputation (MI) by imputing values for participants with missing data on alcohol consumption. The imputation was performed using standard methods available in the SAS package, with appropriate model specifications to reflect the structure of the data. Importantly, the resulting MI estimates are valid if the missing data are 'Missing At Random' (MAR) [240, 312]. However, MI may produce biased estimates if the data are 'Missing Not At Random' (MNAR), which occurs when the study participants with missing data differ from the study participants with complete data in a manner that cannot be explained by the observed data in the study [240, 312]. Unfortunately, alcohol consumption itself could determine the rate of missingness for level of consumption thus potentially violating the MAR assumption.

In addition, although the data collection processes are similar in Icona and Hepaicona cohorts there were important differences in the protocols. For example, maximum level of education achieved is collected at baseline in Icona cohort but not in the Hepaicona cohort. Consequently, in analyses involving participants of both cohorts and in which education was a likely confounder of the association of interest it was not possible to control for this variable. For example in chapter 7

were education was identified as a potential confounder (effect of region of care on probability of HCV-RNA testing).

8.5.3 Confounding bias

Observational cohort studies are useful in assessing the usual or natural course of condition, and including multiple outcomes and exposures. They are fundamentally different from RCTs which are essentially experiments carried out under controlled conditions.

Although no study is likely on its own to prove causality, randomization reduces bias and provides a rigorous tool to examine cause-effect relationships between an intervention and outcome. This is because the act of randomization balances participant characteristics (both observed and unobserved) between the groups allowing attribution of any differences in outcome to the study exposure or intervention. This is not possible in observational studies as exposed and not exposed individuals are typically different for several other characteristics besides the intervention which can also affect the chance of developing the outcome.

Accounting for all source of confounding is certainly a challenge in observational studies. An example is ‘confounding by indication’ which can occur when prescription of a particular treatment is based on severity of disease which can also affect the outcome [456]. For example, in the pre universal access to DAA era, HCV treatment was prioritised to people with advanced liver disease. Therefore, in the analysis of an observational study comparing treated and untreated individuals, those who received treatment were likely to have advanced disease. Individuals with advanced disease are also likely to show poorer outcomes which may bias the treatment comparison [456].

Another example is ‘residual confounding’ which can occur if there is measurement error of a confounder included in the model [457]. For example, the mode of HIV

transmission variable includes the category PWID, however the data does not allow us to distinguish between ex-PWID or current PWID, and these categories are likely to have different probability of, say, achieving SVR post DAA so that bias due to misclassification of the exposure is likely.

Finally, the fundamental problem of the analysis of data collected in the observational setting is ‘unmeasured confounding’. This occurs when confounding is introduced by a factor which has not been collected.

In the last two chapters I made clear assumptions regarding the underlying causal structure of the data and I used a sophisticated algorithm written in R to identify minimal set of confounding variables for the various associations of interest (via the webpage DAGitty.net). These assumptions were illustrated by means of the transparent language of a direct acyclic graph (DAG). The use of this novel methodology increases the rigour of the analysis and decrease the chance of introducing bias in the estimate of the associations of interest.

8.5.4 Model building

At the start of my PhD, statistical model building in chapters 4 and 5 was approached by fitting sequential adjusted models including potential confounders identified to be predictors of the outcome in the univariate analyses. In Chapters 6 and 7 I have switched to the use of DAGs for model specification. The use of DAGs has exponentially increased in publications in recent years for analyses aiming to establish a causal link between exposure and outcome. The method is heavily reliant on strong, mainly untestable, assumptions especially when background knowledge is sparse.

Construction of DAGs is challenging in nature as wrong specifications can lead to incorrect inferences. The classic case being misclassifying confounders, colliders and mediators. However, one of the main strengths of using DAGs lie in the

transparency of these assumptions by visual representation and the possibility to change the model specifications should new information become available. The other key advantage of using DAG is that by focussing on a single exposure at the time, I was able to identify potential confounders for such an exposure of interest and reported results separately from those of other models, thus avoiding what is indicated by *Sanders Greenland et al* as 'the fallacy of Table 2' [401].

Another advantage of using the 'back door path' rule to remove confounding is the identification of minimal sets of factors which are sufficient to block all the biasing paths, which reduces the risk of overfitting the model. A strength of the analyses included in this thesis, is the fact that similar results were obtained when I used alternative set of confounders indicated by the DAGitty software as such a result would not be expected under a mis-specified model. The main findings were often in line with those reported in the literature in similar settings.

DAGs also helps the identification of possible sources of unmeasured confounding which seriously limit the possibility of establishing causal links in the observational setting. For example, when assessing the causal relationship between region of care and the probability of achieving SVR in chapter 7, adherence was one of the unmeasured factors that needed to be considered. Fortunately, under our set of assumptions, adherence was not a confounder for the association of interest so it was still possible to identify a set of measured confounders which was sufficient to establish conditional exchangeability and estimate the causal effect of region care on probability of achieving SVR.

Furthermore, as mentioned previously, another challenge is that the directionality assumptions for the relationships between variables are not always known or easy to establish especially in the cross-sectional context. For example in chapter 7, I specified a causal relationship between region of care and probability of HCV-RNA but this relationship may be bidirectional. Specifically, the assumption was

employment may influence where you receive care, but it would also the other way around.

