TOWARDS ELIMINATION OF HEPATITIS C IN VIETNAM

Barnaby F Flower

A thesis submitted to Imperial College London, U.K

For the degree of Doctor of Philosophy in the field of Medicine

Oxford University Clinical Research Unit

Hospital for Tropical Diseases

Ho Chi Minh City, Viet Nam

November 2022

Abstract

Vietnam has a high burden of viral hepatitis. This thesis strives to advance its elimination, addressing important gaps in the literature that I hope will contribute to treatment guidelines and health policy, both in Vietnam and internationally.

Firstly, to define the hepatitis epidemic in Vietnam, I assimilate all published seroprevalence data since 1990 to estimate pooled prevalence of HBV, HCV and HDV in high and low risk populations. I show that although blood safety has improved, and HDV is largely confined to high-risk populations, a renewed focus on birth dose HBV vaccination and targeted HCV screening and treatment of people who inject drugs, is urgently required to meet elimination targets.

The next chapters address HCV therapy, namely predictive factors for selecting individuals who could be treated for shorter duration, treatment failure in relation to rare HCV subtypes, and the clinical importance of resistance mutations. I describe a prospective clinical trial evaluating the efficacy of shortened sofosbuvir and daclatasvir therapy, based on early virological response: firstly, in genotype 1 or 6-infected individuals with mild disease (chapter 3) and then in genotype 6-infected individuals with advanced liver fibrosis (chapter 4). I show that shortened therapy, with retreatment if needed, can reduce antiviral use while maintaining high cure rates, but that day 2 virologic response alone is not an adequate predictor of cure. I demonstrate that a high frequency of putative NS5A inhibitor resistance mutations in genotype 6 infection does not impact cure rates, negating the need for costly genotyping in Vietnam.

In my final data chapter, I explore an innovative means of decentralising HCV care. In two independent study populations from Vietnam and the UK, I show that an increase in routinely taken alanine transaminase after HCV therapy is a reliable screen for treatment failure that could substantially reduce reliance on nucleic acid testing in remote and resource-limited settings.

Acknowledgements

I would like to thank my supervisors, Professor Graham Cooke and Professor Jeremy Day. Without their inspiration and encouragement, I would not have embarked on this thesis, let alone completed it. Graham initiated and devised the SEARCH study, long before I arrived in Vietnam and has been a motivational influence throughout. Over the last four years he has invited me into several successful collaborations on COVID-19 and viral hepatitis, and I will always be grateful for the faith he showed in me as a fledgling academic. He went out of his way to support me and my growing family through various turbulent periods of the COVID-19 pandemic and everything I have achieved during my time in Vietnam can be attributed to his trust, support, creative thinking, and broad knowledge of the field.

Jeremy, despite being a distinguished expert in cryptococcal meningitis, welcomed our viral hepatitis work in Vietnam and has proved a sensitive and intelligent leader. I have benefited enormously from his clinical trial expertise and his long-standing relationship with the Hospital for Tropical Disease (HTD). I will cherish our conversations on trial-design, p values, and the broader impacts of research, which have frequently happened spontaneously over a strong *cà phê nóng* (coffee) or a watery lager with bobbing ice cubes. Jeremy invited me and my wife into his house on our very first day in Vietnam, and increasingly became a role-model and mentor, with whom I have appreciated sharing many laughs.

I would also like to thank the Wellcome Trust, who funded this research, and the patients of the HTD who embraced our clinical trials with enthusiasm and patience. The SEARCH study was only made possible through the hard work of colleagues at OUCRU and the HTD, too many to mention, who I have always found to been kind, highly competent and impressively conscientious. Special mention must be made of Thuan Dang Trong, our clinical trials coordinator; Hang Vu Thi Kim, who helped with the data collection in chapter two; Duc Du Hong, Leanne McCabe, and Phuong Nguyen Thi Ngoc, who supervised all the statistical analysis; and Azim Ansari who helped me navigate the field of whole genome sequencing and led the genomics work in Oxford. I am extremely grateful for the time and expertise these people have shared with me.

I also thank Professors Sarah Fidler and Graham Taylor, who conducted my Early-Stage Assessment and Late Stage Review: their comments and encouragement were invaluable. Finally, I would like to acknowledge the support I have received from my wife Vanny, and my children Unni and Vincent, who have provided welcome joy and distraction throughout.

Declaration

The work presented here is my own except where explicitly stated otherwise. Chapter two, a systematic review and meta-analysis of seroprevalence studies, was designed by me, with supervision from Graham Cooke. It was registered it in my name with the Centre for Reviews and Dissemination (CRD) in September 2020 (PROSPERO CRD42020202567) and conforms to its original protocol. Some aspects of the methodology were adapted from a 2015 paper evaluating Hepatitis C seroprevalence and HIV co-infection in sub-Saharan Africa[1]. Duc Du Hong, an OUCRU statistician, helped me generate the code for the DerSimonian-Laird random-effects model on R version 4.1.

The SEARCH study (described in chapters 3 and 4) was a collaboration of multiple co-investigators from OUCRU, the HTD, Imperial College London, Oxford University, University College London (UCL), and Mahidol Oxford Tropical Medicine Research Unit (MORU) in Bangkok. It was conceived by Graham Cooke and Professor Sarah Walker in 2017, with the latter responsible for statistical aspects of trial design. As clinical research fellow at OUCRU, I oversaw delivery of the trial, analysed and summarised the data and wrote the manuscripts for both resulting papers. Leanne McCabe (UCL) curated the data and performed statistical analysis with supervision from Sarah Walker. Azim Ansari (Oxford) a group leader in the Experimental Medicine Division of Nuffield Department of Medicine and expert in statistical genomics, performed the viral sequencing and host polymorphism analysis described in chapter 3. Professor Joel Tarning (MORU) designed the pharmacokinetic study and its analysis was performed by Richard Hoglund.

For chapter 5, the idea to conduct performance analysis of post-treatment ALT and AST changes in detecting HCV treatment failure was entirely my own. Jeremy Day and Graham Cooke supervised study design. Transaminase data from STOPHCV1 was provided by Leanne McCabe with permission of STOPHCV1 investigators. Phuong Nguyen Thi Ngoc helped me generate receiver operator curves on IBM® SPSS®.

Copyright

Abbreviations

&	And
Δ	Delta (difference)
µg	Microgram
µM	Micromole
µL	Microliter
AASLD	American Association for the Study of Liver Disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
APASL	Asian Pacific Association for the Study of the Liver
DAA	Direct Acting Antiviral
DCV	Daclatasvir
DNA	Deoxyribose nucleic acid
EASL	European Association for the Study of the Liver
EOT	End of treatment
EOT+12	12 weeks after end of treatment
Gt1-6	Genotype 1-6
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
HCMC	Ho Chi Minh City
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HCVcAg	Hepatitis C virus core antigen
HDV	Hepatitis D virus
HIV	Human immunodeficiency virus
HTD	Hospital for Tropical Disease
INR	International Normalised Ratio
LDV	Ledipasvir

LFT	Liver function test
LLOQ	Lower limit of quantification
MORU	Mahidol Oxford Tropical Medicine Research Unit
NS5A-i	Non-structural protein 5A inhibitor
NS5B-i	Non-structural protein 5B inhibitor
OUCRU	Oxford University Clinical Research Unit
PEG-IFN	PEGylated Interferon
PK	Pharmacokinetics
PD	Pharmacodynamics
PI	Protease inhibitor
RAS	Resistance-associated substitution
Rbv	Ribavirin
RNA	Ribonucleic acid
RT-PCR	Reverse transcription Polymerase Chain Reaction
SOF	Sofosbuvir
SVR	Sustained virological response
SVR12	SVR 12 weeks after end of treatment
VEL	Velpatasvir
VL	Viral load
VND	Vietnamese Dong (currency)
WHO	World Health Organisation

Table of Contents

List of Figures

List of Tables

Introduction

Hepatitis C virus (HCV) is a major global health problem. The World Health Organisation (WHO) estimate that in 2019, 58 million people were living with HCV and 290,000 people died of chronic infection[2]. While this mortality estimate is high and comparable with other major infections, such as all-cause meningitis[3], it belies the true disease burden: HCV infection was responsible for some 15·3 million disability adjusted life years (DALYs) in 2019 (95% uncertainty interval 13·3–17·5), constituting 0·6% (0·5–0·7) of total global DALYs[4].

This morbidity is a consequence of HCV's ability to evade and supress the host immune response, such that around 50-85% of infections result in chronic viral replication in the liver. Up to 75% of infected individuals develop immune- or inflammatory-mediated extrahepatic manifestations of HCV[5], and approximately 10-20% develop cirrhosis or hepatocellular cancer over a 20-30 year period[5,6]. Disease progression is highly variable, but, overall, the quality of life of individuals with chronic HCV is lower than that of the general population[7]. The Global Burden of Disease (GBD) initiative estimates acute hepatitis, cirrhosis, and liver cancer contribute 1·7% (0·9–2·5), 79·5% (76·1–82·7), and 18·9% (15·9–22·2) to HCV-related DALYs, respectively[4]. Cirrhosis and liver failure in working age adults is therefore the main driver of HCV-morbidity and continues to have devastating consequences for families, society, and health systems worldwide.

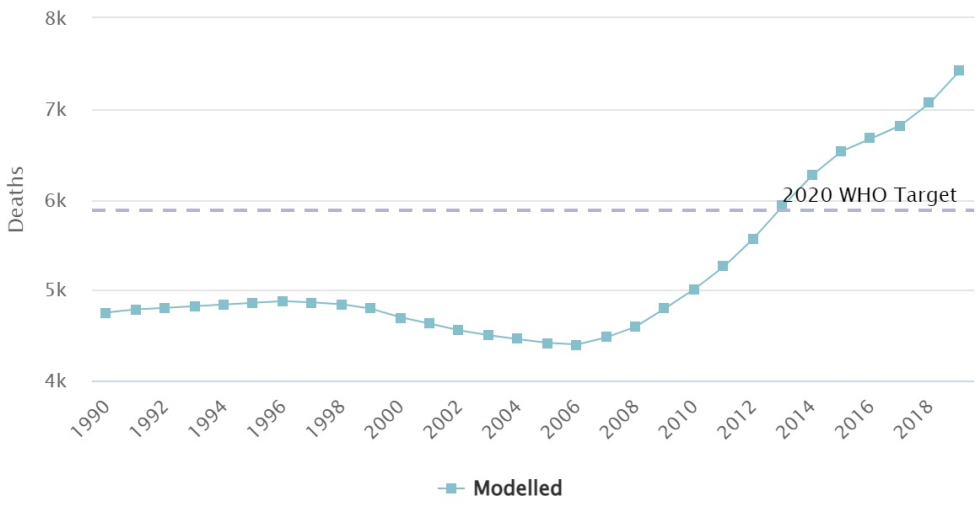
Hearteningly, these sequelae are easily averted. The emergence of oral, well-tolerated, highly efficacious direct acting antiviral (DAA) therapy since 2014 has revolutionized HCV treatment, such that cure rates of over 95% are achievable with just 8-12 weeks of once-daily tablets. This development prompted the WHO in 2016, to adopt a global hepatitis strategy to eliminate viral hepatitis as a public health threat by 2030, targeting a 90% reduction in incident cases of hepatitis B and C and a 65% reduction in mortality[8]. To meet this objective, 80% of treatment-eligible individuals with HCV will need access to care.

This target is hugely ambitious. Worldwide, an estimated 58 million persons were living with HCV in 2019, but only around 21% were diagnosed and only 13% had access to treatment[9]. To achieve the goals of the WHO global strategy over the next eight years, new infections from HCV need to be reduced from around 1.5 million new cases (20 per 100,000) in 2020 to 350,000 (5 per 100,000) by 2030, and deaths from HCV must be reduced from 290,000 (5 per

100,000) to less than 140,000 deaths (2 per 100,000). This requires massive expansion in the availability of prevention, diagnostic and treatment services in low and middle-income countries (LMICs)[8].

Vietnam, population 97.3 million[10], is one of 31 countries reported to shoulder 80% of the global burden of HCV[11], and remains a long way off WHO's 2030 elimination targets. In 2019 there were an estimated 7,415 (95% C.I 5218 – 10,185) HCV-related deaths[12]. This figure continues to climb year on year, far exceeding WHO's 2020 target of 5850 (figure 1). Data on diagnosis and linkage to care are sparse, but less than 20% of active infections are thought have been diagnosed, and far fewer treated.

Figure Intro-1-1 Modelled HCV-related deaths in Vietnam, 1990-2019.



From the Coalition for Global Hepatitis Elimination. www.globalhep.org

Of added importance, Vietnam is virtually unique as a high-burden country in its high prevalence of genotype 6 infection. With 29 confirmed subtypes and 21 unassigned subtypes, genotype 6 is the most genetically diverse genotype, increasing the probability of naturally occurring resistance mutations which could affect treatment outcomes. Estimates of genotype 6 prevalence in Vietnam range from 34 – 55%[13–15], but it is rare outside of Southeast Asia,

and clinical trial data for DAAs are lacking. In 2018 WHO highlighted an urgent need for research into rare HCV genotypes and subtypes[16].

Screening, diagnosis, linkage to care, and access to affordable DAA drugs will all be fundamental to achieving 2030 elimination targets. In Vietnam and other resource-limited, high-burden settings, these issues are now principally political: the onus is on health ministries to invest in screening and procurement of generic antivirals, decentralise health services, and expand prevention services, such as opioid substitution therapy and needle exchange programmes for people who inject drugs (PWID). The WHO HCV treatment guidelines were updated in 2022 with a call to 'urgently simplify hepatitis care, while using innovative diagnostics to make care more accessible to more people in need'[17].

Two of the studies described in this thesis are firmly grounded in these present concerns: in chapter two, I present a systematic review and meta-analysis of HBV, HCV and HDV seroprevalence in Vietnam, that describes the viral hepatitis epidemic in different population groups, with a view to informing elimination policy. In chapter five I investigate whether monitoring liver enzyme levels in blood after HCV treatment could offer a low-cost means of screening for HCV treatment failure in remote and resource-limited settings, reducing reliance on centralised, costly, nucleic acid testing.

The intervening chapters are focused on the HCV epidemic's endgame, which will be increasingly defined by difficult-to-treat infections in populations under-served by existing models of care, such as persons who inject drugs, and marginalised communities with limited access to health care and high rates of loss to follow-up. The SEARCH study is a prospective, two-strata pilot study, which explores important research questions raised in the 2018 WHO guidelines[16] relating to HCV treatment: namely, predictive factors for selecting individuals who could be treated for shorter duration, treatment failure in relation to rare HCV subtypes, and the clinical importance of resistance-associated mutations.

In the following section I will discuss what is known about HCV infection, including its origins, discovery, epidemiology, natural history, virology and the evolution of HCV therapeutics. I will review the literature on predictors of response to DAA therapy, before describing what is known about the viral hepatitis epidemic in Vietnam, including the important contributions made by HBV and HDV.

Chapter 1

Literature Review

HCV Origins

The precise origins of HCV, (genus Hepacivirus, family Flaviviridae) as a human pathogen are unknown, but like most human viruses it appears to have entered the human population from one or multiple zoonotic events with subsequent diversification through human to human transmission[18]. Up until 2011 HCV was the only confirmed member of the Hepaciviruses genus, but others have since been identified in several domestic and wild mammals, with the largest viral diversity observed in bats[19] and rodents[20] and the closest relatives of HCV found in horses/donkeys and dogs[21]. It has been proposed mechanical transmission by biting insects such as tabanids could have originally connected dogs, horses and human hosts but this particular cross-species transmission remains speculative[22].

The existence of genetically diverse endemic HCV strains in specific geographic locations, (such as rare genotype 4 subtypes in in Central and West sub-Saharan Africa, and genotype 6 lineages in South and Southeast Asia) implies a long-standing association of HCV with human populations. Selection-informed evolutionary models, date the common ancestor of current circulating HCV genotypes to at least 3000 years ago (95% CI: 3192–5221 years ago), with the oldest, most genetically diverse genotypes endemic to Asia[18]. However, the worldwide explosion of the HCV epidemic occurred more recently, in the 1930s–1940s driven by unsafe administration of parenteral medical treatments, immunisations, blood transfusion and haemodialysis, and increasing injecting drug use in the community[23].

Discovery

HCV's discovery dates to the 1970s, when a virus other than Hepatitis A (HAV) or B (HBV), was suspected to be causing transfusion-associated hepatitis with a chronic progressive course[24]. For several years this phenomenon was labelled as 'non-A, non-B hepatitis' (NANBH). Work by Harvey Alter and colleagues at the National Institutes of Health (NIH)

showed that acute infection was generally asymptomatic and rarely severe or fatal, but would lead to chronic infection in most patients[25]. They deduced that chronic infection was generally asymptomatic but accompanied by chronic hepatitis that could, typically after decades of infection, result in cirrhosis, end-stage liver disease, and hepatocellular carcinoma. By 1978 Alter and others had shown that, NANBH was transmissible to chimpanzees[26,27] causing clinically apparent hepatitis 2–10 weeks after the injection with histological evidence of hepatitis on liver biopsy. They observed the infectious agent had characteristics of an enveloped virus. However, approaches that had worked to detect the likes of Hepatitis A (HAV) and HBV, such as immunofluorescence, immunodiffusion, enzyme-linked immunoassay, radioimmunoassay, immune electron microscopy, and cell culture, were unsuccessful in isolating the pathogen. In the absence of a specific test, NANBH remained a diagnosis of exclusion. It soon became evident that disease transmission was not limited to blood transfusions and could occur via other blood-derived products, such as heat-treated factor VIII concentrate given to haemophiliacs[28], as well as via intravenous drug use[29], and haemodialysis[30].

The breakthrough came in 1989, when Michael Houghton and colleagues at the Chiron Corporation used a technique that combined the power of molecular biology with the specificity of antigen–antibody reactions, that would ultimately pave the way to modern virology diagnostics. They created a library of all DNA and RNA found in infected Chimpanzee plasma and then used bacteriophage-expressed clones to screen serum from non-A, non-B-infected patients - who they anticipated would possess antibodies to the virus. More than one million clones were screened before they identified a RNA molecule, consisting of at least 9,000 nucleotides, that was specific for non-A non-B hepatitis[31]. The specificity of their approach was proven when serologic assays using phage-expressed polypeptide were further evaluated with Harvey Alter's collection of samples from posttransfusion hepatitis[32]. The virus was named Hepatitis C and designated a flavivirus. Comparisons of different patients' clones revealed a high genetic diversity with differences of up to 33% of nucleotides between individual clones, leading to the classification of HCV into different genotypes[33].

HCV's precise structure and function didn't become clear until a couple of years later, when Charles Rice, a virologist at the University of Washington, applied his knowledge of Flaviviridae and molecular expertise to characterise different genetic regions of HCV and ultimately construct a complete cDNA clone of the viral genome[34]. In the absence of a cell culture system, RNA transcribed from the cDNA clone had to be inoculated directly into a

chimpanzee liver. Subsequent studies of a cell-culture–adapted strain of HCV provided a cornerstone for the understanding of the genomic structure and replicative cycle of HCV. In 1999, the first robust in vitro HCV replicon model was established, based on a genotype 1b clone transfected into a hepatoma cell line[35]. But while these early cell culture systems were able to produce subgenomic replicons, they failed to produce infectious viral particles. This limited scientists' ability to directly test candidate therapeutics for their antiviral activity in vitro, and largely explains the 'long' wait for efficacious direct acting antiviral therapy. This limitation was finally overcome in 2005, with the establishment of a replicon model based on a genotype 2 clone, which demonstrated that the produced viral particles could infect a human hepatoma cell line[36]. Highly efficacious pangenotypic antiviral therapy emerged within a decade of this discovery.

In 2020, during the writing of this thesis, Alter, Houghton and Rice were jointly awarded the Nobel Prize in Physiology or Medicine for their collaborative efforts in the discovery of HCV[37].

Implications of discovery

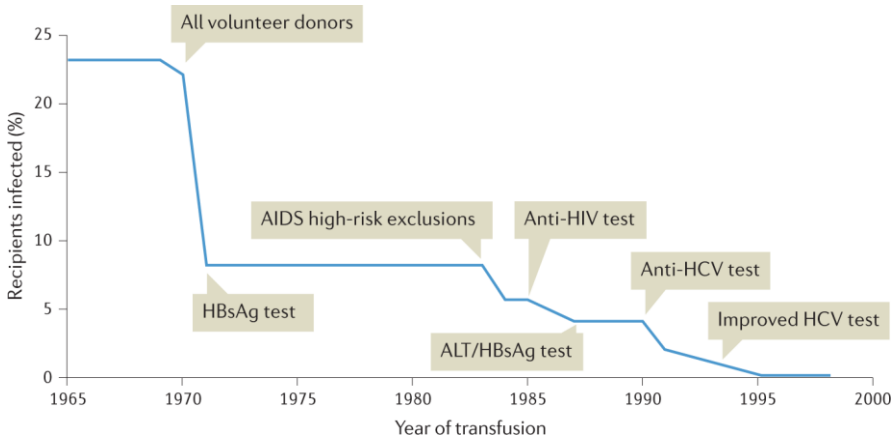
Blood Safety

The first major consequence of HCV's identification was improvement in blood safety. In the UK, between 1970 and 1991, it is estimated 26,800 people were infected with HCV through blood donations, contributing to around 1,820 deaths[38]. Even before HCV was identified, Alter's interest in non-A, non-B hepatitis led to improved screening of blood donors for historical risk factors, serologic evidence of hepatitis B infection (HBsAg and anti-HBc), and elevated serum ALT in serum, leading to a substantial reduction in the rates of post-transfusion hepatitis (figure 2).

Houghton and colleagues helped develop the first HCV antibody test in 1990, which consisted of a fusion protein (C100-3) made up of the HCV polypeptide and the human superoxide dismutase, coated on microtitre plates, where it was capable of capturing circulating HCV antibodies. Additional radioactive antibodies were then used to identify the captured HCV antibodies[39]. Increasingly sensitive commercial enzyme-linked immunosorbent assays soon followed, enabling mass screening of blood. Within months, the incidence of post-transfusion hepatitis C in high-income settings was dramatically reduced[40] (figure 1-1).

The subsequent introduction of minipool nucleic acid amplification screening for HCV RNA in 1999 proved highly effective in identifying HCV in donor blood taken during the window period before seroconversion[41]. This virtually eliminated post-transfusion HCV in high-income settings (figure 1-1). Unfortunately, in resource-limited settings, including Vietnam[42], healthcare-related transmission of HCV continues to be a problem[43].

Figure 1-1 Impact of measures on incidence of post-transfusion NANBH (latterly HCV)



Reproduced from Manns, M.P., Maasoumy, B. Breakthroughs in hepatitis C research: from discovery to cure. Nat Rev Gastroenterol Hepatol 19, 533–550 (2022). with permission via licence with Springer Nature (license number 5398130917788)

Epidemiology

In addition to enabling the provision of safe blood, the development of anti-HCV testing shed light on the epidemiology of HCV. The association with liver cirrhosis and HCC was confirmed[44], organ transplant was implicated for the first time[45], and an extremely high prevalence of chronic infection was revealed in haemophiliacs, patients on haemodialysis and PWID[46,47]. This, and the burgeoning HIV epidemic, led to rapid improvements in healthcare safety. Reuse of needles, syringes, multiple-use medication vials and infusion bags was prohibited, and surgical equipment had to be sterilized to higher standards. Preventative interventions targeting high-risk groups were implemented, such as needle and syringe programmes (NSP) and opioid substitution therapy (OST). These have proven highly effective in reducing HCV transmission[48]. Unfortunately, these strategies remain illegal, unavailable, or regionally limited in many high-burden countries[49].

Sexual transmission was found to be largely limited to heterosexuals with multiple sexual partners and men who have sex with men (MSM), with no increased risk of sexual transmission of HCV among heterosexual couples in regular relationships[50]. Among MSM, rectal shedding of HCV in infected individuals was reported[51]. HIV co-infection, and certain sexual practices (e.g. unprotected receptive anal sex) were shown to carry higher risk of transmission[50,52].

Mother-to-child transmission was shown to be far less common than in HBV, but it remains an important source of infection. Transmission to new-borns is estimated to occur in 6% of HCV-mono-infected mothers and 11% of mothers with HIV-HCV co-infection[53]. Mode of delivery and breast feeding do not appear to influence transmission in mono-infected mothers.

Implementation of good public health and infection control policy in high-income countries has meant the HCV epidemic is now largely confined to individuals with a history of injecting drugs or high-risk sexual activity[43]. In LMICs, however, a high proportion of infections still result of unsafe health-care associated activities including injections[54], dialysis[42], blood transfusions and dental[55] and surgical procedures[42]. Use of contaminated equipment in barbershops, tattoo parlors[56], piercing salons[57] and alternative medicine clinics[58] also contributes to community spread. Understanding HCV prevalence in high- and low- risk groups in Vietnam, and how it has changed in recent decades, is one of the major objectives of chapter one.

Global Distribution

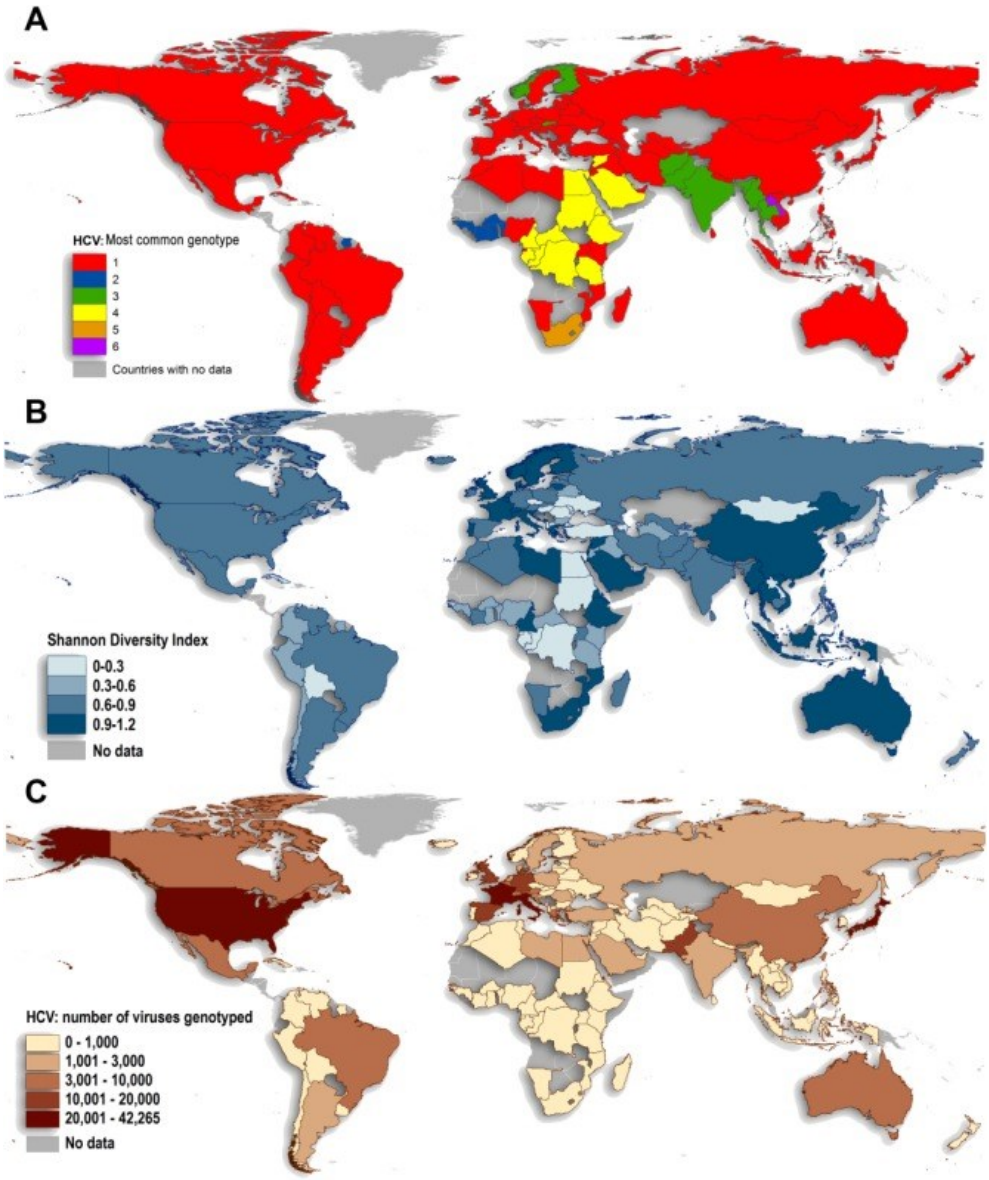
Expansion of HCV antibody testing in the 1990s revealed the global scale of the HCV epidemic. The highest prevalence (>2%) was found in the Eastern Mediterranean region, West Africa (>2%) and Eastern Europe, with lower prevalence in the Americas, Southeast Asia and the Western Pacific. Egypt was noted to have an unusually high seroprevalence of genotype 4 infection, which would later be attributed to healthcare-associated transmission through a national parenteral anti-schistosomal treatment programme[59].

Advances in molecular sequencing shed light on HCV's high genetic diversity and enabled regional mapping of HCV genotypes and subtypes. Six major genotypes of HCV were originally defined[33,60], and this has subsequently been expanded to eight[61]. More than 86 subtypes have been sequenced[5].

HCV genotype 1 is the most prevalent worldwide, and in 2013 was estimated to comprise 46.2% of all HCV cases[62]. Genotype 2 comprises around 9.1% globally, with a higher prevalence in West Africa, central Latin America and Southeast Asia. Genotype 3 (30.1% of global infections) predominates in India, East Asia and Australia and Genotype 4 (8.3%) is the most common lineage in North Africa and increasingly found among PWID in the southern Mediterranean. Genotype 5 is the rarest of the original six HCV lineages (<1% of total infections) and is largely confined to South Africa. Genotype 6 comprises around 5.4% of infections globally but is highly prevalent in Vietnam (34.6%), Laos (95.6%), Cambodia (45.7%) and Myanmar (27%)[63]. Genotype 7 was identified in the Democratic Republic of the Congo in 2014[64] and genotype 8 was first reported in 2018 in Canada in 4 patients originally from the Punjab in India[61].

Figure 1-2 panel A shows the most common genotypes by country. Panel B displays the Shannon Diversity Index by country, ranging from 0 (where the epidemic is comprised of larger proportions of a few genotypes) to 1.2 (where the epidemic is comprised of smaller proportions of many genotypes). Panel C shows the number of viruses genotyped in 2013.

Figure 1-2: Global HCV genotype distribution and diversity



Reproduced from Messina JP et al. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015 Jan;61(1):77-87. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Natural history

Our understanding of the natural history of HCV infection has advanced greatly since Alter's initial observations regarding transmission of non-A, non-B hepatitis following blood transfusion.

Acute HCV

Once inoculated into the bloodstream, HCV enters the liver through sinusoidal capillaries, where it penetrates hepatocytes through a complex multi-step process, involving several cellular factors that trigger virus uptake[65]. Viral replication begins within days, leading to detectable HCV RNA in peripheral blood within a week of infecton[66]. Its arrival is readily detected by host sensing machinery, leading to production of type 1 interferons (IFN-1) and the activation of downstream viral targets[67]. However, an adaptive, HCV-specific immune response and its associated clinical sequelae, take weeks to develop. The reasons for this delay are not well understood but it is apparent that the magnitude, diversity, and quality of the adaptive immune response during incubation, ultimately determines symptomatology and progression to chronic disease.

Acute infection, which refers to the first six months following exposure, is typically anicteric and asymptomatic, with less than a quarter of cases clinically apparent[68]. Symptoms, when they do occur, emerge between 2 and 26 weeks post-exposure (mean 7-8 weeks) and include malaise, nausea, anorexia, right upper quadrant abdominal pain, jaundice and dark urine[69]. Such illness may last weeks to months. Acute infection is typically associated with elevated transaminase levels (indicative of liver inflammation), which may be 10-20 times the upper limit of normal. Fulminant hepatitis is very rare (<1% cases of HCV) but occurs more frequently in individuals with HBV co-infection[70], HIV and in the context of immunosuppression[71].

Acute HCV (following first exposure) is diagnosed by the detection of HCV RNA in blood with undetectable anti-HCV antibodies (which only become detectable around 12 weeks after exposure). In those with anti-HCV antibodies, newly detectable HCV RNA with documentation of undetectable HCV RNA within the preceding six months is also diagnostic of acute infection. In the absence of recent results, the distinction between acute HCV

infection in a previously exposed individual and newly discovered chronic infection can be problematic, since in both cases patients have detectable HCV RNA, HCV antibodies, and elevated serum aminotransferases.

Persistence of HCV beyond six months defines chronic infection and is estimated to occur in 50-85% of infections. This contrasts importantly with HBV, which is cleared from blood and liver in >95% of individuals exposed in adulthood (with development of lasting protective immunity), and HIV, which becomes chronic in all cases.

The precise mechanism underlying HCV's tendency to persist is not well known, but it is thought to be a consequence of both virus and host factors. With regards to the virus, a high mutation rate (see HCV genome and viral replication below) leads to substantial genetic diversity, which helps the virus escape immune recognition. A range of host factors have been shown to determine spontaneous clearance of HCV. Chief among these is a genetic polymorphism close to the chromosomal locus IFNL4 (previously known as IL28B). In one study of 1008 patients, a favourable allele at this locus was associated with clearance in approximately 50% cases, compared with only 16-20% in patients with an unfavourable allele[72]. Favourable alleles are more common in patients of European and Asian ancestry compared with those of African ancestry[72,73].

Other factors associated with spontaneous clearance of HCV include being female[6], infection during childhood[74] and a more overt inflammatory response to acute infection[75]. The latter is characterised by clinical signs and symptoms such as fever, jaundice, and higher levels of transaminases[76]. Immune responses favouring clearance are high titres of neutralizing antibodies against HCV structural proteins[77], host neutralizing responses that target viral entry[78], persistence of an HCV-specific CD4 T-cell response[79], and high interferon γ-induced protein-10 concentrations in blood[76].

Chronic HCV

Ongoing HCV replication induces a dramatic decrease in the activity of CD8+ cytotoxic T lymphocytes and CD4+ Th cells in the liver without achieving viral clearance. T cell dysfunction appears to be restricted to HCV-specific CD8+ T cells, with influenza-specific CD8+ cells shown to remain functional in individuals with chronic HCV infection[80].

Chronic infection, once established, is slowly progressive and frequently does not result in clinically apparent liver disease. Around 5-30% chronically infected individuals develop cirrhosis over a 20- to 30-year period. A 2008 systematic review of 111 studies evaluating the natural history of HCV infection estimated that the prevalence of cirrhosis 20 years after infection was 16% overall (95% CI 14-19%)[81]. However, estimates were significantly influenced by whether studies were retrospective (17-55%) or prospective (7-16%) or conducted in clinical or non-clinical settings. Historically, studies involving patients presenting with clinical signs of chronic hepatitis, referred to specialist clinics, report high risks of progression to cirrhosis and HCC[82] while more inclusive studies report better outcomes. A large French study of 2235 HCV-infected individuals found around a third never progress to cirrhosis or do not progress for at least 50 years[83].

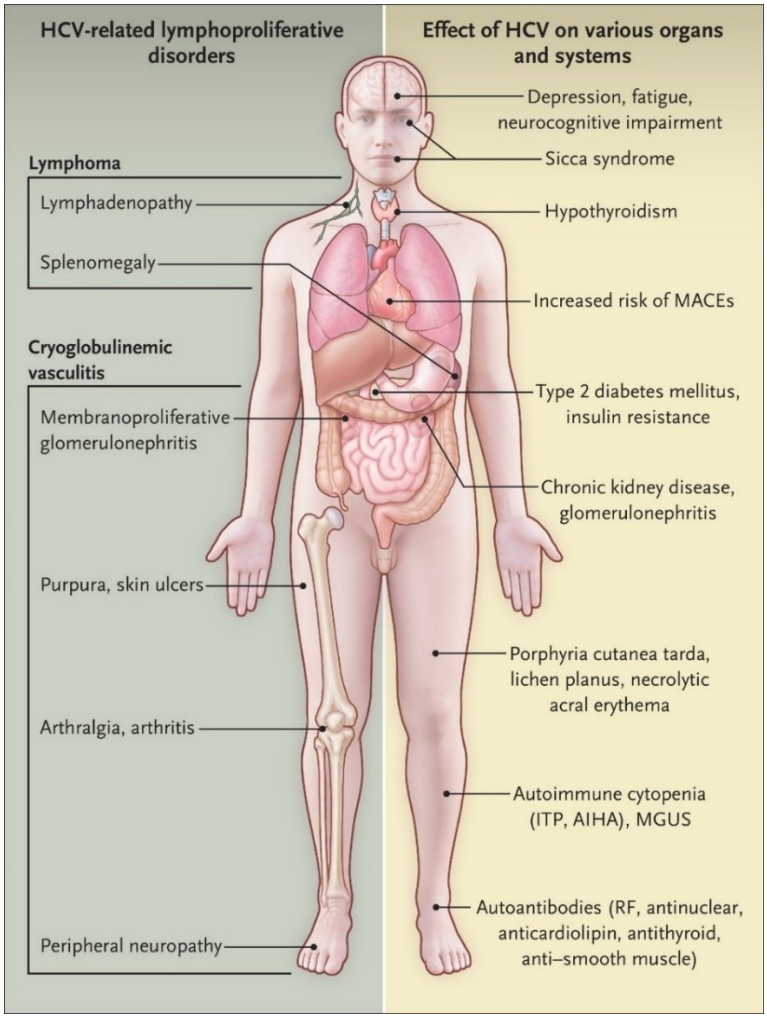
Liver disease is accelerated in certain subgroups of the population including those acquiring HCV later in life, males, the immunosuppressed, and those with HIV-infection, obesity, or history of alcohol excess[81]. Once an individual develops advanced fibrosis, the risk of progression to cirrhosis increases significantly, at around 10% per year[84]. Those who develop cirrhosis are at increased risk of infections and complicating events such as variceal haemorrhage, ascites, and encephalopathy. These associated morbidities are substantial and place a high burden on the health system as well as the individual.

Five-year risk of hepatocellular carcinoma (HCC) varies from 1%, in individuals with mild liver disease, to 13% in patients with cirrhosis and is also known to be increased in males, older individuals[83], HBV co-infection, diabetes, and both alcoholic and non-alcoholic hepatic steatosis. Infection with HCV genotypes 3 and 6 has also been implicated with increased risk of HCC but this remains controversial[71,85].

Patients diagnosed with chronic HCV report a high symptom burden, and the quality of life of individuals with chronic HCV is lower than that of the general population[86]. However, as with many chronic diseases, the extent to which HCV infection itself, rather than comorbid conditions, contributes to symptoms is not clear. The most frequent complaints are fatigue, sleep disturbances, nausea, anorexia, myalgia, arthralgia, diarrhoea, abdominal pain, weight loss and neuropsychiatric symptoms (e.g. depression and anxiety)[87]. Chronic HCV infection has also been associated with cognitive impairment, independent of the severity of liver disease, but the mechanism of this is not well understood[88].

A broad range of extrahepatic manifestations and immune- or inflammatory-mediated events occur in up to 75% of those with chronic infection, including atherosclerotic cardiovascular disease[89], renal disease (type 1 membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis, and interstitial nephritis), mixed cryoglobulinaemia vasculitis, type 2 diabetes, lymphoproliferative disease (non-Hodgkin lymphoma and hepatosplenic T-cell lymphoma), skin disease (porphyria cutanea tarda and lichen planus), thyroid disease (Hashimoto's thyroiditis and Graves' disease), and eye disease (Mooren's ulcers and Sjögren's syndrome)[5,90]. Numerus studies have shown a reduction in both the incidence and severity of virtually all extrahepatic manifestations of HCV infection after cure of HCV infection[91].

Figure 1-3: Extrahepatic manifestations of chronic HCV infection



Reproduced with permission from Cacoub et al, 2021[91], Copyright Massachusetts Medical Society.

Laboratory abnormalities

Liver transaminase (ALT and AST) levels fluctuate over time in individuals with chronic HCV and are normal in around one third of patients[71]. Elevated levels of ALT have been associated with progressive increase in risk of liver-related death, while patients with normal transaminases are more likely to have mild fibrosis on liver biopsy[92]. However there is generally a poor correlation between ALT and liver histology[93]. Acute increases in ALT may be observed without other obvious cause but it is not clear how frequently this occurs.

HCV RNA levels in blood are the major marker of active viral replication, but there is little correlation between viral load and serum aminotransferase levels or severity of liver disease[94]. Following the acute phase of infection HCV RNA levels remain broadly constant, although substantial fluctuations can occur. In one study that evaluated changes in HCV RNA over five years in a cohort of 818 mostly HIV-co-infected individuals, variations in HCV viral load >1 log occurred in just 15% and variations >0.5 log occurred in 44%[95].

Other laboratory abnormalities associated with chronic HCV infection are generally related to liver disease or extrahepatic manifestations, and include thyroid and kidney function abnormalities, reduced platelets (secondary to liver disease or immune mediated thrombocytopenia), proteinuria and microscopic haematuria.

Reinfection

Another important element of HCV's natural history is its ability to reinfect individuals who have cleared the virus, due to a lack of protective immunity afforded by anti-HCV antibodies and memory T cells. Although individuals who spontaneously eliminate acute HCV infection are less likely to become persistently infected upon re-exposure, and tend to have lower peak HCV RNA levels when reinfected than observed in primary infection[96], they still remain vulnerable to chronic reinfection. There is currently no evidence that previously treated HCV infection provides meaningful protection from reinfection. This phenomenon is largely a consequence of HCV's genetic variability, and the incomplete reversion of T cell exhaustion after DAA therapy[97]. Studies in mice have shown how TOX, a critical transcription factor that determines CD8+ T cell exhaustion, is highly detectable in HCV-specific CD8+ T cells during chronic infection and after DAA-mediated cure, but not after spontaneously resolved HCV infection[98].

Reinfection therefore presents a major obstacle to the elimination of HCV as a public health threat. After curative therapy, reinfection among people injecting drugs regularly has been estimated at 3·1 reinfections per 100 person-years (incidence rate ratio [IRR] 6·7, 95% CI 1·9–23·5), dropping to 1·4 reinfections per 100 person-years (3·7, 1·1–12·9) in individuals with a past history of injecting drugs and 0·3 reinfections per 100 person-years in those with no history of injecting drugs[99].

Above I have shown how the Nobel-prize winning work of Alter, Houghton and Rice, among others, laid the foundation for our expanding knowledge of HCV's epidemiology and natural history, and the implementation of highly effective preventative measures to interrupt transmission. However, arguably the greatest impact of their work was in laying the ground for development of highly efficacious HCV therapeutics, which have made HCV the easily treatable infection it is today. I will now discuss the evolution of HCV therapeutics, including our increased understanding of HCV virology that made DAA therapy possible.

Evolution of therapeutics

The first attempts at treating HCV came three years before the virus had even been identified. Interferon alfa (IFNα) started life as a cancer treatment, but by the mid-eighties, evidence was emerging of its ability to induce a nonspecific resistance to viral infections by several mechanisms, including inhibition of protein synthesis, inactivation of viral RNA, and enhancement of phagocytic and cytotoxic mechanisms[100]. By 1985 IFNα was the established treatment of chronic Hepatitis B infection. In 1986, Hoofnagle et al trialled it in 10 patients with non-A, non-B hepatitis and noted a reduction in transaminases over a 12 month course of treatment[101]. Based on larger studies, intramuscular injections of IFNα three times/week became the standard of care for HCV. Cure rates did not exceed 25% and came with high treatment costs, unpleasant side effects and a gruelling treatment schedule.

By 1998 evidence for a second non-specific anti-viral, ribavirin, had emerged. As a nucleoside analogue, ribavirin mediates direct antiviral activity against both DNA and RNA viruses. While its mechanism of action is not well understood, some of its antiviral effect is postulated to be driven by it increasing the mutation frequency in viral genomes via inhibition of the RNA dependent RNA polymerase. Increasing availability of HCV RNA assays during

the 1990s helped establish standardised virological endpoints by which to test ribavirin's efficacy, and the concept of sustained virological response (SVR), 24 weeks after cessation of therapy, soon became an accepted measure of cure. Although efficacy of ribavirin monotherapy was disappointing, dual therapy with IFNα was proven to be better than either agent alone[102], with superior outcomes in genotype 2 and 3 infections compared to genotype 1. From 2001, advancements in biotechnology enabled pegylation of IFNα, which allowed simplification of the HCV treatment schedule to once weekly injections. This improved antiviral efficacy in genotype 1 infection, which was considered hardest to treat in the era of interferon-based treatment[103].