8.6 Conclusion

In conclusion, the data presented in this thesis suggest that in the current DAA era the population of HIV/HCV coinfected individuals in Italy are receiving satisfactory levels of care in terms of HCV screening and treatment. The Icona and Hepaicona data indicate that although there is variability across regions, WHO targets for HCV testing are met in most areas of the country [11]. Moreover, the analyses have also identified important risk factors as well as regional differences in terms of patients' access to HCV testing and care.

In addition, the analyses have highlighted that late HCV presentation was common even in the DAA era and did not show a decrease in recent years suggesting that more efforts are also needed to improve education about HCV testing especially in those identified to be at higher risk (older people, males, those with CD4≤ 200 cells/mm^3 and PWID) of presenting late with HCV. Because HCV can now be eradicated in the HIV/HCV co-infected individuals, late diagnosis should be minimised to reduce the risk of all-cause mortality and enable a faster route to HCV elimination. Finally, despite a national health system with universal coverage of DAA, Italy still needs to improve the HCV cascade of care from testing to treatment.

Important clinical questions remain that were not addressed in this thesis. For example, evaluating re-infection rates of HCV and how much this could impact on the time to achieve the WHO HCV global elimination targets. Additional studies are also needed to evaluate the long-term risk of HCC or other hepatic and extra-hepatic comorbidities in individuals cured with DAA. Similarly, monitoring adherence of treatment and the investigation of long-term effects of DAA use on outcomes of interest such as progression of liver disease also warrant further

studies. Additionally, following the introduction of PREP, it will be interesting to evaluate the impact of HCV on the rate of HIV transmission.

Finally, given the current situation that we have been living over the past 1.5 year with the COVID-19 pandemic, a key question is how this and other future pandemics might impact on the HCV continuum of care of PLWH. Indeed, there are data showing that the pandemic has had an effect on HCV testing and DAA uptake since March 2020 in a number of countries[400].

CHAPTER 9

9 APPENDICES

Figure 9.1 Publication from chapter 4

Shanyinde et al. BMC Public Health (2019) 19:1291
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RESEARCH ARTICLE Open Access

Is physician assessment of alcohol consumption useful in predicting risk of severe liver disease among people with HIV and HIV/HCV co-infection?

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Abstract

Background: Alcohol consumption is a known risk factor for liver disease in HIV-infected populations. Therefore, knowledge of alcohol consumption behaviour and risk of disease progression associated with hazardous drinking are important in the overall management of HIV disease. We aimed at assessing the usefulness of routine data collected on alcohol consumption in predicting risk of severe liver disease (SLD) among people living with HIV (PLWHIV) with or without hepatitis C infection seen for routine clinical care in Italy.

Methods: We included PLWHIV from two observational cohorts in Italy (ICONA and HepaICONA). Alcohol consumption was assessed by physician interview and categorized according to the National Institute for Food and Nutrition Italian guidelines into four categories: abstainer; moderate; hazardous and unknown. SLD was defined as presence of FIB4 > 3.25 or a clinical diagnosis of liver disease or liver-related death. Cox regression analysis was used to evaluate the association between level of alcohol consumption at baseline and risk of SLD.

Results: Among 9542 included PLWHIV the distribution of alcohol consumption categories was: abstainers 3422 (36%), moderate drinkers 2279 (23%), hazardous drinkers 637 (7%) and unknown 3204 (34%). Compared to moderate drinkers, hazardous drinking was associated with higher risk of SLD (adjusted hazard ratio, aHR = 1.45; 95% CI: 1.03–2.03). After additionally controlling for mode of HIV transmission, HCV infection and smoking, the association was attenuated (aHR = 1.32; 95% CI: 0.94–1.85). There was no evidence that the association was stronger when restricting to the HIV/HCV co-infected population.

Conclusions: Using a brief physician interview, we found evidence for an association between hazardous alcohol consumption and subsequent risk of SLD among PLWHIV, but this was not independent of HIV mode of transmission, HCV-infection and smoking. More efforts should be made to improve quality and validity of data on alcohol consumption in cohorts of HIV/HCV-infected individuals.

Keywords: HIV-infected, HIV/HCV co-infection, Alcohol consumption, Severe liver disease