Pegylated IFNα (PEG IFNα) is associated with major side effects, including an initial flu-like syndrome in approximately 90% patients, fatigue, anorexia, nausea, diarrhoea, weight loss, hair loss, emotional lability, depression, bone marrow suppression, and induction of autoantibodies, which can result in thyroid abnormalities in up to 30% of patients, or enhancement of autoimmune diseases[104,105]. Furthermore, interferon is contraindicated in decompensated liver disease. Ribavirin is teratogenic and causes haemolytic anaemia, requiring regular monitoring and in some cases corrective blood transfusion. Despite these issues, and the difficulties inherent in delivering weekly injections to typically marginalised populations, pegylated IFNα (PEG-IFNα) with ribavirin became standard treatment of HCV for a decade from 2001-2011.

Predictors of response with PEG-IFNα based therapy

During this era of PEG-IFNα based therapy, various patient and viral factors were found to be associated with inferior treatment responses. Host factors such as advanced liver fibrosis, male gender, Black race, high body mass index (BMI), IFNL4 genotype[106], a history of treatment failure, and specific comorbidities such as diabetes and HIV co-infection were all associated with worse outcomes[107]. Viral factors such as a high baseline viral load, non-genotype-2 infection, and unfavourable viral kinetics on treatment (such as a delayed or fluctuating virological response) were also predictive of failure[107,108].

One of the most effective predictors of a sustained virological response from treatment with PEG-IFNα and ribavirin was found to be a so-called rapid virological response (RVR), defined as undetectable HCV RNA four weeks after commencing treatment[108]. RVR allowed

PEG-IFNα-based therapy to be shortened to 12–16 weeks for infections with favourable genotypes (genotypes 2 and 3), without compromising rates of cure[109]. Intuitively, in (unfavourable) genotype 1 infections, improved cure rates were achieved by extending PEG-IFNα and ribavirin therapy to 72 weeks in patients who demonstrated slower virological responses[110]. In addition, continuation of PEG-IFNα was determined to be futile in participants with less than a 1–2 log viral decline from pre-treatment level by week 12.

As a consequence of these developments, HCV therapy became increasingly personalised: each patient underwent careful appraisal to determine a) whether treatment should be initiated or deferred b) what duration of PEG-IFNα or ribavirin dose was appropriate and c) whether treatment should be continued on the basis of on-treatment virological response[107]. Unsurprisingly, PEG-IFNα-based treatment costs escalated. Treatment became increasingly specialised and was centralised to tertiary treatment centres, falling far outside the remit of general physicians.

Direct Acting Antivirals

While clinical trialists optimised the efficacy and duration of PEG-IFNα therapy, scientists were building on Charles Rice's characterisation of HCV, with the aim of developing more efficacious treatments with less adverse effects. A detailed understanding of the HCV life cycle led to the development of an entirely new generation of antiviral compounds to treat HCV infection.

HCV Genome and viral replication

The HCV genome consists of a positive-sense RNA molecule of approximately 9500 nucleotides. Highly conserved 5' and 3' untranslated regions (UTRs) flank an approximately 9000 nucleotide single open reading frame which encodes a large polyprotein of about 3000 amino acids. This central polyprotein undergoes post-translational processing by host and viral enzymes to form the structural and non-structural (NS) proteins and enzymes of the virus (figure 3).

The 5'UTR contains the essential components of viral protein synthesis and is highly conserved across genotypes, serving as a useful target for amplification in polymerase chain

reaction. The NS3 protein encodes a viral protease and cleaves the link between NS3 and NS4[111]. This is an integral part of viral replication and mediates the cleavage of virally encoded polyprotein to mature proteins. NS4A provides a co-function in this process. NS5A protein has an important role in viral replication, packaging, assembly, and NS5B encodes the RNA-dependent RNA polymerase[112].

Figure 1-4: Structure and genome of HCV

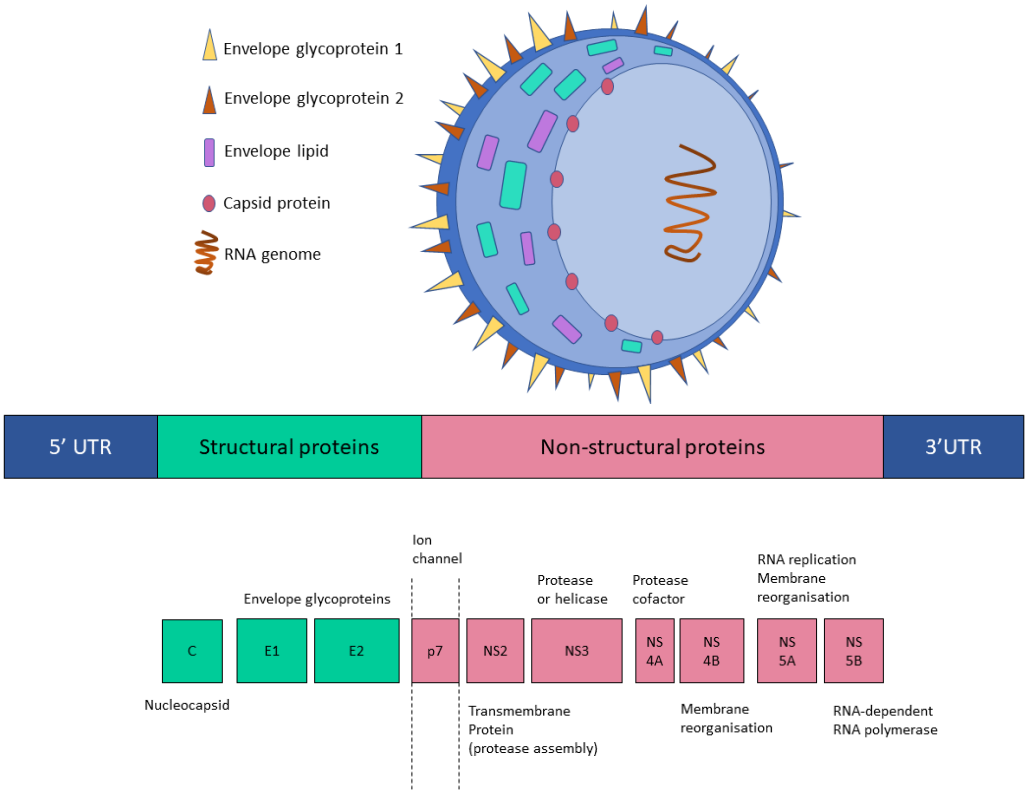


Diagram drawn by B. Flower. Adapted from Expert review in Molecular Medicine © 2003 Cambridge University Press. Abbreviations: HCV, hepatitis C viral; IFN, interferon; NTR, non-translated RNA.

The RNA dependent RNA polymerase is a defining feature of HCV. The polymerase's inability to proofread and correct the frequent copying errors made during viral replication is ultimately the cause of HCV's substantial genetic diversity. While most nucleotide changes result in non-functional or lethal mutants, some persist and replicate. Within the individual, this results in of millions of quasi-species (highly similar strains within with >95% sequence homology). This ever-moving target of non-identical strains helps the virus evade the host

immune response and resist eradication. At the population level, over hundreds of years, these replicative errors have led to the evolution of distinct HCV genotypes (defined as having less than 80% sequence homology[33]). Significant variability in expression of non-structural proteins between genotypes means they each respond differently to treatment. For many years this meant a genotype-tailored approach to therapy was required.

HCV's non-structural proteins were described in 1993[111] but it took nearly two decades before the first DAAs were licenced. As described above, the key step came around 2005, with the creation of a working *in vitro* HCV replication model, based on a genotype 2 clone[36], which allowed candidate molecules to be directly tested in vitro for their antiviral activity. These agents finally made it into phase 2 clinical studies around 2010 and a series of novel antivirals soon emerged over the course of a few dizzying years for Hepatitis C clinical trials.

Boceprevir and Telaprevir

The first DAA drugs to make it as far as phase 3 trials were the protease inhibitors boceprevir[113] and telaprevir[114] which bind to the NS3 protein's active site. Monotherapy with either of these tablets, which needed to be taken three times a day, led to a rapid decrease in HCV replication and elimination of detectable HCV RNA from blood. However, it was already apparent from in vitro work that protease inhibitors have a low barrier to resistance, and so it proved that resistance-associated substitutions (RAS) were rapidly selected during therapy[115]. Initially, therefore, these drugs were only suitable to be used in combination with PEG-IFNα, with which treatment was licenced in 2011.

Triple therapy with PEG-IFNα + ribavirin + protease inhibitor (PI) increased SVR rates by approximately 30% compared with the standard PEG-IFNα + ribavirin dual therapy in treatment-naive patients, with high proportions achieving rapid virological response and qualifying for shorter therapy[116,117]. Significant improvements in outcome were also observed in genotype-1 infected individuals who had previously relapsed on standard therapy[118]. However, treatment remained long and burdensome. Boceprevir was associated with intolerable dysgeusia and telaprevir with skin rash. In one cohort of 1587 patients with advanced liver fibrosis or cirrhosis treated with telaprevir triple therapy, 59% developed grade 1–4 anaemia, 21% required erythropoietin and 10% needed blood transfusion[119]. Thrombocytopenia and hypoalbuminaemia were also common in patients with advanced liver

disease, as were drug interactions, on account of PIs being substrates and inhibitors of cytochrome P450.

NS5A and NS5B inhibitors

By the time boceprevir and telaprevir were licenced, the DAA pipeline was gathering pace and an entirely new class of drug was soon available. NS5A inhibitors were shown to be effective at supressing viral replication *in vitro*, and in 2012, Lok et al showed that previously treated HCV could be cured with 24 weeks of an all-oral regimen of the first generation NS5A inhibitor daclatasvir in combination with a newly developed PI, asunaprevir[120].

Before this was trialled further, sofosbuvir (SOF) emerged as the first NS5B inhibitor, changing the face of HCV therapy forever. SOF was originally developed by Pharmasset, which was purchased by pharmaceutical giant Gilead Sciences in a multibillion dollar take-over in 2011[121]. The major advantage of SOF was that it demonstrated a high barrier to resistance, with most emerging NS5B-associated RAS exhibiting poor viral fitness[122]. Furthermore, the drug could be taken once daily, was well tolerated, had few drug interactions, and no requirement for adjustment in mild to moderate renal failure or in any degree of hepatic impairment. Cure rates of >90% were observed when SOF was used in combination with PEG-IFNα + ribavirin in genotype 1 infection and with ribavirin alone in genotype 2 infection[122].

Within months of sofosbuvir being approved in the USA and Europe, the next guideline-changing development was being published. Gilead combined SOF with their own NS5A inhibitor, Ledipasvir (LDV), in a fixed dose combination tablet, with dramatic results. Efficacy of SOF/LDV+/- ribavirin for 12 or 24 weeks was evaluated in a randomised trial of 440 genotype-1 infected patients (20% with cirrhosis), who had previously failed PEG-IFNα based therapy[123]. SVR12 rates of 94% (95% CI 87-97) were observed in patients treated with 12 weeks SOF/LDV and 99% in those treated with 24 weeks (95% CI, 95-100). In treatment naïve genotype-1 infection, outcomes were even better, with SVR12 rates of 99% (95% CI 95-100) with 12 weeks SOF/LDV[124]. A third *New England Journal of Medicine*-published trial in as many weeks compared efficacy of 8 or 12 weeks of SOF/LDV +/- ribavirin in 645 genotype 1-infected patients with mild liver disease[125]. Non-inferiority of the shorter regimen was demonstrated, with cure rates of 94% (95% CI 90-97) with 8 weeks SOF/LDV (without ribavirin) and 95% (95% CI, 92 to 98) with 12 weeks. No additional benefit was associated

with the inclusion of ribavirin in the regimen or with extension of the duration of treatment to 12 weeks.

For a time, it was assumed 8-week therapy with SOF/LDV would become the standard for patients without cirrhosis, but a sub-analyses subsequently revealed there was a higher rate of virological relapse among males given shorter treatment (8% (10/121) vs 2% (3/127) for the 8- and 12-week regimens, respectively) and among participants with a high baseline HCV viral load >6,000,000 IU/ml (10% (9/92) with 8 weeks vs 1% (1/83) with 12 weeks. This prompted US and European guidelines to recommend that 8 weeks SOF/LDV should be reserved for patients with most favourable predictors of response (i.e. mild liver disease, HIV-uninfected, no history of treatment failure, and a low baseline viral load)[126]. This decision was partly influenced by a concern over generation of resistant virus should patients experience virological relapse, and is considered by some as conservative, especially given SVR12 of >98% were achieved with 8 weeks therapy in females and those with favourable genetic polymorphisms (rs12979860 allele)[127].

Gilead's development of the NS5B inhibitor sofosbuvir has been the biggest game changer in HCV therapy, but important contributions have been made by other drugs. In the same month in 2014 that Gilead announced their SOF/LDV data, AbbVie pharmaceuticals reported similar impressive efficacy for their novel fixed dose combination of the NS5A inhibitor ombitasvir with the PI paritaprevir boosted by ritonavir (once daily), and the non-nucleoside NS5B inhibitor dasabuvir (twice daily) – so called 'AbbVie 3D'[128]. This combination was licensed around the same time as SOF/LDV and, as an independent competitor, had a positive impact on driving down DAA drug prices. It also found an important niche in treating HCV-infected patients with end-stage renal failure - sofosbuvir initially being considered unsafe for that population. Unfortunately, efficacy of AbbVie 3D in treating genotype 1a infection was inferior to that reported for SOF/LDV. In addition, co-treatment with ribavirin was advised, pill burden was higher, and drug interactions and adverse events were more common.

Additional antivirals soon emerged, including the second generation PI grazoprevir in fixed dose combination with the NS5A inhibitor elbasvir (developed by Merck) which had high efficacy in genotype 1 and genotype 4 infections[129]. Given the vast costs of the first licenced DAAs, this market competition was welcome: in 2015 SOF/LDV had a US list price of $84,000 (around $1000/tablet). Prices were so high that even high-income countries were inclined to restrict use to individuals with evidence of liver fibrosis and those at low risk of

reinfection[130]. Treatment decisions became highly complex: as well as factoring in stage of liver disease, treatment history, genotype, subtype and viral load, clinicians had to consider procurement costs and local contract details. Guidelines changed frequently as international societies attempted to keep pace with a rapidly changing field. In most LMICs, the new drugs were scarcely available, and most patients were being advised to await the advent of affordable tablet therapy.

Pan-genotypic therapy

A major draw-back to the first generation of DAAs was that they were tailored to genotype 1 infection, the most prevalent genotype worldwide and the dominant lineage in the high-income countries where antiviral development was centred[62]. Their efficacy against other genotypes was less reliable: ledipasvir, ombitasvir and elbasvir had lower efficacy against genotypes 3 in particular[129,131], and dasabuvir was ineffective in genotype 4 infection.

A breakthrough came in 2015, when Gilead introduced their second generation NS5A inhibitor velpatasvir, in fixed dose combination with their NS5B inhibitor sofosbuvir. In a randomised, placebo-controlled study, among 624 patients with a range of genotypes treated with SOF/VEL, 99% achieved SVR12 (95% C.I, 98-99%). Although inferior outcomes were reported in genotype 3-infected individuals with cirrhosis or history of treatment failure[132], rates of SVR generally exceeded 90% such that SOF/VEL was declared the first pan-genotypic DAA treatment for HCV.

One year later, a second pan-genotypic fixed dose combination was approved – AbbVie pharmaceutical's combination of the PI glecaprevir with NS5A inhibitor pibrentasvir (G/P). Like SOF/VEL, this combination exhibited a high barrier to resistance. In treatment-naive patients without cirrhosis, 8 weeks of G/P was sufficient to achieve cure rates >95% regardless of genotype[133]. In patients with cirrhosis, the balance of evidence favours 12 weeks treatment in non-genotype 3 infections and 16 weeks treatment in genotype 3 infections[134,135].

Sofosbuvir with Daclatasvir

As mentioned above, daclatasvir (developed by Bristol Myers Squibb) was one of the first DAAs to emerge, and its use with Gilead's NS5B inhibitor sofosbuvir (SOF/DCV) was one of the first combinations with broad genotypic activity to be evaluated in late stage clinical

trials[136]. The combination showed good efficacy and tolerability, with SVR rates exceeding 96% in individuals without cirrhosis treated for 12 weeks, in genotypes 1 through 4[136,137]. Inferior rates of cure were observed in patients with cirrhosis, particularly in genotype 3 in which SVR rates with 12 weeks therapy ranged from 80-86%[138,139].

Regrettably, SOF/DCV's availability in high-income settings was to be short-lived. Once Gilead had developed its own second generation NS5A inhibitor (velpatasvir) the combination of SOF/DCV was no longer commercially viable and this combination was withdrawn from high-income countries, who were obliged to pay a premium for novel, patented antivirals.

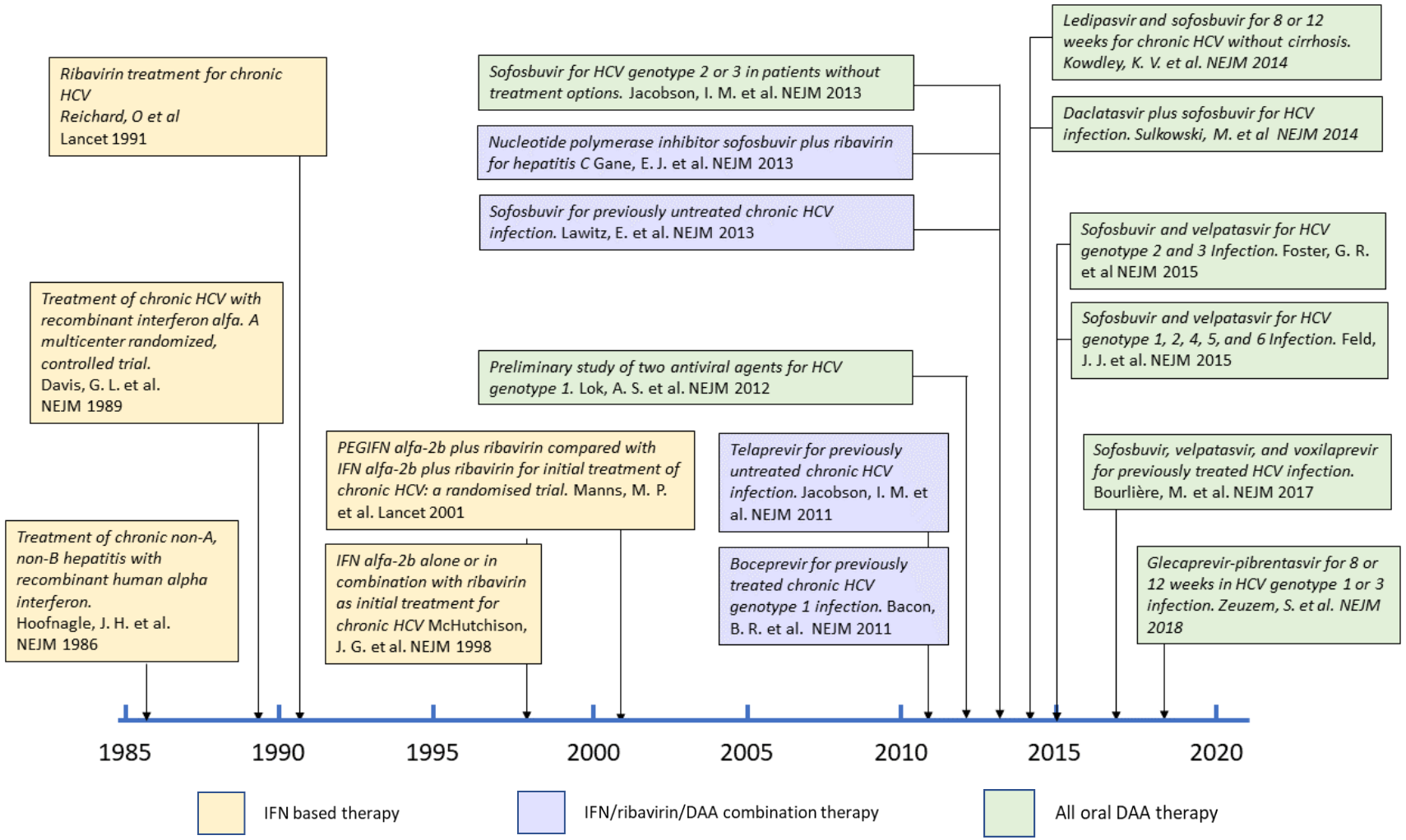
Nonetheless, WHO recognised the potential of SOF/DCV, which had not been shown to be inferior to SOF/VEL. Learning from mistakes made in the global distribution of antiretroviral therapy for HIV in the early 2000s[140], WHO took measures to include SOF/DCV in international HCV treatment guidelines[16] and ensured daclatasvir was on the WHO Essential Medicine List. This encouraged competition amongst generic manufacturers to supply treatment to LMICs through voluntary licences. Consequently, SOF/DCV has become the lowest priced and most widely available DAA option globally, with the most generic manufacturers worldwide[141].

Retreatment

The most recent significant drug development in HCV therapy came in 2017 with the licencing of the fixed dose combination of SOF/VEL plus voxilaprevir (a protease inhibitor) for retreatment after virological relapse. While most DAA combinations proved highly effective at treating individuals who had failed previous interferon-based treatment, or interferon therapy with a PI, concerns remained over how to manage the small proportion of individuals who experienced treatment failure after NS5A/NS5B combination therapy. Mounting evidence showed NS5A resistance mutations that emerged after standard durations of therapy were durable, persisting for several years after cessation of treatment[142]. The POLARIS studies were randomised controlled trials in which patients who had previously experienced treatment failure with an NS5A inhibitor (POLARIS-1) or non-NS5A-based therapy (POLARIS-4) were randomised to retreatment with SOF/VEL/VOX or placebo (in POLARIS-1) or SOF/VEL (in POLARIS-4). With SOF/VEL/VOX retreatment, patients who

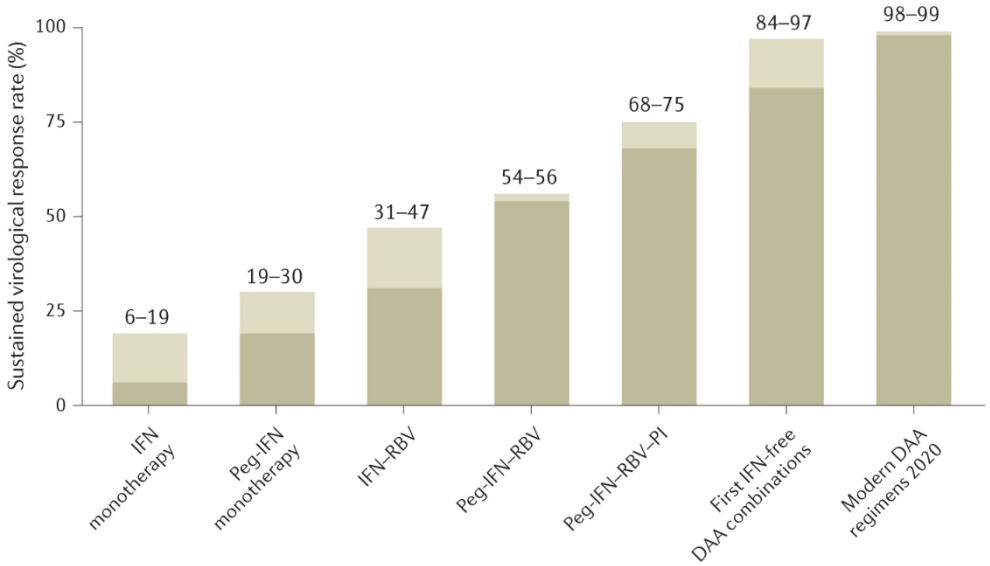
had previously failed NS5A therapy achieved SVR12 rates of 96% (vs 0% with placebo) and patients who had previously failed non-NS5A therapy achieved SVR12 rates of 98% (vs 90% with SOF/VEL alone).

Figure 1-5: Key clinical trials in HCV therapeutics



From 2011 and 2018 HCV was thus transformed from a difficult-to-treat infection, with multiple pre-treatment considerations relating to host factors and viral genotype/subtype, to an easily curable infection with effective retreatment options if necessary. A summary of the current EASL treatment guidelines is shown in table 1. Improvement in SVR rates with the evolution of HCV therapy are summarised in figure 1-6.

Figure 1-6: Improvement in SVR rates with evolution of HCV therapy



Reproduced from Manns, M.P., Maasoumy, B. Breakthroughs in hepatitis C research: from discovery to cure. Nat Rev Gastroenterol Hepatol 19, 533–550 (2022). with permission via licence with Springer Nature (license number 5398130917788)

In 2018, in response to overwhelming evidence of the efficacy and safety of these novel pan-genotypic therapies, falling drug prices, and expanding access to care, WHO radically simplified international HCV treatment guidelines. Firstly, they advised that *all* HCV-infected individuals should be treated, regardless of stage of liver disease or ongoing exposure risks. Secondly, they recommended use of one of three pan-genotypic regimens for first line therapy: SOF/VEL, SOF/DCV or G/P, advising that genotyping was no longer required to make treatment decisions (table 2). Thirdly, they called for urgent roll out of DAA treatment in LMICs.

This thesis was conceived around the release of those guidelines and responds to key gaps in the literature highlighted at that time. The SEARCH study, which forms the basis of chapter 3 and 4, specifically explores predictive factors for selecting persons who could be treated for a shorter duration, the clinical importance of NS5A (daclatasvir) resistance and the relationship between rare HCV subtypes and treatment failure. Here I will discuss what is already known.

Impact of genotypes and subtypes on DAA treatment outcomes

Genotype 1

As described above, the first DAA drugs were targeted to genotype 1 infection, on account of its global predominance and relative resistance to interferon-based therapy. A pattern soon emerged of marginally lower SVR rates in genotype 1a infection compared with 1b. This was observed with the first generation PIs (boceprevir and telaprevir) in combination with PEG-IFNα and ribavirin[113,114], and with ombitasvir, paritaprevir-ritonavir plus dasabuvir (AbbVie 3D), in which 1b-infected individuals with mild liver disease had cure rates of 99% compared with 90% in 1a -infected individuals, (rising to 97% with the addition of ribavirin)[128]. This subtype effect was also seen with elbasvir and grazoprevir, in which pre-existing resistance-associated substitutions (RAS) were associated with inferior outcomes in 1a-infection but not 1b[129]. As a consequence, NS5A resistance testing is still recommended in genotype 1a patients being considered for elbasvir/grazoprevir therapy in high-income settings. If baseline substitutions at amino acid positions 28, 30, 31, or 93 are discovered, an alternative regimen is recommended[126]. Reassuringly, differences in outcome between 1a and 1b infections appear to be negligible with the newer pan-genotypic drugs SOF/VEL[143], SOF/DCV[144] and G/P[133].

Genotypes 2 and 3

Genotype 2 subtypes and their associated resistance-associated substitutions do not significantly impact on treatment outcomes with the WHO-approved pan-genotypic agents. However, genotype 3, which makes up around 30% of global infections, is considered the

hardest-to-treat HCV variant and the presence of baseline resistance mutations affects outcome. In one landmark SOF/VEL study evaluating its efficacy in genotype 2 and 3 infections, the presence of baseline NS5A or NS5B resistance-associated variants was not associated with virologic failure in genotype 2 infection. But among patients with HCV genotype 3, an SVR rate of 88% was observed among patients with NS5A resistance-associated variants at baseline compared with 97% among those who had no evidence of such NS5A resistance mutations[132]. Lowest rates of cure (84%) were recorded in patients with the Y93H variant at baseline. As consequence, US guidelines recommend that NS5A resistance testing is performed before treating genotype 3-infected cirrhotic individuals with SOF/VEL. If Y93H is present, the addition of ribavirin or an alternative regimen is advised[134,145].

With regards to SOF/DCV, 12 weeks of therapy achieves high rates of cure in genotype 3 infection without cirrhosis, but SVR rates fall below 90% in individuals with liver disease[138,139]. Given SOF/DCV is predominantly used in LMIC settings where NS5A resistance testing may be unavailable, WHO guidelines recommend the addition of ribavirin to 12-week SOF/DCV regimens for all genotype 3-infected infected individuals with cirrhosis, or extension of treatment to 24 weeks where ribavirin is contraindicated[16] (table 2).

Genotype 4

Genotype 4 infection is easily treated with most DAAs, regardless of the stage of liver disease. An important exception is genotype 4r. In a single arms study of 300 genotype 4-infected individuals in Rwanda, treated with 12 weeks sofosbuvir and ledipasvir, SVR12 was observed in 27/48 (56%, 95% CI 41-71) participants with 4r infection, versus 234/252 (93%, 90-96) individuals with other subtypes[146]. A subsequent study investigating RASs before and after DAA failure in 195 patients in Europe with different genotype 4 subtypes confirmed 4r was frequently sequenced after treatment failure (30%) compared with other subtypes. The number of NS5A RASs was significantly higher in subtype 4r compared to 4a or 4d (median three RASs vs no or one RAS, respectively, $P < .0001$)[147]. The is currently no consensus on whether subtype 4r should be treated differently to other genotype 4 subtypes such that routine genotype 4 subtyping prior to therapy is not currently recommended.

Genotypes 5 and 6

Data relating to treatment failure in genotype 5 and 6 infection are limited. This was a major motivation for conducting the SEARCH study in Vietnam, where genotype 6 is highly prevalent. Genotype 6 constitutes around 5% of HCV infections globally[62] but is reported to be responsible for around 35% infection in Vietnam[63,73]. Prior to the advent of modern direct methods of genotyping, diagnosis of genotype 6 infection was inconsistent, due to identical 5'UTR between genotypes 6 and genotype 1b[148]. This has meant that regional genotype 6 prevalence estimates have likely been underestimated.

With 29 confirmed subtypes (6a to 6xf) and 21 unassigned subtypes, genotype 6 is the most genetically diverse genotype, raising concerns about the potential for naturally occurring resistant variants that may impact treatment outcomes[149,150]. In vitro data suggests that certain genotype 6 subtypes have a lower barrier to DAA resistance, particularly NS5A inhibitors. Pre-existing NS5A RAS are very common, at two residues in particular (L28 & R30). In the largest assessment of its kind, these RAS were detected in 6b, 6f and 6r sequences, and were associated with significantly reduced susceptibility in vitro against all tested NS5A inhibitors in comparison with a genotype 6a consensus replicon.

The clinical relevance of these mutations in vivo is less clear. In a study exploring the impact of pre-existing RAS on treatment outcomes with SOF/VEL in genotypes 1-6, the highest rates of VEL-specific RAS were found in genotype 6[151]. Single class NS5A resistance to VEL was detected in a few patients with virologic failure, but not to the NS4A inhibitor sofosbuvir. Overall, high rates of SVR were achieved irrespective of the presence of NS5A RAS.

Data relating to daclatasvir is sparce. In a 2019 systematic review of genotype 6 outcomes with different DAA therapies, only two clinical studies were identified that used SOF/DCV. No RAS data relating to SOF/DCV treatment failure were reported[152]. A key objective of the SEARCH study, described in chapters 3 and 4, is therefore to assess the prevalence of putative NS5A- and NS5B-inhibitor RAS in genotype 6 subtypes, and evaluate their clinical relevance.

Table 1: summary of 2020 EASL HCV treatment guidelines

Genotype	Liver disease	Prior treatment	SOF/DCV	G/P	SOF/VEL	SOF/VEL/VOX
Unavailable	No cirrhosis	Naive	12 weeks	8 weeks	12 weeks	No
		PEGIFN + rbv	12 weeks + rbv			
	Compensated cirrhosis	Naive	12 weeks			
		PEGIFN + rbv	12 weeks + rbv or 24 weeks	12 weeks		
1b	No cirrhosis	Naive	12 weeks	8 weeks	12 weeks	
		PEGIFN + rbv				
	Compensated cirrhosis	Naive				
		PEGIFN + rbv		12 weeks		
1a, 2, 4, 5, 6	No cirrhosis	Naive	12 weeks	8 weeks	12 weeks	
		PEGIFN + rbv	12 weeks + rbv or 24 weeks			
	Compensated cirrhosis	Naive	12 weeks			
		PEGIFN + rbv	12 weeks + rbv or 24 weeks	12 weeks		
3	No cirrhosis	Naive	12 weeks + rbv or 24 weeks	8 weeks	12 weeks	No
		PEGIFN + rbv		12-16 weeks		
	Compensated cirrhosis	Naive		8 weeks	12 weeks*	12 weeks
		PEGIFN + rbv		16 weeks		
All genotypes	No cirrhosis	NS5A-I experienced	No	No	No	12 weeks
	Compensated cirrhosis					
	Decompensated cirrhosis	Naive	12 weeks + rbv (G1,2,4,5,6) or 24 weeks +rbv (G3)		12 weeks + rbv or 24 weeks	
		PEGIFN + rbv				
	End-stage renal disease	Naive	Possible	Preferred	Possible	Possible
		PEGIFN + rbv				

** In patients with cirrhosis who have the Y93H RAS, addition of ribavirin (rbv) or an alternative regimen is recommended.*

Table 2 : summary of 2018 WHO HCV treatment guidelines

	SOF/DCV	G/P	SOF/VEL	SOF/VEL/VOX
No cirrhosis	12 weeks	8 weeks	12 weeks	No
Compensated Cirrhosis	24 weeks*	12 weeks**	12 weeks	No
Decompensated cirrhosis	24 weeks	No	24 weeks	No

**12 weeks may be considered in countries where genotype 3 distribution is known and prevalence is <5%. This is based in estimation that in a population of persons with cirrhosis where 5% of persons would be infected with genotype 3 HCV, the SVR would be 80% in the 5% infected with genotype 3 and 93% in the 95% infected with other genotypes, leading to an overall SVR rate of (0.05x0.80)+(0.93x0.95) = 92%.*

***Persons with HCV genotype 3 infection who have received interferon and/or ribavirin in the past should be treated for 16 weeks*

Predictors of response to standard duration therapy

As outlined in the section above 'Evolution of HCV therapeutics', in the era of PEG-IFNα-based treatment, various host factors and viral factors were used to predict treatment response and determine duration of therapy. These included the presence of liver cirrhosis, host polymorphisms in the IFNL4 gene, viral genotype and subtype, baseline viral load and the achievement of a rapid virological response[106,153]. Male gender, black race, high BMI, older age, HIV co-infection, diabetes mellitus and a high degree of HCV genome heterogeneity (quasispecies), were also considered negative predictors of response, even if not formally used to determine duration of therapy[153–155].

With pangenotypic DAA therapy, such are the high rates of cure with standard durations of treatment (8-12 weeks), most of these predictors are no longer relevant. The most important exception to this is liver fibrosis, which is still used to determine DAA treatment choice and duration, particularly in the hardest to treat infections with genotypes 1a and 3. Cirrhosis has long been recognised as an important negative predictor of treatment response, but the mechanisms underlying this association are poorly understood. Slower viral decline on treatment and lower rates of SVR have been variously attributed to impaired drug delivery, uptake and metabolism, and the deficient host immune response associated with chronic liver disease[156].

Previous treatment failure also remains an important negative predictor of response in the era of DAA therapy. This is intuitive given the high potential for persistent NS5A-RAS complicating retreatment after NS5A therapy. However, since patients who do not achieve SVR with pan-genotypic therapy likely have several negative predictors of response, it is often difficult to define the contribution of NS5A RAS per se on first-line treatment failure.

Age also appears to influence DAA treatment outcomes but is not factored into treatment guidelines. In a retrospective single-centre cohort study done in the US, among 551 patients treated with DAAs from 2014-2016, SVR12 was achieved in 81% of patients who were >70 years at treatment initiation as compared with 95% in the non-elderly group. Binary logistic regression revealed age >70 years to be the strongest predictor of treatment failure (odds ratio = 3.4), along with diagnosis of cirrhosis (odds ratio = 2.4), when corrected for gender, race, prior treatment experience, genotype, and presence of hepatocellular carcinoma[157].

Finally, as discussed above, certain genotypes and subtypes are associated with worse DAA outcomes. Even in this era of pan-genotypic therapy, these must still be factored into treatment decisions, especially with regards to genotype 3 infection.

Other traditional predictors of response to PEG-IFNα therapy are generally considered obsolete with DAAs. These include, viral kinetics, baseline viral load, IFNL4 polymorphisms and HIV co-infection.

Viral Kinetics

Whereas an HCV viral load during week 4 of treatment was a well-evidenced timepoint at which to determine duration of PEGINFα-based treatment, HCV RNA kinetics on DAAs appear to be poorly predictive of SVR with standard durations of NS5A/NS5B combination therapy[158–160].

Detailed kinetic modelling has shown that HCV RNA exhibits a biphasic viral decline in response to DAA therapy, with a rapid first phase (from day zero to day 2), followed by a slower second phase. Fastest first phase viral decline is seen with NS5A inhibitors (such as daclatasvir[161], ledipasvir[162], and velpatasvir), which has been attributed to the fact that these drugs efficiently block two distinct stages of the viral lifecycle, namely viral RNA synthesis and virion assembly/secretion. A faster second phase has been described in protocols that include a protease inhibitor[163].

The utility of rapid virological response with PEGIFN treatment and the differing speed of virological response with different classes of antivirals led some to hypothesise that viral kinetics (at an earlier timepoint than 4 weeks), may be used to predict therapeutic outcomes with DAA therapy. However, in 152 individuals with genotype 3 infection treated with SOF/DCV, on-treatment response was found to be a limited indicator of outcome[160]. HCV RNA levels declined rapidly during the first week of treatment in both treatment-naive and -experienced cohorts. Individuals with liver cirrhosis had a slower initial virological response as measured by the proportion of patients with HCV RNA below the lower limit of quantification at day 7, but responses converged thereafter and SVR12 rates were not

impacted by time to first undetectable HCV RNA[160]. Likewise low levels of detectable HCV RNA at end of treatment do not necessarily herald treatment failure. In one study of 69 patients treated with SOF/LDV, 6/6 patients with low level viraemia at end of treatment (range, 14-64 IU/mL) achieved SVR[164].

Reluctance to use viral kinetics to predict DAA treatment outcomes is also partly pragmatic: the velocity of the virological response to DAA therapy is such that most patients have undetectable virus in peripheral blood within a few days of starting treatment. Therefore, any kinetic monitoring needs to occur in the first days after starting antivirals which is rarely practical, especially in the context of high rates of cure (>95%) with standard durations of therapy. It remains to be determined whether viral kinetics could help achieve high rates of cure with shortened therapy (see below).

Baseline viral load

When efficacy of sofosbuvir and ledipasvir combination therapy in mild liver disease was first reported in 2014[125], it was noted that, amongst participants with baseline HCV RNA level >6 million international units/mL (as determined using the COBAS Taqman HCV Test, version 2.0) cases of virologic relapse were numerically higher in individuals treated with 8 weeks than those given 12 weeks (5% vs 1%). As a consequence this threshold was incorporated into treatment guidelines[126]. However, this cut-off was subsequently questioned based on the lack of any statistical difference between all viral load strata. The impact of baseline viral load on treatment outcome appears modest and is no longer considered relevant with standard duration therapy according to WHO treatment guidelines[16].

IFNL4 polymorphisms

Neither race, nor polymorphisms in the IFNL4 gene have been consistently associated with lower SVR rates in multiple trials and cohort studies of standard DAA combination regimens. Although some studies have found a modest association with race or IFNL4 polymorphisms and SVR[127,165], the impact appears to be small when standard durations of pan-genotypic DAAs are used, and not sufficient enough to recommend IFNL4 genotype testing in routine clinical practice. One large retrospective study of 21,095 hepatitis C virus–infected patients in

the US found that among genotype 1–infected patients treated with ledipasvir/sofosbuvir, Black patients had significantly lower SVR than white patients when treated for 8 weeks but not when treated for 12 weeks. This might suggest that host polymorphisms become more relevant with shorter durations of therapy[165] but evidence for this is not strong.

HIV

HIV co-infection also has less impact on treatment response in the era of DAA therapy. In a study of 151 HIV-co-infected individuals with genotypes 1-4, treated with 8- or 12-weeks SOF/DCV (of which 14% had cirrhosis), 96.4% (95% C.I, 89.8 - 99.2) of those treated with 12 weeks achieved SVR[136]. Of note, only 75.6% (95% CI, 59.7 -87.6) treated for 8 weeks achieved SVR, raising the possibility that HIV co-infection becomes a more important negative predictor of response with shortened therapy. However, of the 12 patients who had a relapse, 9 were receiving concomitant darunavir–ritonavir. Based on pharmacokinetic (PK) data available at the time, daclatasvir doses were halved to 30 mg in these patients. Subsequent PK data has shown this dose adjustment is unnecessary, lessening the importance of this result.

Drug levels

There is currently no evidence that altered DAA metabolism or plasma drug levels impact on DAA outcomes with standard duration therapy. Although multiple factors are known to affect levels of SOF and DCV, and some degree of inter-host variation in drug levels is typical, the antiviral effect (measurable by a rapid decline in HCV RNA in peripheral blood), is remarkably consistent[166].

SOF is a nucleotide prodrug that is converted intracellularly from monophosphate form to an active triphosphate, GS-461203[167]. Dephosphorylation of GS-4611203 leads to production of GS-331007, its inactive metabolite, which is eliminated mainly via the kidneys. SOF's absorption is not affected by food and no dose adjustment is required in hepatic or renal impairment, (although its safety has not yet been established in patients with severe renal

impairment). Pharmacokinetics are not significantly altered by age, race, or gender[167], but SOF area under the curve (AUC) is increased in moderate (Child–Pugh class B) and severe (Child–Pugh class C) hepatic impairment by 126% and 143%, respectively, compared to patients with mild liver disease. In patients with mild, moderate, and severe renal impairment, GS-331007 AUCs are increased by 56%, 90%, and 456%, respectively, compared with healthy controls. SOF and its metabolites can be subject to drug-drug interactions. Medicinal products that are potent P-gp inducers in the intestine (i.e. rifampicin, rifabutin, St. John's wort (Hypericum perforatum), carbamazepine, phenobarbital and phenytoin) may significantly decrease SOF plasma concentration leading to reduced therapeutic effect of SOF. Co-administration of these drugs with SOF is therefore contraindicated. Medicinal products that are moderate P-gp inducers in the intestine (e.g. oxcarbazepine and modafinil) may decrease SOF plasma concentration leading to reduced therapeutic effect. Co-administration with SOF is not contraindicated but it is not recommended.

DCV is highly selective NS5A replication complex inhibitor that reaches peak plasma concentrations a median of 1–2 h after administration and has potent antiviral activity against all HCV genotypes. Oral DCV 60 mg once daily achieves a mean Cmin of 255 ng/mL by day 14, which is substantially higher than the median effective concentration values for genotype 1a (0.283 ng/mL) or genotype 1b (0.036 ng/mL) HCV. Steady state is achieved after 3–4 days of once-daily administration. DCV is metabolized by cytochrome P450 (CYP) 3A isoenzymes and nearly 90% is eliminated in the faeces. High-fat meals have been shown to reduce the Cmax and AUC by 28% and 23%, respectively, but meal timing is not considered critical in daily dosing[167]. The AUC of DCV is increased in patients with creatinine clearance values of less than 30ml/min by 39%, and less than 15 mL/min by 51%, but no dose adjustments are recommended in renal impairment. DCV AUC is decreased in subjects with mild, moderate, or severe hepatic impairment, but hepatic impairment has no clinically apparent effect on unbound DCV concentrations[168] such that dose adjustments are not required. Gender, race, and age also have no clinically significant effect on DCV pharmacokinetics[168,169]. With regards to drug interactions, DCV is a substrate of CYP3A4 and P-gp, so administration of is not recommended with strong inducers of CYP3A4. This includes but is not limited to phenytoin, carbamazepine, oxcarbazepine, phenobarbital, rifampicin, rifabutin, rifapentine, systemic dexamethasone, and the herbal product St. John's wort (Hypericum perforatum). DCV dose may require alteration when taken in conjunction

with other drugs which affect CYP3A4 metabolism (including ritonavir and azole drugs such as itraconazole).

There are therefore multiple factors that could influence SOF and DCV drug levels during therapy, and detailed pharmacokinetics are a valuable addition to any treatment shortening study.

Predictors of response to shortened therapy

Although some of the traditional predictors of response described above have become obsolete with standard-duration DAA treatment, they may still have a role in determining which individuals could successfully cure with shorter durations of therapy. 12 weeks of daily tablets presents a significant barrier to successful engagement in care for some populations[170,171] and novel, shorter treatment strategies may be desirable for individuals under-served by existing models of care, such as people who inject drugs and those of no fixed abode.

In 2018 we conducted a systematic review and meta-analysis of treatment optimisation strategies with DAA therapy, separating strategies into those aiming to maintain SVR in the absence of predictors of failure, and those aiming to improve SVR in the presence of predictors of failure[172]. Predictors included past treatment failure, genotype 3 infection, pre-existing RAS, liver fibrosis, obesity, high baseline viral load, and unfavourable on-treatment viral kinetics. In individuals lacking such negative predictors, pooled SVR for DAA regimens of ≤4 weeks duration was 63.1% (95% C.I 39.9-83.7), 6 weeks duration was 81.1% (75.1-86.6%), and 8 weeks duration was 94.2% (92.3-95.9%). A subgroup analysis found higher SVR rates were achieved with strategies that used ≥3 personalised treatment factors (92.9% [90.4-95.1%]) compared to one (81.4% [71.1-90%]) or two (87.2% [82.1-91.6%]). In individuals with unfavourable predictors of response, pooled SVR for 12 weeks duration was 97.7% (94.9-99.5%), 16 weeks duration was 95.1% (91-98.2%), and 24 weeks duration was 96.3% (93.5-98.5%). However, these figures should be interpreted with caution, given that longer durations of therapy were mainly reserved for DAAs known to have poor efficacy when used for 12 weeks in patients with negative predictors of response.

Response guided therapy

There is limited evidence to support response guided therapy (RGT) in the era of DAA therapy. The most promising results were reported by Lau et al in 2016[173]. In a small Phase IIb, open label, single centre proof-of-concept study in Hong Kong, they investigated the efficacy of ultrashort RGT with three DAA drug classes (NS5A-i + NS5B-i + PI), based on day 2 viral load. Participants were randomised to one of three triple therapy regimens: SOF, ledipasvir (LDV), and asunaprevir; SOF, DCV, and simeprevir; or SOF, DCV, and asunaprevir. Those with day 2 viral load <500IU/ml received 3 weeks and those >500 IU/ml received 8 or 12 weeks SOF/LDV. Two thirds of participants were female, and all had low BMI, mild liver disease and genotype 1b infection (i.e., a highly selected population with few negative predictors of response). Among 26 patients enrolled, 18 suppressed HCV RNA <500 IU/mL after 48 hours and received 3 weeks triple-drug therapy. All 18 (100%) achieved SVR12. By way of comparison, in an open label US study which used 4 weeks triple therapy (SOF/LDV + PI) to treat genotype 1-infected patients (68% genotype 1a, 32% 1b) with early stage liver fibrosis without using early virological response to guide treatment duration, SVR was achieved in just 30% (15/50) individuals[174]. Amongst genotype 1b infected individuals SVR12 rate was 50% (8/16).

No studies have used response guided therapy with dual antiviral therapy (NS5B-i + NS5A-i) of less than 6 weeks duration. A 2017 study in Egypt explored RGT in HCV genotype 4 infected adults with mild disease, using day 14 viral load to determine treatment duration[175]. Participants in the intervention group received 8 weeks of SOF/DCV therapy if HCV viral load at 2 weeks was less than the lower limit of quantification (LLOQ; <20 IU/ml) and 12 weeks if it was above this threshold. Overall, 48/60 (80%) received 8 weeks treatment and of the 47 patients who completed the 8 weeks of therapy, 100% achieved SVR12. In total 59/60 (98.3%) in the intervention group achieved SVR, which was similar to the standard therapy group.

More recently a group in Israel evaluated the feasibility of using a RGT approach to reduce the duration of DAA therapy based on mathematical modelling of early viral kinetics. They treated 29 patients (8 of which had cirrhosis) with a variety of DAAs, and measured HCV RNA on day 2 and weeks 1, 2 and 4 after treatment initiation to determine the trajectory of viral clearance. Their mathematical model recommended shortening treatment in 11/29

(38%), of which two received 6 weeks treatment, eight received 8 weeks and one received 10 weeks. Overall 97% achieved SVR. Virological relapse was observed after end of treatment in a single case of a non-cirrhotic male with genotype 3, who was treated with SOF/VEL for 6 weeks. Given the high rates of cure we would expect with 8 or 10 weeks of DAA therapy in patients with mild liver disease, and the small numbers in this study, this can't, on its own, be taken as a strong endorsement for the efficacy of RGT.

Baseline viral load

The efficacy of using baseline viral load to determine treatment duration was evaluated in a randomised controlled trial, 'STOPHCV1' conducted across 14 UK NHS Hospital Trusts from 2016-2018 in patients with mild liver disease. The study assessed variable ultrashort-course DAA treatment for HCV (4 to 8 weeks based on pre-treatment viral load) vs 8 weeks fixed duration therapy[176]. The DAAs used were ombitasvir/paritaprevir/ritonavir +/- dasabuvir depending on infecting genotype, +/- ribavirin (1:1). Towards the latter half of recruitment ombitasvir/paritaprevir/ritonavir +/- dasabuvir was replaced with glecaprevir/pibrentasvir. First-line SVR12 was 91% [86%-97%] (92/101) after 8 weeks fixed-duration therapy vs 48% [39%-57%] (47/98) after 4-8 weeks variable-duration therapy. This finding indicates that baseline viral load, on its own, is not a helpful predictor of response to ultra-short therapy.

Drug levels and IFNL4 polymorphisms

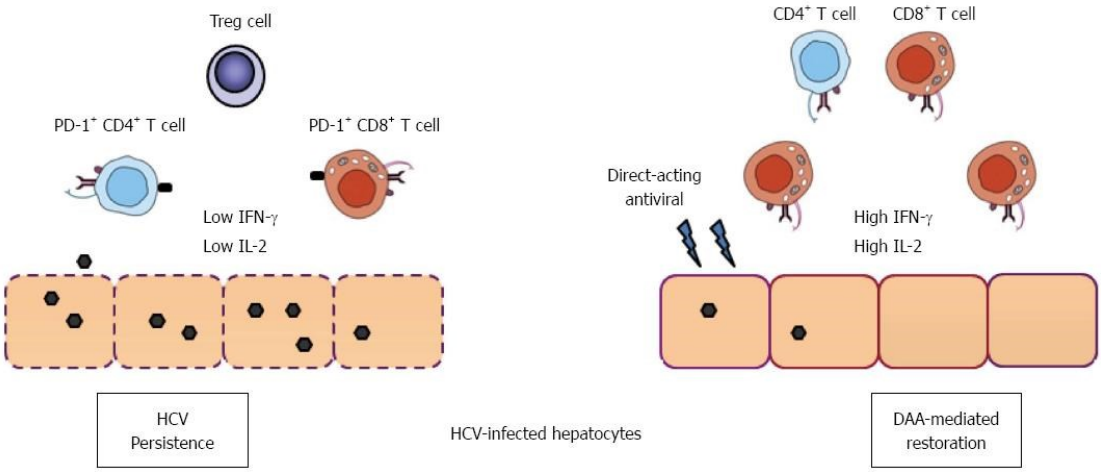
There is no evidence for the effect of drug levels or IFNL4 polymorphisms on treatment outcomes when DAA therapy is shortened to less than 8 weeks.

Host antiviral immunity

As discussed in the section on natural history of HCV, strong and persistent $CD8^+$ and $CD4^+$ T-cell responses and cytokine-induced factors (that directly inhibit virus replication) are critical for spontaneous HCV clearance[177]. In chronic infection, prolonged and excessive

stimulation of T cells by HCV antigens leads to progressive T-cell exhaustion. This is characterized by expression of several inhibitory receptors, including 'Programmed Cell Death-1' (PD-1) on virus-specific T cells, which reduce the quantity and functional capacity of the HCV-specific immune response[178]. In addition to this direct effect on HCV-specific T cells, chronic infection results in global immune dysregulation, altering vaccine response[179] and inducing autoimmune disease[91].

Figure 1-7: Immune function restoration after DAA therapy for HCV infection



In chronic HCV infection, upregulation of inhibitory receptors means T cells have a narrow repertoire, mounting a weak response to HCV antigens. CD8+ and CD4+ T cells express low levels of IFN-γ and IL-2 accompanied by up-regulation of PD-1 molecules in the liver. Development of T regulatory cells and compromised dendritic cell functions also contribute to T cell functional impairment. DAA's swiftly eliminate HCV antigen, resulting in down-regulation of PD-1, and a rapid restoration of virus-specific CD8+ T cell function[180]. HCV: Hepatitis C virus; IFN: Interferon; IL: Interleukin; PD-1: Program death-1; TCR: T cell receptor. Figure reproduced from Sun J, Rajsbaum R, Yi M. Immune and non-immune responses to hepatitis C virus infection. World J Gastroenterol 2015; 21(38): 10739-10748 [PMID: 26478666 DOI: 10.3748/wjg.v21.i38.10739] which has Creative Commons Attribution-Non-commercial (CC BY-NC 4.0) License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non-commercial and is otherwise in compliance with the license.

T cell recovery after removal of HCV antigen, or HCV-specific immune reconstitution, is increasingly recognised as a key determinant of SVR after HCV therapy[177,180–182] (figure 1-7). During the era of interferon therapy, disentangling the role of immune reconstitution from interferon induced immunomodulation was problematic. DAAs, unlike interferon, have no direct immunomodulatory effect, but instead directly inhibit viral assembly and secretion,

resulting in rapid removal of HCV antigen. DAA-mediated antigen removal has been shown to result in reduced PD-1 expression and improved proliferation of HCV specific CD8+ T cells[180]. Suppression of HCV is also associated with a decline in other T-cell exhaustion markers (CD57; Tim3; PD1) and augmentation of HCV-specific T-cell IFN-gamma responses after treatment[181].

The degree and speed at which this immune reconstitution occurs may be integral to achieving SVR, particularly with shortened therapy. In an aforementioned 4 week treatment study[174], in which genotype-1 infected adults achieved SVR rates of 30% with 4 weeks SOF/LDV+PI (Vedroprevir), higher HCV specific CD8+ T cell response and circulating PD-1+CD8+ or CD4+ T cell subset frequencies at baseline were identified as potential biomarkers of successful response to 4 weeks DAA therapy in ~90% patients[182]. While this suggest pre-treatment and end-of-treatment host immune function may be integral to ultimate clearance of HCV with short duration therapy, the analysis was conducted on peripheral blood mononuclear cells of just 26 patients, and there is currently no recognised immune biomarker that reliably predicts response to shortened HCV therapy.

In the final section of the introduction, I consider what is known about the HCV epidemic in Vietnam. I will describe what steps are been taken to expedite HCV's elimination before focusing on HBV and HDV and the contribution these viruses make to hepatitis-related morbidity and mortality.

Hepatitis C in Vietnam

It is likely HCV has been endemic in Vietnam for thousands of years, evolving in spatially restricted local epidemics which have resulted in enormous regional viral genetic diversity[183]. Subtypes 6a, 6e, 6h, 6k, 6l, 6o, 6p are thought to be the indigenous strains, with relatively recent introduction of genotypes 1b and 2a in the first half of the 20th century (likely from East Asia), and genotype 1a in the 1960s (likely from the USA). In the 20th century, HCV

prevalence rose exponentially, in parallel with increased use of parenteral healthcare interventions and intravenous drug use. The American war (1955-1975) likely accelerated the spread of non-6 genotypes in Vietnam[184] and may have contributed to propagation of the virus elsewhere: the highest rates of HCV among US veterans are found in those who served in Vietnam between 1964 to 1975[185]. The true burden of HCV in Vietnam today remains poorly understood. One reason for this is that, for more than two decades after its discovery, there was limited capital or motivation to screen large numbers of people for a disease which was notoriously difficult and expensive to treat.

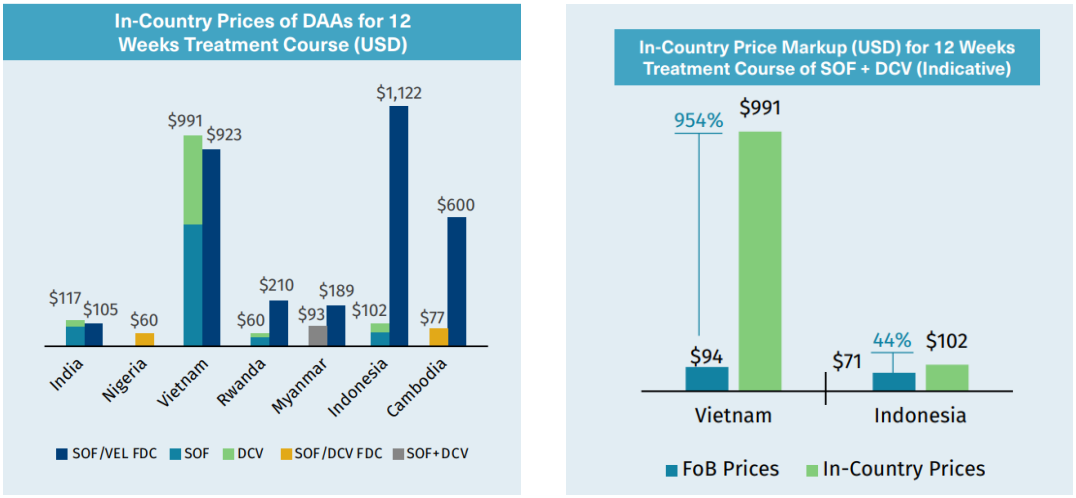
HCV treatment in Vietnam

Pegylated Interferon and ribavirin treatment became available in specialist centres, for those able to afford it, from around 2003. However, it was only officially approved by the Vietnam Ministry of Health (MoH) in 2013, when it was formally introduced into national HCV treatment guidelines. An analysis of treatment costs from 2017 revealed the total cost of Peg-IFN treatment was between US$2,156 and US$5,887[186], with patients incurring substantial out-of-pocket costs (even if receiving the maximum level of support from the national health insurance programme).

DAAs first became available through specialised treatment centres in 2016, but again, most patients were priced out of treatment: a standard course of DAA therapy costing between $2000-2500 at the Hospital for Tropical Disease in HCMC[187]. An additional barrier was that outpatient treatment was initially only available in three cities in Vietnam: HCMC, Hanoi, and Hai Phong. In 2019 the Vietnam MoH announced that DAA therapy would be 50% subsidised by national insurance, and treatment was expanded to most major cities. Unfortunately, HCV care remains prohibitively expensive for many of those infected due to the exceptionally high cost of drugs. The Clinton Health Access Initiative market report on HCV diagnostics and treatments, which provides key updates on global supply and pricing trends, singles out Vietnam as the most expensive place in the world to procure DAAs amongst LMICs with access to voluntary licences. Generic SOF/DCV is procured from foreign manufacturers for US$94 but undergoes a staggering in-country mark-up of 954% (figure 1-7). This is variously attributed to insurance costs, taxes and duties, logistics, and

distributor margins[188]. Until this is seriously addressed, access to treatment will remain limited.

Figure 1-8: In country prices of DAAs for 12 week treatment course (US$)



Reproduced from the Hepatitis C Market Memo, July 2022[188], produced by Clinton Health Access Initiative: an interim update on the market for hepatitis C virus (HCV) diagnostics and treatment, providing key updates on supply and pricing trends from January 2021 to April 2022.

HCV prevention in Vietnam

Prevention of community acquisition of HCV has benefited from a rapid expansion of HIV programmes in Vietnam in the early 20th century, driven by donations from The Global Fund[189] and the US Presidents Emergency Plan for AIDS Relief (PEPFAR)[190] among others. Opioid substitution therapy (OST) and needle and syringe programmes were introduced in 2007, followed by universal antiretroviral treatment (ART) for HIV-infected PWID. Since then additional community-based organisations (CBO) have emerged in most cities, delivering HIV and hepatitis screening and distributing free syringes[191]. In 2015 the Ministry of Health (MoH) expanded methadone treatment to at least 30 provinces with the aim of providing treatment for more than 80,000 drug users[192]. According to data from the Department of HIV/AIDS Prevention and Control, by 2017, more than 50,000 people had received opiate replacement therapy[193]. However, while these initiatives have been effective in reducing the incidence of HIV, their impact on the incidence of HCV seroconversions in

PWID seems to have been more limited[194]. In recent years, international funding for harm reduction intervention programs in Vietnam has decreased significantly[193].

Healthcare associated transmission of HCV has also been a major problem in Vietnam until relatively recently. A systematic review of HCV infection in haemodialysis patients found that prior to 1999, seroprevalence of HCV antibodies was 54.8% (95% CI 48–61.5%) in the south of Vietnam, falling significantly to 15.5% (95% CI 8.1–22.9%) from 2008, ($p < 0.001$)[195]. Seroprevalence is also high in individuals who have received multiple blood transfusions, estimated at around 6% (32/529) in one study that used data from prior to 2010[42]. In 2012, the Vietnam MoH formally introduced national infection prevention and control guidelines. These aimed to establish infection control teams and standardised operating procedures in all healthcare facilities, with mandatory monitoring and audit of healthcare associated infections. While this has been effectively implemented in large tertiary centres in major cities, a 2017 government-commissioned report acknowledged that implementation of safe injections, and sterilization of procedural and surgical instruments has not been addressed in smaller healthcare facilities. According to the report, 20% of medical facilities with over 150 beds were yet to establish an infection control department with active screening and surveillance of blood born viruses[193].

Antibody screening of blood donations for HBV, HCV and HIV was first implemented in Vietnam in 1992 with additional pooled nucleic acid screening from 2013. While blood safety is likely to have improved significantly in the last thirty years, longitudinal data are lacking. In a 2021 report on blood safety the Vietnam MoH cites a lack of standardised quality control on blood product screening and incomplete surveillance as important areas for improvement[193].

Hepatitis B

While this thesis is primarily focused on HCV, no discussion of viral hepatitis elimination should ignore Hepatitis B virus (HBV), which has similar sequelae to HCV (chronic hepatitis,

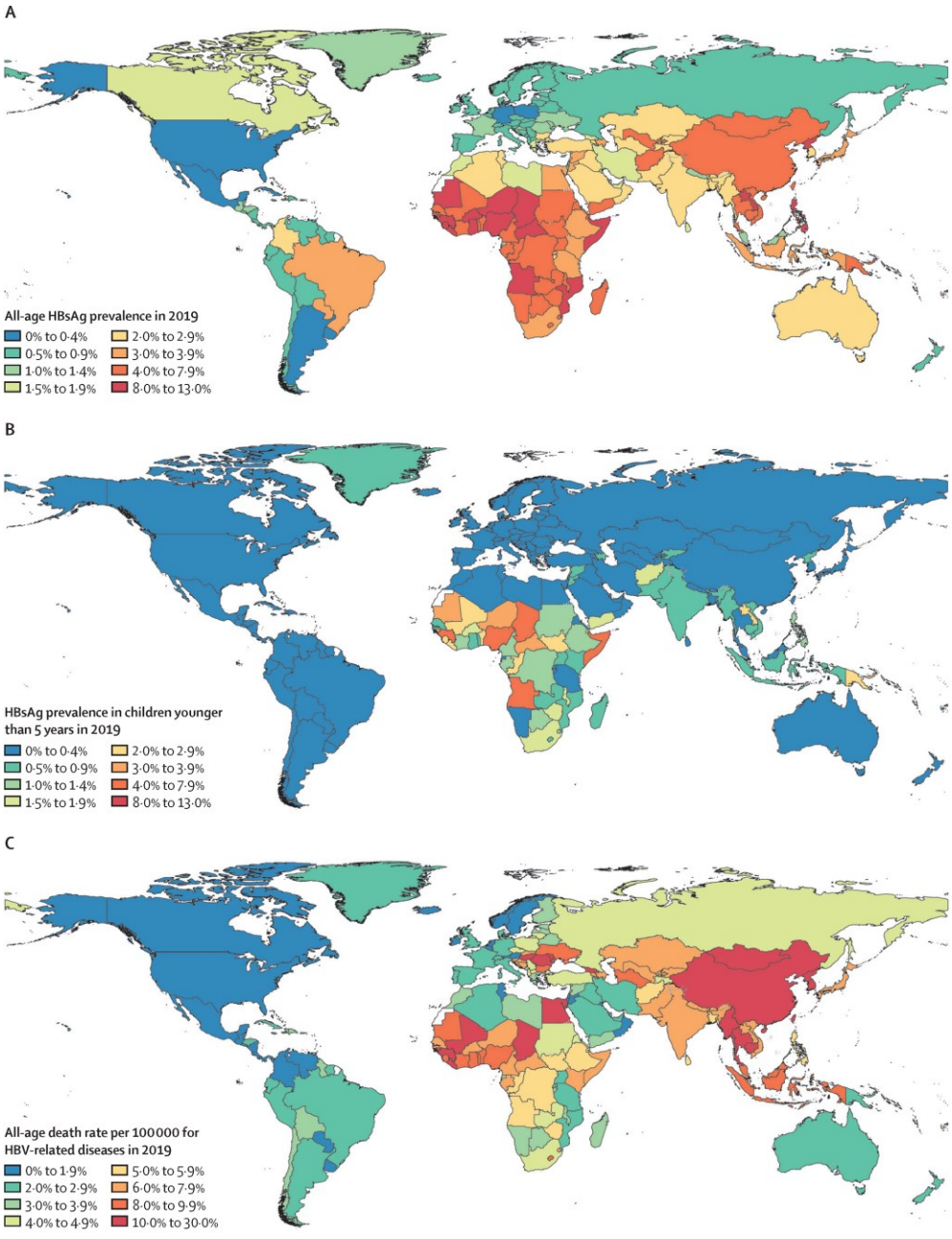
liver cirrhosis and cancer), is transmitted through many of the same routes (unsafe healthcare activities, and intravenous drug use), but dwarfs HCV in terms of prevalence, morbidity and mortality.

HBV is a *hepadna* virus that is estimated to have infected around two billion people worldwide, resulting in chronic carriage in approximately 316 million[196]. Regional prevalence is highly variable (figure 1-8), but some of the highest rates of infection in the world are observed in the Western Pacific, including Vietnam. The Vietnam MoH estimates around 8-25% of the population are Hepatitis B surface antigen positive[197], but in the absence of large cross sectional serosurveys or effective surveillance, the precise burden is unknown. Around 7,603 (95% C.I. 5,418 - 10,480) HBV-related deaths are estimated to occur each year.

Unlike HCV, HBV infection is most frequently acquired in childhood, through vertical mother-to-child transmission (MTCT), or through horizontal transmission from infected close contacts. The wide range in prevalence of chronic infection in different parts of the world largely reflects differences in the age at exposure, which is inversely related to risk of chronicity. Progression from acute to chronic HBV infection is estimated to be around 90% for perinatally acquired infection, 20-50% for infections between the age of one and five years, and <5% percent for adult-acquired infections[198–200].

Figure 1-9 Geographical distribution of the prevalence and death rate of hepatitis B in 2019.

(A) All-age HBsAg prevalence in 2019. (B) HBsAg prevalence in children younger than 5 years in 2019. (C) All-age death rate per 100 000 for HBV-related diseases in 2019. HBV=hepatitis B virus.



Reproduced from Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019[196] *available through Creative Commons Attribution (CC BY 4.0).*

Hepatitis B natural history

HBV has a complex natural history, that progresses from HBeAg-positive chronic infection to HBeAg-positive hepatitis, to HBeAg-negative chronic infection over a period of 10-40 years (figure 1-8).

Figure 1-10 Hepatitis B disease phases and treatment indications

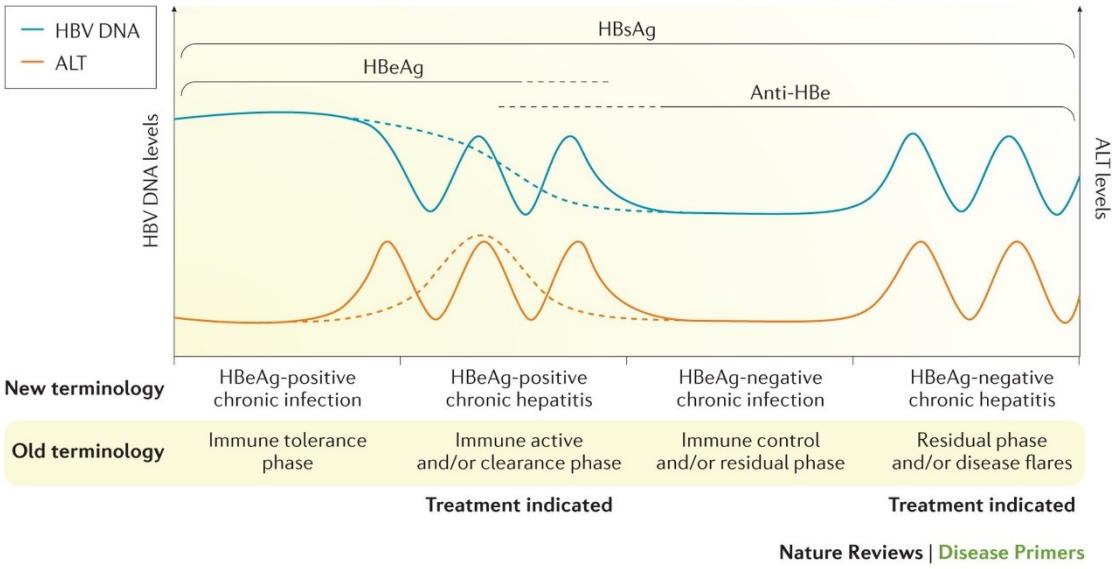


Diagram adapted from Hepatitis B virus infection, Man-Fung Yuen et al, Nature Reviews (with permission under licence number 5420670585922, Nov 02, 2022) showing the relationship between hepatitis B virus (HBV) DNA and alanine transaminase (ALT) levels and the relation of these levels to different phases of chronic HBV infection using new and old terminology. Some patients (solid lines) experience intermittent flares in HBV DNA and ALT levels before achieving HBeAg seroconversion. Other patients (dashed line) may have a less frequent flares. Treatment is indicated when the HBV DNA levels are >2,000 or >20,000 international units (IU) per litre and ALT levels are higher than one or two times the upper limit of normal according to different regional guidelines. anti-HBe, antibodies against Hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

Variability in the age at which HBeAg to anti-HBe seroconversion occurs partly explains why the risk of MTCT is especially high in South-East Asia and the Western Pacific: late HBeAg seroconversion means mothers are frequently still HBeAg positive (with high HBV viral loads) at time of delivery. In the absence of any preventive interventions, the risk of transmission from mother to child ranges from 70-90% for HBeAg mothers with high viral loads and from 10-40% for those that are HBeAg negative[201–203]. Historical epidemiological

studies estimate 50% of women in Southeast Asia are HBeAg positive at the time of delivery in comparison with less than 15% in African cohorts, where perinatal transmission is proportionately less common[204].

Age of seroconversion is influenced by viral and host factors, with HBV genotype thought to play an important role. HBV is classified into 10 genotypes (A to J) based upon a genetic divergence of greater than 8% in the complete nucleotide sequence. Although genotypes are not tested in routine HBV care, there is growing evidence that they influence clinical outcome and HBeAg seroconversion rates. In Vietnam genotypes B and C predominate[205,206], and genotype C, in particular, is associated with later age and lower rate of HBeAg seroconversion[207]. This may partly explain the exceptionally high rates of MTCT of HBV in the region.

HBV prevention in Vietnam

Regardless of genotype, HBV infection is still largely preventable through a highly efficacious vaccine, that has been available for over 30 years. According to WHO guidelines, infants born to mothers who are HBsAg positive should receive active and passive immunization (i.e. hepatitis B vaccine and hepatitis B immune globulin [HBIG]) as soon as possible and preferably within 12 hours of birth. This intervention reduces perinatal transmission by at least 95%. In addition, nucleoside analogue therapy (tenofovir) is recommended in the third trimester of pregnancy for mothers with high HBV viral loads. HBV vaccination is also recommended for high-risk groups such as PWID, sex workers and people on haemodialysis.

HBV was added to standard childhood immunisation programme in Vietnam in 1997 and birth dose vaccination was introduced in 2004. However, despite immunisation campaigns at district obstetric facilities in recent years, HBV birth-dose vaccination remains below WHO targets, at around 80% nationally[193]. HBV antivirals have been covered by government insurance since 2015, but passive immunisation of new-borns with HBIG must still be paid by parents and costs around US$100. Blood donor screening, implementation of infection control in hospitals and dialysis units, and promotion of community interventions for PWID

have also play a part in reducing community and healthcare associated transmission of HBV in recent decades.

Data on the HBV cascade of care in Vietnam is lacking, but in 2017 WHO estimated just 1.34% (43,230/3,220,000) of treatment eligible patients were on antiviral therapy[197].

Hepatitis D

Hepatitis D (HDV or delta virus), discovered in 1977, is a defective virus that requires the presence of HBV for complete virion assembly and secretion. Consequently, individuals with HDV are always dually infected with HDV and HBV. The virion comprises an RNA genome, a single HDV encoded antigen, and a lipoprotein envelope courtesy of HBV.

Transmission can occur simultaneously with HBV (co-infection), after which chronicity is determined by persistence or clearance of HBV. Alternatively HDV infection occurs when a HBsAg positive individual is exposed to HDV (superinfection), after which HDV chronicity is the norm[208]. Clinical sequelae of HDV infection are similar to HBV and HCV, varying from an inactive carrier state to fulminant hepatitis with liver failure.

While acute HDV infection is characterised by direct cytopathic damage in the liver, the natural history of chronic infection is highly variable and is determined by a complex interplay between HBV activity, the host immune response and viral factors, including HDV genotype and expression of Hepatitis D antigen (HDV Ag). HDV genotype is increasingly recognised as a key determinant of prognosis[209]. Eight HDV genotypes have been described, but most existing data concerns genotypes 1 and 2. Genotype 1 is dominant in Europe and North America and is associated with increased risk of liver failure and progression to cirrhosis[210]. Genotype 2 HDV is more common in East and Southeast Asia and generally has a more benign course.

The global burden of HDV is poorly understood, on the basis that most infections remain undiagnosed. A recent systematic review estimates the prevalence is around 13.02% (95% C.I 11.96–14.11%) among HBV carriers, with far higher rates among HBV-infected individuals with cirrhosis (25.77% (20.62–31.27%) and HCC (9.80% 10.97–30.45%)[211]. Investigators of

that study estimate that overall, HDV infections progress to cirrhosis within 5 years and to HCC within 10 years.

HDV in Vietnam

HDV seroprevalence data in Vietnam is especially sparse. HDV does not feature in current hepatitis guidelines or elimination strategy and most clinics do not test for it. This is partly because the optimal treatment of HDV is uncertain, and the current therapeutic options are expensive and frequently unavailable: first line treatment for chronic HDV is either pegylated IFN alfa-2a or pegylated IFN alfa-2b administered for one year, but these therapies are no longer recommended in national HBV or HCV treatment guidelines, making procurement impossible. New HDV therapeutics are forthcoming[212], so understanding the true burden and impact of HDV in Vietnam will be vital.

Chapter 2

Seroprevalence of Hepatitis B, C and D in Vietnam

Background

Vietnam is one of twenty countries reported to shoulder 75% of the world's burden of viral hepatitis[213]. Around 96% of viral hepatitis deaths globally are attributable to Hepatitis B virus (HBV) and Hepatitis C virus (HCV)[213] but the prevalence of these infections in Vietnam is poorly characterized and published estimates vary widely.

Reliable prevalence data is considered a central policy indicator by which to measure a country's progress towards elimination[213,214]. In a recent analysis of policy scores and rankings of 66 countries with the highest burden of viral hepatitis, Vietnam was singled out as scoring poorly[214]. Mandatory reporting of newly diagnosed hepatitis infections was introduced in 2015 but implementation of the online surveillance system has been problematic: by 2019 just 52,086 cases of hepatitis B virus and 6,792 cases of hepatitis C had been formally reported to the national surveillance system, representing less than 1% of total estimated cases of both infections from past surveys. The Vietnam Ministry of Health blames this under-reporting on software glitches and inadequate awareness of notifiable disease surveillance at the local level[215]. In the absence of reliable surveillance or regular large cross-sectional surveys, meta-analysis of small scale seroprevalence studies can provide prevalence estimates to focus elimination efforts.

To formally assess this gap in the literature I searched PubMed and Embase for systematic reviews published between January 1st 1990 (when HCV antibody testing became available) and 31st December 2021 using the terms 'Hepatitis' and 'Vietnam' and 'prevalence'. I found two relevant publications: a 2019 meta-analysis of 16 studies relating to HBV and HCV infections in dialysis patients[195], and a 2017 systematic review of HCV control efforts[216]. The latter identified basic epidemiological and public health data as a significant gap in the literature and concluded there is an urgent need for an up-to-date assessment of hepatitis disease burden in Vietnam.

HBV prevalence estimates

Morbidity from viral hepatitis in Vietnam is largely driven by Hepatitis B virus (HBV), with most chronic infections acquired through mother-to-child transmission (MTCT)[217] and horizontal transmission in early childhood[218]. Vietnam's Ministry of Health (MOH) approximates the prevalence of chronic HBV infection to range from 8-25%[197]. WHO Vietnam Office estimates there were 7,697,525 chronic infections in 2017 (8.1% prevalence)[219], and the most recent Global Burden of Disease (GBD) modelling from 2019 estimates a prevalence of 6.6% (95% C.I. 6.30 - 6.92)[12] based on data from eight studies[220]. The Vietnam Viral Hepatitis Alliance concedes that since these models are based on a small number of studies, the true burden of HBV in Vietnam remains uncertain[197].

HCV prevalence estimates

Prevalence estimates for HCV are similarly variable. Since 2015 WHO have reported HCV prevalence in terms of individuals with active infection (based on detection of HCV RNA), rather than detectable anti-HCV antibodies alone, to better reflect the numbers requiring treatment. WHO estimate that approximately one million Vietnamese (~1%) have chronic active infection[197], while the most recent GBD modelling suggests this figure may be over 60% higher (1.66% [95% C.I 1.35 – 2.0])[12]. A major reason for this discrepancy is that a high proportion of HCV infections are believed to result from unsafe health-care associated activities[42,54,55] and community services[56–58], making it difficult to accurately assess the size of the population at risk of chronic infection. Prevalence estimates from both low- and high-risk populations and data relating to co-infection with HIV are needed to better characterize the epidemic and plan elimination strategy.

HDV prevalence estimates

HDV is not currently screened for in Vietnam and is not included in national treatment guidelines. It therefore unclear what contribution HDV makes to hepatitis-related morbidity and mortality. Worldwide HDV prevalence is estimated to be around 4.5% (95% CI 3.6-5.7) among all HBsAg-positive individuals and around 16.4% (14.6-18.6)[221] in those attending

hepatology clinics. However, prevalence is highly variable and does not parallel rates of HBV infection[221]. In some regions of the world HDV is endemic, with high rates of infection recorded in Pakistan[222], Mongolia and some countries in Eastern Europe and West Africa[221]. However, in other countries that report high rates of HBV and might be expected to have high rates of HDV co-infection, prevalence of HDV is low and largely confined to high-risk individuals such as PWID. Improved HDV therapeutics are forthcoming[212], so HDV prevalence estimates for Vietnam are urgently needed.

Trends in seroprevalence

In the last thirty years Vietnam has undergone unprecedented change[10]. Economic and political reforms under 'Doi Moi', launched in 1986, established Vietnam as the fastest-growing economy in the world[223]. It is estimated that between 2002 and 2018, 45 million people were lifted out of poverty[10], bringing remarkable improvements in public health. Notable progress has been made in access to HBV vaccination, blood donor screening, healthcare infection control and, more recently, government subsidization of HBV and HCV therapy, altering the shape and scope of the hepatitis epidemic. The study described in this chapter evaluates viral hepatitis trends over the last 30 years with the aim of informing future hepatitis elimination strategy.

Objectives

1. Conduct a systematic review of all published HBV, HCV and HDV prevalence studies in Vietnam since 1990

2. Assess study quality and heterogeneity to determine if meta-analysis of pooled seroprevalence of each infection in low- and high-risk populations is viable

3. Evaluate geographical and chronological trends in viral hepatitis prevalence in Vietnam

Methods

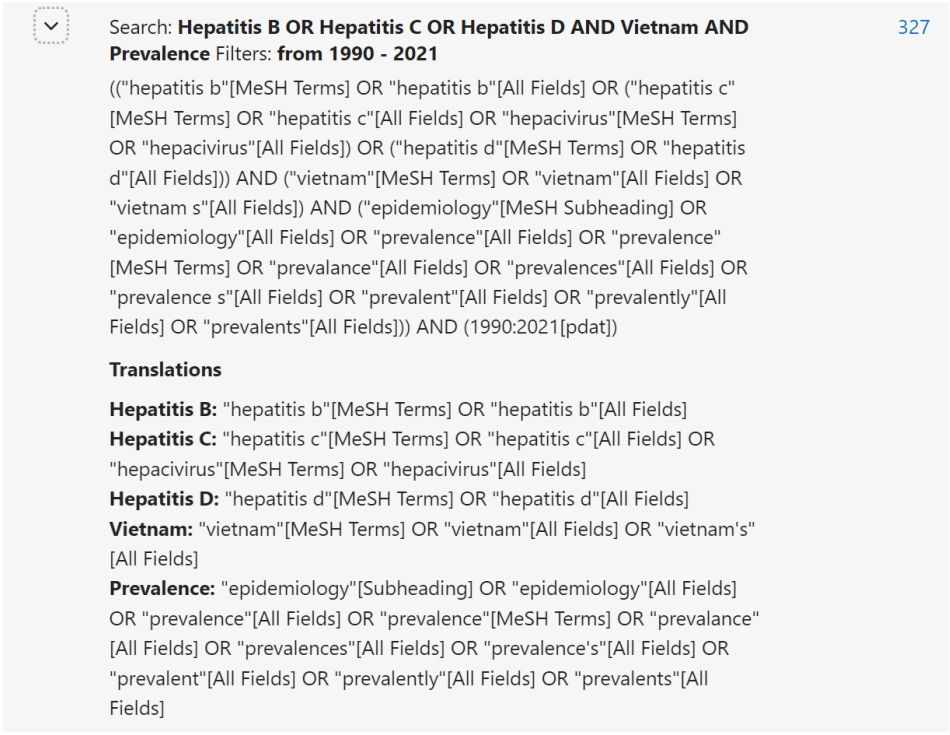
I conducted this systematic review in accordance with PRISMA guidance[224]. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) provides an evidence-based minimum set of items for reporting systematic reviews and meta-analyses and has become the standard by which most journals expect such studies to be reported. While PRISMA primarily focuses on the reporting of reviews evaluating the effects of interventions, it can also be used for reporting systematic reviews with alternative objectives such as prevalence, diagnosis or prognosis[224]. A full checklist for this systematic review is provided at the end of the chapter.

I registered the study with the Centre for Reviews and Dissemination (CRD) on 4th September 2020 (PROSPERO CRD42020202567). PROSPERO is an international database detailing the key aspects of prospectively registered systematic reviews for which there is a health-related outcome. By providing a comprehensive listing of systematic reviews registered at inception, PROSPERO helps avoid duplication of work and reduces reporting bias by enabling comparison of the completed review with what was planned in the protocol[225]. This study conforms to its original protocol, with subsequent addition of chronological and HIV co-infection analyses (neither of which affect the original study objectives).

I searched Medline, Embase and Global Health - Ovid® (Wolters Kluwer) from 1st January 1990 to 31st December 2021 for all reports that contained data for HBV, HDV and HCV seroprevalence in Vietnam. Using a free text search strategy, I entered the search terms [‘Hepatitis B’ OR ‘Hepatitis C’ OR ‘Hepatitis D’] AND [‘Vietnam’] AND [‘Prevalence’] (figure 2-1).

Figure 2-1: PubMed Free text advanced search

'Hepatitis B' OR 'Hepatitis C' OR 'Hepatitis D' AND 'Vietnam' AND 'Prevalence' with date filter 1990 – 2021 elicits 327 results.



Search: **Hepatitis B OR Hepatitis C OR Hepatitis D AND Vietnam AND Prevalence** Filters: **from 1990 - 2021** 327

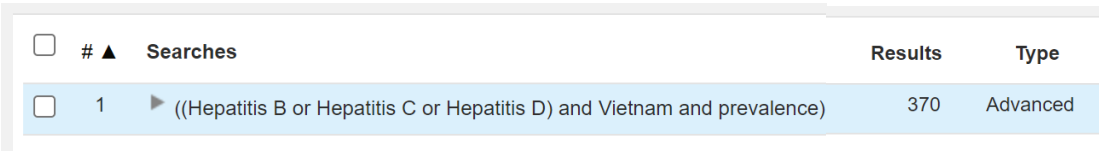
(("hepatitis b"[MeSH Terms] OR "hepatitis b"[All Fields] OR ("hepatitis c"[MeSH Terms] OR "hepatitis c"[All Fields] OR "hepacivirus"[MeSH Terms] OR "hepacivirus"[All Fields]) OR ("hepatitis d"[MeSH Terms] OR "hepatitis d"[All Fields])) AND ("vietnam"[MeSH Terms] OR "vietnam"[All Fields] OR "vietnam s"[All Fields]) AND ("epidemiology"[MeSH Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms] OR "prevalance"[All Fields] OR "prevalences"[All Fields] OR "prevalence s"[All Fields] OR "prevalent"[All Fields] OR "prevalently"[All Fields] OR "prevalents"[All Fields])) AND (1990:2021[pdat])

Translations

Hepatitis B: "hepatitis b"[MeSH Terms] OR "hepatitis b"[All Fields]
Hepatitis C: "hepatitis c"[MeSH Terms] OR "hepatitis c"[All Fields] OR "hepacivirus"[MeSH Terms] OR "hepacivirus"[All Fields]
Hepatitis D: "hepatitis d"[MeSH Terms] OR "hepatitis d"[All Fields]
Vietnam: "vietnam"[MeSH Terms] OR "vietnam"[All Fields] OR "vietnam's"[All Fields]
Prevalence: "epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms] OR "prevalance"[All Fields] OR "prevalences"[All Fields] OR "prevalence's"[All Fields] OR "prevalent"[All Fields] OR "prevalently"[All Fields] OR "prevalents"[All Fields]

Figure 2-2: Embase Free text advanced search

Hepatitis B' OR 'Hepatitis C' OR 'Hepatitis D' AND 'Vietnam' AND 'Prevalence' with date filter 1990 – 2021 in Embase Classic+Embase (1947 to 2022 March 11), Global Health (OVID) (1973 to 2022 Week 10) elicits 370 results.



# ▲	Searches	Results	Type
1	((Hepatitis B or Hepatitis C or Hepatitis D) and Vietnam and prevalence)	370	Advanced

I included both prospective and retrospective studies with manuscripts published in English, French or Vietnamese language. This included published surveys from screening programmes, antenatal clinics, blood donations, sexual health and HIV clinics, needle and

syringe programmes for people who inject drugs (PWID), commercial sex worker initiatives, and inpatient, outpatient and community serosurveys.

For HBV I included studies reporting HBsAg, the diagnostic marker of infection. For HCV and HDV, I separated studies reporting antibody (a marker of past exposure, but not necessarily active infection), from those reporting antigen or RNA (markers of active infection) for separate analyses. HCV core antigen assays are around 90%-95% sensitive and 98-100% specific in diagnosing active HCV infection versus gold standard HCV RNA testing by polymerase chain reaction (PCR)[226–229]. For HCV I additionally noted any studies that included genotyping to estimate pooled prevalence of genotypes 1-6.

I excluded non-peer reviewed conference abstracts, and studies not stating sample size or HBV, HCV or HDV seroprevalence. I also rejected studies involving Vietnamese patient populations from outside of Vietnam (e.g. USA) and studies exclusively published in Vietnamese without evidence of peer review.

I performed the literature search, screened all abstracts, and extracted all required data from prevalence studies. Hang Vu Thi Kim (HVTK), an OUCRU colleague and study doctor, independently reviewed the abstract search, reviewed all manuscripts written in Vietnamese, checked the 49 full-text articles that I had excluded, and assisted with data entry. Discrepancies regarding study eligibility were resolved through discussion between myself and HVTK.

I documented study title, authors, study type & design and seroprevalence data for extraction and synthesis on a spreadsheet (with predetermined dropdown lists where applicable). I recorded year of publication, year(s) of data collection, region of Vietnam, study population, study type, exposure risk, sample size, HBsAg and/or HCV antibody seroprevalence, HCV antigen and/or PCR prevalence, HCV genotypes, HDV antibody and HDV RNA prevalence, and prevalence of HIV co-infection. I also recorded HBV and HCV co-infection prevalence in HIV infected populations.