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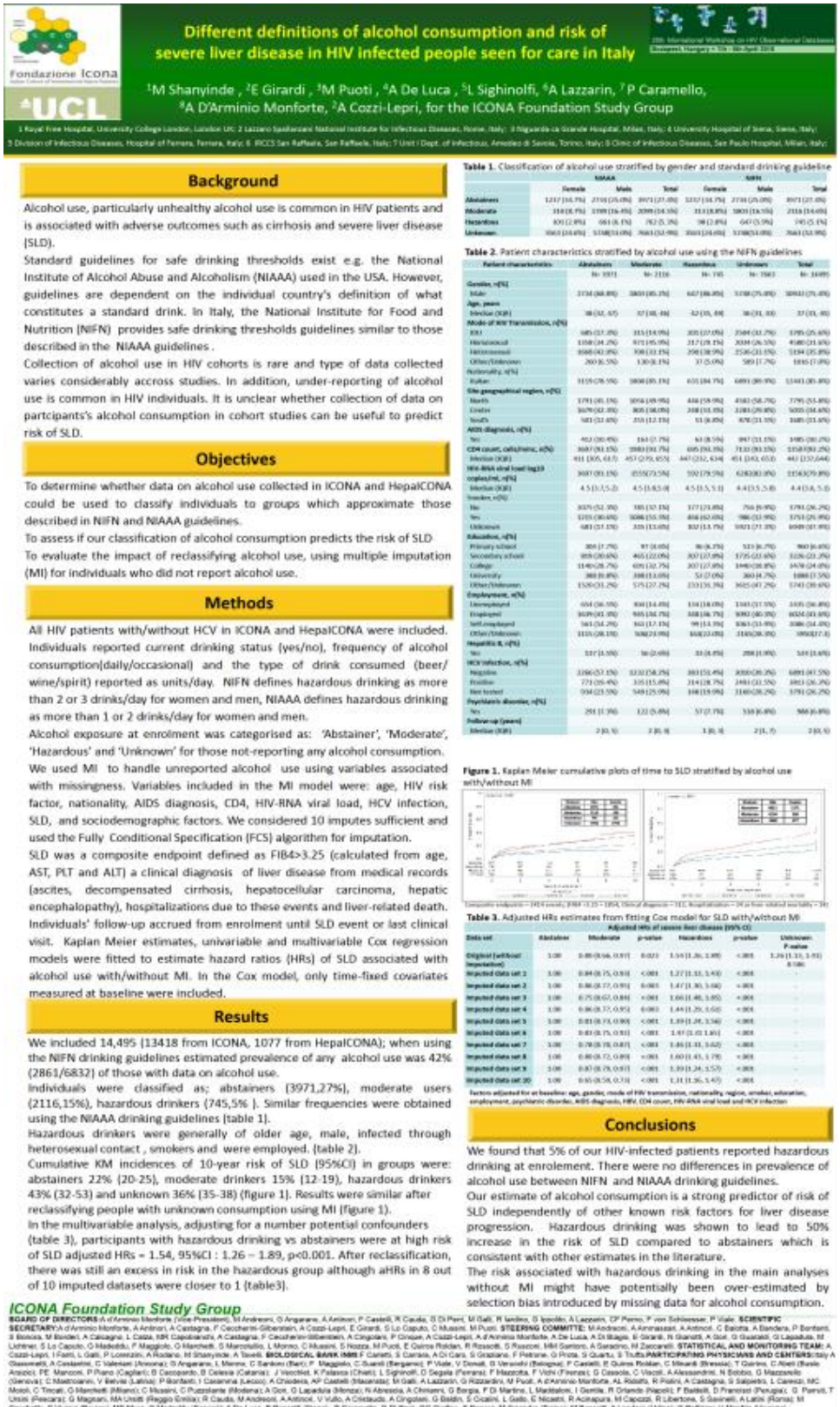
Figure 9.2 Poster publication for IWHOD 2016



Different definitions of alcohol consumption and risk of severe liver disease in HIV infected people seen for care in Italy

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Background

Alcohol use, particularly unhealthy alcohol use is common in HIV patients and is associated with adverse outcomes such as cirrhosis and severe liver disease (SLD).

Standard guidelines for safe drinking thresholds exist e.g. the National Institute of Alcohol Abuse and Alcoholism (NIAAA) used in the USA. However, guidelines are dependent on the individual country's definition of what constitutes a standard drink. In Italy, the National Institute for Food and Nutrition (NIFN) provides safe drinking thresholds guidelines similar to those described in the NIAAA guidelines .

Collection of alcohol use in HIV cohorts is rare and type of data collected varies considerably accross studies. In addition, under-reporting of alcohol use is common in HIV individuals. It is unclear whether collection of data on partcipants's alcohol consumption in cohort studies can be useful to predict risk of SLD.

Objectives

To determine whether data on alcohol use collected in ICONA and HepaICONA could be used to classify individuals to groups which approximate those described in NIFN and NIAAA guidelines.

To assess if our classification of alcohol consumption predicts the risk of SLD

To evaluate the impact of reclassifying alcohol use, using multiple imputation (MI) for individuals who did not report alcohol use.

Methods

All HIV patients with/without HCV in ICONA and HepaICONA were included. Individuals reported current drinking status (yes/no), frequency of alcohol consumption(daily/occasional) and the type of drink consumed (beer/ wine/spirit) reported as units/day. NIFN defines hazardous drinking as more than 2 or 3 drinks/day for women and men, NIAAA defines hazardous drinking as more than 1 or 2 drinks/day for women and men.

Alcohol exposure at enrolment was categorised as: 'Abstainer', 'Moderate', 'Hazardous' and 'Unknown' for those not-reporting any alcohol consumption.

We used MI to handle unreported alcohol use using variables associated with missingness. Variables included in the MI model were: age, HIV risk factor, nationality, AIDS diagnosis, CD4, HIV-RNA viral load, HCV infection, SLD, and sociodemographic factors. We considered 10 imputes sufficient and used the Fully Conditional Specification (FCS) algorithm for imputation.

SLD was a composite endpoint defined as FIB4>3.25 (calculated from age, AST, PLT and ALT) a clinical diagnosis of liver disease from medical records (ascites, decompensated cirrhosis, hepatocellular carcinoma, hepatic encephalopathy), hospitalizations due to these events and liver-related death. Individuals' follow-up accrued from enrolment until SLD event or last clinical visit. Kaplan Meier estimates, univariable and multivariable Cox regression models were fitted to estimate hazard ratios (HRs) of SLD associated with alcohol use with/without MI. In the Cox model, only time-fixed covariates measured at baseline were included.

Results

We included 14,495 (13418 from ICONA, 1077 from HepaICONA); when using the NIFN drinking guidelines estimated prevalence of any alcohol use was 42% (2861/6832) of those with data on alcohol use.

Individuals were classified as; abstainers (3971,27%), moderate users (2116,15%), hazardous drinkers (745,5%). Similar frequencies were obtained using the NIAAA drinking guidelines (table 1).

Hazardous drinkers were generally of older age, male, infected through heterosexual contact , smokers and were employed. (table 2).

Cumulative KM incidences of 10-year risk of SLD (95%CI) in groups were: abstainers 22% (20-25), moderate drinkers 15% (12-19), hazardous drinkers 43% (32-53) and unknown 36% (35-38) (figure 1). Results were similar after reclassifying people with unknown consumption using MI (figure 1).

In the multivariable analysis, adjusting for a number potential confounders (table 3), participants with hazardous drinking vs abstainers were at high risk of SLD adjusted HRs = 1.54, 95%CI : 1.26 – 1.89, p<0.001. After reclassification, there was still an excess in risk in the hazardous group although aHRs in 8 out of 10 imputed datasets were closer to 1 (table3).

Table 1. Classification of alcohol use stratified by gender and standard drinking guideline

	NIAAA			NIFN		
	Female	Male	Total	Female	Male	Total
Abstainers	1217 (34.7%)	2710 (25.0%)	3971 (27.4%)	1217 (34.7%)	2710 (25.0%)	3971 (27.4%)
Moderate	314 (8.7%)	1799 (16.6%)	2099 (14.5%)	313 (8.8%)	1803 (16.5%)	2116 (14.6%)
Hazardous	83 (2.8%)	661 (6.1%)	762 (5.3%)	98 (2.8%)	647 (5.9%)	745 (5.1%)
Unknown	1963 (54.8%)	5768 (53.3%)	7661 (52.9%)	1963 (54.8%)	5768 (53.3%)	7661 (52.9%)

Table 2. Patient characteristics stratified by alcohol use using the NIFN guidelines

Patient characteristics	Abstainers	Moderate	Hazardous	Unknown	Total
	N= 3971	N= 2116	N= 745	N= 7661	N= 14495
Gender, n(%)					
Male	2710 (68.8%)	1803 (85.2%)	647 (86.8%)	5768 (75.3%)	10923 (75.4%)
Age, years					
Median (IQR)	38 (32, 47)	37 (30, 44)	42 (35, 49)	38 (31, 46)	37 (31, 45)
Mode of HIV Transmission, n(%)					
IDU	685 (17.3%)	315 (14.9%)	205 (27.5%)	2584 (33.7%)	3795 (26.2%)
Heterosexual	1358 (34.2%)	971 (45.9%)	217 (29.1%)	3034 (39.6%)	5580 (38.5%)
Heterosexual	1048 (41.9%)	708 (33.5%)	298 (38.9%)	2536 (33.1%)	5194 (35.8%)
Other/Unknown	260 (6.5%)	130 (6.1%)	37 (5.0%)	509 (6.7%)	1036 (7.0%)
Nationality, n(%)					
Italian	3159 (79.5%)	1808 (85.1%)	655 (84.7%)	6891 (89.9%)	12441 (85.8%)
Site geographical region, n(%)					
North	1791 (45.1%)	1056 (49.9%)	444 (59.6%)	4561 (59.7%)	7791 (53.8%)
Centre	1679 (42.3%)	805 (38.0%)	258 (34.6%)	2181 (28.4%)	5035 (34.6%)
South	501 (12.6%)	255 (12.1%)	43 (5.8%)	919 (12.0%)	1669 (11.6%)
AIDS diagnosis, n(%)					
Yes	402 (10.4%)	164 (7.7%)	63 (8.5%)	847 (11.1%)	1485 (10.2%)
CD4 count, cells/mmc, n(%)	3687 (93.1%)	1980 (93.7%)	695 (93.3%)	7133 (93.1%)	13558 (93.2%)
Median (IQR)	411 (305, 613)	457 (329, 653)	447 (332, 634)	451 (343, 653)	442 (327,644)
HIV-RNA viral load log10 copies/ml, n(%)	3697 (93.1%)	2155(73.5%)	592 (79.5%)	6282(82.0%)	12436(79.8%)
Median (IQR)	4.5 (3.5,5.2)	4.5 (3.8,5.0)	4.5 (3.5, 5.1)	4.4 (3.5, 5.0)	4.4 (3.6, 5.1)
Transfer, n(%)					
No	3079 (52.3%)	585 (37.1%)	177 (23.8%)	756 (9.9%)	3791 (26.2%)
Yes	1255 (30.6%)	1086 (55.3%)	466 (62.6%)	986 (13.9%)	3753 (25.9%)
Unknown	681 (17.1%)	335 (13.6%)	102 (13.7%)	5921 (71.3%)	6949 (47.9%)
Education, n(%)					
Primary school	304 (7.7%)	97 (4.6%)	36 (4.7%)	533 (6.7%)	960 (6.6%)
Secondary school	959 (30.6%)	465 (22.0%)	207 (27.8%)	1775 (22.6%)	3126 (22.2%)
College	1140 (28.7%)	695 (32.7%)	207 (27.8%)	1440 (18.8%)	3478 (24.0%)
University	360 (9.8%)	388 (13.6%)	51 (7.0%)	360 (4.7%)	1888 (13.5%)
Other/Unknown	1509 (31.2%)	575 (27.2%)	233 (31.3%)	3605 (47.2%)	5743 (39.6%)
Employment, n(%)					
Unemployed	654 (16.5%)	304 (14.4%)	134 (18.0%)	1383 (17.5%)	2335 (16.8%)
Employed	1639 (41.3%)	945 (44.7%)	348 (46.7%)	3092 (40.3%)	6024 (41.6%)
Self-employed	561 (14.2%)	362 (17.1%)	99 (13.3%)	3063 (13.9%)	3086 (14.4%)
Other/Unknown	1115 (28.1%)	504(23.9%)	164(22.0%)	3160(28.9%)	3950(27.3)
Hepatitis B, n(%)					
Yes	137 (3.5%)	56 (2.6%)	33 (4.4%)	288 (3.8%)	514 (3.6%)
HCV infection, n(%)					
Negative	2266 (57.1%)	1132 (58.2%)	383 (51.4%)	3930 (51.3%)	6893 (47.5%)
Positive	771 (19.4%)	335 (15.8%)	214 (28.7%)	2491 (32.5%)	3811 (26.3%)
Not tested	934 (23.5%)	549 (25.9%)	148 (19.9%)	3160 (28.2%)	3791 (26.2%)
Psychiatric disorder, n(%)					
Yes	251 (7.3%)	121 (5.8%)	57 (7.7%)	538 (6.8%)	966 (6.8%)
Follow-up (years)					
Median (IQR)	2 (0, 5)	2 (0, 5)	1 (0, 3)	2 (1, 7)	2 (0, 5)