Given the heterogeneity in study populations included in viral hepatitis seroprevalence surveys, I classified each population by risk. I subcategorized ‘high risk’ populations into patients with known liver disease (acute hepatitis, chronic hepatitis, hepatocellular carcinoma (HCC)), and patients with high-risk exposure to blood borne viruses. In the latter group I

included i) people who inject drugs (PWID), ii) commercial sex workers (CSW) iii) men who have sex with men (MSM) attending sexual health services, iv) dialysis patients v) individuals who have had multiple transfusions or major surgery and vi) (for HBV only) children of HBsAg positive mothers.

I defined 'low risk' as absence of any of the above risk factors. To limit sampling bias I further subdivided low-risk groups into blood donors and non-blood donors in recognition that blood donors are risk-assessed prior to testing and may not be representative of the general population. I further subcategorised non-blood donors into i) antenatal patients, ii) adults in the general population (including community studies, outpatient studies and occupational surveys), iii) children in the general population and iv) inpatients with non-hepatic illness.

Assessment of methodological quality

Methodological quality of all selected studies was assessed using the Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence data[230], a validated tool specifically designed to assess methodological quality of prevalence studies. It is comprised of nine criteria to assess whether a study should be included in a review.

1. Was the sample frame appropriate to address the target population?
2. Were study participants recruited in an appropriate way?
3. Was the sample size adequate?
4. Were the study subjects and setting described in detail?
5. Was data analysis conducted with sufficient coverage of the identified sample?
6. Were valid methods used for the identification of the condition?
7. Was the condition measured in a standard, reliable way for all participants?
8. Was there appropriate statistical analysis?
9. Was the response rate adequate, and if not, was the low response rate managed appropriately?

Each study is given a score out of nine and a decision is made on whether it is suitable for inclusion in the meta-analysis. There is no agreed standard for assessing methodological quality of prevalence studies. A 2020 analysis of systematic reviews of prevalence studies identified an urgent need for the development and validation of a standardised tool that could

be widely endorsed and accepted by the research community. The authors conclude that until that has been achieved, the JBI critical appraisal tool is the optimum choice for assessing study quality on the grounds that it has been formally evaluated and remains the most popular method[231].

Meta-analysis

Not all data harvested from a systematic review is suitable for meta-analysis. To determine whether data was eligible for pooled analysis I held that study populations, years of data, locations, diagnostic tests, and sampling strategies should all be adequately described. While most studies met these criteria, there was substantial qualitative heterogeneity between the blood donor studies and other low-risk populations, such that it was not possible to include blood donor cohorts pooled prevalence estimates for all low-risk populations.

Statistical Analysis

I determined point estimates and 95% CIs for the proportion of people with HBsAg, HCV antibody, HCV core antigen or PCR, HDV antibody and HDV RNA where available. In light of substantial between-study heterogeneity, data from each study were pooled with a DerSimonian-Laird random-effects model[232], which estimates between-study variance, allowing that the true effect size may vary between studies. Pooling describes the practice of gathering together small sets of data that are assumed to have a shared value or characteristic (e.g. 'antenatal populations, PWID) and using the combined larger set (the "pool") to obtain a more precise estimate of that characteristic. I combined different populations from the same study provided they were from same decade, population and geographical region. From this I estimated the overall pooled prevalence of HBsAg, HCV antibody and HCV antigen in low-risk groups (blood donors and other low risk populations), those at high risk of exposure and those with liver disease. I further determined prevalence of each virus by population subgroup.

For the chronological analysis, I assessed pooled prevalence by decade (1990-2000; 2001-2010; 2011-2020). In high-risk populations I used a different approach for each virus. For

HBV the difference in risk between various high-risk exposures is small, and it is recommended that all high-risk individuals are vaccinated. Therefore, I estimated pooled prevalence by decade in all high-risk exposures combined. For HCV there is no vaccine, and PWID are at highest risk of infection by far. Therefore, I restricted chronological and regional analysis of HCV to PWID populations, in which between study heterogeneity was minimal.

I utilised the t2 statistic to assess between-study heterogeneity for the estimates of pooled prevalence by population and by decade. The variance of raw proportions was stabilised with a Freeman-Tukey type arcsine square root transformation[233]. There are several methods available for pooling proportions; the Freeman-Tukey method works well with both fixed-effects and random-effects meta-analysis[234]. Statistical analysis was performed on R version 4.1[235].

Results

I analysed 72 studies in total, representing 22 different locations in Vietnam (figure 1). This included 501,543 individuals tested for HBsAg in 120 cohorts, 448,765 individuals tested for HCV (antibody or antigen/RNA) in 114 HCV cohorts, and 7055 individuals tested for HCV or HBV co-infection in 13 HIV cohorts. Most studies included populations from the three largest cities in Vietnam, HCMC (29), Hanoi (24) and Hai Phong (16), while rural representation was low (6 studies). A full list of included studies in shown in table 2-1 and appendix A.

A summary of all included studies, with study quality assessment, is shown in table 3. Breakdown of the quality assessment is shown in appendix A, table V. Study quality was generally good, but only 8 studies met all nine critical appraisal checklist criteria for prevalence data[230]. The most frequently identified deficiency was in sampling, with 82% (59/72) studies relying on non-random consecutive, or response-driven sampling or including an entire single centre population. Sampling was most rigorous in the low-risk general population, with 6/14 studies apparently truly cross sectional in nature.

Figure 2-3: Study selection

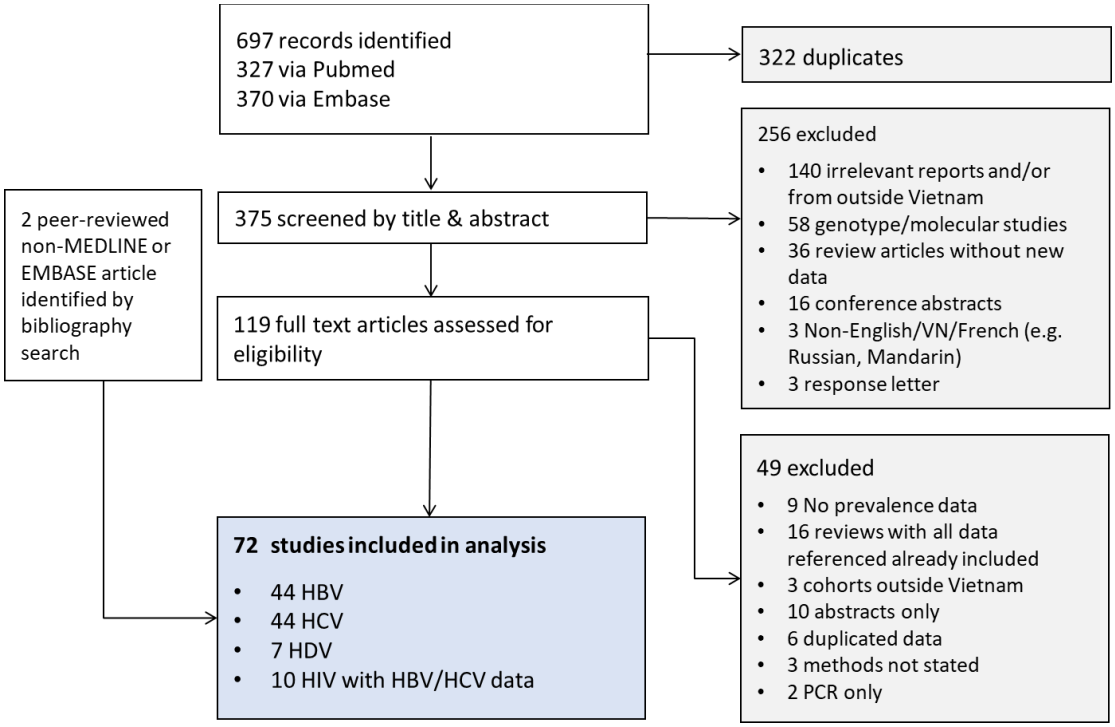


Table 3: Summary of 72 included studies with JBI quality assessment

Study	Year(s) of data	Region(s)	Study population(s)	Population categories	Prevalence data	JB score*	Potential bias
Barcus et al 2002	1992-96	HCMC	Inpatients with severe malaria	Low risk	HBsAg	7	Non-random consecutive sampling, non-representative sample (severe malaria)
Binh et al 2018	2013-15	Hanoi	HBV positive outpatients	HDV	HDV RNA	7	Non-random consecutive sampling, non-representative sample (85% male)
Boettiger et al 2015	1998-13	Hanoi	HIV outpatients	HIV population	HBsAg, HCV Ab	8	Non-random consecutive sampling
Buchy et al 2004	2002	Nha Trang	Inpatients with hepatitis	High risk (liver disease)	HBsAg, HCV Ab	7	Non-random consecutive sampling, underpowered
Bùi et al 2014	2005-11	Hanoi	HIV outpatients	HIV population	HBsAg, HCV Ab	8	Non-random; entire centre's population
Chau et al 2002	1991-96	HCMC	Inpatient PWID with malaria, inpatient non-	High risk (exposure), low risk	HBsAg, HCV Ab	8	Non-random consecutive sampling

			PWID with malaria				
Clatts et al 2009	2005-6	Hanoi	PWID	High risk (exposure)	HCV Ab, HIV coinfection	7	Non-random sampling; non-representative sample (male IVDU only)
Clatts et al 2015	2010-11	Hanoi, HCMC, Nha Trang	MSM sex workers	High risk (exposure)	HCV Ab	8	Non-random; non-representative (sex workers)
Colby et al 2016	2014	HCMC	MSM sex workers	High risk (exposure)	HCV Ab, HIV coinfection	8	Non-random sampling; non-representative (sex workers)
Cordier et al 1993	1989-92	Hanoi	Inpatients, patients with HCC	Low risk, high risk (liver disease)	HBsAg, HCV Ab	8	Non-random, non-representative, male HCC only
Corwin et al 1996	1993-95	Hanoi	Adults in general population, inpatients with hepatitis	Low risk, high risk (liver disease)	HBsAg, HCV Ab	8	Non-random consecutive sampling
Dang et al 2020	2019	Hanoi	MSM with HIV and non-MSM with HIV	HIV population	HBsAg, HCV Ab	8	Non-random consecutive sampling
Do et al 2015	2012	Binh Thuan	Adults in general population	Low risk	HBsAg, HCV Ab, HCV Ag	9	
Dunford et al 2012 (HBV)	2008-9	Hanoi, Haiphong, Danang, Khanh Hoa, Can Tho	Military, antenatal, adults in general population, blood donors, PWID, dialysis, CSW, multiple transfusions, surgical	Low risk, high risk (exposure), HDV	HBsAg, HDV Ab	8	Non-random consecutive sampling
Dunford et al 2012 (HCV)	2008-9	Hanoi, Haiphong, Danang, Khanh Hoa, Can Tho	Military, antenatal, adults in general population, blood donors, PWID, dialysis, CSW, multiple transfusions, surgical	Low risk, high risk (exposure)	HCV Ag/RNA	8	Non-random consecutive sampling
Duong et al 2009	2006	Thai Nguyen	Adults in general population	Low risk	HBsAg	8	Cross sectional but non-representative rural sample
Duong et al 2015 i	2012-2013	HCMC	Dialysis	High risk (exposure)	HCV Ag/RNA	8	Non-random consecutive sampling
Duong et al 2015 ii	2012-2013	HCMC	Dialysis	High risk (exposure)	HBsAg, HCV Ag	8	Non-random consecutive sampling
Duong et al 2016	2012-2014	HCMC	Dialysis	High risk (exposure)	HBsAg, HCV Ag	8	Non-random; entire centre population
Duong et al 2018	2014	Hai Phong	PWID	High risk (exposure)	HCV Ab	8	Non-random consecutive sampling
Duong et al 2019	2012-2014	HCMC	Dialysis	High risk (exposure)	HCV Ab, ACV Ag, HCV RNA	8	Non-random; entire centre population

Follezou et al 1999	1996	HCMC	PWID	High risk (exposure)	HBsAg, HCV Ab	6	Non-representative sample (very high rates HIV)
Goto et al 2005	2003	Nghe An	Antenatal	Low risk	HBsAg	8	Non-random consecutive sampling
Hall et al 2015	2010-11	Hanoi, Hai Phong, Da Nang, Khanh Hoa, Can Tho	HBV positive PWID	High risk (exposure)	HDV Ab, HDV RNA	7	Non-random consecutive sampling, lacks baseline characteristics
Hipgrave et al 2003	1990-99	Thanh Hoa	Children in general population, adults in general population	Low risk	HBsAg	9	
Hoang et al 2015	2009	Hai Phong, HCMC	PWID	High risk (exposure)	HBsAg, HCV Ab	8	Non-random consecutive sampling
Ishizaki et al 2017	2007-2012	Hai Phong	Blood donors, antenatal, PWID, CSW	Low risk, high risk (exposure)	HBsAg, HCV Ag, HIV coinfection	8	Non-random consecutive sampling
Kakumu et al 1998	1994-96	HCMC, Da Lat	Chronic hepatitis; adults in general population	Low risk, high risk (liver disease)	HBsAg, HCV Ab	8	Non-random consecutive sampling of hepatitis patients. Details of sampling strategy for general population lacking
Katelaris et al 1995	1993	Dong Nai	children in general population	Low risk	HBsAg, HCV Ab	8	Under powered for HCV prevalence
Kha To et al 2020	2017-19	HCMC	blood donors	Low risk	HBsAg, HCV Ab	8	Non-random consecutive sampling
Lan et al 2008	2006	Hanoi	Adults in general population	Low risk	HBsAg	8	Non-representative sample (married women age 18-49)
Lien et al 1997	1994	HCMC	Adults general population, CSW, PWID, HIV patients	Low risk, high risk (exposure) HIV population	HCV Ab	8	Non-cross sectional sampling
Linh-Vi et al 2019	2013	Hanoi, Hai Phong, HCMC	CSW	High risk (exposure)	HBsAg, HCV Ab, HCV Ag, HIV coinfection	9	
Minh et al 2021	2018-20	Hue	Adults in general population	Low risk	HBsAg	7	Non-random, non-representative sample (males from infertile couples)
Miyakawa et al 2021	2009-12	Khan Hoa	antenatal, children in general population	Low risk	HBsAg	7	Non-random sample, high drop out >30%
Mohan et al 2017	2012-15	Hanoi, Pho Yen, Thai Nguyen	HIV outpatients	HIV population	HBsAg, HCV Ab	8	Non-random retrospective chart review
Molès et al 2020	2014	Hai Phong	PWID	High risk (exposure)	HCV Ab	8	Non-random response-driven sampling

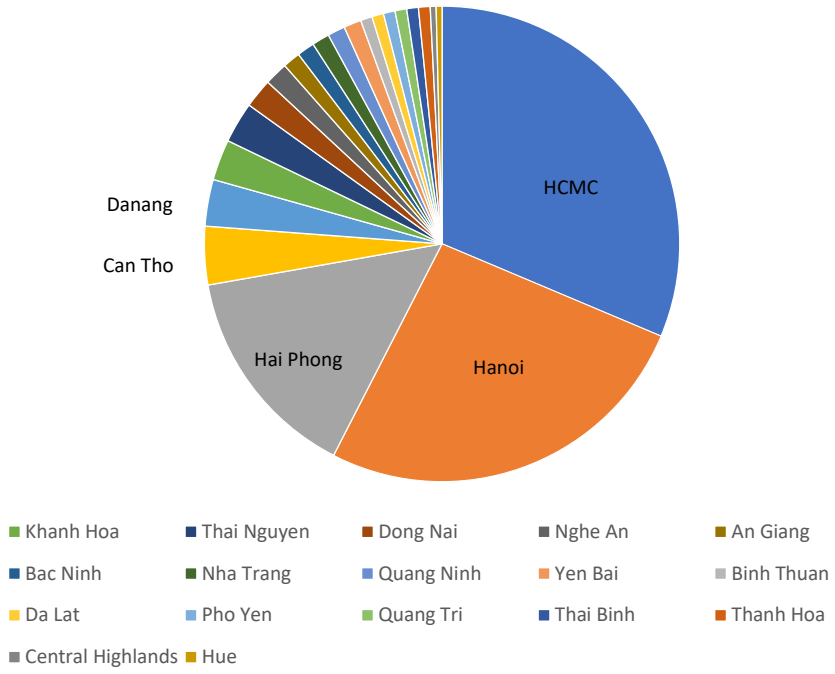
Nadol et al 2015	2009-10	Hanoi, Hai Phong, Quang Ninh, Nghe An, Yen Bai. Da Nang, Dong Nai, HCMC. Can Tho, An Giang	PWID	High risk (exposure)	HBsAg, HCV Ag, HIV coinfection	8	Non-random response-driven sampling
Nadol et al 2016	2009-10	Hanoi, Hai Phong, HCMC, Can Tho	MSM	High risk (exposure)	HBsAg, HCV Ag, HIV coinfection	8	Non-random response-driven sampling
Nakata et al 1994	1993	Hanoi, HCMC	inpatients, multiple transfusions, PWID. Dialysis, CSW, prisoners	Low risk, high risk (exposure)	HBsAg, HCV Ab	8	Non-random consecutive and retrospective sampling
Nerurkar et al 1999	1997-98	Hanoi	PWID	High risk (exposure)	HCV Ab, HIV coinfection	7	Non-random sampling, diagnostics were combo of sera or filter paper-blotted whole blood
Nghiem et al 2021	2018-19	Hanoi	HBV positive outpatients	HDV	HDV RNA	8	Non-random, consecutive sampling
Ngo et al 2009	2007	HCMC	General inpatients	Low risk	HBsAg, HCV Ab	6	Non-random, consecutive sampling, non-representative sample (inpatients and outpatients), minimal baseline characteristics
Nguyen et al 1997	1995	HCMC	Inpatients with Dengue	Low risk	HBsAg, HCV Ab	6	Non-random consecutive sampling, non-representative sample (patients with severe Dengue), under-powered for HCV
Nguyen et al 2006	2002	Thai Binh	Adults general population	Low risk	HBsAg	9	
Nguyen et al 2007	2002	Thai Binh	Adults general population	Low risk	HCV Ab	9	
Nguyen et al 2011	2007	Hai Phong	Adults in general population, antenatal, blood donors	Low risk	HBsAg	8	Non-random consecutive sampling
Nguyen et al 2014	2011	Vietnam (national)	Children in general population	Low risk	HBsAg	9	
Nguyen et al 2017	2015	Da Nang	HBV positive outpatients	HDV	HDV RNA	7	Non-random consecutive sampling, non-representative
Nguyen et al 2021	2017	Thai Nguyen	MSM in sexual health clinics, PWID	High risk (exposure)	HCV Ab	7	Non-random sampling, Oraquick diagnostics
Nguyen-Dinh et al 2018	2010-16	HCMC	Patients with HCC	High risk (liver)	HBsAg, HCV Ab	8	Non-random retrospective sample
Pham et al 2020	2017-18	Hai Phong	Antenatal, children of HBV infected mothers	Low risk, high risk (exposure)	HBsAg	9	

Pham et al 2020 ii	2018	Central Highlands	Adults in general population	Low risk	HBsAg	9	
Quan et al 2009	2003	Bac Ninh	PWID	High risk (exposure)	HBsAg, HCV Ab, HIV coinfection	8	Non-random snowball sampling using peer recruiters
Quesada et al 2015	1994-05	HCMC	Adults in general population	Low risk	HCV Ab	8	Non-representative sample (females only)
Rangarajan et al 2016	2013-14	HCMC	HIV outpatients	HIV population	HBsAg, HCV Ab	8	Non-random consecutive sampling
Riondel et al 2020	2016-17	Hai Phong	PWID	High risk (exposure)	HCV Ab	8	Non-random response-driven sampling
Sinh et al 2012	1992-09	HCMC	Dialysis	High risk (exposure)	HCV Ab	7	Non-random consecutive sample, lacking baseline characteristics
Son et al 2014	2006-09	Hai Phong	HIV inpatients	HIV population	HBsAg, HCV Ab	6	Non-random consecutive sampling, non-representative (inpatients with penicilliosis), under powered for HBV/HCV prevalence
Song et al 1994	1992	HCMC, Hanoi	blood donors	Low risk	HBsAg, HCV Ab	8	Non-random sampling
Sy et al 2013	2000-09	Hanoi	HBV positive outpatients	HDV	HDV RNA	7	Non-random consecutive sampling, non-representative (HCV and HIV positive patients excluded)
Tanimoto et al 2010	2007	Hai Phong	PWID	High risk (exposure)	HCV Ab, HCV PCR	8	Non-random response driven sampling
Tanuma et al 2017	2007-13	Hanoi	HIV outpatients	HIV population	HBsAg, HCV Ab	8	Non-random consecutive sampling
Terakawa et al 2011	2009-10	HCMC	Adults in general population	Low risk	HBsAg, HCV Ab	7	Non-random sampling, non-representative (healthy workers at major companies)
Thanh et al 2020	2016-17	HCMC	Hepatitis outpatients	High risk (liver)	HBsAg	7	Non-random consecutive sample, non-representative (HCV-infected outpatients)
Tran et al 2003	1998-02	HCMC	Adults in general population, patients with liver disease	Low risk	HCV RNA, HDV Ab	7	Non-random sampling, non-representative (healthy outpatients)
Truong et al 2016	2014	Hai Phong	HIV outpatients	HIV population	HBsAg	8	Non-random consecutive sample
Trung et al 2010	2006-08	HCMC	Inpatients with dengue and non-dengue acute infections	Low risk	HBsAg	8	Non-random consecutive sampling
Van Be et al 1992	1989-91	HCMC	Blood donors, adults in general population, inpatients, prisoners, CSW, PWID	Low risk, high risk (exposure)	HBsAg	6	Non-random sampling, unclearly defined study populations, no baseline characteristics

Van Quang et al 2019	2010-17	Hanoi	Patients with HCC	High risk (liver)	HBsAg	8	Non-random, retrospective sample
Viet et al 2012	2007	Quang Tri	Blood donors	Low risk	HBsAg, HCV Ab	7	Non-random sampling; non-representative (*potential* blood donors, HBV-vaccinated individuals excluded)
Zhang et al 2015	2005-07	Thai Nguyen	PWID	High risk (exposure)	HCV Ab, HIV coinfection	8	Non-random sampling

HBsAg = Hepatitis B surface antigen; HCV Ab = Hepatitis C antibody; HCV Ag = Hepatitis C antigen; HDV Ab = Hepatitis D antibody; RNA = ribonucleic acid

Figure 2-4: Study populations by location



Blood donors

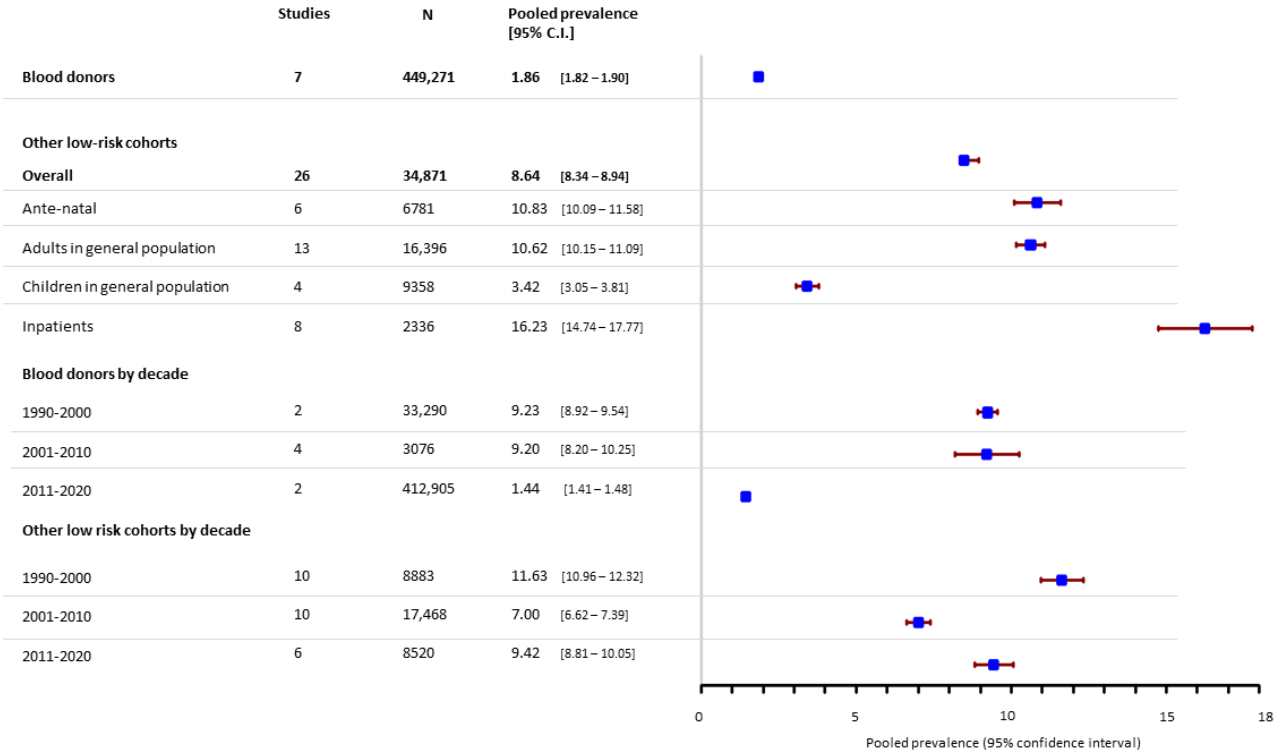
Blood donor screening studies contributed the largest study populations, such that 90% of all included individuals tested for HBsAg and 93% tested for HCV were blood donors. Overall infection rates in this population were lower than is reported in the general population (see next section), with an HBsAg point prevalence of 1.86% (95% C.I. 1.82 - 1.90) (figure 2-5),

an HCV antibody prevalence of 0.18% (0.16 - 0.19) and HCV antigen prevalence of 0.31% (0.10 – 0.61) (figure 2-6). Pooled HBsAg prevalence in blood donor cohorts from prior to 2011 was around 9%, with apparent improvement in pre-screening in the last decade, when it fell to 1.44% (1.41 – 1.48). HCV prevalence in blood donors was extremely high when it was first discovered in the 1990s (7.6% [6.1 – 9.4]) but is around 0.2% overall in studies since 2001.

Low risk (non-blood donors)

Overall prevalence of HBsAg in non-donor low-risk groups was 8.6% (8.3 – 8.9). It was lowest in children in the general population (3.4% (3.1 – 3.8)) but was 10.8% [10.1-11.6] in antenatal women and 10.6% [10.2-11.1] in adults from the general population. HBsAg prevalence was high in inpatients presenting with non-hepatic illness (16.2% (14.7 - 17.8)), which included patients admitted with Dengue[236] and Malaria[237,238]. Pooled prevalence of HBsAg in low-risk non-donors fluctuated from 11.6% (11.0 – 12.3) in studies from 1990 to 2000, 7.0% (6.6 – 7.4)) in 2001-2010 and 9.4% (8.8 – 10.1) in studies since 2011.

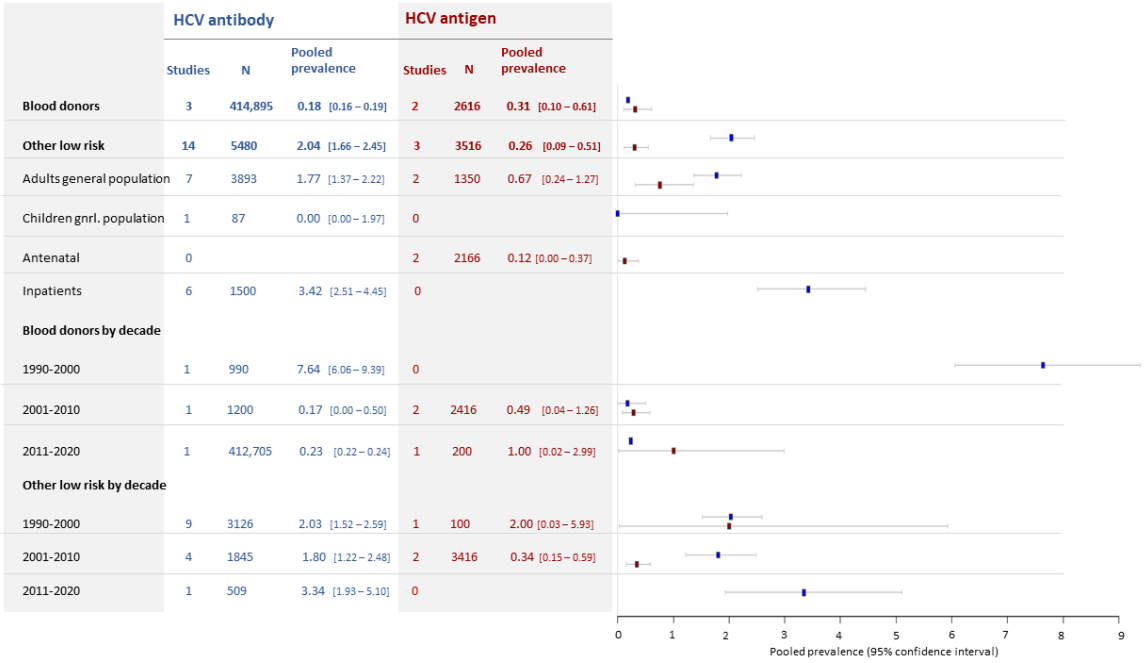
Figure 2-5: Estimated pooled seroprevalence of HBsAg in low-risk populations



Studies = number of separate study populations included. N = total number tested. Children defined as aged 0-16 years

For HCV, pooled antibody prevalence in non-blood donor low-risk populations was 2.0% (1.7 – 2.5) and HCV antigen prevalence was 0.26% (0.09 - 0.51). We found no significant change in prevalence of HCV antibody in non-blood donor low-risk populations by decade. There was insufficient data to assess whether prevalence of HCV antigen has changed (Figure 2-6).

Figure 2-6: Estimated pooled seroprevalence of HCV antibody (blue) and HCV antigen or PCR (red) in low-risk populations



Studies = number of separate study populations included. N = total number tested. Children defined as aged 0-16 years

High-risk

In high-risk groups, overall pooled prevalence of HBsAg was 13.3% (12.8 – 13.8) (figure 2-7). Prevalence of HBsAg in individuals undergoing haemodialysis, blood transfusion or surgery was similar or lower than observed in non-blood donor low-risk groups (8-10%). In contrast, rates of HCV infection were significantly elevated in these populations, with 16.8% (14.7-18.9) of dialysis patients showing evidence of active HCV infection (figure 2-8).

Overall, for HCV in high-risk groups, pooled prevalence was 49.3% [48.3 – 50.3]) for HCV antibody and 31.4% (30.6 – 32.2) for HCV antigen (figure 2-8). These figures were heavily influenced by very high prevalence of HCV in PWID, with evidence of past HCV exposure in 72.5% (71.4 – 73.6) of PWID tested, and active infection in 57.8% (56.5 – 59.1) (figure 2-8). Extremely high rates of active HCV infection were reported in in the northern provinces of Yen Bai (87.4% [83.7 – 90.7])) and Quang Ninh (84.6% [80.3 – 88.5]))[239] (figure 2-9) and the southern metropolis of Ho Chi Minh City (92.2% [73.0 -100] HCV antibody, five studies[238,240–243] and 71.0% (65.8 – 75.9) HCV antigen, one study[239]) (figure 2-9).

Only six studies described both HCV antibody and antigen/RNA prevalence in the same individuals (table 2-10). In one PWID cohort[244] 79.3% (74.4 – 83.6) of individuals testing positive for HCV antibody had evidence of current infection. This proportion was lower among individuals with liver disease (60.9% [48.3 – 72.4])[245] and in sex workers (58.5% [52.4 – 64.4][246], and in adults the general population (50.0% [26.0 – 74.0][247] and 44.4% [13.70 - 78.8][245], which likely reflects less frequent exposure. A fifth study in individuals undergoing haemodialysis[248] found HCV antigen prevalence exceeded antibody prevalence (12.9% [8.6 – 18.4] vs 5.5% [2.8 - 9.6]). This surprising finding may reflect a high number of acute HCV infections associated with the dialysis unit concerned, or defective antibody generation in the context of frequent exposure to HCV from haemodialysis. However, numbers were small.

Table 4: Studies reporting HCV antibody and HCV antigen or HCV RNA in same population

Study	Population	Proportion of HCV antibody positive individuals with HCV RNA or antigen	95% C.I.
Duong et al 2019	Dialysis	100%	[71.5 - 100]
Tanimoto et al 2010	PWID	79.30%	[74.4 – 83.6]
Kakumu et al 1998	Liver disease	60.90%	[48.3 – 72.4]
Le et al 2019	CSW	58.50%	[52.4 – 64.4]
Do et al 2015	General population	50.00%	[26.0 – 74.0]
Kakumu et al 1998	General population	44.40%	[13.70 -78.8]

Figure 2-7: Pooled seroprevalence of HBV in high-risk populations

	Studies	N	Pooled prevalence [95% C.I.]
Overall	**15**	**17,401**	**13.32 [12.81 – 13.83]**
PWID	9	7740	17.07 [16.23 – 17.92]
CSW	5	3899	8.77 [7.89 – 9.70]
Dialysis	5	1110	10.76 [8.95 – 12.70]
MSM sexual health	2	2174	13.50 [12.09 – 14.98]
Multiple transfusions/surgery	2	902	8.47 [6.65 – 10.46]
Prisoners	1	1400	13.00 [11.29 – 14.81]
Infants of HBV infected mothers	1	176	13.07 [8.45 – 18.48]
High risk exposures by decade			
1990-2000	4	3394	13.22 [10.77 – 15.88]
2001-2010	7	11,074	15.57 [11.88– 19.65]
2011-2020	6	2933	8.63 [7.35 – 10.01]
Liver disease overall	**9**	**27,495**	**52.70 [52.10 – 53.29]**
Acute hepatitis	1	188	23.94 [18.09 – 30.32]
Chronic hepatitis	5	5151	14.04 [13.10 – 15.01]
Hepatocellular carcinoma	4	22,156	62.13 [62.13 – 63.41]

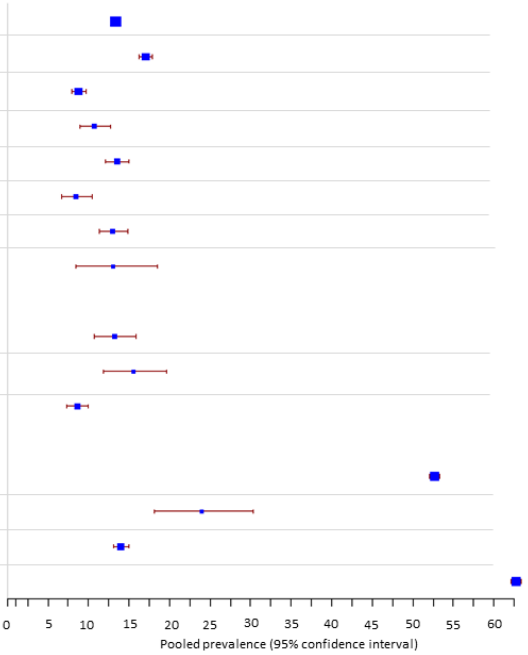
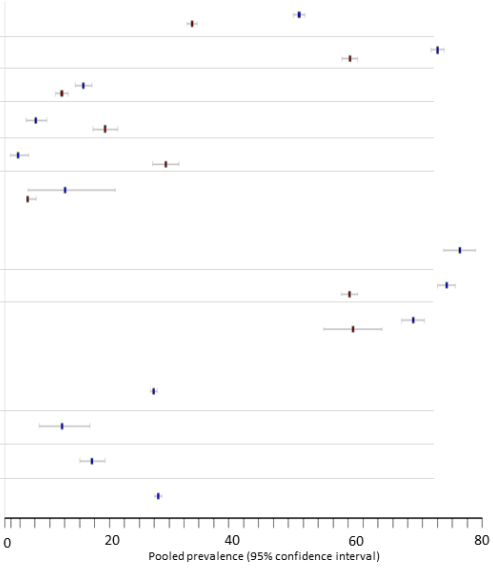


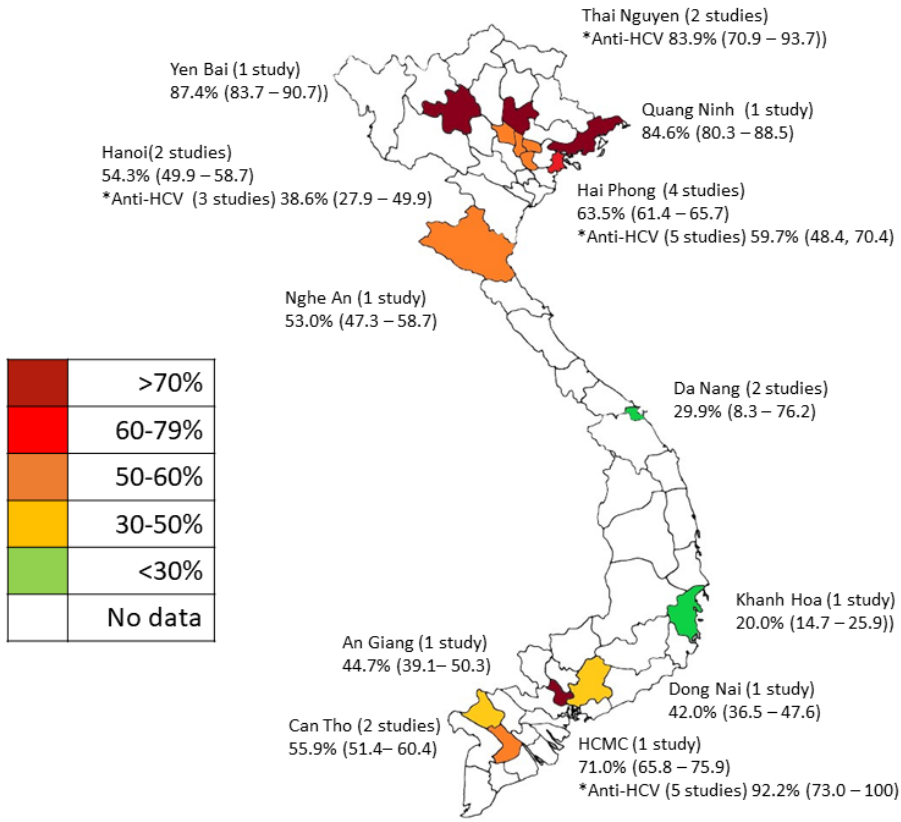
Figure 2-8: Pooled prevalence of HCV Ab and HCV antigen or PCR in high-risk groups

	HCV antibody			HCV antigen		
	Studies	N	Pooled prevalence	Studies	N	Pooled prevalence
Overall	17	10,150	49.30 [48.32-50.28]	9	12,108	31.37 [30.54-32.20]
PWID	14	6666	72.51 [71.42-73.58]	3	5461	57.80 [56.48 -59.11]
CSW	3	2294	13.14 [11.78-14.57]	3	3066	9.51 [8.48-10.59]
Dialysis	3	713	5.18 [3.56-7.03]	5	1232	16.75 [14.70-18.91]
MSM	2	404	2.20 [0.91 – 3.94]	1	1520	26.91 [24.71-29.18]
Blood transfusion	1	73	10.09 [3.83-18.45]	1	529	3.76 [2.51 - 5.23]
PWID by decade						
1990-2000	5	1008	76.23 [73.52-78.84]	0		
2001-2010	5	3371	74.00 [72.50-75.47]	3	5072	57.76 [56.39-59.12]
2011-2020	4	2287	68.4 [66.51-70.33]	1	389	58.35 [53.41-63.21]
Liver disease						
Overall	8	22,986	24.94 [24.38-25.51]	0		
Acute hepatitis	1	188	9.57 [5.74-14.23]	0		
Chronic hepatitis	4	1065	14.58 [12.5-16.8]	0		
HCC	2	21,733	25.69 [25.11-26.28]	0		



Studies = total number of study populations included. N = total individuals tested. CSW = commercial sex worker; MSM = Men who have sex with men; PWID = People who inject drugs. HCC = hepatocellular carcinoma

Figure 2-9: HCV antigen prevalence (and antibody where available) in PWID by region



Prevalence pooled for locations with more than one study. HCMC = Ho Chi Minh City. All prevalence is HCVAg/PCR unless otherwise stated to be anti-HCV.

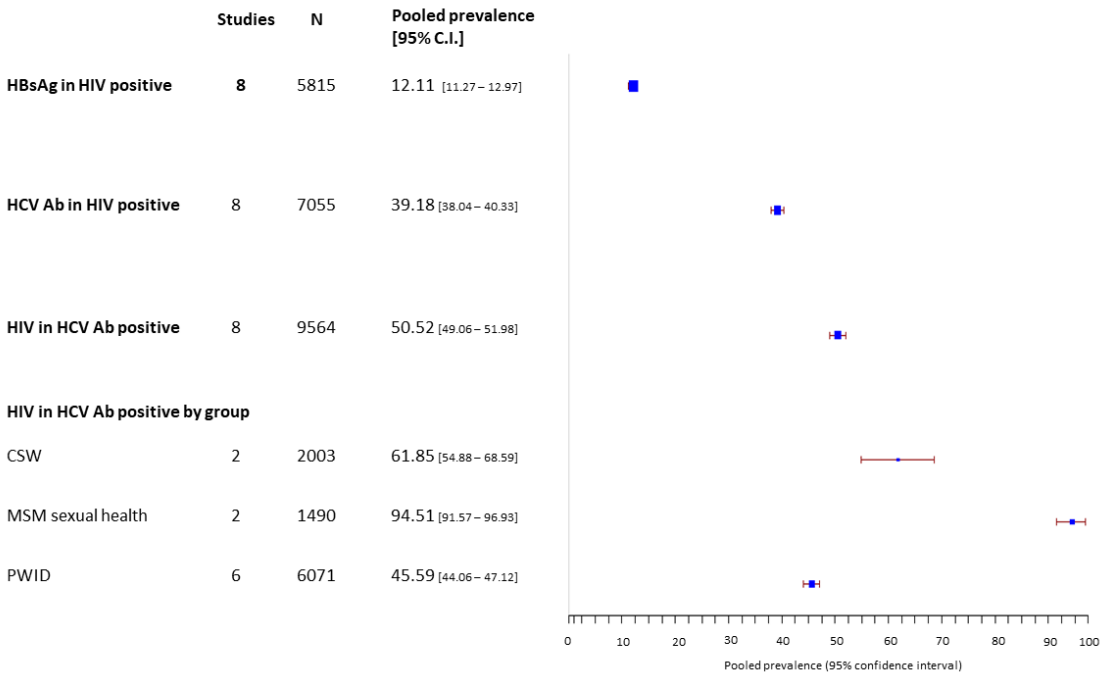
In HIV positive cohorts, 12.1% (11.3 – 13.0) were HBsAg positive and 39.2% (38.0 – 40.3) were HCV antibody positive (figure 7). Although baseline characteristics were available, it was not possible to ascertain specific risk factors in coinfected individuals, such as past injecting drug use or high-risk sexual activity. Only one study compared HCV co-infection in HIV positive MSM vs HIV positive heterosexual men attending the same HIV service[249]. Injecting drug use was more prevalent in HIV positive heterosexuals than in MSM (46.8% vs 2.4%). Consequently, HIV-HCV co-infection was higher in heterosexual males (55%) than MSM (4.9%).

Among 4676 HCV-infected individuals in 27 high-risk cohorts screened for HIV, 50.52% (49.1 – 51.2) were HIV co-infected. HIV co-infection was extremely prevalent in HCV antibody positive MSM, with 94.5% (91.6 – 97.0) testing positive. It was less prevalent in

PWID (45.6% (44.1 – 47.1), reflecting different routes of exposure: HCV is more likely to be accompanied by HIV when sexually acquired.

Among over 20,000 individuals with Hepatocellular carcinoma (HCC), a very high prevalence of both HBsAg (62.8% (62.1 – 63.4)) (figure 4) and HCV antibody (25.7% (25.1 – 26.3)) (figure 5) was observed, highlighting the devastating consequences of these infections.

Figure 2-10: Estimated pooled prevalence of i) HBsAg and ii) HCV antibody in HIV positive populations and iii) HIV co-infection in HCV-antibody positive populations



Studies = total number of study populations included. N = total individuals tested. CSW = commercial sex worker; MSM sexual health = Men who have sex with men attending clinic; PWID = People who inject drugs.