Figure 1. Kaplan Meier cumulative plots of time to SLD stratified by alcohol use with/without MI

Table 3. Adjusted HRs estimates from fitting Cox model for SLD with/without MI

	Adjusted HRs of severe liver disease (95% CI)					
Data set	Abstainer	Moderate	p-value	Hazardous	p-value	Unknown P value
Original (without imputation)	1.00	0.89 (0.66, 0.97)	0.021	1.54 (1.26, 1.89)	<.001	1.26 (1.11, 1.41) 0.586
Imputed data set 1	1.00	0.84 (0.75, 0.93)	<.001	1.27 (1.11, 1.43)	<.001	-
Imputed data set 2	1.00	0.86 (0.77, 0.95)	0.003	1.47 (1.30, 1.66)	<.001	-
Imputed data set 3	1.00	0.75 (0.67, 0.84)	<.001	1.66 (1.48, 1.85)	<.001	-
Imputed data set 4	1.00	0.86 (0.77, 0.95)	0.003	1.44 (1.29, 1.60)	<.001	-
Imputed data set 5	1.00	0.81 (0.73, 0.90)	<.001	1.39 (1.24, 1.56)	<.001	-
Imputed data set 6	1.00	0.83 (0.75, 0.91)	<.001	1.47 (1.31, 1.65)	<.001	-
Imputed data set 7	1.00	0.78 (0.70, 0.87)	<.001	1.46 (1.31, 1.62)	<.001	-
Imputed data set 8	1.00	0.86 (0.72, 0.89)	<.001	1.60 (1.43, 1.79)	<.001	-
Imputed data set 9	1.00	0.87 (0.79, 0.97)	<.001	1.39 (1.24, 1.57)	<.001	-
Imputed data set 10	1.00	0.85 (0.78, 0.73)	<.001	1.31 (1.16, 1.47)	<.001	-

Factors adjusted for at baseline: age, gender, mode of HIV transmission, nationality, region, smoker, education, employment, psychiatric disorder, AIDS diagnosis, HBV, CD4 count, HIV-RNA viral load and HCV infection

Conclusions

We found that 5% of our HIV-infected patients reported hazardous drinking at enrolement. There were no differences in prevalence of alcohol use between NIFN and NIAAA drinking guidelines.

Our estimate of alcohol consumption is a strong predictor of risk of SLD independently of other known risk factors for liver disease progression. Hazardous drinking was shown to lead to 50% increase in the risk of SLD compared to abstainers which is consistent with other estimates in the literature.

The risk associated with hazardous drinking in the main analyses without MI might have potentially been over-estimated by selection bias introduced by missing data for alcohol consumption.

Figure 9.3 Publication from chapter 5

European Journal of Clinical Microbiology & Infectious Diseases (2018) 37:871–881
https://doi.org/10.1007/s10096-017-3180-8

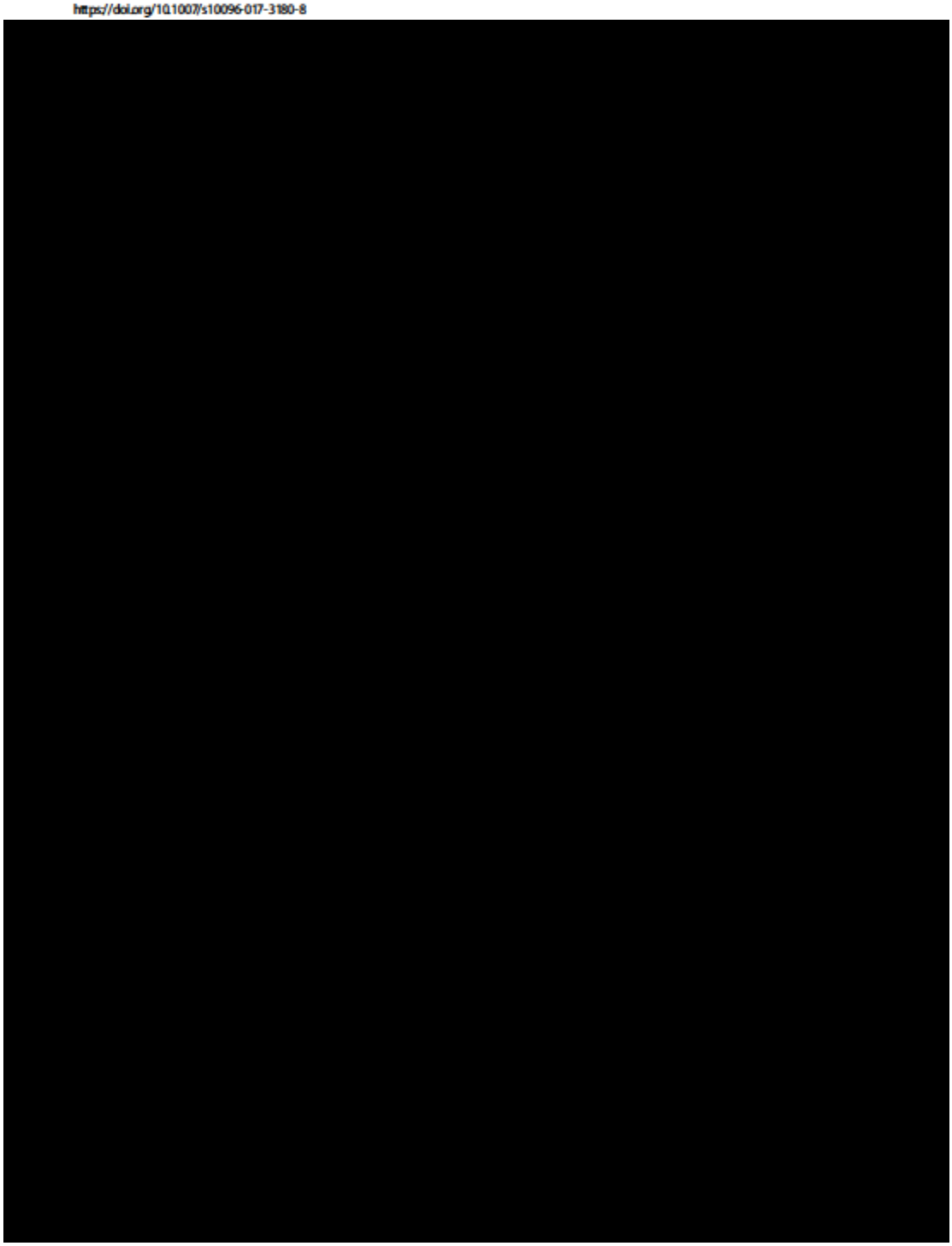

Figure 9.4 Other publications I collaborated in (I did the analysis)

PLOS ONE

RESEARCH ARTICLE

HIV-1 co-receptor tropism and liver fibrosis in HIV-infected patients

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Abstract

Background

In vitro, gp120 of both X4 and R5 HIV-1 strains activates human hepatic stellate cells, but if it can promote liver fibrosis *in vivo* is unknown. We aimed to evaluate if patients carrying X4 or R5 strains have a different liver fibrosis (LF) progression over time.