HCV genotypes

HCV genotype data was available for 8,707 individuals from 17 separate study populations in 14 studies (table 5). Overall 47.7% (95% C.I 46.6, 48.8) had genotype 1, 37.7% (36.7, 38.7) had genotype 6, 11.4% (10.7, 12.1) had genotype 2. A very low prevalence of genotype 3

(0.01%) was reported across studies. Amongst PWID, pooled prevalence of genotype 1 was higher (68.5% [95% C.I 63.2, 73.5]) and genotype 6 was lower (26.5% [21.8, 31.6]) than among low-risk individuals (37.5% [34.8, 40.2] genotype 1 and 48.4% [45.6, 51.2] genotype 6). Subtype data was available for 1,993 individuals from 13 populations in 10 studies. The most prevalent subtypes were 1a (22.0%) [20.1, 23.9]), 1b (20.3% [18.5, 22.2]), 6a (19.2% [17.5, 21.1]) and 6e (13.9% [12.3, 15.5]). The only other subtype found at greater than 1% prevalence was 2a (2.7% [1.9, 3.6].

Table 5: HCV genotypes

	Studies	N	Gt1	Gt2	Gt3	Gt6
Overall	17	8707	47.7% [46.6, 48.9]	11.4% [10.7, 12.1]	0.01% [0.0, 0.03]	37.7% [36.7, 38.7]
Acute hepatitis	1	322	56.5% [51.1, 61.9]	7.5% [4.8, 10.6]	2.2% [0.8, 4.1]	33.9% [28.8, 39.1]
Chronic hepatitis	3	7343	47.8% [46.6, 49.0]	14.5% [13.6, 15.3]	0.15% [0.07, 2.7]	36.8% [35.7, 38.0]
PWID	3	320	68.5% [63.2, 73.5]	0.15% [0.00, 1.15]	4.2% [2.2, 6.8]	26.5% [21.8, 31.6]
CSW	1	33	63.6% [46.4, 79.4]	0.0% [0.0, 5.2]	3.0% [0.0, 12.6]	33.3% [18.1, 50.5]
Dialysis/multiple transfusions	4	144	53.0% [44.3, 61.5]	0.0% [0.0, 1.5]	0.09% [0.0, 2.4]	44.9% [36.3, 53.5]
HIV positive	1	93	68.8% [59.0, 77.9]	0.0% [0.0, 1.8]	5.4% [1.5, 11.1]	25.8% [17.4, 35.2]
Low risk*	4	452	37.6% [34.9, 40.3]	11.3% [9.5, 13.2]	0.0%	48.4% [45.6, 51.2]

*N = total number of individuals genotyped; Gt = genotype; PWID = People who inject drugs; CSW = Commercial sex worker; *Low risk includes blood donors, general population surveys, general inpatients.*

Figure 2-11: HCV genotypes

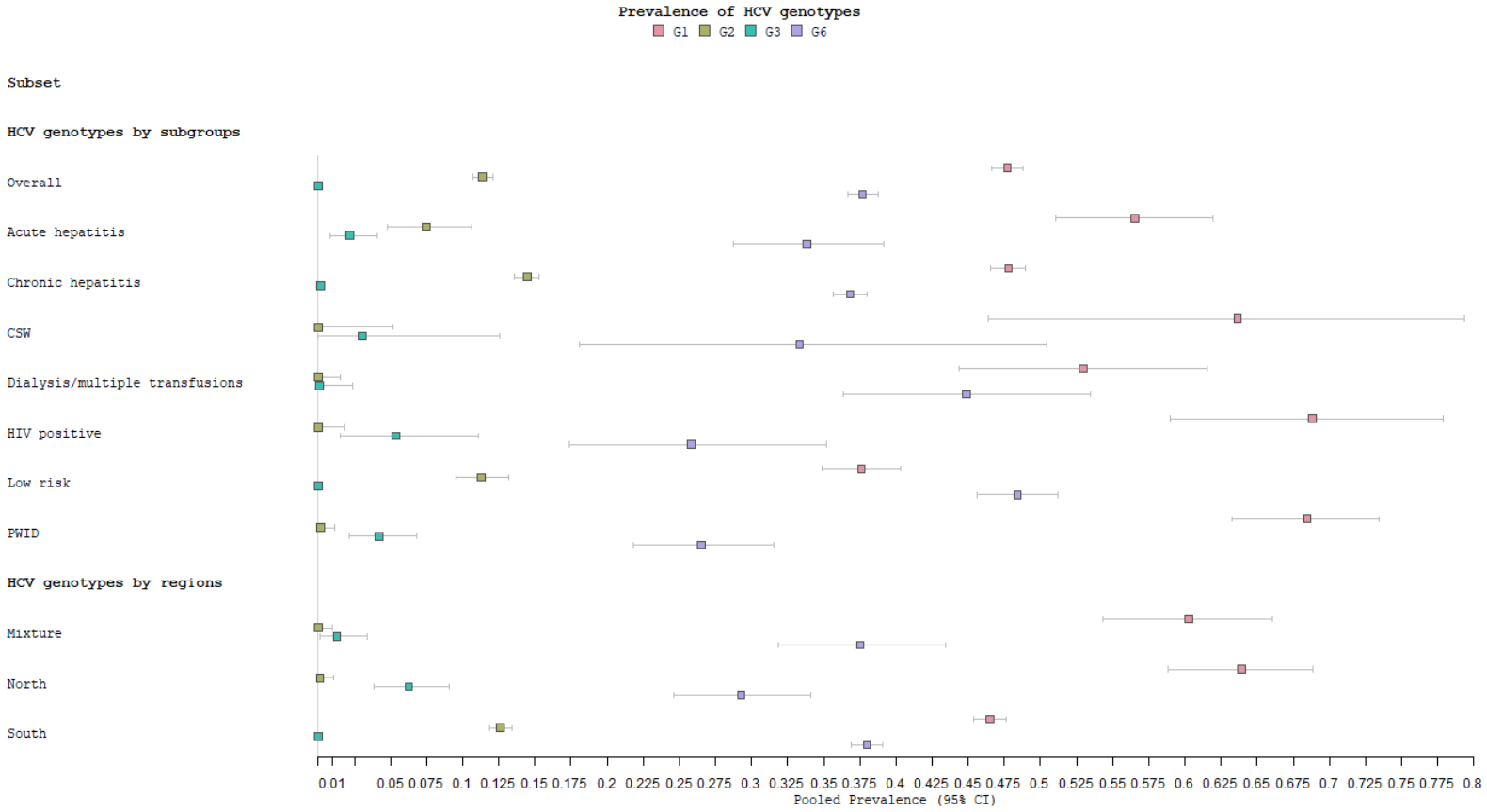
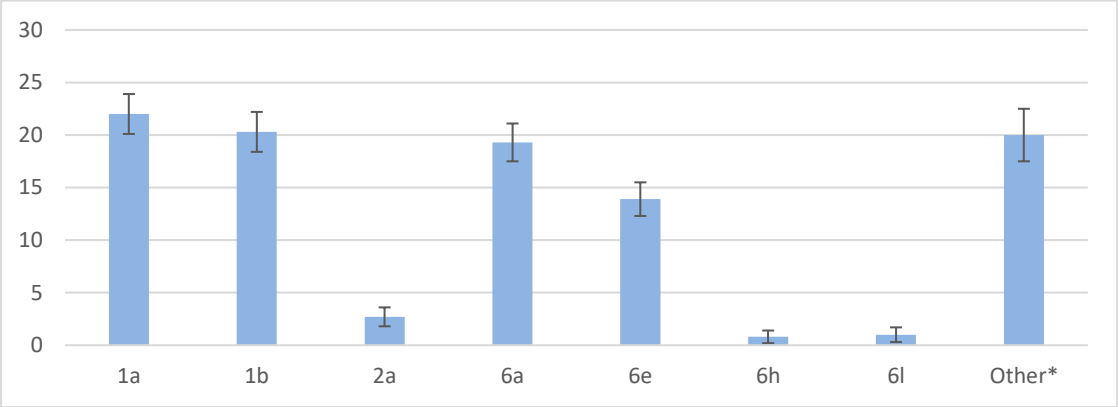


Figure 2-12: Pooled prevalence of HCV subtypes among 1,993 individuals genotyped

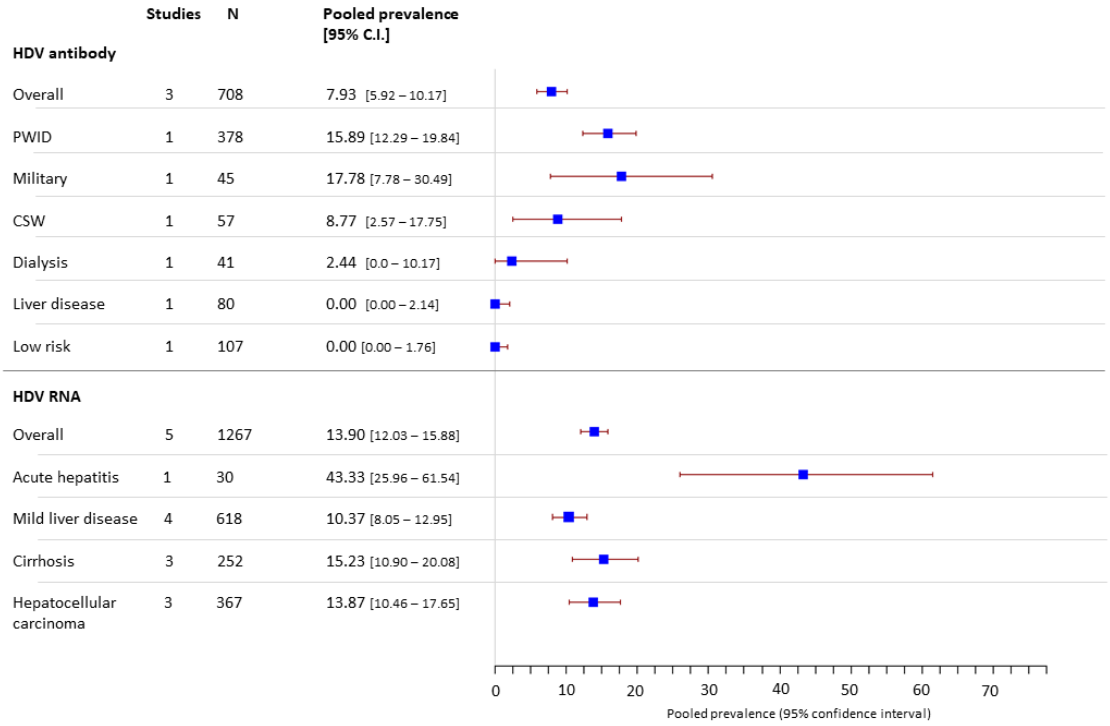


**'Other' includes genotypes 1e, 2b, 2c, 2i, 2j, 2k, 2m, 3a, 3b, 6a, 6c, 6e, 6f, 6h, 6k, 6l, 6n, 6o, 6p, 6r, 6t (all with prevalence <1%) and unidentifiable genotypes.*

Hepatitis D

We included 1975 individuals tested for HDV antibody or HDV RNA in 23 HDV cohorts. Of 708 HBsAg positive individuals tested for HDV antibodies in 12 cohorts, 7.9% (5.9 – 10.2) were positive (figure 2-11). Highest rates of HDV infection were seen in PWID. One study of 45 HBsAg positive military recruits found 17.20% (8.0 - 32.1) were HDV antibody positive[250] but no cases of HDV were detected in other larger low risk cohorts. We found highest prevalence of HDV RNA in individuals presenting with acute hepatitis (43.3% [26.0 – 61.5]), suggesting HDV may be an under-recognised cause of this presentation in Vietnam. Only 4 studies included genotype data in 115 individuals. Of these 74% had genotype 1 infection and 26% had genotype 2.

Figure 2-13: Estimated pooled prevalence of HDV antibody and HDV RNA in HBsAg positive cohorts



Studies = total number of study populations included. N = total individuals tested. CSW = commercial sex worker; MSM sexual health = Men who have sex with men attending clinic; PWID = People who inject drugs.

Discussion

This study is the most comprehensive review of HBV, HCV and HDV seroprevalence in Vietnam and provides important detail on the distribution of the hepatitis epidemic in one of the highest burdened countries in the world.

Hepatitis B

I found that pre-screening of blood donors in Vietnam has improved significantly in the last 30 years, with very low rates of both HBV and HCV infection detected in blood donors

compared with the general population. This improvement may be attributable to the prohibition of paid and family/replacement blood donation since 2013[251], with a successful switch to voluntary unpaid blood donation, supported by an annual "All People's Voluntary Blood Donation Day"[252]. In addition, in the last decade rapid HBsAg testing has become mandatory for all new blood donors prior to blood donation[251].

In in other low-risk populations I found that the pooled prevalence of HBV was high, exceeding 10% in all non-donor adult cohorts. The overall estimate (8.64% [95% C.I 8.34 – 8.94%]) is broadly consistent with that of a large, unpublished seroprevalence survey conducted by the Vietnam MoH in collaboration with the US Centers for Disease Control and Prevention (US CDC) and Abbott Diagnostics from 2018 - 2019. Amongst 25,649 adults in 32 provinces, they recorded a HBsAg prevalence of 9.2% (95% C.I 8.9 – 9.6%)[193].

The high HBsAg prevalence observed in inpatients with non-hepatic illness may reflect the increased all-cause morbidity associated with chronic liver disease. HBV prevalence in groups at high-risk of exposure was similar, or only modestly elevated compared to low-risk populations. This may be explained by high rates of vaccination in high-risk groups and a reduced risk (<10%) of chronic infection when exposed to HBV in adulthood[253].

The lower HBsAg prevalence in children (3.4% (3.1 – 3.8)) is somewhat reassuring. HBsAg positivity in children (defined as 0-16 years of age) was >15% in two studies from the 1990s[254,255], compared to 2.7% (2.2 - 3.3) in a national study from 2011[256] and 1.9% (1.2 - 2.7) in a study in infants from Central Vietnam with data from 2009-2012[257]. This change is a direct consequence of vaccination, which has been included in Vietnam's national vaccine program since 1997 and was expanded to a cost-free 4-dose schedule for all new-borns in 2004, including birth dose vaccination within 24h of delivery. Scale up of this vaccine series has had a profound impact on horizontal transmission in early childhood[256], which will become apparent in future surveys of the adult population. However, this has made vertical transmission proportionally more dominant[258].

Despite a concerted effort in the last decade to improve delivery of birth dose vaccine, coverage is not yet perfect, being below the WHO target of 90%. A 2019 study found that only 63% of children in Vietnam received birth dose vaccine, with lowest uptake seen in poor, rural communities and among ethnic minorities[259]. The WHO has stated that among countries in the Western Pacific, Vietnam ranks 34/37 in terms of timely delivery of birth

dose vaccination and is one of only five countries in which HBsAg prevalence is estimated to be greater than 1% among children under 5 years of age[193] (infections in this age group being a surrogate indicator of the cumulative incidence of chronic HBV). In a 2021 National Action Plan for the elimination of viral hepatitis, the Vietnam MoH cites vaccine hesitancy and distribution issues as major obstacles to improving birth dose vaccination rates[193]. Recent data from Haiphong showed that 13.1% (8.5-18.5) of children of HBV-infected mothers are HBsAg positive[217]. Given the high rates of chronic infection resulting from HBV acquired in infancy (>90%)[253], more therefore needs to be done to reduce the perinatal transmission driving Vietnam's HBV epidemic.

Prophylactic antiviral treatment for HBsAg positive expectant mothers in the final trimester of pregnancy has been recommended by Vietnam's MOH since 2014, and tenofovir is now covered by health insurance. However, many pregnant women lack this basic cover, and antenatal care in rural settings is frequently inadequate, with one study indicating only one fifth of rural women receive sufficient core antenatal services according to national recommendations[260].

Hepatitis B immunoglobulin (HBIG) is also recommended at birth for all children born of HBsAg-positive mothers. However, HBIG is a blood product that requires infection screening and a cold chain, making delivery to resource-poor regions problematic. Where available, HBIG costs around US$100/dose, which must be fully paid by the parents and access is very limited. Even when these preventative measures are implemented appropriately, vertical transmission rates from HBeAg-positive women are estimated to range from of 8–30%,[261–263] indicating research into additional interventions is still warranted.

Hepatitis C

In contrast to HBV, our findings indicate that HCV infection is probably less common in the general population than previous estimates suggest. One possible reason for this is that studies prior to 2012 were generally limited to measuring HCV antibody - a marker of past HCV exposure but not active infection. Estimates for the number of active infections were inferred from population studies measuring both antibody and antigen, in which high-risk individuals from high-income settings are over-represented[264]. Given 15-45% of acute HCV infections

spontaneously resolve without treatment[2], the proportion of antibody positive individuals with active infection will be lower in low-risk groups compared to those with repeated exposure, such as PWID. Our data illustrate this point, with very low prevalence of active HCV infections in general population cohorts, and a high prevalence of active infections in PWID, MSM and dialysis patients. The risk of over-estimating prevalence of active HCV infection may be greater in LMICs, in which a greater proportion of antibody positive individuals are low-risk, and repeat exposure risk is hard to quantify. This has important implications for health policy, which is currently insufficiently loaded towards those at highest risk.

I identified an extremely high prevalence of HCV in PWID, with antibody positivity (72.5% [71.4 – 73.6]) 39% higher than global estimates from a 2017 meta-analysis (52.3% [42·4–62·1])[265]. PWID represent a key target population for ending the HCV epidemic and are estimated to contribute to nearly 40% of disability-adjusted life-years (DALYs) due to HCV worldwide[8]. In Vietnam preventative interventions have been implemented since 2008, including opioid substitution therapy, universal antiretroviral treatment (ART) for HIV-infected PWID, and financing of community-based organisations to deliver harm reduction and distribute free syringes[191]. In 2015 the MOH expanded methadone treatment to at least 30 provinces to provide treatment for more than 80,000 drug users[192]. While these initiatives have been effective in reducing the incidence of HIV, incidence of HCV seroconversions in PWID remains very high[194] and I found no significant reduction in HCV antigen prevalence in the last decade.

Accumulating evidence shows that PWID can achieve high cure rates with DAA drugs, comparable with other populations[266], reducing the risk of onward transmission of HCV in the process. In 2019 the government began subsidizing 50% HCV treatment costs for those with health insurance. However, many PWID lack coverage and treatment remains expensive - a 12 week course of sofosbuvir and daclatasvir currently costing US$ 1347[141]. In 2021 the Global Fund committed to providing free DAA therapy to 16,000 HIV/hepatitis C co-infected patients at HIV treatment facilities across Vietnam[267]. This represents a positive step, but given less than 50% of HCV antibody positive PWID in our study are co-infected with HIV, most won't be eligible for free treatment through this scheme, highlighting important limitations in current global funding. Provision of free HCV screening and treatment for PWID would have a major impact on reducing the scale of the HCV epidemic in Vietnam[268].

My finding of a high prevalence of active HCV infection among patients on dialysis is concerning. Given that chronic HCV infection is associated with a range of renal pathologies including type 1 membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis, and interstitial nephritis, some of these patients may have end-stage renal failure because of HCV acquired some years previously. However, given the high rates of HCV antigen in this population it is likely many individuals are acquiring HCV from dialysis, highlighting a need for improved infection control and an HCV vaccine.

HCV genotypes

My findings regarding genotype prevalence are broadly consistent with a 2021 meta-analysis of HCV genotypes and subtypes in Southeast Asia which found that genotypes 1, 2 and 6 accounted for 56.1%, 10.6% and 34.6% HCV infections in Vietnam, respectively[63]. That study used different search criteria (including manuscripts in Russian and non-prevalence studies) and identified 5 additional studies from Vietnam with HCV genotype data. My finding of a relatively higher prevalence of genotype 6 in low-risk individuals is consistent with data from previous phylogenetic analyses, which have shown that genotype 6 is the endemic lineage, and therefore more likely to be associated with infections acquired through one-off exposures decades ago[184]. In contrast genotype 1a is estimated to have emerged in Vietnam as recently as 1963, and appears to be more prevalent is those with more recent high-risk exposures, such as PWID and CSW. None of the genotype studies were truly cross-sectional in design, and methodology, geography and sampling date will all have impacted these subtype prevalence estimates. Historically the 5' untranslated region (5'UTR) sequences of subtype 6e were noted to be indistinguishable from those of genotype 1b such that genotyping performed prior to 2010 likely underestimated 6e prevalence in Vietnam.

Hepatitis D

HDV infection in Vietnam remains poorly characterised. I found just seven studies assessing HDV prevalence and most cohorts were small. The largest HDV antibody cohort surveyed just 97 HBV infected individuals across five provinces, and the largest RNA cohort included

just 250 individuals. Across all studies, only 115 HDV infections were genotyped, with genotype 1 appearing dominant.

The finding that 7.9% (5.9 – 10.7) of individuals with HBV may have HDV co-infection is highly skewed by the nature of the cohorts surveyed, with >50% individuals tested for HDV antibody coming from PWID cohorts. Globally HDV prevalence is frequently over-estimated, as surveys tend to be performed in patients with known HBV infection and liver disease, representing the more severe end of the HBV disease spectrum[221]. Despite a high prevalence of HBV in Vietnam, from the limited data available, it appears HDV is not endemic and seems to be concentrated in high-risk groups at risk of repeated exposure to blood borne viruses. This is in keeping with the HDV distribution in Japan, Hong Kong and parts of Europe and quite different to the high community prevalence reported in other parts of Asia such as Pakistan[222] and Mongolia[221].

HDV is not routinely screened for in Vietnam, partly because of its perceived rarity, but also because drugs licensed to treat HDV (PEGylated IFN alfa-2a, PEGylated IFN alfa-2b and bulevirtide) are not available. My findings would not support mass screening. However, given the high prevalence in individuals with acute hepatitis, I suggest HDV diagnostics should be available and HDV should be included in future treatment guidelines. Given the high prevalence of HBV in Vietnam, the most effective measure to minimise the impact on HDV will be vaccinating high-risk HBV-susceptible individuals against HBV and improving birth dose vaccine coverage.

Study strengths & Limitations

The major strength of this study is in its breakdown of population groups. The significant difference in HBV and HCV prevalence between blood donors and other low-risk adult populations, highlights the bias inherent in including pre-screened blood donors in prevalence estimates. By estimating pooled prevalence of both HCV antibody and antigen I was also able to identify the groups most in need of screening and treatment.

This study has some important limitations. Over 70% of study populations analysed came from just three large cities (figure 2-4): Ho Chi Minh City, Hanoi and Hai Phong. While these are the most populous cities, this urban focus limits generalisability to the entire country,

particularly rural areas where there may be increased risk of community transmission of HCV and lower rates of HBV vaccination. In addition, my search was limited to peer-reviewed articles retrieved from three databases and published in one of three languages; an expanded search including grey literature and non-peer reviewed Vietnamese manuscripts, including the aforementioned MoH data[193], could strengthen the meta-analysis. Equally, the lack of methodological detail in the grey literature, such as the sampling strategy or disclosure of diagnostics, could introduce bias that is harder to quantify. HCVAg testing platforms, in particular, vary in their sensitivity for detecting HCV RNA[226].

The quality of studies included was generally good, with Joanna Briggs Institute Systematic Review Index scores of 6-9. However, most were not randomized cross-sectional surveys, and only 11% (8/72) met all nine quality criteria. Low-risk 'general population' groups are at highest risk of selection bias, but I mitigated this to some extent by separating adults from children and inpatient studies from community surveys. In addition, MSM studies were universally high-risk populations attending sexual health clinics or engaged in commercial sex work, such that pooled prevalence should not be extrapolated to the wider MSM community. Finally, the DerSimonian–Laird random effects model has been criticized for producing confidence bounds that are too narrow when the number of studies is small or when there are substantive differences among study estimates[269]. This is because it fails to capture uncertainty in estimations of between-study and within-study variance when few studies are available for comparison. This is especially relevant to my smallest subgroups, such as pooled prevalence of HCV in PWID by region and HDV by population.

These limitations are important when extrapolating data to the population level. The numbers in a meta-analysis such as this can be large, generating prevalence estimates with narrow confidence intervals that appear accurate and precise. However, the lack of information regarding the sampled populations' age distribution, gender, ethnicity, occupation and address limits the generalisability of findings. A good example of this is the pooled prevalence estimate for HCV RNA in 'low risk populations' of 0.26% (0.09 – 0.51). This estimate comes from just 3 studies (Tran et al 2003, Dunford et al 2012 and Ishizaki et al 2017), which included 2166 antenatal patients and 1350 adults in the general population identified through non-random consecutive sampling at the involved study sites. Antenatal populations are, by definition, young and female and over-represented by individuals living in urban settings with good access to healthcare. This estimate therefore likely underestimates HCV prevalence in

the low risk adult population of Vietnam. In this respect there is no replacement for large, well-conducted serosurveys which use randomised sampling strategies to select more representative study populations.

Conclusion

In this chapter I have shown that although blood safety has improved in Vietnam, and HDV appears to be largely confined to high-risk populations, a renewed focus on birth dose HBV vaccination and targeted HCV screening and treatment of people who inject drugs, is urgently required to meet elimination targets. Larger randomised cross-sectional surveys using high-quality HBV, HCV and HDV PCR as well as serological markers in both urban and rural settings will provide more robust prevalence estimates to inform future hepatitis strategy. Likewise, mandatory reporting of newly diagnosed infections to a national database would provide incidence and prevalence data in real time. Improved surveillance is a major objective of the Vietnam MoH's 2021 National Action Plan for Elimination of Viral Hepatitis[193]. A surveillance program was piloted at the National Hospital for Tropical Diseases, Hanoi in 2017 and expanded to Thai Nguyen Central Hospital (north of Hanoi) and Cho Ray Hospital (in HCMC) in 2018. It aims to collect information on risk factors, disease stage and mortality from acute and chronic viral hepatitis and hepatocellular carcinoma to further inform interventions. The government are committed to further expansion and development of this project, which should, in time, reduce reliance on modelled data from small studies of variable quality.

Chapter 3

Efficacy of ultra-short, response-guided sofosbuvir and daclatasvir therapy for Hepatitis C: a single arm mechanistic pilot study

Background

As discussed in chapter 1, directly acting antiviral (DAA) therapy for hepatitis C (HCV) offers high cure rates (>95%) to those able to adhere to standard durations of treatment. In low- and middle-income countries, where treatment is limited to second generation NS5A/NS5B-inhibitor combinations, standard treatment is at least 12 weeks. This duration presents a barrier to successful engagement in care for some populations[170,171], hampering the elimination of HCV as a public health threat. Novel treatment strategies are required for individuals under-served by existing models of care, such as people who inject drugs and those of no fixed abode.

In Vietnam DAA therapy remains prohibitively expensive for many of those infected. A standard twelve-week course of sofosbuvir and daclatasvir (SOF/DCV) was priced at US$2417 - 2472 in Ho Chi Minh City in 2019[187]. Despite the government subsidising 50% of drug costs since 2019, the Ministry of Health estimate only 1000 individuals accessed DAA treatment through health insurance in 2019, and 2700 in 2020[193].

In 2018 the World Health Organisation called for research into predictive factors for selecting persons who could be successfully treated with shorter durations of therapy[16], on the grounds that this could help expand access to treatment and reduce drug costs. Studies evaluating short course therapy are challenging for infectious diseases where there are significant clinical risks of failure (e.g. TB, sepsis). However, HCV provides a model where treatment failures can be successfully retreated[176] allowing exploration of mechanisms underlying successful therapy.

In chapter 1 I showed how shortened DAA therapy is generally associated with disappointing rates of cure (see section on '*Predictors of response to shortened therapy'*), such that it could never be recommended routinely. Our 2019 systematic review and meta-analysis of treatment optimisation for HCV revealed that for individuals with favourable predictors of

response, pooled sustained virological response (SVR) for regimens of ≤4 weeks duration was 63.1% (95% C.I. 39.9-83.7), 6 weeks duration was 81.1% (75.1-86.6) and 8 weeks duration was 94.2% (92.3-95.9)[172]. However improved rates of cure were seen with an increased number of individual-level factors known (or assumed) to be favourable, such as non-genotype 3 infection, lower body mass index (BMI), lower baseline viral load, mild liver disease, absence of prior treatment failure, and a rapid virological response to treatment[172].

Use of rapid virological response may offer a promising means of shortening treatment duration while maintaining high rates of cure. Evidence supporting response guided therapy (RGT) with DAAs is emerging[173,175,176], notably using day 2 (D2) viral load to determine treatment duration in genotype 1b infection. In this population, high cure rates were observed with just three weeks triple therapy (protease inhibitor (PI), NS5A inhibitor and NS5B inhibitor)[173]. In a UK treatment shortening study, which used 4-8 weeks ombitasvir, paritaprevir, dasabuvir and ritonavir based on baseline viral load, all 10 individuals who became undetectable at day-3 of treatment achieved first-line SVR12 regardless of treatment duration[176]. There is currently no data for RGT durations less than 8 weeks with sofosbuvir and daclatasvir (SOF/DCV), which remains the lowest-priced and most widely available treatment option globally[141].

Drug resistance in association with particular viral genotypes and subtypes is also known to influence treatment outcome[270,271] and may predict who can be treated with shortened therapy. Vietnam has a high burden of genotype 6 HCV infection (around 35%)[63], which is rare outside South East Asia and under-represented in clinical trials. Since genotype 6 is the most genetically diverse HCV lineage[149], legitimate concerns remain about the potential for emergence of resistant variants[150].

Shortening therapy to less than 8 weeks may reveal additional predictors of treatment response. The human *IFNL4* di-nucleotide polymorphism rs368234815 (ΔG/TT) controls generation of the IFNL4 protein and is also associated with impaired clearance of HCV[272] and inferior responses to pegylated interferon-alpha/ribavirin therapy[273] and SOF-based treatment[274,275]. The impact of host *IFNL4* genotype in shortened DAA therapy is not well understood but merits investigation. It is also unknown how serum levels of SOF, its metabolite GS-331007, and DCV might impact treatment success with shortened therapy.

The SEARCH study, described in this chapter, is double strata, single arm, mechanistic pilot study which aimed to address these research questions. Stratum A, described in this chapter, concerns patients with mild liver disease.

Objectives

1. Test the hypothesis that early on-treatment virological response to treatment with SOF/DCV can identify a group of individuals able to achieve high cure rates with shortened (4 week) courses of treatment.
2. Evaluate efficacy of standard duration (12 weeks) retreatment with the same antiviral combination (SOF/DCV) in individuals who do not achieve SVR with shortened therapy.
3. Explore whether genotype 1 and genotype 6 subtypes and associated resistance-associated substitutions impact treatment outcomes with shortened therapy.
4. Explore whether IFNL4 genotype, drug levels or alternative host (age, gender, BMI) and viral factors (baseline viral load) impact treatment outcomes with shortened therapy.

Methods

Study population

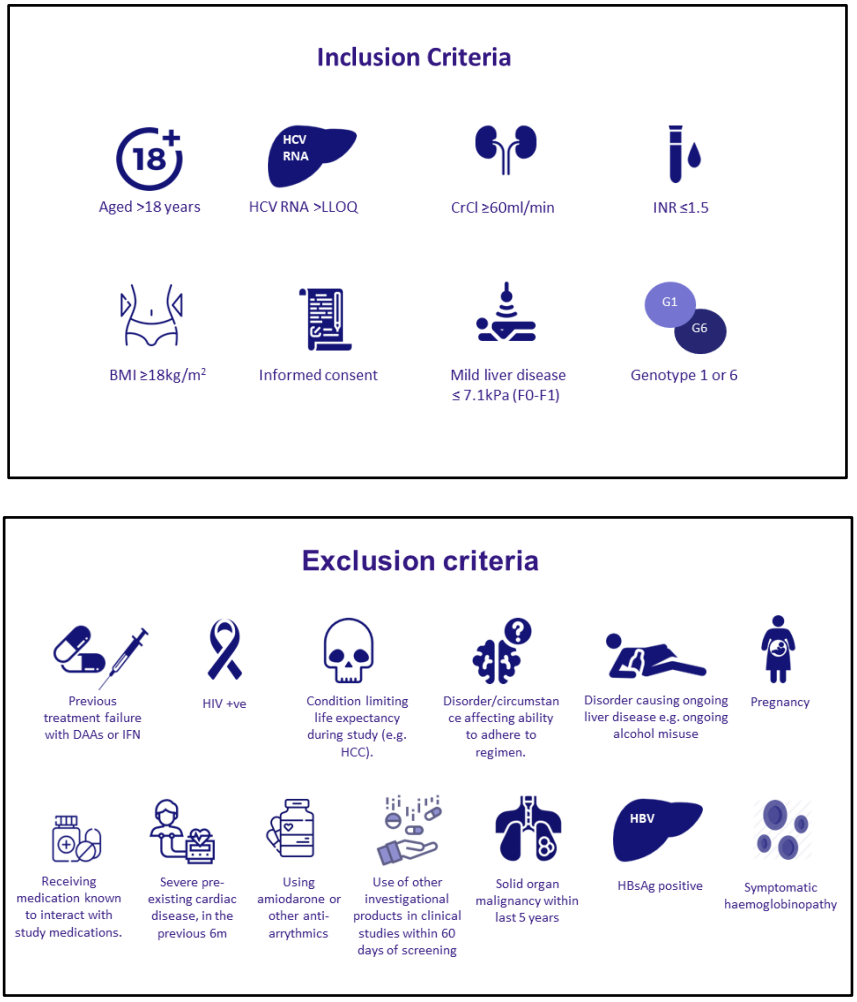
Participants were recruited from the outpatient hepatitis clinic of the Hospital for Tropical Diseases (HTD) in HCMC, between February 2019 and June 2020. The HTD is a 650-bed referral hospital for infectious diseases for southern Vietnam. In 2018, it was estimated approximately 1000 patients with viral hepatitis were attending the clinic each day, of which approximately 10-15% had hepatitis C.

For this study, eligible patients were ≥18 years and had chronic infection with HCV genotype 1 or 6 without evidence of liver fibrosis (defined as a FibroScan® score ≤7.1kPa, equivalent to F0-F1 disease)[276]. In addition, participants were required to be HCV-treatment naïve, have

a BMI ≥18kg/m^2, a creatinine clearance ≥60ml/min, with no evidence of HIV or Hepatitis B coinfection, or solid organ malignancy in the preceding 5 years. Full eligibility criteria are provided in the online protocol available at https://doi.org/10.1186/ISRCTN17100273 and a graphical representation is shown in figure 3-1.

Patients referred to the trial were initially enrolled into an observational study at the HTD, which involved FibroScan® assessment and genotyping. Individuals in this cohort found to be potentially eligible for the trial were invited for further screening. All patients provided written informed consent.

Figure 3-1: SEARCH stratum A Eligibility Criteria



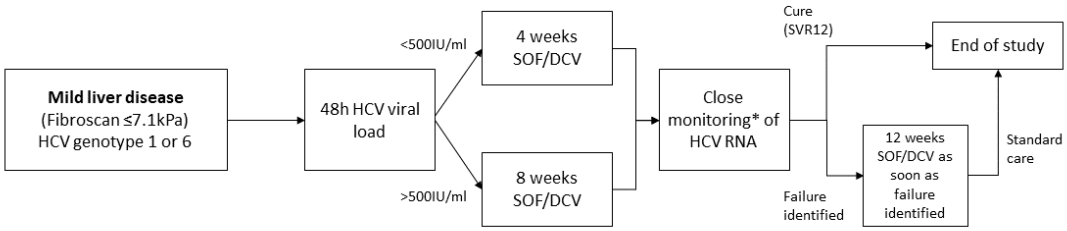
Study design

All participants were treated with sofosbuvir 400mg and daclatasvir 60mg (Pharco Pharmaceuticals, Egypt) administered orally as two separate tablets, once daily. Individuals requiring dose adjustment for any reason were excluded.

Treatment duration was determined using hepatitis C viral load measured two days after treatment onset (D2). Participants with viral load <500 IU/ml at D2 (after second dose of SOF/DCV) were treated with 4 weeks of SOF/DCV. Those with HCV RNA ≥500 IU/ml received 8 weeks (figure 3-2). This threshold was taken from the aforementioned Lau study[173], which had found 100% SVR12 following 3 weeks triple therapy (NS5A-i, NS5B-i, PI). A minimum 4-week duration was chosen for this study based on the broader inclusion criteria (any subtype of genotypes 1 and 6) and the use of dual class therapy (SOF/DCV - NSA5B-i/NS5A-i).

To determine viral kinetics on treatment (and on occasion of any failure), HCV viral load was measured at baseline (day 0) and at all subsequent follow up visits on day 1, 2, 7 and then twice weekly until end of treatment (figure 3-2). Visits after end-of-treatment (EOT) were scheduled twice weekly in the first month after completion of treatment, and then at 8 and 12 weeks after EOT.

Figure 3-2: Study design



**HCV RNA on day 0, 1, 2, 7, 10, 14, 17, 21, 24, 28, (42, 56), EOT+3, EOT+7, EOT+10, EOT+14, EOT+17, EOT+21, EOT+24, EOT+28s, EOT+56, EOT+84*

The primary endpoint was sustained virological response (SVR12) defined as plasma HCV RNA less than the lower limit of quantification (<LLOQ) 12 weeks after the end of treatment without prior failure. Failure of first-line treatment was carefully defined to incorporate individuals who fully suppressed HCV RNA (<LLOQ) on therapy with late virological rebound, as well as those who never fully supressed HCV viral load. In both cases two consecutive viral loads >LLOQ, taken at least one week apart, were required to confirm failure, with the second >2000 IU/ml. This higher threshold for determining failure was used because patients have been observed to achieve cure despite having low-level viraemia at the EOT or shortly after. In practice any patient with low-level viraemia <2000 IU/ml either cures or viral load rises above this level. This same failure criteria was used in a previous short-treatment trial and found to be robust[176]. Once failure was confirmed, participants commenced retreatment with standard duration SOF/DCV within 2 weeks (figure 3-2).

Secondary endpoints were lack of initial virological response (<1 log10 decrease in HCV viral load from baseline), serious adverse events (SAE), grade 3/4 clinical adverse events (AEs), adverse events of any grade leading to change in treatment (SOF, DCV or any other concomitant medication) and adverse reactions (AR). Severity of all AEs and ARs were graded using the Common Toxicity Criteria for Adverse Events gradings[277].

Sample size justification

We set a target cure rate of ≥90%, and an unacceptably low cure rate of 70%. Assuming 90% power and one-sided alpha=0.05, 37 participants were required to exclude the null hypothesis that cure was <90%. Assuming 5% loss to follow-up, and that, based on the study by Lau et al[173], 65% would suppress viral load <500IU/ml by day 2 and receive 4 weeks (rather than 8 weeks) of therapy, the final target population was 60 participants, pooling genotypes 1 and 6.

Study assessments

At each visit patients were assessed by a study doctor. AEs and ARs were recorded and graded according to a standardised scale[277] and medication adherence and use of healthcare facilities were recorded on case report forms.

HCV RNA was measured in the hospital using the available commercial platform. At start of study (for the first 41 participants enrolled), this was the Abbott Architect® (LLOQ = 12 IU/ml). This was subsequently replaced with the COBAS AmpliPrep®/COBAS TaqMan® HCV Quantitative Test, version 2.0 (Roche Molecular Systems, LLOQ = 15 IU/ml). Standard laboratory tests - including full blood count, renal function, and liver function tests – were performed in the hospital laboratory at baseline, EOT and EOT+12.

Virus sequencing

At screening, HCV genotype and subtype were determined using NS5B, Core and 5' UTR sequencing, according to the method described by Chau et.al[13]. To evaluate the impact of HCV subtypes and resistance associated substitutions on treatment outcome, whole genome sequencing (WGS) was additionally performed on all enrolled participants' virus at baseline, and upon virological rebound and at start of retreatment in participants who did not achieve SVR with shortened treatment. WGS of the HCV viral genome was attained using Illumina MiSeq platform as described previously[278–281]. The de novo assembled nucleotide sequences were translated into amino acids and were aligned to H77 HCV reference (GenBank ID: NC_038882.1) and the NS5A and NS5B protein regions were extracted. We only looked for RAS that were present in at least 15% of the reads in the sample and had a read count of greater than 10.

We used the Public Health England (PHE) HCV Resistance Group's definition for resistance associated substitutions (RAS)[282]. For genotype 1 we looked for RASs defined specifically for genotype 1 as they are well studied. For genotype 6 we looked for all RASs defined across all genotypes, as little work has been done on RASs in genotype 6.

For DCV we looked for 24R, 28T, 30E/K/T, 31M/V, 32L, 58D, and 93C/H/N/R/S/W in genotype 1 infection and additionally looked for 28S, 30R and 31F in genotype 6 infection. For SOF we looked for 159F, 237G, 282T, 315H/N, 321A/I in genotype 1 infection and additionally looked for 289I in genotype 6 infection[274,275].

In addition to viral sequencing, we evaluated host genetic polymorphisms within the interferon lambda 4 (*IFNL4*) gene of all participants at baseline. Genotyping of *IFNL4*

rs368234815 was performed on host DNA using the TaqMan® SNP genotyping assay and primers described previously[272] with Type-it Fast SNP Probe PCR Master Mix (Qiagen).

Pharmacokinetics & pharmacodynamics (PK/PD)

To assess pharmacokinetics (PK) and pharmacodynamics (PD), we measured the plasma drug levels of SOF, its inactive metabolite GS-331007, and DCV at baseline, at day 14 and at EOT (day 28 or 56) in all participants. In addition, we conducted intensive drug level sampling in a subset of 40 participants, who were sequentially invited to join an ancillary PK study. In this subgroup, five samples were collected in each participant after the first dose of SOF/DCV and at day 28, according to one of two randomly assigned sampling schedules (A and B). In sampling schedule A, drug levels were measured at 0.5, 2, 4, 6, and 24 hours post-dose; in sampling schedule B, drug levels were measured at 1, 3, 5, 8 and 24 hours post-dose.

Drug quantification was performed using liquid chromatography tandem mass-spectrometer at Mahidol Oxford Tropical Medicine Research Unit, Bangkok. Two separate analytical assays were developed and validated to quantify SOF plus its metabolite GS-331007, and DCV, respectively. Full methodological details of PK/PD analysis are provided in appendix B.

Statistical analysis

Primary and secondary outcomes

Analysis were performed under intention-to-treat (the per-protocol analysis, defined as including all participants taking 90-110% of prescribed treatment, was equivalent to the intention-to-treat analysis) with an additional post-hoc analysis excluding those who were non-G1/6 from WGS. Where possible, proportions and 95% confidence intervals (CIs) were estimated from the marginal effects after logistic regression. Where no events were recorded and models would not converge, we used binomial exact 97.5% CIs. Absolute HCV VL was analysed using interval regression (incorporating censoring at the LLOQ) adjusting for baseline HCV VL. Differences between baseline means and medians in 4-weeks cures vs 4-week failures were analysed with unpaired t-tests and Wilcoxon rank sum tests respectively;

differences in proportions were assessed using chi-squared tests or Fisher's exact tests as appropriate. Analyses were performed using Stata v16.1[283].

Virus genomics

Fisher's exact test was used to test for association between presence and absence of each RAS and treatment outcome. To test for association between outcome and number of RAS we used logistic regression.

PK/PD

Intensive drug levels of SOF, its metabolite GS-331007, and DCV from the subset of 40 patients at day 0 and day 28, together with any EOT samples at day 28, were analysed using non-compartmental analysis in PKanalix version 2020R1[284]. Two separate analyses were performed to characterise the pharmacokinetic properties of the study drugs.

In the first, naïve pooled analyses were performed separately on data from day 0 and day 28 (not including end of treatment samples) to derive median pharmacokinetic parameters at each day. In these analyses, the median concentration at each protocol time were calculated. Individual concentration measurements below the LLOQ was set to LLOQ/2 when calculating the median values. It was assumed that the participants had no drug concentrations at time 0.

In the second analysis, data from day 0 and day 28 were pooled for each individual. This resulted in a full pharmacokinetic profile for each subject, which was analysed with a non-compartmental approach. The mean value of drug concentrations were used if patients had two or more samples taken at the same time point. These derived individual drug exposures were used to evaluate the relationship between drug exposure and therapeutic outcome. It was assumed that the participants had no drug concentrations at time 0. In this analysis the first measurement below LLOQ in a series of LLOQ samples were imputed as LLOQ/2 and the later measurements were ignored. In both approaches, SOF samples taken at ≥24 hours post-dose were excluded. SOF is a prodrug and has a very short half-life of less than 1 hour, which make concentrations at 24 hours after dose extremely unlikely[285].

In addition, outcome variables and the relationship between outcome variables and drug exposure were evaluated. Additional detail of the PK/PD analysis (performed at MORU in Bangkok by my co-investigator Richard Hoglund) is provided in appendix B.

Ethical approval

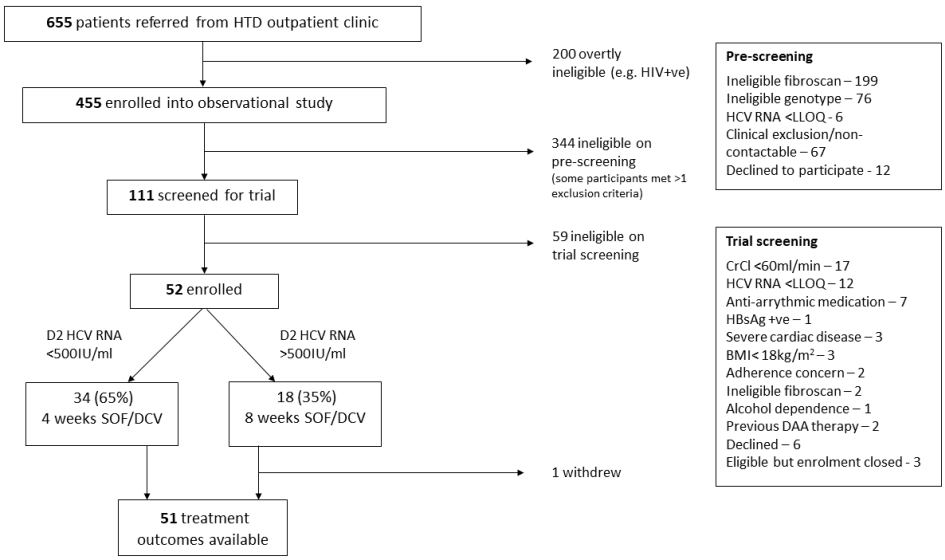
The trial was approved by the research ethics committees of The Hospital for Tropical Diseases[286], Vietnam Ministry of Health[287], Imperial College London[288], and Oxford University Tropical Research Ethics Committee[289]. The study's conduct and reporting is fully compliant with the World Medical Association's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects.[290] The trial was registered at ISRCTN, registration number is ISRCTN17100273[291].

Results

Baseline characteristics

Of 455 patients screened, 52 were enrolled and one subsequently withdrew (figure 3-3). Most exclusions were on account of either FibroScan score of >7.1kPa (with cirrhotic patients enrolled into a parallel study[292]), or ineligible genotype. 22/51 were initially identified as genotype 1 infection and 30 as genotype 6. With the benefit of WGS data, it was confirmed that 22 (43%) had genotype 1 infection, 27 (53%) had genotype 6, one had genotype 2 and another had genotype 4 infection. The latter two individuals were included in the intention-to-treat analysis but excluded from a post-hoc analysis of G1 and G6 infections only. Recruitment was completed short of the initial target of 60 due to severe COVID-19-related restrictions in Vietnam from February 2020. These included clinic closures, travel restrictions and repurposing of the HTD as a COVID-19 treatment centre.

Figure 3-3: Screening and enrolment



Baseline and clinical characteristics are described in Table 5. One participant, a male with genotype 1b infection who was cured with 4 weeks of therapy, had an HCV viral load of 618 IU/ml on day 0 which may have been consistent with spontaneously resolving acute infection,

but could equally reflect fluctuating viraemia. Baseline viral load was >10,000 IU/ml in all other participants, who were all assumed to have chronic infection.

Table 6: Baseline characteristics

	N/ median	%/range
Total participants	52	
Age in years	49.5	(25.0, 67.0)
Female	29	(56%)
Body-mass index in kg/m^2	23.3	(18.7, 30.6)
Genotype 1	22	(43%)
1a	11	
1b	12 (1 withdrew)	
Genotype 6	27	(53%)
6a	12	
6e	10	
6h	2	
6l	2	
6u	1	
Genotype 2(m)	1	
Genotype 4(k)	1	
Baseline HCV viral load in IU/ml	1,932,775	(618, 11,200,000)
HCV viral load – log10 IU/ml (range)	6.3	(2.8, 7.0)
Past medical history:		
Illicit drug use	4	(8%)
Alcohol dependence (historic; current excluded)	4	(8%)
Diabetes	2	(4%)
Hypertension	7	(13%)
Ischaemic heart disease	1	(2%)
Tuberculosis	2	(4%)
Current smoker	18	(35%)
Previous spontaneous clearance of HCV with re-infection	2	(4%)

Treatment duration, adherence and efficacy outcomes

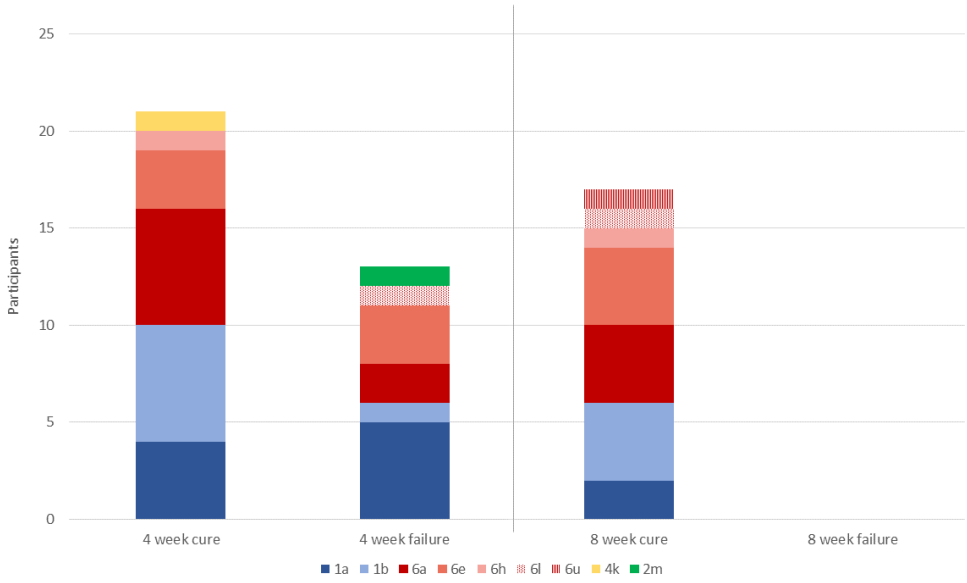
By day two, 34 participants (65%) had HCV viral load below the threshold of 500 IU/ml (figure 3-3; table 2), so received 4 weeks of treatment. 18 participants were above the threshold at this timepoint, of which one withdrew after 9 days of treatment, meaning 17 completed 8 weeks therapy. Adherence was good, with 96% completing the full prescribed

course of SOF/DCV (as assessed by patient reporting and physician pill counting). 18 (35%) participants missed at least one visit because of COVID-19-related restrictions.

Of the 51 participants with outcome data, 38 (75% [95% CI (63, 86)]) achieved SVR12 while 13 failed therapy and required retreatment. All treatment failures occurred in individuals who received 4 weeks therapy, translating to an SVR12 of 62% (21/34; 95% CI (44, 78)) in rapid responders who received 4 weeks therapy, and 100% (17/17; 97.5% CI (80, 100)) in slower responders who received 8 weeks SOF/DCV (figure 3-4; table 6).

Of the 13 participants who underwent retreatment, 100% were cured. The mean first-line SOF/DCV treatment duration was 37 days (standard deviation, SD 13.7), with a first-line cure rate of 75%. The mean (SD) total SOF/DCV duration (i.e. including 12 weeks retreatment where required), was 58 (34.2) days per patient, with a 100% cure rate. There was no evidence of differences in age, gender, BMI, IFNL4 genotype, transaminases or baseline HCV viral load between patients who achieved cure with 4-weeks of treatment versus those who experienced treatment failure with 4 weeks of treatment (table 7).

Figure 3-4: Primary outcome, with HCV subtypes



All 13 individuals who experience treatment failure with 4 weeks SOF/DCV were cured with 12 weeks SOF/DCV retreatment

Table 7: Treatment outcome

	N/ median	%/range
Detectable HCV viral load (HCV VL) at day 2	50	96%
Abbot	39/41	95%
COBAS	11/11	100%
Median (IQR) HCV VL at day 2 in IU/ml	269	(104, 690)
Abbot	217	(101, 690)
COBAS	459	(209, 832)
Below threshold - for 4 weeks therapy	34	(65%)
Abbot	31	(66%)
COBAS	3	(60%)
Above threshold – for 8 weeks therapy	18	(35%)
Abbot	16	(34%)
COBAS	2	(40%)
Mean (SD) duration of first-line therapy received in days	37	(13.7)
Mean (SD) duration of all therapy received in days	58	(34.2)
Median weeks from enrolment to last visit (range)	20	(1, 42)
Primary Outcome		
Outcome available	51	
SVR12 by intention-to-treat analysis and per protocol analysis	38	(75% [95% CI 63, 86])
SVR12 by sensitivity analysis (i) [missing results = failure]	38	(73% [95% CI 61, 85])
SVR12 by post-hoc analysis (ii) [G1 and G6 only]	37	(76% [95% CI 63, 88])
Secondary Endpoints		
Lack of initial virological response	0	(0% [97.5% CI 0, 7])*
Serious adverse events	0	(0% [97.5% CI 0, 7])*
Grade 3/4 clinical adverse events	0	(0% [97.5% CI 0, 7])*
Non-serious adverse reactions	18	(35% [95% CI 22, 48])
Adverse events or reactions leading to change in study medication	0	(0% [97.5% CI 0, 7])*

For each result, I have indicated whether HCV RNA was tested on Abbott or COBAS. No samples were tested on more than one platform.

Viral kinetics and timing of treatment failure

All participants had an initial virological response ((i.e. ≥1 log10 decrease in HCV viral load from baseline) (figures 3-5, 3-6). There was no evidence of association between time to complete virological suppression (HCV RNA <LLOQ) and treatment outcome (table 7; figure 3-7). In an exploratory analysis, we estimated first-line cure rates based on suppression below the LLOQ at other timepoints which could be used for RGT. At day 7, 9/21 cures and 1/12 treatment failures (one missed visit) had HCV RNA <LLOQ (p=0.054; table 8), translating to 90% sensitivity (95% CI [56, 100]) for predicting cure with 4 weeks treatment. However, by day 10, 9/21 cures and 9/13 failures had HCV RNA <LLOQ (p=1.00), making a rapid virological response 50% [26, 74] sensitive in predicting cure with 4 weeks treatment. HCV RNA kinetics in all participants treated with 4 weeks SOF/DCV are shown in figure 3-6, with

cures (blue lines) distinguished from those experiencing treatment failure (red lines). Even though the numbers are small, this helps illustrate that early on-treatment response alone may be of limited value in determining cure with ultra-short therapy.

Figure 3-5: Mean (95% C.I) HCV RNA (log10) by visit day

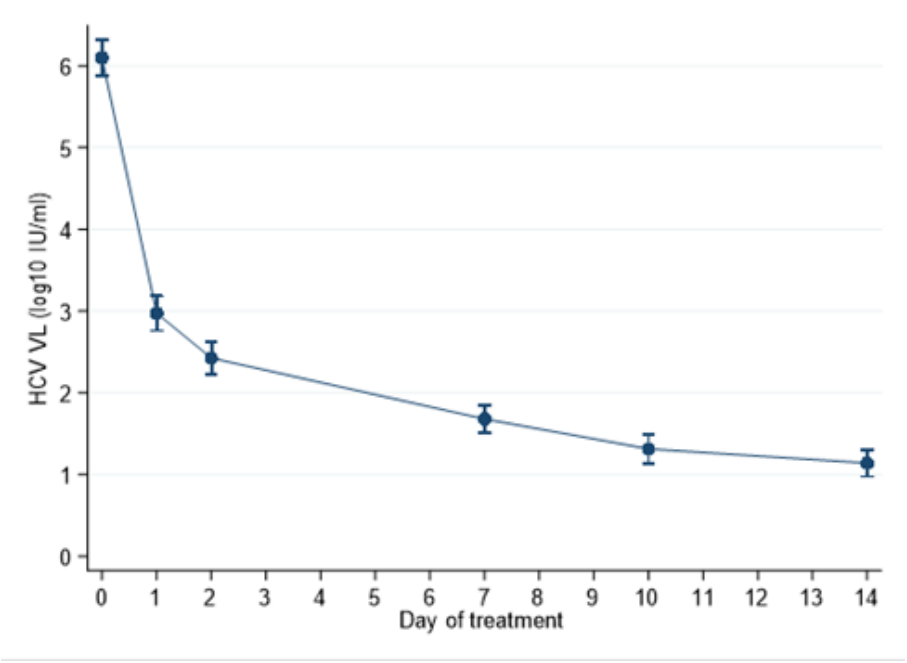


Figure 3-6: HCV RNA (log10) kinetics in participants treated with 4 weeks SOF/DCV

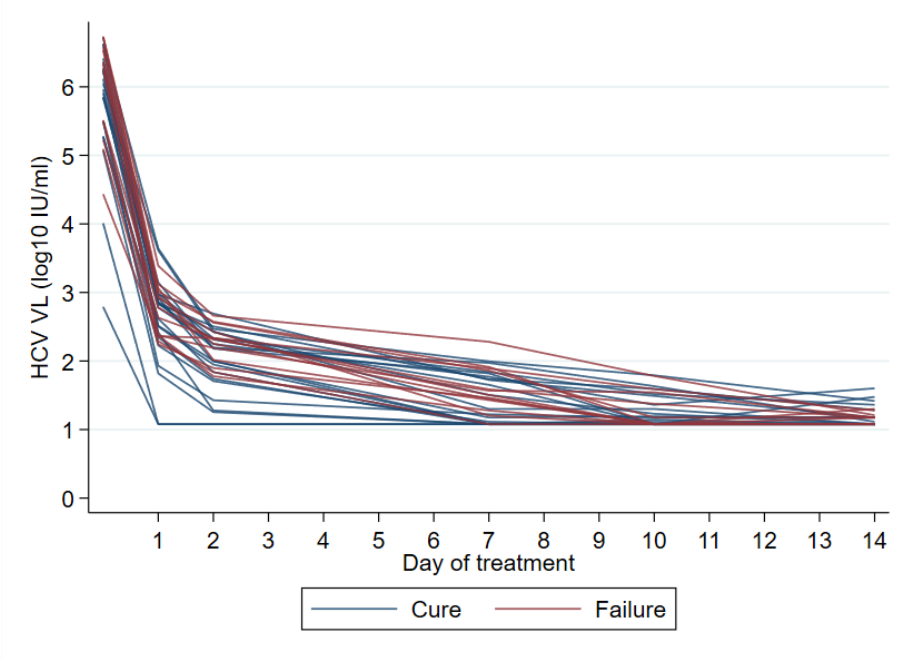
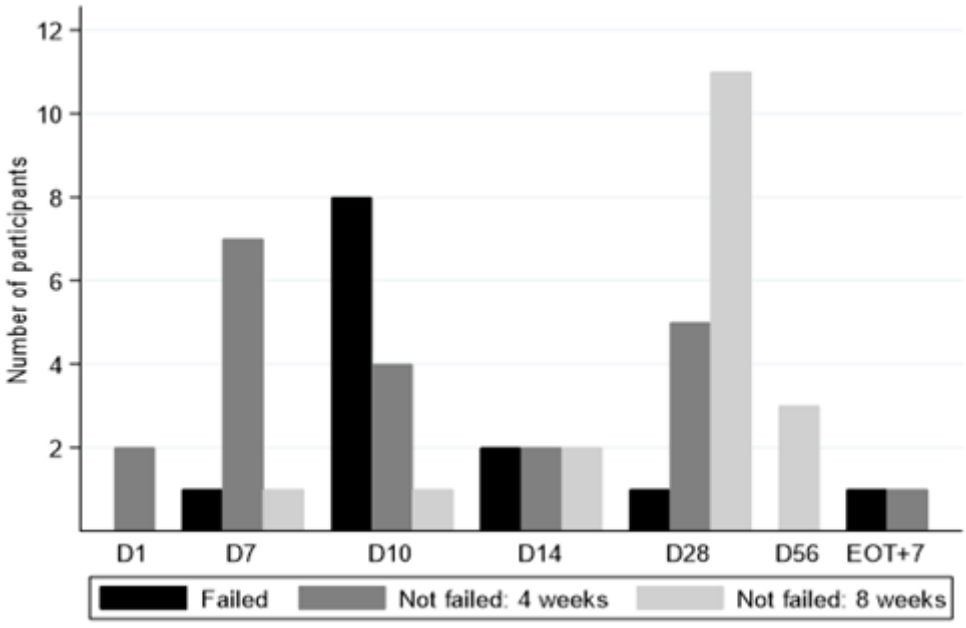


Figure 3-7: Time to HCV RNA suppression <LLOQ and treatment outcome



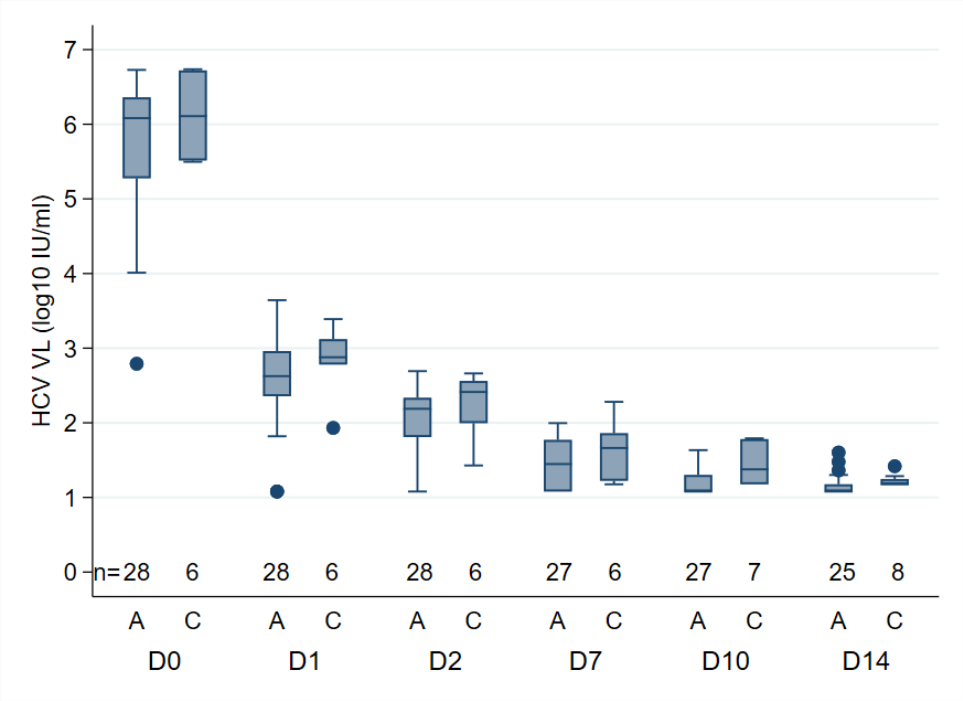
D = day; EOT+7 = 7 days after end of treatment

Since the two HCV assays used in our study have previously been shown to yield different HCV RNA results in the same individuals on therapy[293], we conducted additional analyses of

viral kinetics stratified by platform. We found no evidence of a difference between platforms in terms of proportion of participants with undetectable viral load at different timepoints (table 6, table 7), or in terms of first phase (day 0 to day 2) or second phase (day 2 to first HCV RNA < LLOQ) viral decline on treatment (figure 3-8). However, numbers were small meaning we may have lacked power to detect effects.

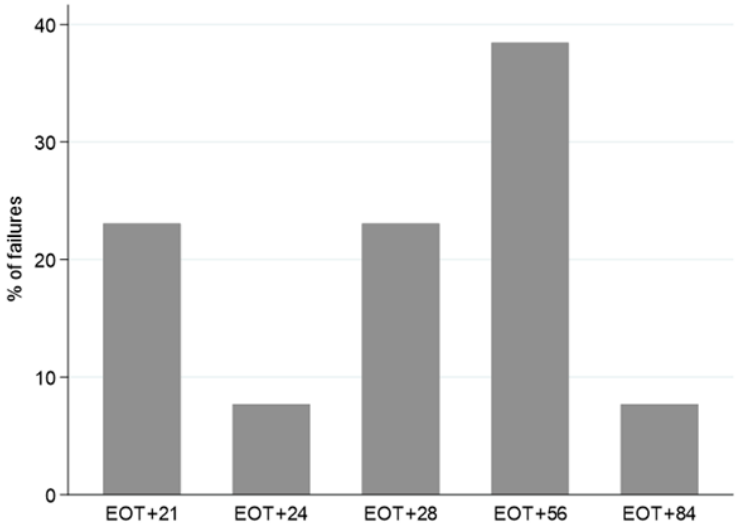
All treatment failures occurred during follow-up after EOT. Despite intensive twice weekly sampling from EOT to EOT+28d, the earliest virologic rebound occurred 3 weeks after completion of therapy (figure 3-9).

Figure 3-8: Median HCV RNA (log10), by PCR assay, at different timepoints in participants treated with 4 weeks SOF DCV



Box plot with range ('whiskers' indicating minimum and maximum values in data set), IQR (box indicating Q_1 and Q_3), and median (line within box). (A = Abbott Architect® (LLOQ = 12 IU/ml). C = COBAS AmpliPrep®/COBAS TaqMan® HCV Quantitative Test, version 2.0 (Roche Molecular Systems, LLOQ = 15 IU/ml).

Figure 3-9: Timing of treatment failure



Treatment failure defined as second of two consecutive viral loads >LLOQ, taken at least one week apart, with the second >2000 IU/ml. EOT = End of Treatment.

Table 8: Comparison of baseline factors, drugs levels and virological response in individuals who failed to achieve SVR12 with 4 weeks therapy vs those who cured with 4 or 8 weeks therapy

	4-week cures (n=21)	4-week failures (n=13)	*p*	8-week cures (n=17)
Host factors				
Male (%)	62%	38%	*0.18*	29%
Mean age	45	48	*0.23*	55
Mean BMI	23	23	*0.40*	24
Median ALT	54	36	*0.10*	31
Median AST	34	28	*0.44*	33
IFNL4 delG/TT and TT/TT genotypes (rs368234815)	71%	58%	*0.47*	69%
Virus factors				
Median D0 HCV VL	916,000	2,139,258	*0.20*	4,982,889
Abbot	960,913	1,972,841	*0.47*	4,625,118
COBAS	916,000	5,260,000	*0.40*	4,605,000
D2 VL <LLOQ	2/21 (10%)	0/13 (0%)	*0.51*	0%
Abbot	2/18 (11%)	0/10 (0%)	*0.41*	0/13 (0%)
COBAS	0/3 (0%)	0/3 (0%)	-	0/5 (0%)
D7VL <LLOQ	9/21 (43%)	1/12 (8%)*	*0.054*	0%
Abbot	8/18 (44%)	1/9 (11%)	*0.09*	0/13 (0%)
COBAS	1/3 (33%)	0/3 (0%)	*1.00*	0/5 (0%)
D10 VL <LLOQ	9/21 (43%)	9/13 (69%)	*0.17*	6%
Abbot	8/17 (47%)	8/10 (80%)	*0.12*	1/10 (10%)
COBAS	1/4 (25%)	1/3 (33%)	*1.00*	0/6 (0%

D14 VL <LLOQ	14/21 (68%)	9/13 (69%)	*1.00*	18%
Abbot	11/16 (69%)	6/9 (67%)	*1.00*	1/11 (18%)
COBAS	2/4 (50%)	3/4 (75%)	*1.00*	1/6 (17%)
HCV genotype 1	10/21 (48%)	6/13 (46%)	*1.00 (vs Gt 6)*	6/17 (35%)
1a	4/21 (19%)	5/13 (38%)	*0.15 (vs 1b)*	2/17 (12%)
1b	6/21 (24%)	1/13 (8%)		4/17 (24%)
HCV genotype 6	10/21 (48%)	6/13 (46%)		11/17 (65%)
6a	6/21 (29%)	2/13 (15%)	*0.58 (vs 6e)*	4/17 (24%)
6e**	3/21 (14%)	3/13 (23%)		4/17 (24%)
Resistance associated substitutions				
Median (range) SOF-RAS	0 (0-1)	0 (0-2)	*0.76*	0 (0-1)
Median (range) DCV-RAS	2 (0-2)	1 (0-2)	*0.17*	2 (0-4)
Median (range) SOF- & DCV-RAS combined	2 (0-3)	2 (1-2)	*0.12*	2 (0-4)
Drug Exposure (n=37)****	n=15	n=8		n=14
Median $AUC_{last,}$ SOF (h×ng/mL) ***	1,250 (594-2,410)	1,170 (496-2,070)	*0.975*	1,120 (755-1,380)
Median AUC_{last} GS-331007 (h×ng/mL) ***	3,050 (2,190-3,670)	3,920 (2,400-5,140)	*0.023*	3,640 (2,670-4,540)
Median $AUC_{last,}$ DCV (h×ng/mL) ***	9,610 (5,020-16,500)	9,720 (4,900-19,900)	*0.728*	10,500 (6,800-12,600)

**n=12, no HCV VL data for one participant's day 7 visit*

*** h, l and u subtypes excluded from the table/analysis due to small numbers (≤2)*

**** Results presented as median (5^{th}-95^{th} percentile)*

***** Complete d0 and d28 data only available for 37 participants*

***** AUC_{last} is the total exposure to the last time point (8 hours for SOF and 24 hours for GS-331007 and DCV)*

P value represents differences between baseline means and medians in 4-weeks cures vs 4-week failures, analysed with unpaired t-tests and Wilcoxon rank sum tests respectively; differences in proportions were assessed using chi-squared tests or Fisher's exact tests as appropriate. A p value of <0.05 was considered statistically significant.

Whole genome sequencing was attempted on all participants' virus at baseline, but consensus sequences could not be assembled in two individuals (who had low baseline viral load and were both cured with first line therapy). This left 50 patients with baseline sequences, of which 49 had outcome data.

We found nine discrepancies between lab genotyping and sequencing-based genotyping. Five of these differences were at the level of subtypes for genotype 6 samples, highlighting difficulties inherent in classifying this rare and genetically diverse lineage using an amplicon approach for genotyping (lab genotyping). Two samples were called 6a/e using lab genotyping and whole genome sequencing classified them as 6e. One sample was classified as 6e on lab genotyping, but whole genome sequencing showed that it was a genotype 2m sample. Whole genome sequencing revealed another patient to have mixed infection with genotype 1a and genotype 6a; this had been classified by laboratory genotyping as a genotype

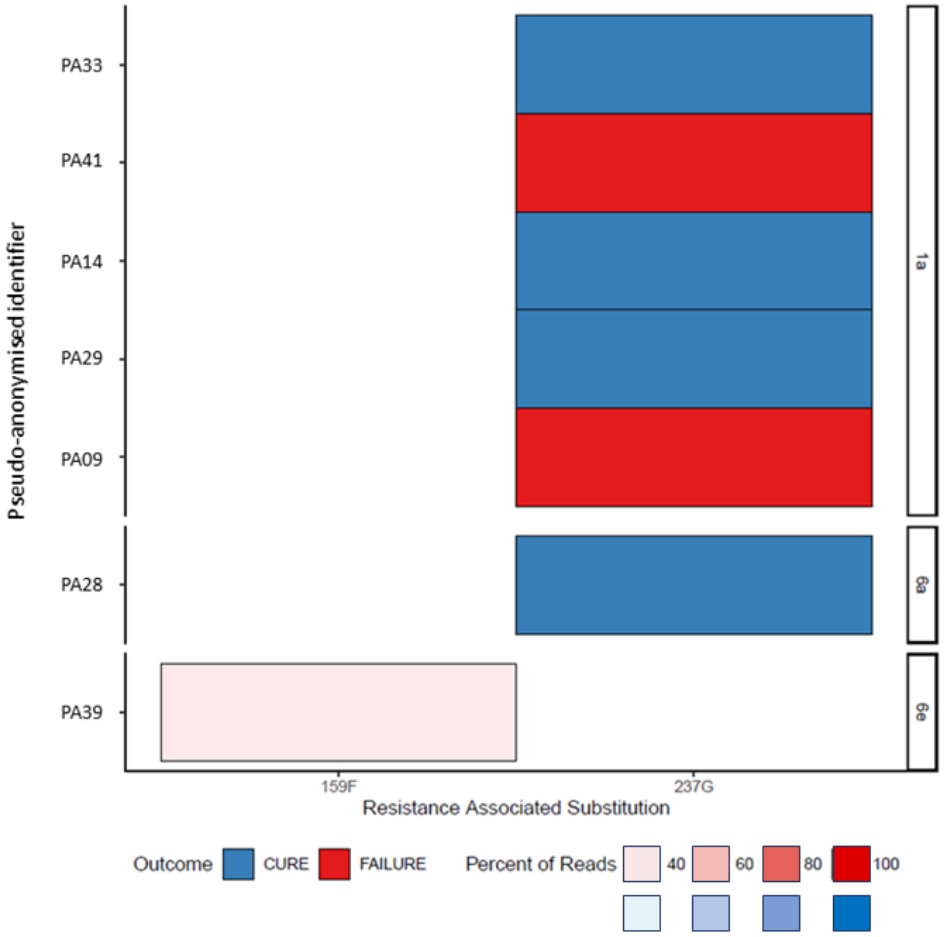
6a mono-infection. The individual with mixed infection received 4 weeks of SOF/DCV but cure was not achieved, with relapse of the genotype 1a infection. They subsequently responded to 12 weeks retreatment.

We found no evidence of differences between genotypes or subtypes with regards to rates of treatment failure (table 7), however the study was not powered for this analysis. Among genotype 1-infected individuals, 1/7 subtype 1b infections experienced treatment failure with 4 weeks therapy compared with 4/8 subtype 1a infections (including the mixed infection case) (p=0.15). Among genotype 6-infected individuals, 1/8 subtype 6a infections were not cured with 4 weeks SOF/DCV compared with 3/6 subtype 6e (p=0.58), 0/1 subtype 6h and 1/1 subtype 6l (figure 3-4).

At baseline, the 159F SOF RAS was identified in one patient, and the 237G putative SOF RAS was identified in six patients (figures 3-10 and 3-11). The DCV RAS 24R, 30R, 31M, 93H and 93S were detected at baseline (figures 3-12 and 3-13).

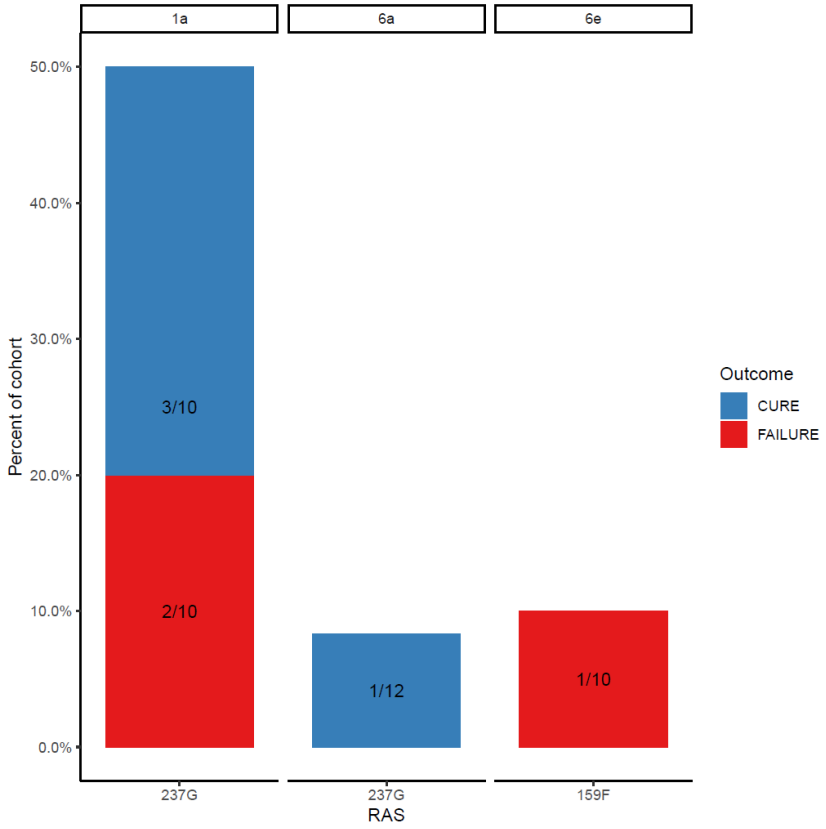
In the assessment of SOF RAS (figure 3-10), the one patient who had 159F at baseline failed treatment, although this was a minority variant making up 20% of the sequencing reads (figure 3-14; black box). 237G was identified as a majority variant in two individuals where treatment failed, but was also seen in four individuals who were cured (three received 4 weeks treatment (figure 3-10).

Figure 3-10: All SOF RAS at baseline with treatment outcome



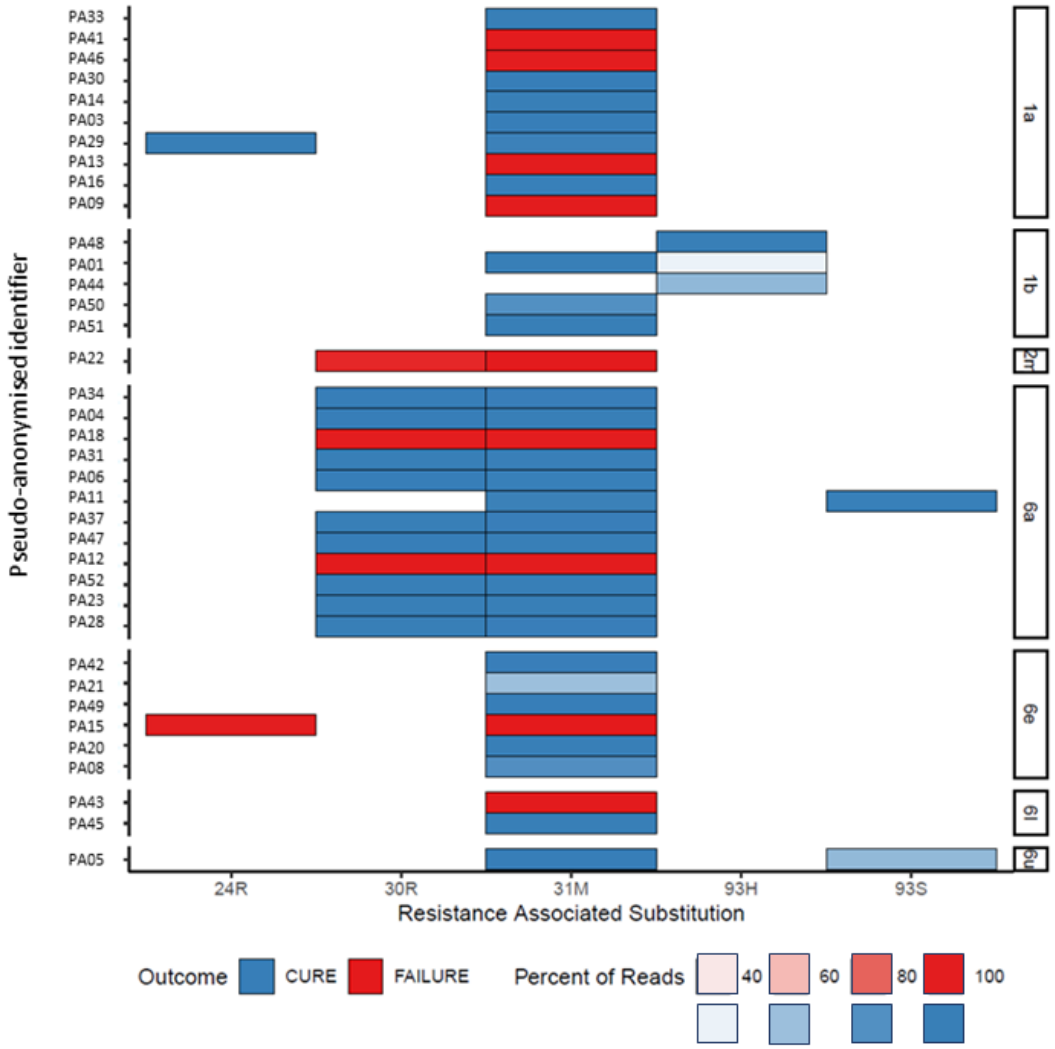
PA33, PA41 etc. represent pseudo-anonymised patient identifiers. SOF-RAS are shown on the x axis. Genotypes and subtypes are displayed on the right of the figure. Shade of red/blue indicates percent of reads with specified SOF-RAS.

Figure 3-11: Proportion of each subtype with each SOF RAS at baseline



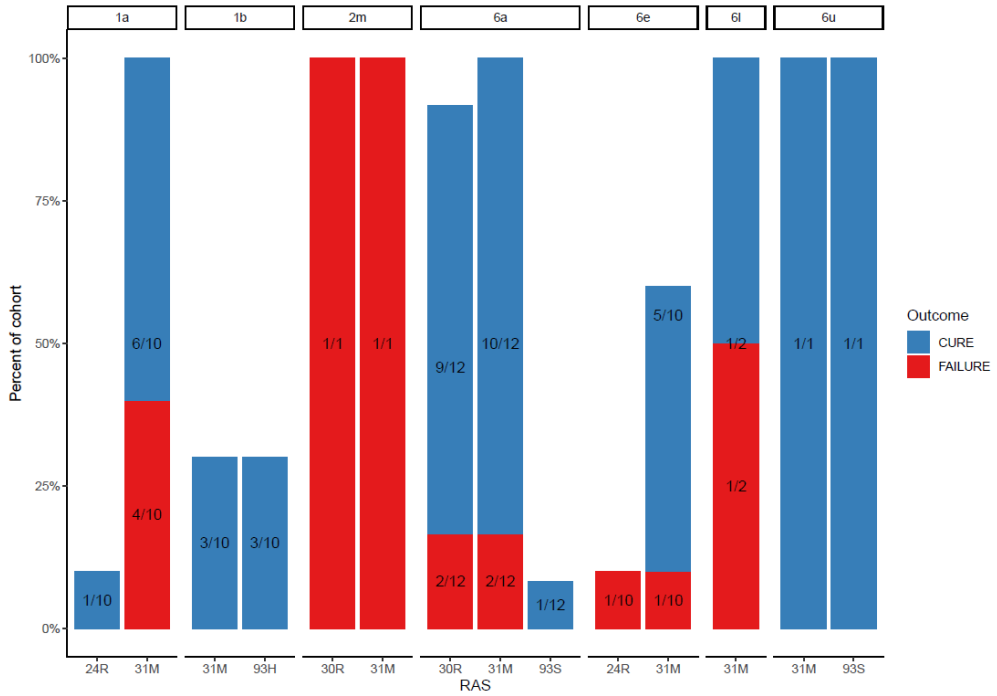
The most prevalent DCV RAS was 31M, present in 9 participants where treatment failed after 4 week first-line therapy (figure 3-12, figure 3-13). However, 31M was also found in 13 individuals cured with 4 weeks treatment, and 13 cured with 8 weeks. The next most prevalent RAS was 30R, present at baseline in 3 patients who had treatment failure, in 5 individuals cured with 4 weeks treatment and in 4 patients cured with 8 weeks treatment. 30R RAS was present in 11/12 6a genomes and 1/1 2m genomes but was absent in other subtypes. 31M RAS was present in 10/11 1a genomes and 12/12 6a genomes and was also found in other subtypes. Additionally almost all of the subtype 6a samples carried both 30R and 31M RASs while other subtypes did not carry this combination (apart from the 2m sample).

Figure 3-12: All DCV RAS at baseline (with treatment outcome)



PA33, PA41 etc. represent pseudo-anonymised patient identifiers. DCV-RAS are shown on the x axis. Genotypes and subtypes are displayed on the right of the figure. Shade of red/blue indicates percent of reads with specified DCV-RAS.

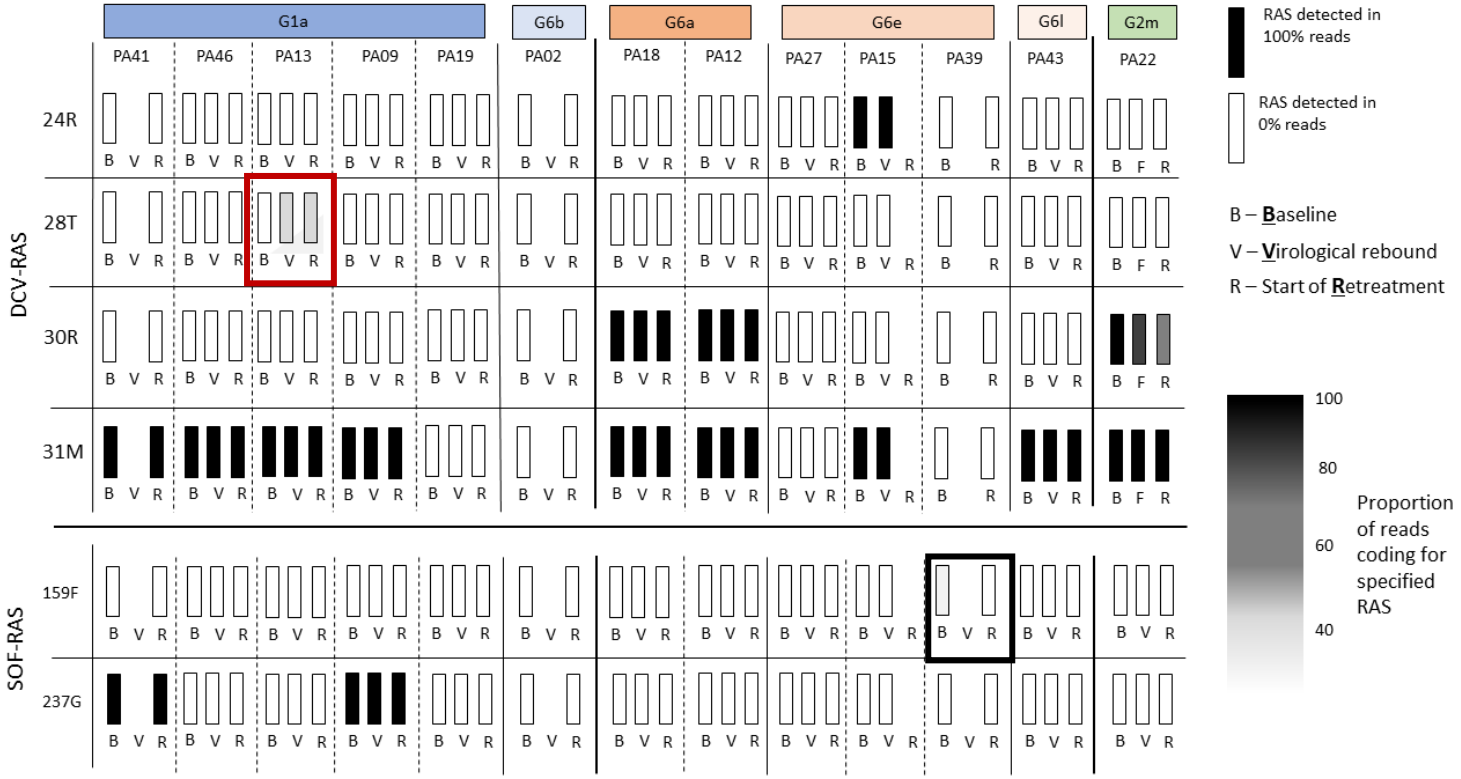
Figure 3-13: Proportion of each subtype with DCV RAS at baseline



Viral genomics in participants who did not achieve SVR with shortened therapy

Among 13 individuals who experienced treatment failure, we compared the emerging viral genome with baseline virus (figure 3-14). Full genome sequences could not be assembled for three participants at time of virological relapse, however, we were able to generate whole viral genomes using samples from the start of retreatment for two of these individuals. No new genomes were identified at treatment failure (ruling out any new infections). No new SOF RAS were identified on virologic rebound. DCV 28T RAS (not present at baseline) was identified in one participant who did not achieve SVR with shortened therapy (see red box figure 3-14) as a minority variant at time of virological rebound and start of retreatment (at 30% and 25% of reads respectively). Given 100% of retreated individuals achieved SVR12 with standard duration of therapy we found no evidence to suggest this emerging RAS was clinically significant. There was no evidence of differences in the number of combined SOF- and DCV-RAS at baseline in those who did not achieve SVR with 4 week therapy (median 2, range 0-3) vs those who cured with 4 weeks (median 2, range 1-2), (p=0.12) or in those with a slower initial virological response, who received 8 weeks (median 2, range 0-4).