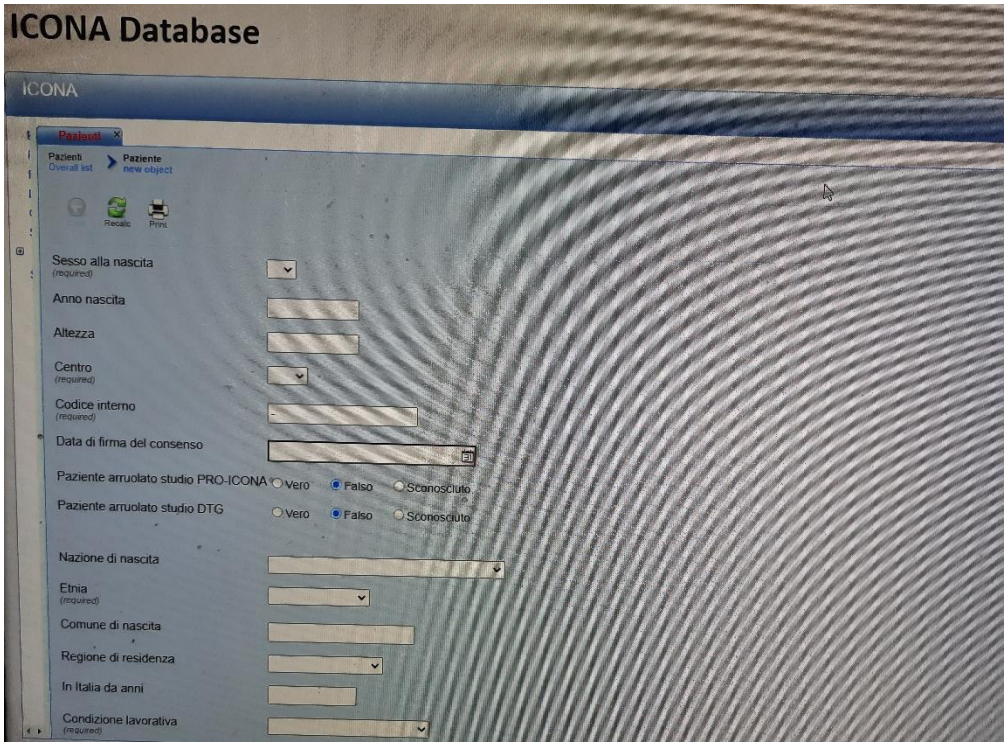
Methods

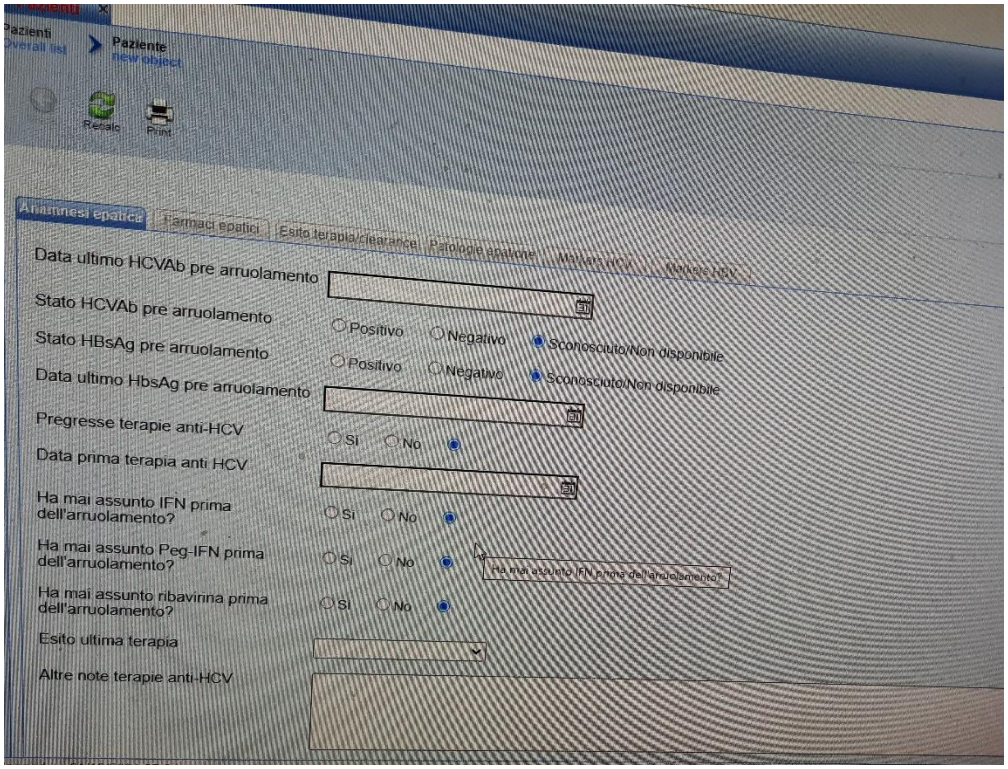
A total of 1,137 HIV-infected patients in ICONA cohort (21% females, 7% HCV co-infected) with an available determination of HIV-1 co-receptor tropism (CRT), a Fibrosis-4 Index for Liver Fibrosis (FIB-4) <3.25 and at least one-year follow-up were included. CRT was assessed by gp120 sequencing on plasma RNA and geno2pheno algorithm (10% false positive rate) or by Trofile. LF was assessed by means of FIB-4. LF progression was defined as an absolute score increase or a transition to higher fibrosis stratum and/or occurrence of liver-related clinical events.