Figure 3-14: SOF RAS and DCV RAS at baseline, at virological rebound, and at start of retreatment in all participants who did not achieve SVR with first line treatment.



PA41, PA46 etc. represent pseudo-anonymised patient identifiers of the 13 participants who did not achieve SVR with shortened therapy. DCV-RAS and SOF-RAS are shown on the left of the figure. Genotypes and subtypes are displayed at the top of the figure. Each shaded box represents virus sequenced. B = at Baseline, V = when virological rebound identified, R = at start of retreatment (commenced within 2 weeks of virological rebound). Boxes are absent where sequencing failed. Black boxes indicate 100% reads coding for specified RAS. White boxes indicate specified RAS was identified in 0% of reads. Gray shades indicate approximate proportion of reads coding for specified RAS. Solid vertical grid lines separate subtypes/genotypes. Solid horizontal lines separate RAS. Red box (PA13) indicates participant in which 28T SOF-DCV RAS was absent at baseline but detected upon virological rebound and persisted at start of retreatment. Black box (PA39) indicates participant in which 159F RAS was present at low read frequency at baseline but was absent at time of retreatment (sequencing at time of virological rebound failed).

Pharmacokinetics and pharmacodynamics

Pharmacokinetic parameters derived from the naïve pooled analysis (based on 40 patients on day 0 and 37 patients on day 28) are presented in table 8. Exposure after the individual analysis as well as outcome measurements are presented in table 9. In the individual analysis and the linear regression between outcome measurements and drug exposure, 3 patients were excluded as they did not have dense samples collected at day 28 (n=37). In the analysis of outcome variables, data from all 40 patients were used. No significant relationship between outcome variables and drug exposure was found using linear regression (table 10).

In the subset of 37 patients who underwent dense PK analysis at day 0 and 28, 23 patients received 4 weeks of SOF/DCV and 14 patients received 8 weeks of therapy. There was no significant difference between total drug exposure (AUC_{last}) for SOF and DCV in 4 week cures (n=15) vs 4 week treatment failures (n=8); (table 7). GS-331007 exposures were significantly higher in the patient group with treatment failures (p=0.023) although numbers were small.

Table 9: Pharmacokinetic parameters from the naïve-pooled analysis

	Sofosbuvir		GS-331007		Daclatasvir	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Cmax (ng/mL)	1,320	1,070	988	1,230	1,170	1,110
Tmax (h)	1.00	1.00	3.00	4.00	3.00	3.00
t1/2 (h)	0.670	0.650	9.20	12.4	7.31	8.18
AUClast (h×ng/mL)*	1,550	1,600	10,500	14,600	11,400	12,400
AUCINF (h×ng/mL)*	1,550	1,600	12,700	20,400	12,800	14,400

C_{max} is the maximum observed concentration, t_{max} is the time to reach the maximum concentration, $t_{1/2}$ is the terminal elimination half-life (calculated using the 3-6 last concentration measurements, depending on drug and day), AUC_{last} is the total exposure to the last time point (8 hours for SOF and 24 hours for GS-331007 and DCV), AUC_{inf} is the total exposure extrapolated to infinity.

**Extrapolation based on the last observed concentration measurement*

Table 10: Pharmacokinetic exposure from the individual analysis & pharmacodynamic parameters

Pharmacokinetics			
	Sofosbuvir	GS-331007	Daclatasvir
AUClast (h×ng/mL)	1,140 (598-2,150)	3,430 (2,200-4,720)	9,770 (5,080-16,200)

Pharmacodynamics	
AUC (days×IU/mL)	252,000 (19,200-1,370,000)
t1/2 (days)	2.25 (0.986-5.22)
%ReductionEnrolment-Day1	99.9 (99.0-100)
%ReductionEnrolment-Day7	100 (100-100)

Data is presented as median (5^{th} -95^{th} percentile). AUC_{last} is the total exposure to the last time point (8 hours for SOF and 24 hours for GS-331007 and DCV). AUC_{14} is the area under the viral load-time curve from enrolment (day 0) to day 14, $t_{1/2}$ is the terminal viral half-life (estimated using at least three measurements), $\%Reduction_{Enrolment\text{-}Day1}$ is the reduction in viral load from enrolment to day 1, $\%Reduction_{Enrolment\text{-}Day7}$ is the reduction in viral load from enrolment to day 7.

**The half-life could not be determined for one participant due to only one sample above the lower limit of quantification.*

Table 11: Pharmacokinetic-pharmacodynamic analysis

Linear regression						
	Sofosbuvir		GS-331007		Daclatasvir	
	Slope (95% CI)	*p*	Slope (95% CI)	*p*	Slope (95% CI)	p-value
Area under the viral load-time curve	-157 (-423 - 109)	0.239	16.2 (-74.4 - 107)	0.719	-14.2 (-67.1 - 38.6)	0.589
Viral elimination half-life	1.55×10^{-4} (-8.70×10^{-4} - 5.60×10^{-4})	0.662	-3.64×10-5 (-2.74×10^{-4} - 2.01×10^{-4})	0.757	2.17×10^{-5} (-1.16×10^{-4} - 1.60×10^{-4})	0.751
Relative reduction in viral load at day 1	1.31×10^{-6} (-4.54×10^{-6} - 7.16×10^{-6})	0.652	2.67×10-8 (-1.94×10^{-6} - 1.99×10^{-6})	0.978	2.81×10^{-7} (-8.62×10^{-7} - 1.42×10^{-6})	0.621
Relative reduction in viral load at day 7	2.53×10^{-7} (-2.81×10^{-7} - 7.86×10^{-7})	0.343	5.09×10-8 (-1.29×10^{-7} - 2.31×10^{-7})	0.569	$1.44*10^{-8}$ (-9.11×10^{-8} - 1.20×10^{-7})	0.783

Safety

SOF/DCV was well-tolerated and no participants discontinued treatment due to drug side effects. 18 participants (35%; 95% CI 22%, 48%) reported at least one non-serious adverse reaction. The most common of these were insomnia, gastritis and dizziness, which are all consistent with undesirable effects described in the summary of product characteristics of SOF/DCV[169]. There were no serious adverse events or grade 3 or 4 adverse events.

Discussion

In this mechanistic study in individuals with genotype 1 or 6 HCV infection and mild liver disease, treated with 4 or 8 weeks of SOF/DCV depending on HCV viral load 2 days after starting treatment, overall first-line cure rate was 75% [95% CI (63, 86)], with a mean 37 days treatment. This saved 47 days DAA therapy per participant compared with a standard 12 week course, but cure rate fell below our target of ≥90%. For the secondary endpoint - SVR12 after combined first-line therapy or retreatment - cure was 100%, with mean treatment duration 58 days, saving 26 days DAAs per participant.

Effect of shortening therapy

Inferior rates of cure are well described when DAA therapy is shortened below 8 weeks without use of early on-treatment virological response, falling below 50% with ≤4 weeks therapy without stratification[172,294–296]. A few small studies have reported high rates of cure with shortened therapy based early virological response[173,297,298]. The only previous RGT study to use less than 6 weeks treatment, by Lau et al, found a cure rate of 100% with just three weeks of DAA therapy in 18 individuals whose HCV viral load was suppressed below 500 IU/ml after two days of therapy. This was the same threshold and time point used in our study. One important difference was in the treatment regimen, which included a protease inhibitor (simeprevir or asunaprevir). Although NS5A- (DCV) and NS5B- (SOF) inhibitors rapidly eliminate HCV from the blood, second-phase decline in viral load appears to be enhanced by addition of a protease inhibitor[299]. This may be crucial in sustaining high rates of

cure with shortened therapy. Viral kinetics in our participants were broadly similar to those observed in patients treated with DCV-containing regimens in the study by Lau et al, with a rapid first phase viral decline leading to an approximate 4 log10 IU/ml decline in HCV RNA in the first 48h of treatment. However a detailed comparison of viral kinetics is limited by differences in sampling schedule, baseline viral loads and the HCV PCR platforms used. Another key difference relates to infecting genotypes -all participants in the Lau study had genotype 1b infection, compared with just 23% (n=12) in ours. Genotype 1b is associated with favourable outcomes with some DAAs compared with other genotypes[128,300]. Although real world 1b outcomes with standard duration SOF/DCV appear similar to other non-3 genotypes[301], subtype may be more important when treatment is shortened.

Role for response-guided therapy with SOF/DCV

Cure rates with this strategy are too low for it to be routinely recommended. With standard duration therapy, SVR12 is known not to be impacted by time to first undetectable HCV RNA[160] or by the presence of detectable virus at the end of treatment [302]. This also appears to be true of shortened treatment: in one individual who experienced treatment failure, HCV viral load was already <LLOQ by day 7; in five of the 4-week cures, HCV VL was only suppressed to <LLOQ virus for the first time at end of treatment (figure 3-7). Comparison of 4-week cures and 4-week treatment failures indicates that an HCV RNA <LLOQ by day 7 may be a useful discriminator of 4-week treatment outcome (p=0.054). However, neither day 10 nor day 28 HCV RNA<LLOQ was predictive of response to shortened treatment. Day-7 viral load thresholds for shortening DAA therapy are currently being evaluated as part of a large ongoing randomised controlled trial in Vietnam[303].

A case for 8-weeks SOF/DCV therapy

Given the high rates of cure observed with 8 weeks of SOF/DCV in participants with a slow initial virological response (17/17), there is a case for reducing SOF/DCV therapy from 12 to 8 weeks in individuals with mild liver disease. Prior evidence for caution regarding 8 weeks of SOF/DCV comes predominantly from a small 2015 study in HIV-coinfected individuals[136], in which 7/10 treatment failures in the 8-week arm received half-dose daclatasvir (30mg)

because participants were taking concomitant darunavir–ritonavir. This dose adjustment was subsequently deemed unnecessary once drug-interaction data emerged, such that this study is likely to underestimate the efficacy of 8 weeks SOF/DCV. More recent studies corroborate our finding of >90% cure with 8 weeks NS5A/NS5A inhibitor combination[175,297,304]. Larger trials are warranted to evaluate 8 weeks SOF/DCV therapy for patients with mild liver disease (irrespective of speed of virological response). This could save significant costs, particularly in countries where pricing is determined per pill rather than per treatment course, such as Vietnam, and the USA[294,305].

Impact of resistance-associated substitutions and retreatment concerns

To our knowledge this study is the largest assessment of G6 RAS in vivo with SOF/DCV therapy. We hypothesised that a high number of putative RAS at baseline may be associated with higher rates of failure with shortened treatment. However, we found no evidence that number or type of SOF- or DCV-RAS was different at baseline in 4-week cures compared with 4-week treatment failures (table 7, figures 3-10 and 3-12), although numbers were small. Additionally, the excellent retreatment outcomes observed (13/13 SVR12) are reassuring, particularly for low-resource settings where protease inhibitor-based retreatment options are limited. Only one novel RAS was detected after first line treatment failure, and the individual concerned achieved SVR with standard duration retreatment, suggesting this was not clinically relevant.

Impact of drug levels

This was the first assessment of the impact of DAA drug levels on efficacy of shortened therapy. The inactive SOF metabolite GS-331007 is the main circulating metabolite of SOF prior to undergoing renal excretion, and it is frequently used to describe SOF's pharmacokinetics[306]. We hypothesized that accumulation and slow elimination of GS-331007 and DCV in vivo might protect against the re-emergence of HCV viraemia. However we found no evidence of a difference in AUC_{last} between 4-week cures and 4-week treatment failures for SOF or DCV. Total exposure to GS-331007 was higher in treatment failures (3,920 (2,400-5,140) vs 3,050 (2,190-3,670) (p=0.023). This was a surprising result, given

that SOF and GS-331007 AUCs are near dose proportional over the dose range of 200 mg to 1200 mg[306], and higher day 10 concentrations of GS-331007 have been associated with improved rates of cure with SOF/ribavirin treatment[307]. Further PK studies are warranted to better understand if SOF metabolism impacts treatment outcomes.

Limitations

This study has important limitations. Firstly it was powered to determine overall cure rate with 4- and 8- weeks treatment, rather than outcomes with each duration. It is possible that we would have seen patients not achieve SVR with 8 weeks therapy in a larger sample, and our cure estimates may therefore be imprecise. Secondly, the participating cohort did not include any individuals with HIV, Hepatitis B-co-infection or renal impairment and only 4 participants reported a history of injecting drug use, of which none were currently injecting. These populations are known to have an altered immunological response and constitute an important part of the HCV epidemic. Thirdly, in order to identify the timing of failure, the protocol required a visit schedule with many more visits than is standard of care, which many patients would not be able to follow. Consequently, adherence was very high, which may not reflect real world practice.

Another potential limitation relates to our use of two different HCV RNA platforms which have previously been shown to give discrepant results in the same individuals[308]. The Abbott Architect® has a lower LLOQ than the COBAS AmpliPrep®/COBAS TaqMan® and may detect HCV RNA for longer on therapy than the COBAS[293], though we found no evidence of difference in viral decline by platform. With regards to the PK analysis our non-compartmental analysis of drug levels may not adequately account for drug accumulation of sofosbuvir's metabolite GS-331007 and DCV between day 0 and 28, which was observed (see appendix B for more detail).

In summary our findings indicate that shortened SOF/DCV therapy cures a significant proportion of patients with mild liver disease without compromising retreatment with the same drug combination in those who do not achieve SVR with first-line therapy. This study adds to a growing case for shortening SOF/DCV therapy in individuals with mild liver disease from 12 to 8 weeks, and offering retreatment with 12 weeks SOF/DCV when

required. Given the small sample size and high exclusion rate in study screening, this evidence is not, on its own, guideline changing. However, 8 weeks SOF/DCV treatment does now warrant further evaluation (see chapter 6).

I found no evidence that relatively high numbers of putative resistance associated substitutions at baseline were associated with treatment outcomes, suggesting routine sequencing at baseline or prior to retreatment remains unnecessary. I also found no evidence that drug levels affect virological response or influence treatment outcome. Further work is required to understand which factors can reliably predict cure with ultra-short DAA treatment.

Chapter 4

Efficacy of 12 weeks sofosbuvir and daclatasvir therapy for HCV genotype 6-infected adults with advanced liver fibrosis

Background

This chapter concerns the efficacy of SOF/DCV in treating HCV genotype 6 infection in patients with advanced liver fibrosis. As shown in chapter 1, table 2, SOF/DCV is one of just three pangenotypic regimens recommended by WHO for first-line treatment of adults with HCV[16], (the others being the NS5B/NS5A combination SOF/VEL and the PI/NS5A combination glecaprevir/pibrentasvir). As the lowest priced option, with the most generic manufacturers worldwide[309], SOF/DCV has become the most widely available treatment. SOF/DCV can now be procured through voluntary licences in some LMICs for as little as US$60 per treatment course[309].

WHO recommends SOF/DCV for 12 weeks in individuals with mild liver disease, for all HCV genotypes and for 24 weeks in individuals with liver cirrhosis, with the caveat that 12 weeks *'may be considered in countries where genotype distribution is known and Genotype 3 prevalence is <5%'*[16]. While high quality clinical trial evidence exists for SOF/DCV in Genotypes 1-4[138,310–314] , there is little data on the outcomes of DAA treatment in those with genotype 5 or 6 infection, particularly those with cirrhosis. This is acknowledged in WHO Guidelines which advocate for more research into rare genotypes and subtypes[16].

Genotype 6 accounts for around 5% of HCV infections globally but is a dominant genotype in parts of Asia[63,315], including Vietnam, where it is responsible for around a third of infections nationally, with higher prevalence reported in HCMC[63,73,187]. With 29 confirmed subtypes (6a to 6xf) and 21 unassigned subtypes, it is the most genetically diverse lineage[149], raising concerns about the potential for naturally occurring resistant variants that may impact treatment outcome[150].

Observational real-world studies suggest rates of cure of genotype 6 infection with SOF/DCV are high (>90%)[316–320] but may be lower than for genotype 1 or genotype 2 infection, particularly in individuals with cirrhosis.[317,318] Consequently, up until 2020, the Vietnam national guidelines[318] and the Asia Pacific guidelines[321] diverged from WHO, in recommending that ribavirin should be added to the 12-week SOF/DCV regimen in genotype 6-infected individuals with cirrhosis, or, where ribavirin is contraindicated, 24 weeks of SOF/DCV should be given. This is in contrast to sofosbuvir/velpatasvir (SOF/VEL), the other WHO-approved DAA regimen available through voluntary licences in LMICs, which is recommended for 12 weeks only, without ribavirin, in all guidelines[16,318,321]. Given the high prevalence of genotype 6-infected patients with liver cirrhosis in Vietnam, these recommendations had a profound impact of drug procurement, with the Vietnamese MoH opting to prioritise SOF/VEL over SOF/DCV. Consequently SOF/DCV prices in Vietnam remain some of the highest in the world[305].

Table 12: International treatment guidelines in 2018

	SOF/DCV			**SOF/VEL**
Genotype 6	**WHO 2018**[16]	**Vietnam MOH 2016**[318]	**APASL 2016**[321]	All guidelines
Mild Disease	12 weeks	12 weeks	12 weeks	12 weeks
Compensated cirrhosis	24 weeks*	24 weeks OR 12 weeks + RBV	24 weeks OR 12 weeks + RBV	12 weeks
Retreatment	16-24 weeks**	No specific recommendation	No specific recommendation	16-24 weeks**

**May be considered in countries where genotype distribution is known and genotype 3 prevalence is <5%, based on assumption that in a population of persons with cirrhosis where 5% of persons would be infected with genotype 3 HCV, the SVR would be 80% in the 5% infected with genotype 3 and 93% in the 95% infected with other genotypes, leading to an overall SVR rate of (0.05x0.80)+(0.93x0.95) = 92%.*

***Only recommended in the absence of glecaprevir/pibrentasvir or SOF/VEL/VOX, (based on expert consultation).*

Response-guided Therapy

In stratum A of the SEARCH study, described in chapter 3, I evaluated the efficacy of ultra-short, day 2-response-guided SOF/DCV therapy in patients with mild disease. Since liver fibrosis is a well-recognised negative predictor of treatment response, shortening treatment to less than 12 weeks in is not recommended for patients with liver cirrhosis, irrespective of early virological response. Patients with cirrhosis treated with less than 12 weeks of SOF/DCV would be expected to have cure rates <90% in non-genotype 3 infections[139,322,323] and <80% in genotype 3 infections[314]. However, there may still be a role for response guided therapy in determining which patients with cirrhosis could benefit from extension of treatment beyond 12 weeks (i.e. extending therapy to improve moderate rates of SVR rather than shortening therapy to maintain high rates of SVR). This concept was used in the PEGIFN era in genotype 1 infections, where improved cure rates were achieved by extending PEG-IFNα and ribavirin therapy to 72 weeks in patients who demonstrated slower virological responses[110]. In our meta-analysis of treatment optimisation strategies that attempted to improve rates of SVR in the presence of negative predictors of response, we found high pooled SVR rates (95.1-98%) could be achieved in the presence of cirrhosis and/or prior treatment failure, compared with other negative predictors of response such as genotype 3 infection[172]. In subgroup analyses we found no evidence that addition of ribavirin offers any advantage in improving SVR in patients with negative predictors of response (p=0.243).

Stratum B of the SEARCH study addresses whether high rates of cure can be achieved in individuals with genotype 6 HCV-related liver fibrosis, when on-treatment virological response, is used to determine whether they receive 12 or 24 weeks of SOF/DCV (without need for ribavirin).

Objectives

1. Test the hypothesis that high rates of cure can be achieved in genotype 6 infected adults with advanced liver fibrosis by using delayed suppression of HCV RNA by

day 14 of SOF/DCV treatment (>500IU/ml) to identify individuals who should be treated for longer duration (24 weeks).

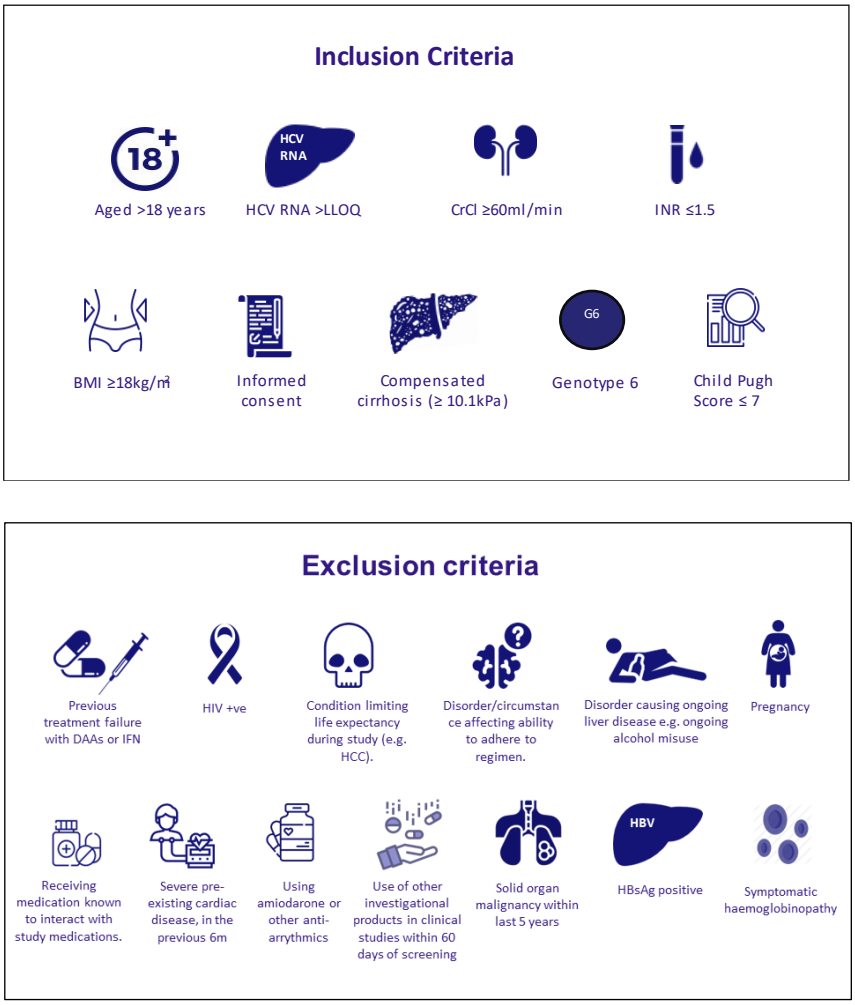
2. Explore whether genotype 6 subtypes and associated resistance-associated substitutions impact treatment outcomes with shortened therapy.

Methods

Study population

As for SEARCH-1 stratum A (chapter 3), participants were recruited from the outpatient clinic of the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam between February 2019 and June 2020. Eligible patients were 18 years or older, had chronic infection with HCV genotype 6 and severe fibrosis or compensated cirrhosis. Severe fibrosis was defined as a FibroScan® score ≥10.1kPa, which has been shown to correlate to histopathological Metavir score ≥F3[324], or a biopsy result consistent with cirrhosis (Ishak score >=5/6 or equivalent). Compensation was defined as Child-Pugh Score ≤7. Participants were required to be HCV-treatment naïve, have a BMI ≥18kg/m^2, a creatinine clearance ≥60ml/min, and no evidence of HIV or Hepatitis B coinfection, or solid organ malignancy in the preceding 5 years. As for stratum A, patients referred to the trial were initially enrolled into an observational study which included FibroScan® assessment and genotyping. Eligible individuals from this cohort were invited for additional screening. Full eligibility criteria are provided in the protocol (available at https://doi.org/10.1186/ISRCTN17100273) and summarised in figure 4-1.

Figure 4-1: SEARCH stratum B Eligibility Criteria



Study design

Participants were treated with sofosbuvir 400mg and daclatasvir 60mg, administered orally as two separate tablets, once daily, with no dose adjustment. Study drugs were procured through Pharco Pharmaceuticals, Egypt.

HCV viral load was measured at baseline and at all subsequent follow up visits. Day 14 viral load was used to guide duration of therapy: participants with viral load <500 IU/ml at day 14 were treated with 12 weeks of SOF/DCV and those with HCV RNA ≥500 IU/ml were to

continue treatment for 24 weeks. The timepoint and viral load threshold were adapted from a study by Yakoot et al, which used an undetectable viral load at day 14 to determine whether genotype 4-infected patients with mild liver disease should receive 8 or 12 weeks of treatment[175]. In that study 48/60 (80%) individuals had HCV RNA < LLOQ at day 14. Since our study concerned patients with liver fibrosis, known to experience slower virological decline on therapy[156], we used a threshold of 500IU/ml at day 14 (the same as that used at day 2 in the treatment shortening study by Lau et al discussed in chapter 3)[173].

Clinic visits were scheduled on day 0, 14, 28, and monthly thereafter until end of treatment, for 12 or 24 weeks depending on day 14 viral load. End of treatment (EOT) follow up visits were scheduled for 4, 8 and 12 weeks after the last tablet for all participants. Unlike in stratum A, no in-study retreatment was proposed for those experiencing treatment failure.

Endpoints

The primary endpoint was sustained virological response (SVR12) defined as plasma HCV RNA <LLOQ 12 weeks after the end of treatment without prior failure. Failure of first-line treatment was defined by the same criteria used in stratum A: i) two consecutive measurements of HCV RNA > LLOQ (taken at least one week apart) after two consecutive visits with HCV RNA <LLOQ, at any time during follow up, with the latter confirmatory measurement also being >2000 IU/ml or ii) two consecutive measurements of HCV RNA that are >1 log10 above the nadir on treatment and >2000 IU/ml, at any time.

Secondary endpoints were lack of initial virological response, serious adverse events, grade 3/4 clinical adverse events (AEs), adverse events of any grade leading to change in treatment, and adverse reactions (AR).

Sample size justification

We set a target cure rate of ≥90%, with an unacceptably low cure rate of 70%. Assuming 90% power and one-sided alpha=0.05, 37 participants were required to exclude the null hypothesis that cure <90%. Assuming 5% loss to follow-up, and that, based on pharmacokinetic

modelling[161], 90% would achieve on-treatment response and receive 12 weeks (rather than 24 weeks) of therapy, the final target population was 43 participants.

Study assessments

HCV RNA was measured using molecular platforms locally. At start of study (for the first 26 participants enrolled), this was the Abbott Architect® (lower limit of quantification (LLOQ) 12 IU/ml). This was subsequently replaced by the COBAS AmpliPrep®/COBAS TaqMan® HCV Quantitative Test, version 2.0 Roche Molecular Systems, (LLOQ 15 IU/ml). HCV genotype and subtype were determined using NS5B, Core, 5' UTR sequencing , according to a method described by Chau et.al[13] (see appendix B). Assessments during treatment included physical examination, standard laboratory testing and serum HCV RNA. Adverse events were recorded and graded according to a standardised scale[277].

Whole genome sequencing (WGS) of the target regions of DCV (NS5A) and SOF, (NS5B), was performed for all patients at baseline, with a plan to also assess this at time of detection of any treatment failures. As in stratum A, for DCV we reported the following RAS (according to European Association for the Study of the Liver (EASL) guidelines for genotype 6[325,326]): Q24H, F/L28A/I/L/M/T/V, R30E/H/N/S, L31I/M/V, P32A/L/S/Q/R/S, T58A/G/H/N/S, E92T and T93A/H/N/S. For SOF we reported S282G/R/T.

WGS of the HCV viral genome was attained using Illumina MySeq platform as described previously[278,327]. The sequence reads were aligned with HCV genotype 6 reference sequence (GenBank accession no Y12083) and NS5A (sequence position 6,212 to 7,567) and NS5B (sequence position 7,568 to 9,340) region was analysed. We reported resistance-associated variants present in more than 1% of sequence reads and analysed the sequence for clinically relevant, in-vitro, primary and secondary drug resistance mutations.

Statistical analysis

Analyses were performed under intention-to-treat; a per-protocol analysis included all participants taking 90-110% of prescribed treatment. Proportions and 95% confidence intervals (CIs) were estimated from the marginal effects after logistic regression where

possible, and calculating proportion with binomial exact 97.5% CIs where not. Absolute HCV VL was analysed using interval regression adjusting for baseline HCV VL. The proportion of participants with undetectable HCV VL at each visit were analysed using binomial exact 95% CIs. Analyses were performed using Stata v16.1, primarily by Leanne McCabe (see thesis declaration).

Results

Baseline characteristics

Of 455 patients screened, 41 were enrolled (figure 4-2). Recruitment was completed short of the initial target of 43 because of COVID-19 related restrictions in Vietnam from February 2020. Baseline and clinical characteristics are described in detail in Table 12. All participants had severe fibrosis or compensated cirrhosis, with a median FibroScan score of 17.3kPa. 95% had the minimum Child-Pugh score. There was a high prevalence of hypertension (44%) and diabetes (20%). Only 2 participants had a history of alcohol dependence and no participants reported illicit drug use. The most prevalent subtype was 6a (51%), followed by 6e (34%). Subtypes 6h, 6k, 6l and 6o were also represented in 1 or 2 participants each.

Treatment duration, adherence to DAA regimen and efficacy outcomes

By day 14, all 41 participants (100%) had a supressed HCV viral load below the threshold of 500 IU/ml (Table 2), meaning everyone in the study was to receive 12 weeks of treatment. 98% participants completed the full prescribed course of SOF/DCV. One participant missed three doses of SOF/DCV in total but intention to treat and per protocol analysis were identical. Eight participants (20%) missed at least one visit. All 41 participants (100% [97.55% CI 91.4; 100]) achieved SVR12.

Figure 4-2: Screening and Enrolment

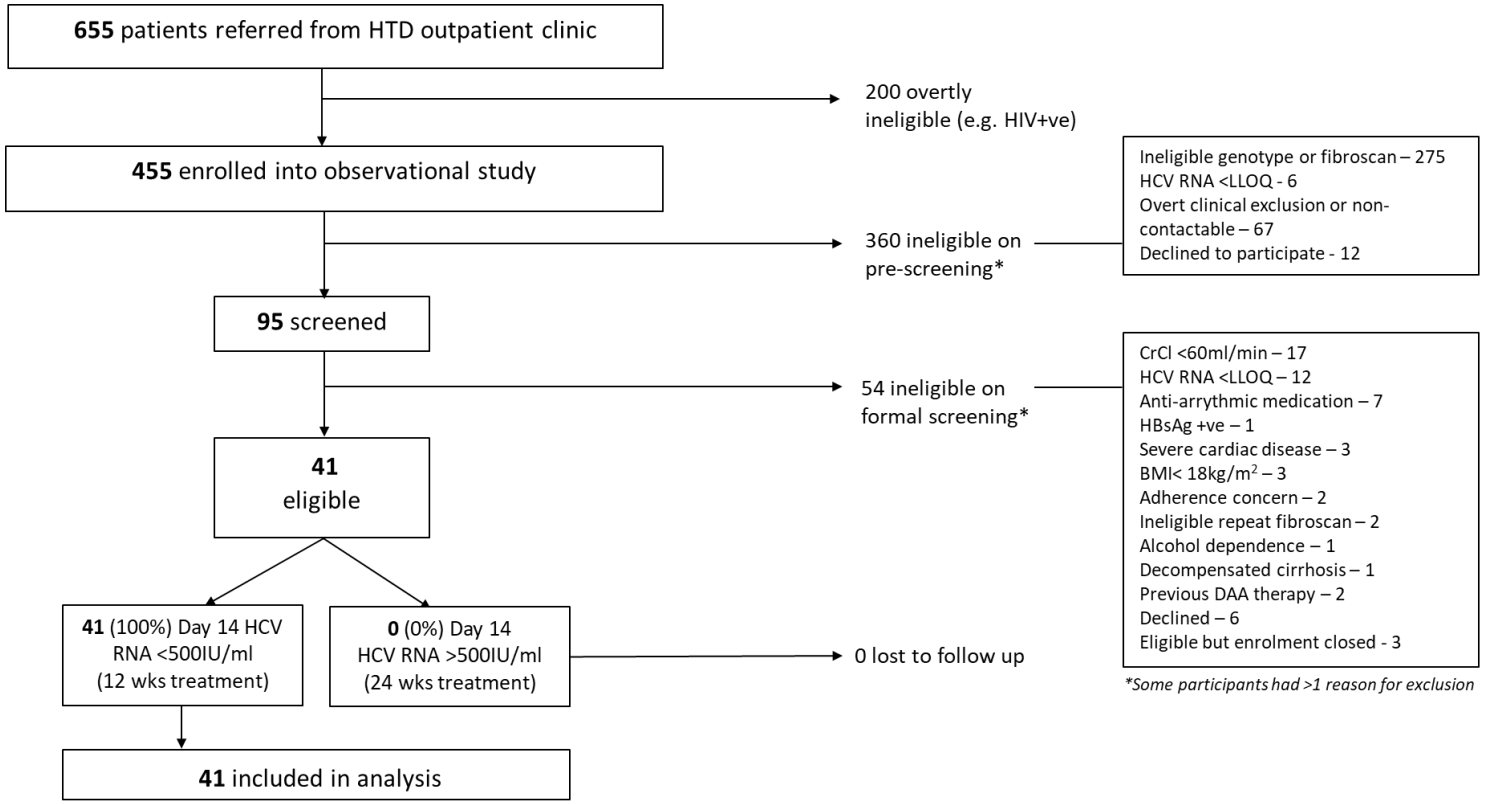


Table 13: Baseline characteristics

Total participants	41	
Age in years	62	(42 – 72)
Female	29	(71%)
Body-mass index in kg/m^2	25.3	(19.5 – 36.5)
Subtype:		
6a	21	(51%)
6e	14	(34%)
6h	1	(2%)
6k	1	(2%)
6l	2	(5%)
6o	2	(5%)
Median FibroScan result (kPa)	17.3	(10.1 – 49.6)
Severe fibrosis by AASLD* criteria[135] (10.1 – 12.5kPa)	10	(24%)
Cirrhosis by AASLD criteria (≥12.5kPa)	31	(76%)
Child-Pugh score		
5	39	(95%)
6	1	(2%)
7	1	(2%)
Baseline HCV viral load in IU/ml	1,030,000	(6258 – 17, 516,779)
HCV viral load – log10 IU/ml (range)	6.0	(3.8 – 7.2)
Previous spontaneous clearance of HCV with re-infection	6	(15%)
Past medical history:		
Illicit drug use	0	(0%)
Alcohol dependence (historic; current excluded)	2	(5%)
Diabetes	8	(20%)
Hypertension	18	(44%)
Ischaemic heart disease	1	(2%)
Stroke	1	(2%)
Malignancy	1	(2%)
Smoking (past, current)	1, 8	(2%, 20%)
Depression	0	(0%)
Chronic obstructive pulmonary disease	0	(0%)
Tuberculosis	0	(0%)
Liver & kidney function:		
ALT in IU/L (range)	61	(19 -216)
AST in IU/L (range)	56	(25 – 200)
ALP in IU/L (range)	91	(64 – 249)
Albumin umol/L (range)	41.6	(30.2 – 47.9)
Bilirubin umol/L (range)	11.5	(5.6 – 34.3)
Creatinine Clearance ml/min (range)	76.2	(60.0 – 176.9)
Platelets K/uL (range)	156	(71 – 282)
INR (range)	1	(1-1.3)

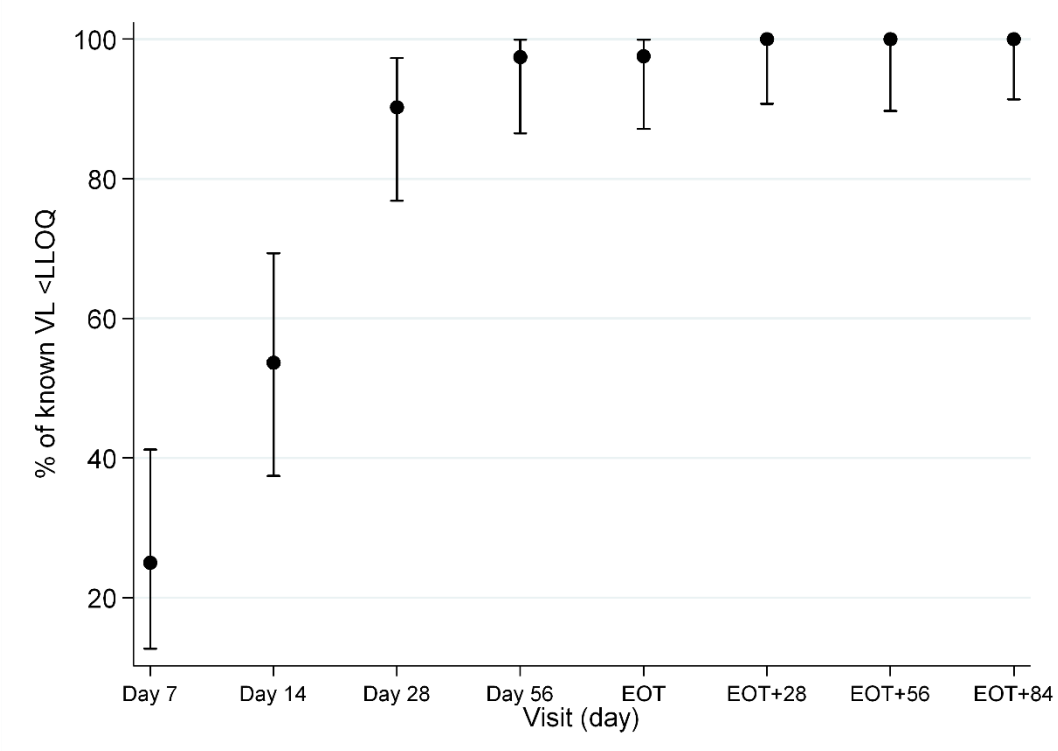
N (%) or median (range) presented.

**AASLD – American Association for the Study of Liver Diseases*

Viral Kinetics

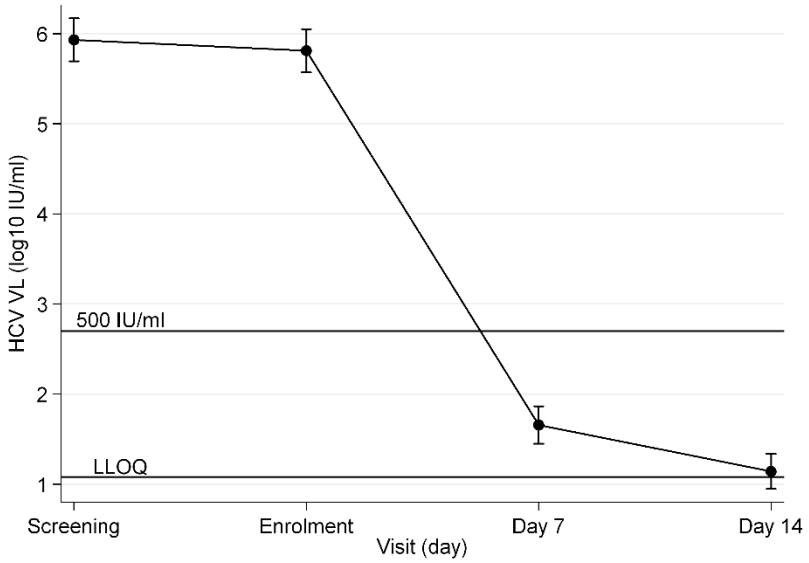
Viral clearance was rapid and no patients showed a lack of initial virological response (97.5% CI 0%, 9%). By day 7, 25% (10/40) had HCV RNA <LLOQ, rising to 54% (22/41) by day 14, 90% at 4 weeks, 97% at 8 weeks and 98% at end of treatment (figure 4-3). Mean (95% CI) HCV viral load log10 fell from 5.93 (5.69, 6.17) at baseline to 1.20 (1.02, 1.38) at day 14 (figure 4-4). At end of treatment, one participant had a detectable viral load (HCV RNA 26 IU/ml, Abbott Architect®) and 2 had detectable virus <LLOQ. All viral loads measured after end of treatment follow up were <LLOQ. All viral loads measured after end of treatment were undetectable except in one participant who had detectable HCV RNA <LLOQ at 4 and 12 weeks after end of treatment, and one who had detectable HCV RNA <LLOQ 8 weeks after end of treatment.

Figure 4-3: Proportion of participants with HCV viral load (VL) < LLOQ).



EOT, end of treatment.

Figure 4-4: Mean log10 hepatitis C virus (HCV) viral load (VL) from day 0 to day 14.



Mean log10 HCV VL at each visit: Screening 5.93 (95% confidence interval [95% CI], 5.69–6.17); Enrolment 5.81 (95% CI, 5.57–6.05); Day 7 1.75 (95% CI, 1.57–1.93); Day 14 1.20 (95% CI, 1.02–1.38).

Table 14: treatment outcome

Detectable HCV viral load (HCV VL) at day 14	19	(46%)
Median (IQR) HCV VL at day 14 in IU/ml	42	(28, 96)
Below threshold for extended (24 weeks) therapy	41	(100%)
Above threshold for extended (24 weeks) therapy	0	(0%)
Mean (SD) duration of therapy received in days	84	(0.4)
Median weeks from enrolment to last visit (range)	24	(23-25)
Primary Outcome		
Sustained viral response 12 weeks after end of treatment	41	(100%; 91%, 100%)
Secondary Endpoints		
Lack of initial virological response	0	(0%; 0%, 9%)
Serious adverse events	1	(2%; 0.1%, 13%)*
Grade 3/4 clinical adverse events	1	(2%; 0.1%, 13%)*
Non-serious adverse reactions	20	(49%; 33%, 64%)*
Adverse events or reactions leading to change in study medication	0	(0%; 0, 9%)

Where not labelled, data presented as n (%; 97.5% confidence interval)
**95% confidence intervals*

Viral genomics

Whole genome viral sequencing of baseline samples revealed seven distinct RAS to DCV (Table 14). The R30S was detected in 100% (14/14) of 6e and the F/L28V RAS were detected in 100% of 6h (1/1), 6k (1/1), 6l(2/2). These RAS almost certainly represent the wild type (WT) amino acids in these genotypes, but may still provide some resistance to DCV. The numbers of 6h, 6k and 6I sequences are low here but when the available sequences on HCV GLUE[328,329] are analysed (8 sequences for 6h, 1 for 6k and 11 for 6I) F/L28V is present in 100% of sequences.

No clinically relevant SOF RAS were detected but the L159F substitution was detected in one 6a sequence. This substitution has been linked to treatment failure (Table 8). Given there were no treatment failures it was not possible to assess the role of these mutations in treatment response. In the four participants who exhibited slower virological response, with HCV RNA persisting >LLOQ by day 28, one was infected with 6e and 3 had 6a. We detected F/L28V in one of the 6a genomes but the remainder had no apparent RAS.

Table 15: Prevalence of genotype 6 RAS in cohort

Subtype	n	Daclatasvir RAS detected (n)	Sofosbuvir RAS detected (n)
6a	21	F/L28V(3); L31M(1)	L159F**(1)
6e	14	F/L28V(7); L28M(5); R30S(14)*; L31I(1); T93S (1)	None Detected
6h	1	F/L28V*(1)	None Detected
6k	1	F/L28V*(1)	None Detected
6l	2	F/L28V*(2);	None Detected
6o	2	F/L28V(1); T58A(1) T93S (1)	None Detected

* RAS is considered WT for this genotype.

** Not considered clinically relevant RAS for genotype 6 but has been shown to be treatment emergent.

(RAS Definitions from EASL Guidelines 2020[134])

Safety

SOF/DCV was well-tolerated and no participants discontinued treatment due to drug side effects. 20 participants (49%; 95% CI 33%, 64%) reported at least one non-serious adverse reaction consistent with those described in the summary of product characteristics (SmPC) for SOF/DCV[169]. One participant with type 2 diabetes developed symptomatic hypoglycaemia, which is a known consequence of DAA therapy due to improved glucose control. There was one serious adverse event, a new diagnosis of diffuse large B cell lymphoma, which was diagnosed after a new splenic mass was identified on an end-of-study routine ultrasound scan. There were no deaths.

Discussion

Pangenotypic DAA combinations are not equally efficacious for all HCV genotypes[138,314] or subtypes[271,330] and have lower rates of cure in patients with cirrhosis[16]. This study provides the most detailed dataset to date for the treatment of genotype 6 HCV with SOF/DCV in patients with severe fibrosis or cirrhosis.

Initial virological response was more rapid than has been observed in other study populations with cirrhosis[159,331,332], with an almost 5-log drop in mean viral load after 7 days of therapy. Previous studies in HCV kinetics have shown a biphasic viral decline in response to DAA therapy, characterised by a rapid 3-4 log viral decline from 12-48h, followed by two slower phases from day two onwards[333]. Slower viral clearance rates from blood have been associated with Child Pugh scores ≥7, and liver stiffness measurements ≥21 kPa[332]. This phenomenon is variously thought to reflect impaired drug delivery in the context of reduced hepatic blood flow and portal systemic shunting in advanced liver disease, altered drug metabolism, and/or a compromised immune response. Participants in this study were well-compensated (95% Child Pugh score = 5) with a median liver stiffness of 17.3kPa which likely explains the rapid virological responses observed.