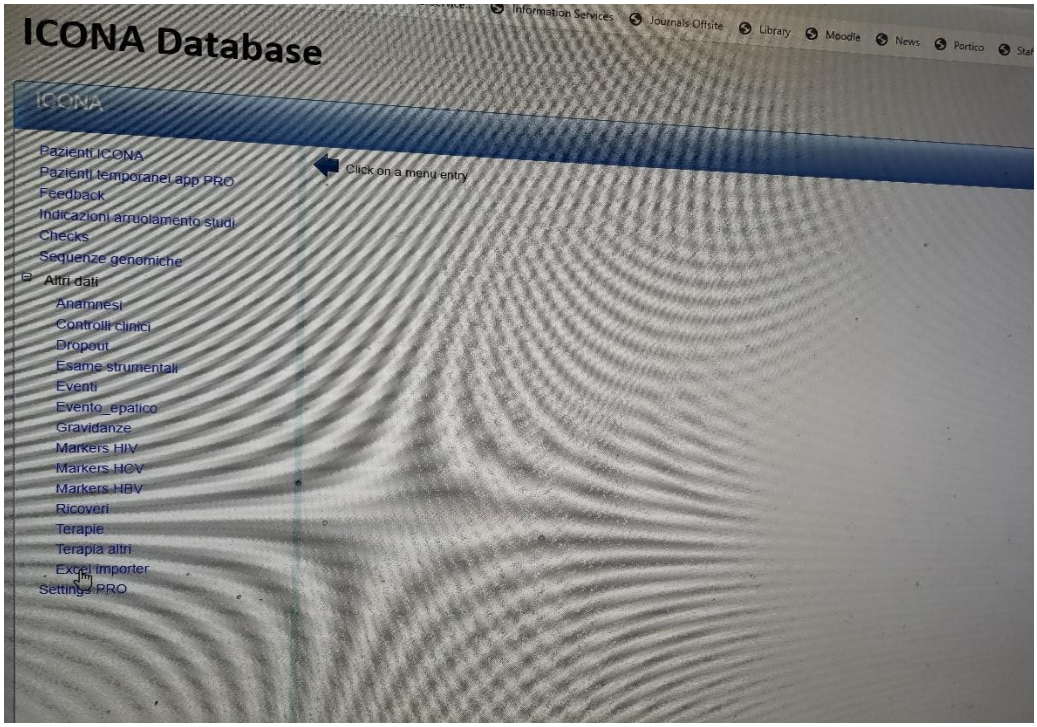
Results

A total of 249 (22%) patients carried X4 strains, which were associated with older age, lower CD4 count, lower nadir CD4, and intravenous drug use. Overall, X4 and R5 patients had similar baseline FIB-4 scores and similar mean FIB-4 slope after a median follow-up of 35 months. There was no difference between X4 and R5 for time to LF progression (p = 0.925). Estimated risk of LF at 24 months (95% CI) after baseline in X4 and R5 was 10.6% (8.3–12.9) and 9.9% (5.9–14.0), respectively. Age, HCV co-infection, diabetes, HIV-duration,

Figure 9.5 A snapshot of some of the eCRFs



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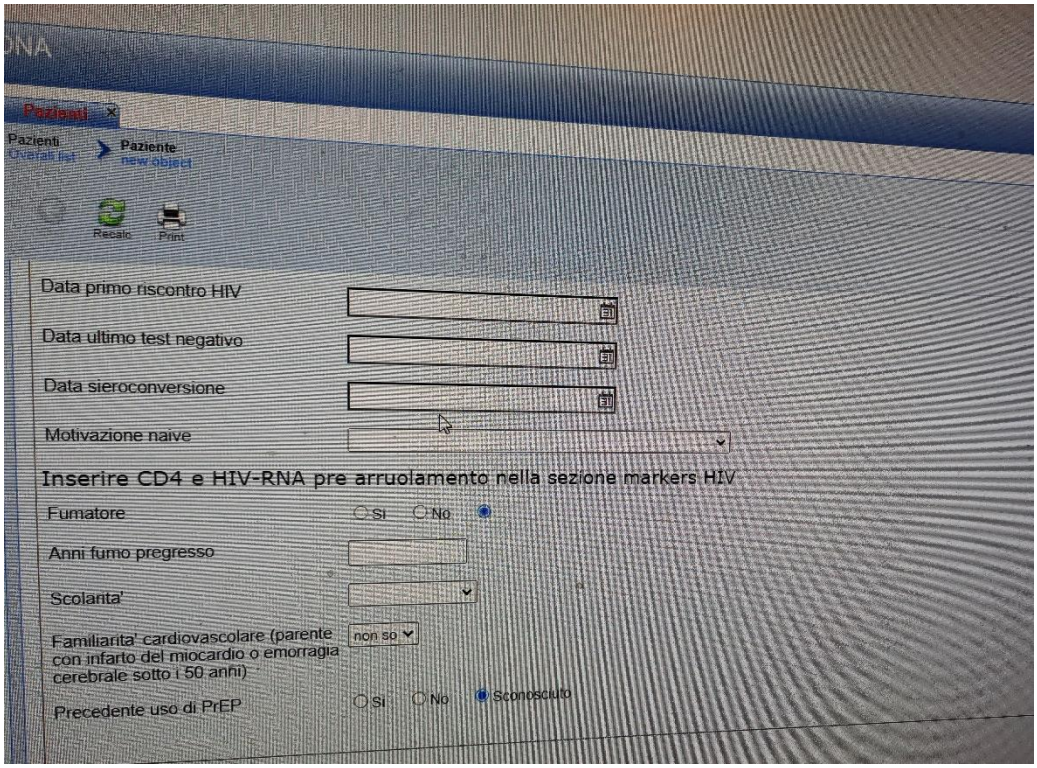
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Inserire CD4 e HIV-RNA pre arruolamento nella sezione markers HIV
Fumatore
Si
No
Anni fumo pregresso
Scolarita'
Familiarita' cardiovascolare (parente con infarto del miocardio o emorragia cerebrale sotto i 50 anni)
non so
Precedente uso di PrEP
Si
No
Sconosciuto

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