The high cure rate observed is consistent with real-world studies[317,318]. In the largest published cohort of genotype 6-infected individuals treated with SOF/DCV, from Phnom Penh in Cambodia, among 1292 patients with a mixture of mild disease and cirrhosis, 95.9% (95% CI [94.7; 96.9]) of patients with a known treatment outcome achieved SVR12 with 12 weeks SOF/DCV (without ribavirin)[317]. A separate analysis from the same study, restricted to patients with compensated cirrhosis (59.7% genotype 6), found a 98.1% cure rate (95% CI [97.5; 98.6]) with this regimen. In Vietnam, 1111/1148 (96.8%) of individuals with genotype 6 infection treated with either SOF/DCV or SOF/ledipasvir achieved SVR with 12 weeks of therapy. Liver fibrosis did not influence outcome.

The added evidence from this study is relevant to current treatment guidelines. Firstly, in most LMICs, SOF/DCV is cheaper than SOF/VEL and easier to procure. There are five WHO prequalified generic suppliers for SOF and three for DCV, contrasting with just one supplier of the SOF/VEL combination[309]. Three DCV products have been registered in Vietnam since the fourth quarter of 2019, which may lead to further decline in DCV price in the future[309]. Data showing high cure rates after 12 weeks treatment duration will promote its use.

Secondly, ribavirin has a problematic side effect profile. It is teratogenic and causes anaemia, so that patients taking it must be monitored for signs of toxicity. International guidelines recommend non-ribavirin based treatment where possible[16,325] and data supporting its obsolescence in the treatment of HCV will be welcomed.

Thirdly, 12 weeks of DAA treatment is cheaper than 24 weeks. In Vietnam DAAs remain expensive, with a 12-week course of SOF/DCV currently priced at $1347[309]. When the additional laboratory tests and healthcare visits involved in long course therapy are factored in, savings are likely to be substantial[309].

Finally, given that WHO recommends that 12 weeks of SOF/DCV treatment “may be considered for patients with cirrhosis, in countries where genotype distribution is known and genotype 3 prevalence is known to be <5%”[16], this data could negate the need for costly genotyping altogether in countries such as Vietnam, where genotype 3 prevalence is around 0.5%[187]. As well as driving down the cost of treatment, this would also help decentralise hepatitis care, ending the reliance on well-resourced laboratories that offer genotyping.

In the absence of slow treatment responders or treatment failures, it is not possible to draw conclusions from this study regarding a role for response-guided therapy. However, while the cost of viral load testing (currently $20-50 in Vietnam) remains well below the cost of drugs, RGT has the potential to provide huge financial savings and appears worth pursuing.

RAS are not well understood in HCV genotype 6, particularly for non-6a subtypes. Six different genotype 6 subtypes were represented in this study (6a, e, h, k, l, o). Most RAS are reported with reference to RAS in other HCV genotypes. We found that several polymorphisms previously reported as RAS for genotype 6 as a whole that are actually WT in some genotype 6 subtypes and their contribution to resistance remains unclear. It has been shown that certain understudied subtypes such as 4r and 3b are inherently resistant to NS5A inhibitors and in-vitro studies have shown that certain genotype 6 subtypes may be inherently resistant to NS5A inhibitors[150]. There was no obvious contribution of RAS to treatment outcomes in this study. However, further clinical studies may be needed to elucidate the role of RAS in genotype 6 infections, especially in the context of other negative predictors of treatment response, such as decompensated cirrhosis[172].

In terms of safety, SOF/DCV was well-tolerated. No patients stopped treatment on account of drug side effects. However, non-severe adverse reactions typical of those described in SOF/DCV SmPC, such as dizziness and gastritis, were reported by nearly half the study population. One participant had a serious adverse event: a new splenic mass detected by ultrasound at end of study, which was ultimately diagnosed as B-cell lymphoma. B-cell non-Hodgkin's lymphoma is a typical extrahepatic manifestation of chronic HCV, and emergence after HCV eradication with DAA therapy has also been described[334]. Three participants were noted to have low levels of detectable virus at end of treatment and all achieved SVR12. In clinical practice, routine testing for HCV RNA at end of treatment is not recommended but this supports recent data showing treatment prolongation is not indicated in this scenario[158,302].

Limitations

Our study has important limitations. Stringent eligibility criteria meant that only 6% of patients screened were enrolled, which could affect the generalisability of our results. The participating study population did not include any people who inject drugs, or individuals

with HIV, Hepatitis B co-infection, or renal impairment. These are important groups which characterise the global HCV population. Adherence was very high and may not reflect real-world practice.

Furthermore, only 31 participants had cirrhosis by AASLD criteria[135]. We did not include patients with decompensated cirrhosis and all but two of the individuals with cirrhosis had the minimum Child- Pugh score. Treatment outcomes in individuals with decompensated cirrhosis are inferior to those in seen in compensated disease[317,318]. In Cambodia, only 66.3% (95% CI [60.2, 72.0] of patients with decompensated cirrhosis who initiated SOF/DCV treatment achieved SVR12, despite 24 weeks of treatment.

Conclusions

Overall, given that this study population is typical of the population attending the outpatient clinic at a large tertiary referral hospital, in terms of age, comorbidities and viral genotypes, and that all participants were confirmed to have severe liver fibrosis (with a median FibroScan score of 17.3kPa), our findings are likely generalizable to genotype 6-infected individuals with compensated cirrhosis. The optimum treatment duration of SOF/DCV in decompensated disease remains uncertain.

In summary this study suggests that excellent outcomes are achievable with 12 weeks of SOF/DCV in the treatment of HCV genotype 6 in individuals with compensated cirrhosis. Further research is required to further assess the utility of early virological response in shortening therapy, and the role of genotype 6-associated RAS in treatment response.

Chapter 5

Performance analysis of post-treatment changes in liver transaminases in detecting HCV treatment failure

Background

To confirm cure from Hepatitis C virus (HCV) infection, the World Health Organisation (WHO) recommends qualitative or quantitative nucleic acid testing for HCV RNA 12-24 weeks after direct acting antiviral (DAA) therapy to confirm sustained virological response (SVR)[16]. This has been shown to be a durable measure of cure[335], associated with significant reduction in all-cause mortality[336–338]. However, nucleic acid testing is often expensive, particularly in resource-limited settings which shoulder the highest burdens of disease. For example, in Vietnam, public sector HCV RNA testing is currently priced at US$37-90[188] per test. While the price of generic DAA drugs is falling[305], the high cost of lab investigations continues to make HCV treatment prohibitively expensive for many of those infected[339].

Nucleic acid testing also involves significant technical expertise, requiring that samples are transported across the country to specialised laboratories. This impedes scale up and decentralisation of HCV care. In updated guidelines published in 2022, WHO calls for more efficient and simplified hepatitis diagnostics that could benefit marginalized populations, such as persons who inject drugs, and communities under-served by existing models of care with high rates of loss to follow-up[17]. Alternative surrogate biomarkers merit evaluation.

The liver enzymes alanine aminotransferase (ALT) and aspartate transferase (AST), measurable in blood, are non-specific markers of liver inflammation which are simple and relatively cheap to test in local facilities. Elevated pre-treatment ALT levels have been associated with slower virological response[298] and pre-treatment levels of both enzymes have been associated with failure to achieve SVR[340], though neither are reliably predictive of treatment outcome. Levels of both enzymes decline on therapy[102,341,342]and, in the pre-DAA era, a ‘sustained biochemical response’ was used as a surrogate for SVR[102,342]. Elevated ALT

levels (greater than upper limit of normal) at EOT and EOT+12 weeks have also been associated with DAA treatment failure[343,344]. In one study, patients were 14% less likely to achieve SVR12 for each one unit increase in ALT at end of treatment versus baseline[343]. I found no published data evaluating how changes in ALT and AST after EOT relate to DAA outcomes or their sensitivity for detecting treatment failure.

Experimental treatment-shortening trials, such as SEARCH stratum A described in chapter 3, have reported cure rates <80%. This relatively high rate of treatment failure provides an opportunity to compare biomarker responses in individuals who achieve SVR versus those that do not. In this chapter I describe changes in ALT and AST observed after 4 or 8 weeks SOF/DCV therapy in SEARCH stratum A. Based on my findings, in a separate population from a larger UK-based short course therapy trial (n=202) I test the hypothesis that any elevation in ALT or AST between EOT and EOT12 is a sensitive marker of treatment failure.

Objectives

1. Describe changes in ALT (ΔALT) and AST (ΔAST) from EOT to EOT+12 weeks in patients that achieve SVR with 4-8 weeks SOF/DCV therapy compared with those experiencing virological rebound (treatment failure).
2. Compare ALT and AST levels at baseline, EOT and change from baseline to EOT in patients who cure versus those who experience treatment failure
3. Evaluate sensitivity, specificity, positive predictive value and negative predictive value of a change in ALT > 0 IU/L and a change in AST > 0 IU/L versus gold standard HCV RNA testing at EOT+12 weeks.
4. Replicate the analysis in a larger, independent study population with different genotypes, patient demographics and DAA therapy.

Methods

Since this work is a retrospective diagnostic accuracy study, I have reported according to STARD 2015 essential items checklist[345], which is provided in appendix C. STARD was initially conceived in 2003 with an objective of improving the completeness and transparency of reporting of studies of diagnostic accuracy. It allows readers to assess the potential for bias in the study (internal validity) and to evaluate its generalisability (external validity). It was last updated in 2015 and is a publishing requirement of all major journals.

SEARCH Stratum A

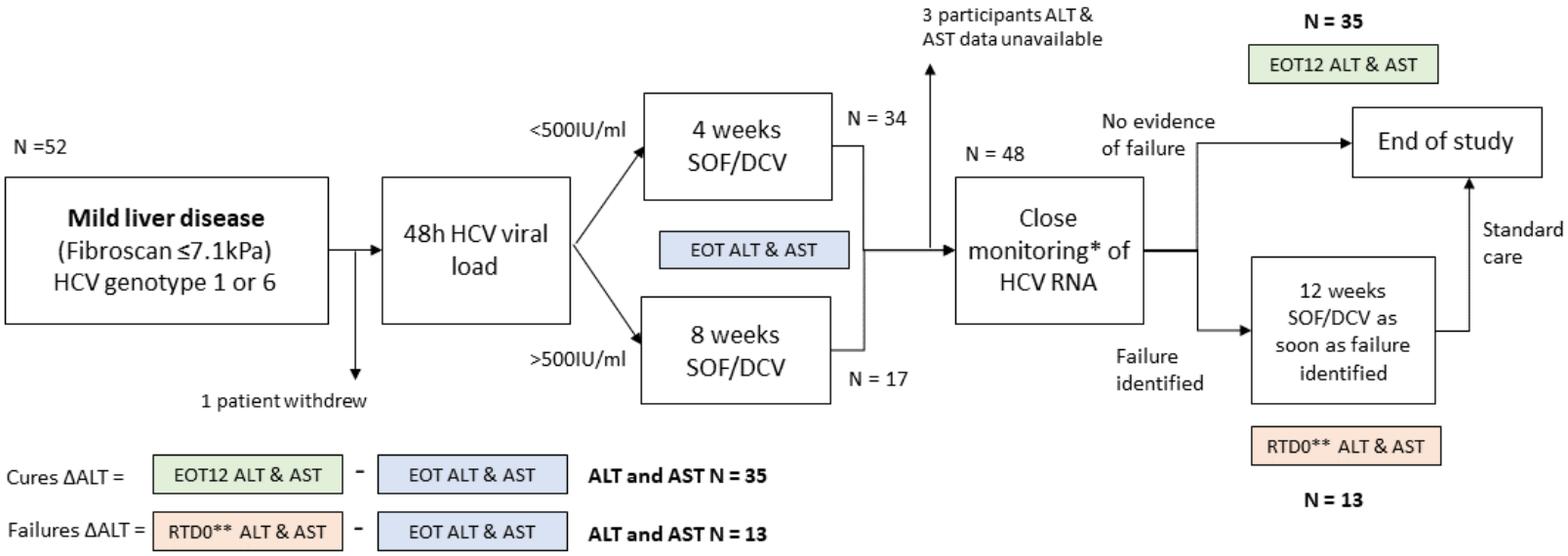
SEARCH Stratum A is described in detail in chapter 3. In summary, genotype 1- or 6-infected adults with mild liver disease (FibroScan score ≤7.1kPa) received 4 or 8 weeks of SOF/DCV therapy according whether HCV RNA was below or above 500 IU/ml after two days treatment. HCV RNA was measured at regular intervals until end of follow up (EOT+12 weeks) or until treatment failure if it occurred first (figure 5-1). Of 52 adults recruited, 34 received 4 weeks SOF/DCV, 17 received 8 weeks, and one withdrew. SVR12 was achieved in 38/51 (75%). 13 (25%) experienced virological relapse (between 21 and 84 days after EOT) and commenced retreatment within 2 weeks. All 13 participants who did not achieve SVR were determined to have virological relapse rather than reinfection on whole genome sequencing.

ALT and AST were measured at baseline and at EOT in all participants, at start of retreatment in those with virological relapse, and at EOT+12 in those without evidence of treatment failure. I analysed change in ALT and AST from EOT to EOT+12 (ΔALT and ΔAST) in participants who cured, and from EOT to retreatment day zero (RTD0) in participants who did not achieve SVR. I chose to exclude patients with ALT or AST greater than twice the upper limit of normal (>2xULN) at EOT on the basis that this would ordinarily prompt HCV RNA testing[145]. I calculated median ΔALT and ΔAST (and interquartile ranges) in patients according to whether their treatment was successful or unsuccessful, and used Wilcoxon's Rank Sum test to compare outcomes. I also compared enzyme levels at baseline, EOT and decline on treatment, and performed genotype-specific analysis. I evaluated sensitivity and

specificity of any increase in ALT (ΔALT >0 IU/L) or any increase in AST (ΔAST>0 IU/L) compared to gold standard of HCV RNA >LLOQ at EOT+12 (or nearest available timepoint).

On the basis of results from this analysis I proceeded to evaluate the performance of an increase in ALT and AST after EOT in detecting treatment failure in a second study population.

Figure 5-1: SEARCH Stratum A flow diagram



Of 52 adults enrolled, 34 received 4 weeks SOF/DCV, 17 received 8 weeks and one withdrew. SVR12 was achieved in 21/34 (62%) treated for 4 weeks, and 17/17 (100%) treated for 8 weeks, equating to 38 cures and 13 treatment failures overall. LFT data were available for 48 participants (35 cures and 13 treatment failures). ΔALT was calculated as change in ALT from EOT to EOT+12 weeks in those without evidence of treatment failure during EOT monitoring (cures; n= 35), and from EOT to retreatment day 0 (RTD0) in those experiencing virological rebound during EOT monitoring (n=13). Timing of RTD0 lay between EOT+6weeks and EOT+14weeks in the 13 participants who did not achieve SVR with shortened treatment.

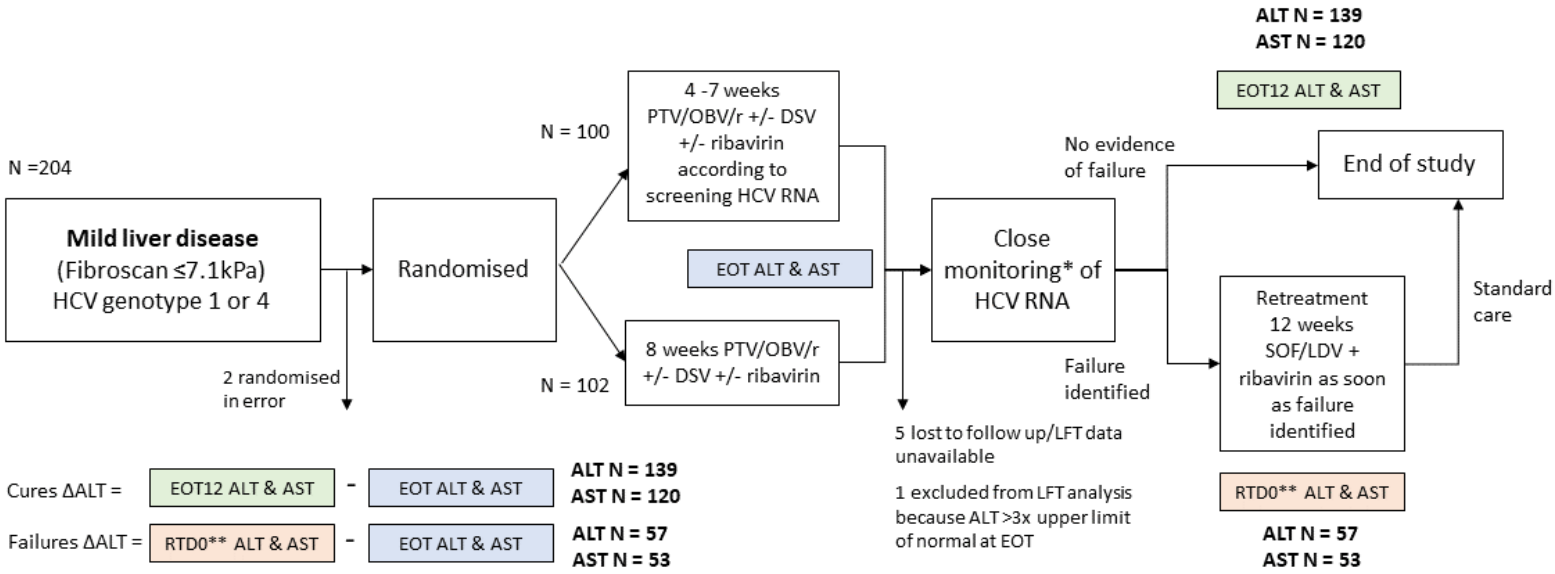
STOPHCV1

STOPHCV1 was a randomised trial, conducted across 14 UK NHS Hospital Trusts from 2016-2018, which assessed variable ultrashort-course DAA treatment (4 to 8 weeks therapy based on pre-treatment viral load according to a continuous scale) versus 8 weeks fixed duration therapy for chronic HCV infection[295]. The DAAs used were ombitasvir/paritaprevir/ritonavir +/- dasabuvir depending on infecting genotype, +/- ribavirin (1:1). Towards the latter half of recruitment ombitasvir/paritaprevir/ritonavir +/- dasabuvir was replaced with glecaprevir/pibrentasvir.

As in the SEARCH-1 study, participants were followed closely after EOT, with viral load testing 2, 4, 8, 12 and 24 weeks after EOT (figure 5-2). Any patients with confirmed virological rebound were retreated as soon as practicable with 12 weeks sofosbuvir 400mg/ledipasvir 90mg once-daily, and weight-based oral ribavirin. Full details are available in the original article[295]. HCV RNA was tested on the platform available in local NHS labs, which included Cobas Amplicor v2, Abbott Realtime, Aptima QuantDX and Roche CAP-CTM.

Of 199 individuals who remained under follow up until EOT week 12, SVR12 was achieved in 141, with 58 individuals experiencing virological rebound at or before this timepoint. ALT and AST were tested on local NHS lab platforms at baseline and EOT in all participants, and at start or retreatment or EOT+12 in those with or without evidence of virological rebound, respectively. I tested the hypothesis that ΔALT >0 IU/L and ΔAST >0 IU/L from EOT to EOT+12 weeks (or nearest available timepoint), would have a high sensitivity for detecting treatment failure, with a high negative predictive value.

Figure 5-2: STOPHCV1 flow diagram



204 participants were enrolled. Two individuals were randomised in error, leaving 202 participants. 100 were randomised to receive variable ultrashort-course treatment with ombitasvir(OBV)/paritaprevir(PTV)/ritonavir(r) +/- dasabuvir(DSV) (49 with ribavirin and 51 without ribavirin), and 102 were randomised to receive 8 weeks fixed-dose therapy with the same antivirals (51 with ribavirin and 51 without ribavirin). Three individuals were lost to follow up and one experienced an increase in ALT on treatment >2xULN so was excluded from this analysis. Of the remaining 196 participants, 139 achieved SVR12 and 57 experience virological rebound during EOT follow up, commencing retreatment with sofosbuvir and ledipasvir as soon as possible. ΔALT was calculated as change in ALT from EOT to EOT+12 weeks in those without evidence of treatment failure during EOT monitoring (cures; n= 139), and from EOT to RTD0 in those with virological rebound (treatment failure; n=57). Timing of RTD0 lay between EOT+7weeks and EOT+42weeks in the 57 participants who did not achieve SVR with shortened treatment.

Statistical Analysis

In each study I plotted ALT and AST at baseline, EOT, and EOT+12 (or retreatment day 0 in those who experienced virological rebound). For STOPHCV1, in which participants received a variable number of days of antiviral therapy, I categorized treatment duration in weeks (+/- 3 days), such that individuals who received 31 days were labelled as receiving 4 weeks DAA (4 weeks +3 days), while those who received 32 days were labelled as receiving 5 weeks (5 weeks -3d) . I calculated median ΔALT and ΔAST and interquartile range in cures vs failures and used Wilcoxon Rank Sum test to compare outcomes. A p-value <0.05 was considered significant. I compared baseline and EOT ALT and AST in cures vs treatment failures in the same way.

I chose to assess the performance of ΔALT or ΔAST >0 IU/L for assessment in STOPHCV1, as the most logical and pragmatic value (i.e. 'any increase'). Evaluation of sensitivity and specificity included all available data for the relevant outcomes. Performance is presented with the corresponding binomial exact 95% confidence interval (95%CI). I calculated positive and negative predictive values for the observed results, and adjusted for a lower prevalence of treatment failure observed with standard durations of therapy (5%). I calculated area under receiver operator curves (AUROC) for both liver enzymes. Finally, I conducted a post-hoc analysis of ΔALT and ΔAST by infecting genotype. In SEARCH-1, in which genotype 6 was predominant, I stratified by genotype 6 and non-6 genotypes. In STOPHCV1, in which 99% of the study population had genotype 1 infection, I stratified by genotypes 1a and 1b. All data were analysed using Stata (version 14.2, StataCorp, Texas, USA).

Results

Baseline characteristics

Patient characteristics at enrolment for each study are shown in table 15. The SEARCH-1 population was made up entirely of Vietnamese adults, with a higher proportion of female participants (56%) and genotype 6-infected individuals (53%). HIV positive individuals were excluded and only 8% reported current or recent illicit substance abuse. In contrast the STOPHCV1 population was predominantly male (69%), of white ethnicity (87%) with genotype 1a predominating (82%). 32% reported current or recent illicit substance abuse and 34% were HIV co-infected. Both study populations had mild liver disease: median FibroScan scores were 6.0kPa and 4.9kPa in SEARCH-1 and STOPHCV1

respectively. In both study populations, mean and median ALT and AST levels were within the normal range.

Table 16: Participant characteristics at enrolment in SEARCH-1 and STOPHCV1

	SEARCH-1 (VN)	STOPHCV1 (UK)
Total participants	52	202
Age (years)	49.5 (39.5, 59.0)	45.5 (37.5, 53.0)
Female at birth	29 (56%)	62 (31%)
Weight	55.4 (51.5, 64.9)	74.0 (66.0, 84.6)
BMI (kg/m^2)	23.3 (20.8, 25.1)	24.9 (22.2, 27.2)
White ethnicity	0 (0%)	176 (87%)
Vietnamese Asian	52 (100%)	
Enrolment HCV viral load (IU/ml)	1,932,775	741,946
(n=199 in STOPHCV-1)	(618, 11,200,000)	(249,097,1872136)
HCV genotype/subtype:		
1a	11 (21%)	166 (82%)
1b	12 (23%)	34 (17%)
2	1 (2%)	0 (0%)
3	0 (0%)	2 (1%)
4	1 (2%)	0 (0%)
6	27 (53%)	0 (0%)
HIV coinfected	0 (HIV excluded)	68 (34%)
Fibroscan result (kPa)	6.0 (5.0, 6.6)	4.9 (4.2, 5.8)
ALT (IU/L)	39 (26, 66)	52 (34, 87)
AST (IU/L) (n=189 in STOPHCV-1)	32 (25, 47)	38 (30, 57)
Current/recent alcoholism/alcohol abuse	4 (8%)	13 (6%)
Current/recent illicit substance abuse	4 (8%)	64 (32%)
Treated with sofosbuvir + daclatasvir	52 (100%)	-
Treated with paritaprevir + ombitasvir + dasabuvir	-	198 (98%)
Treated with paritaprevir + ombitasvir	-	2 (1%)
Treated with glecaprevir + pibrentasvir	-	2 (1%)
Withdrew or lost to follow up before EOT	1	3
ALT data not available	3	2
AST data not available	3	22
ALT or AST >2xULN at EOT warranting exclusion	0	1
Total number with ΔALT analysed	48 (92%)	196 (97%)*
Total number with ΔAST analysed	48 (92%)	173 (86%)
Timing of RTD0 in treatment failures (weeks from EOT)	10 (6,10)	11 (8, 13)

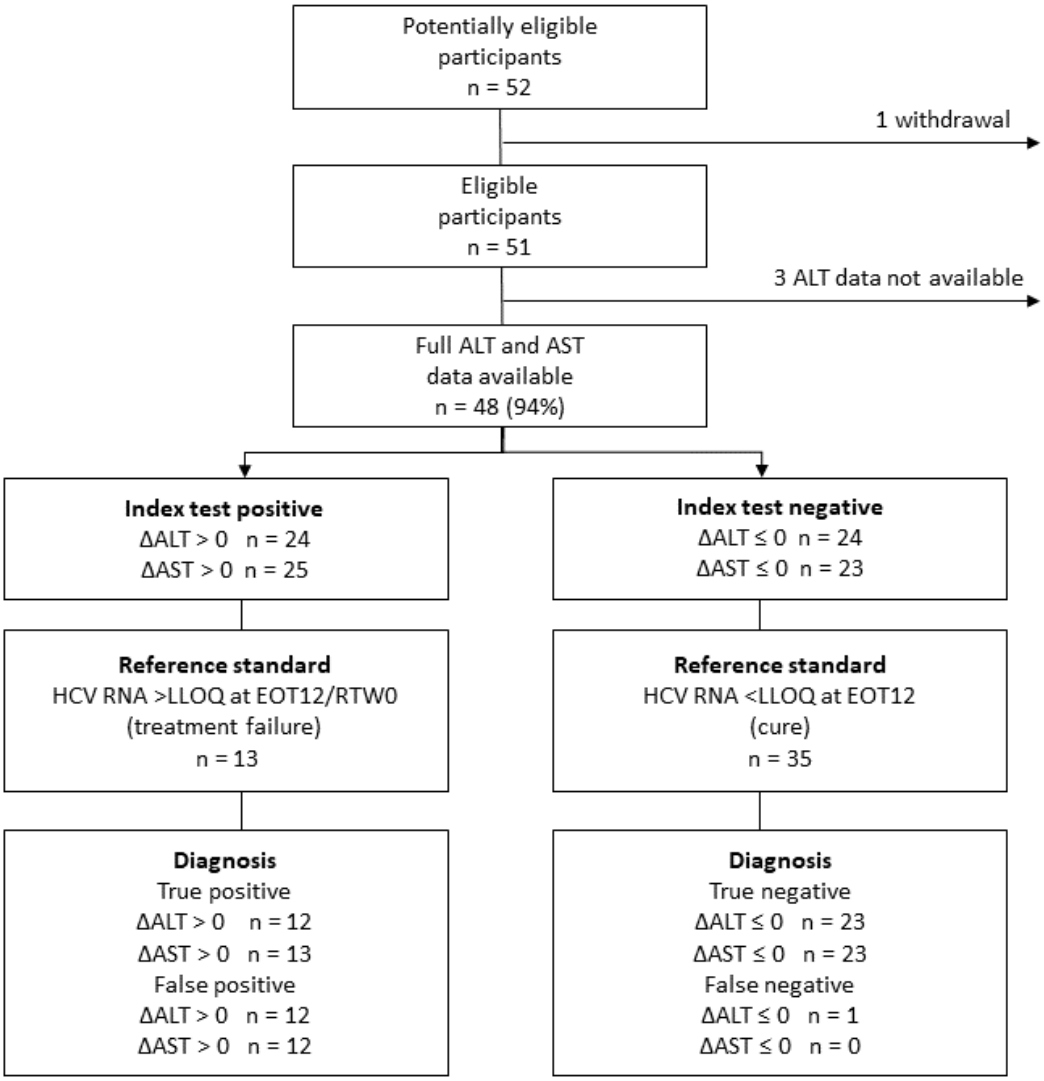
*Note: showing n (%) for categorical factors, or median (IQR) for continuous factors. Missing data indicated by denominators in the row label. *3/135 individuals had EOT24 ALT data but no EOT12 ALT data.*

SEARCH-1

Of 52 patients treated with SOF/DCV in SEARCH-1, 48 (92%) had liver function test data available for analysis (figure 5-3; table 15). All 48 of these individuals experienced a reduction in ALT and AST on treatment, as demonstrated in the spaghetti plots of ALT in figure 5-4, so all were included in the analysis.

ΔALT and ΔAST from EOT to EOT12 (or RTW0 for participants who experienced treatment failure prior to EOT12) are shown in figure 5-4 box and whisker plots. All 35 participants who achieved SVR12 had ALT and AST data from EOT12. For the 13 treatment failures, ALT and AST data was from median 10 weeks after EOT (IQR = 6, 10). Median (IQR) ΔALT from EOT to EOT+12 weeks was -2 IU/L (-6.0, +2.0) in individuals achieving SVR12 and +17 IU/L (+7.5, +38.0) in those who experienced treatment failure ($p<0.001$). Median (IQR) ΔAST was -1 IU/L (-3.0, +1.0) in cures and +12 IU/L (+6.0, +16.0) in treatment failures (table 16). We found no evidence of a difference between baseline ALT or AST, EOT ALT or AST, or change in ALT or AST from baseline to EOT between cures and treatment failures ($p>0.28$; table 16).

Figure 5-3: STARD diagram for flow of participants through SEARCH-1



Study population was treatment naïve adults with chronic HCV infection referred to study screening from the HTD, HCMC. Index test = ΔALT >0IU/ml or ΔAST >0IU/ml. Reference standard = HCV RNA >LLOQ at EOT+12 weeks. LLOQ – lower limit of quantification; EOT12 – end of treatment +12 weeks; RTW0 – retreatment week 0. True positive indicates HCV RNA >LLOQ at EOT+12 weeks (treatment failure) and ΔALT >0IU/ml. False positive indicates HCV RNA <LLOQ) at EOT+12 weeks (SVR12) but ΔALT >0IU/ml. True negative indicates HCV RNA <LLOQ at EOT+12 weeks (SVR12) and ΔALT <0IU/ml. False negative indicates HCV RNA >LLOQ at EOT+12 weeks (treatment failure) but ΔALT <0IU/ml.

Figure 5-4: Serial ALT in cures (blue lines) and treatment failures (red lines) in participants from SEARCH-1

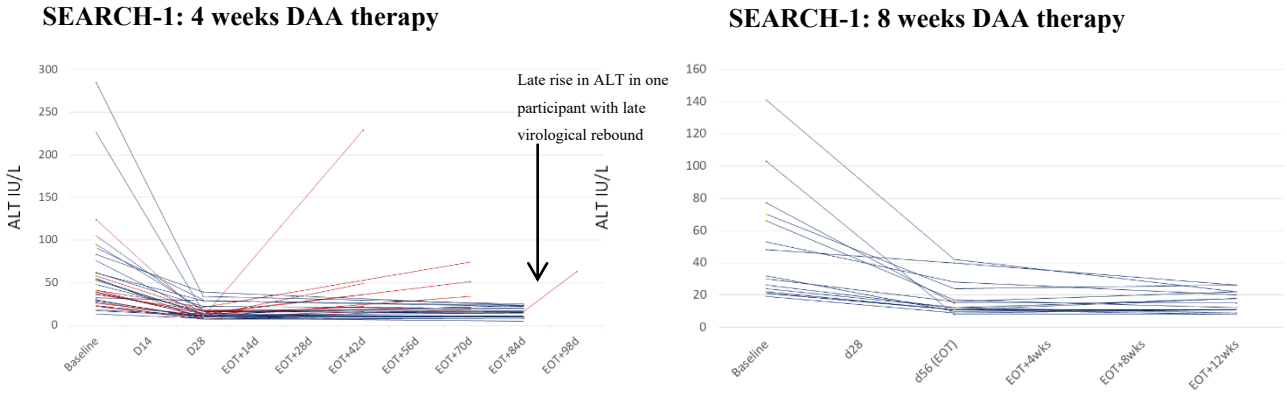
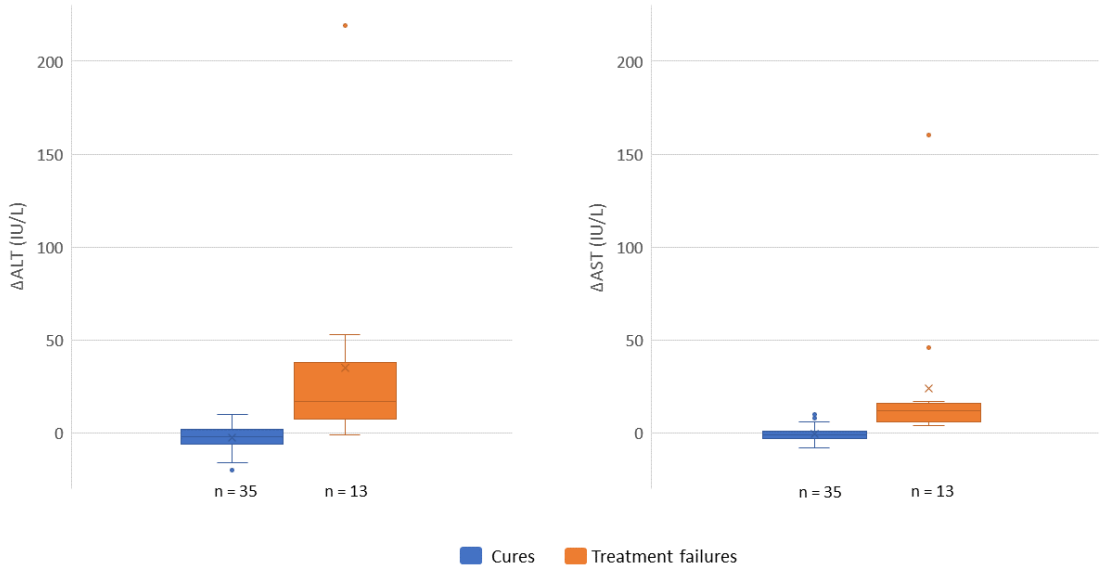


Figure 5-5: ΔALT and ΔAST in cures and treatment failures in all participants from SEARCH-1 (n=48)



'Box plot with range ('whiskers' indicating minimum and maximum values in data set), IQR (bottom of box indicating Q1 and top of box indicating Q3), and median (line within box). Maximum ΔALT and maximum ΔAST recorded in same participant.

Table 17: Performance of ΔALT >0 and ΔAST >0 vs HCV RNA at EOT week 12 in SEARCH-1

SEARCH-1 (n=48)	HCV RNA >LLOQ at EOT12/RTW0 (treatment failures)	HCV RNA <LLOQ at EOT12(cures)	*p*
Timing of ALT & AST in weeks since EOT (median [IQR])	10 (6, 10)	12 (12, 12)	
ALT			
Median ΔALT (IQR) from EOT to EOT+12	+17 (+7.5, +38)	-2 (-6, +2)	<0.001
Median ΔALT (IQR) genotype 6	+12.5 (+5.5 - +95)	-2.5 (-7.25 - +1.25)	<0.001
Median ΔALT (IQR) non-6 genotype	+22 (+12 - +38)	0 (-3 - +2)	<0.001
Participants with ΔALT >0	12	12	
Participants with ΔALT ≤ 0	1	23	
Sensitivity (95% C.I) ΔALT >0	**92% (64.0 – 99.8)**		
Specificity (95% C.I) ΔALT >0	**66% (48 - 81)**		
AUROC (95% C.I)	0.95 (0.87 - 1.00)		
Baseline ALT	36 (24 - 41)	48 (24 - 76)	0.275
EOT ALT	13 (10 - 15.5)	13 (11 - 22)	0.464
Decline in ALT from baseline to EOT	-19 (-28.5, -15)	-28 (-49, -11)	0.437
AST			
Median ΔAST (IQR) from EOT to EOT+12	+12 (+6, +16)	-1 (-3, +1)	<0.001
Median ΔAST (IQR) genotype 6	+12 (7 - 74.5)	-1 (-4 - 1.8)	<0.001
Median ΔAST (IQR) non-6 genotype	+12 (5 - 14)	-1 (-2 - 1.5)	<0.001
ΔAST > 0	13	12	
ΔAST ≤ 0	0	23	
Sensitivity (95% C.I) ΔAST >0	**100% (75 - 100)**		
Specificity (95% C.I) ΔAST >0	**66% (48 - 81)**		
AUROC (95% C.I)	0.96 (0.91 - 1.00)		
Baseline AST	28 (24.5 - 41)	33 (25 - 47)	0.437
EOT AST	19 (14.5 - 21)	18 (15 - 20)	0.472
Decline in ALT from baseline to EOT	-10 (-20.5, -7.5)	-14 (-27, -8)	0.981

Of the 13 participants who experienced treatment failure, 13/13 had an increase in AST between EOT and retreatment, and 12/13 had an increase in ALT (table 16). The exception was a participant who was found to have low level viral rebound (3390 IU/ml) at the EOT+12 week visit having had undetectable virus at preceding visits. At EOT week 12, ΔALT was -1 IU/L, but by RTD0, two weeks later (equivalent of EOT week 14), when HCV RNA had risen to 431,604 IU/ml, ALT and AST had both increased substantially (ΔALT= 46, ΔAST= 44). This participant's ALT profile is marked by the arrow in the 4-week treatment chart in figure 5-4. Of the 35 patients who were cured (blue lines in figure 5-4), ALT and AST both decreased or stayed the same after EOT in 23/35 and increased by >0 IU/L in 12 participants.

Performance analysis of ΔALT > 0 IU/L and ΔAST > 0 IU/L is shown in table 16. An increase in ALT was 92% sensitive (95%C.I [64.0 - 99.8]) for detecting treatment failure vs HCV RNA at

EOT12, and an increase in AST was 100% sensitive (95% C.I [75 - 100]). Both enzymes were 66% specific (48 - 81). The area under the receiver operator curve (AUROC) was 0.95 (95% C.I [0.87 - 1.00]) for ΔALT and 0.96 (95% C.I [0.91, 1.00]) for ΔAST, indicating ΔALT and ΔAST are both very good at distinguishing individuals with virological rebound from those achieving SVR12. A significant difference between cures and failures with regards to both ΔALT and ΔAST was observed in genotype 6-infected individuals (53% study population; median (IQR) ΔALT = 0 IU/L (-3, +2) in cures vs +22 IU/L (+12, +38) in treatment failures ($p<0.001$)) and non-6 infected individuals (43% population; -2.5 IU/L (-7.5, +1.25) in cures vs +12.5 IU/L (+5.5, +94.5) in failures ($p<0.001$; table 16).

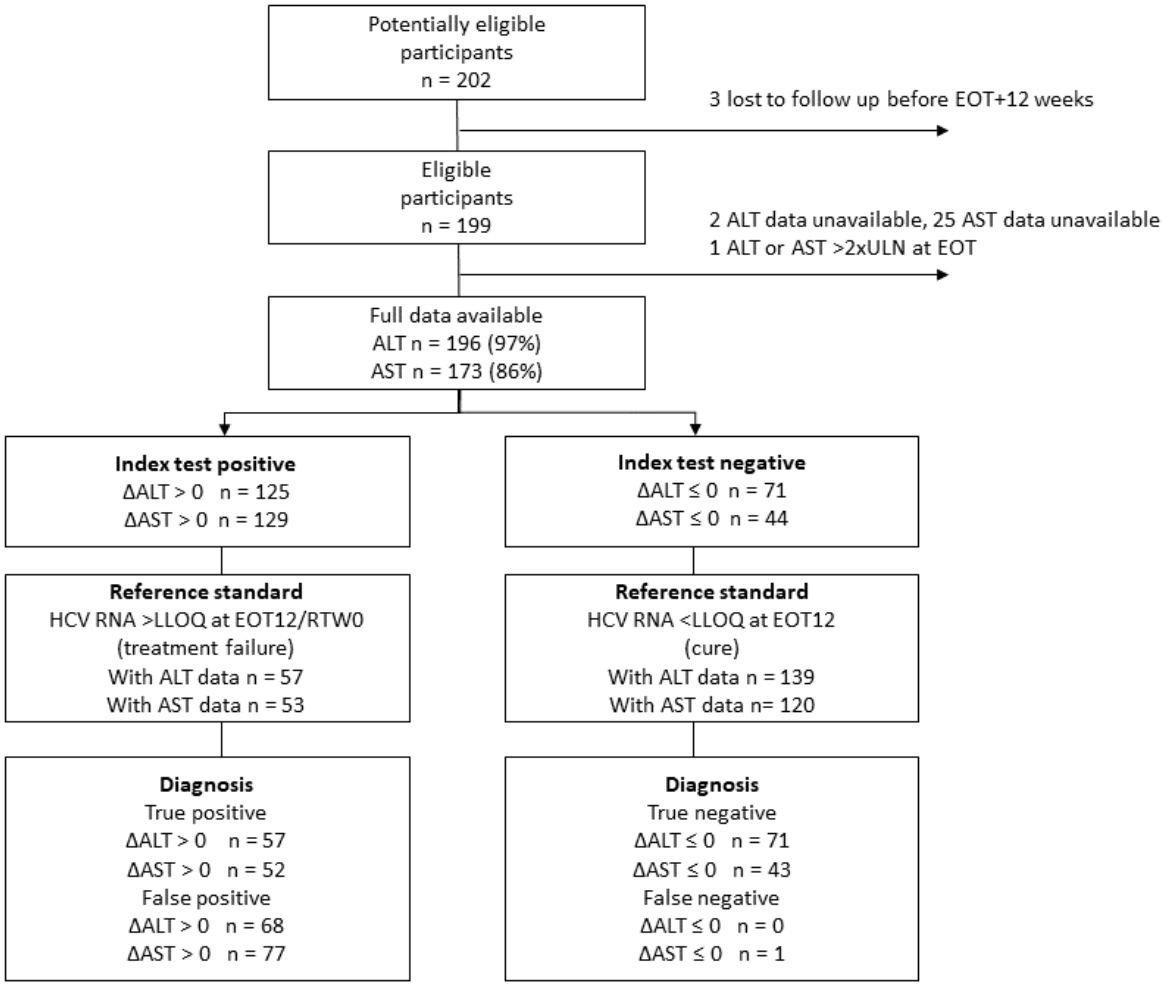
STOPHCV1

ALT and AST data was available for 196 and 173 participants respectively (figure 5-2, figure 5-6; table 15). Figure 5-7 shows the ALT profiles for all 196 participants according to duration of therapy received. One participant experienced an increase in ALT > 3x upper limit of normal by end of treatment so was excluded from performance analysis as per the pre-defined analysis plan (dashed black line in figure 5-7).

ΔALT and ΔAST from EOT to EOT12 (or RTW0 for participants who experienced treatment failure prior to EOT12) are shown in figure 5-8 box and whisker plots. ALT and AST data for the participants who achieved SVR12 was from the EOT+12 week visit in all except 3 participants, in who ALT data was only available from their EOT+24 week visit. ALT and AST data for treatment failures was from median 10 weeks after EOT (IQR = 8, 13).

Median ΔALT from EOT to EOT+12 weeks was 0 IU/L (IQR [-2, +5]) in individuals achieving SVR12 and +41 IU/L (+20, +85) in those who experienced treatment failure ($p<0.001$, figure 5-8, table 17). Median (IQR) AST was +2 IU/L (-1, +5) in cures and +23 IU/L (+13, +49) in treatment failures. As in SEARCH-1, we found no evidence of a difference between those who cured and those who experienced treatment failure in terms of ALT or AST at baseline, EOT or change in ALT or AST from baseline to EOT ($p>0.67$; table 17).

Figure 5-6: STARD diagram for flow of participants through STOPHCV1



"Study population was treatment naïve adults with chronic HCV infection enrolled into STOPHCV-1 study. Index test = ΔALT >0IU/ml or ΔAST >0IU/ml. Reference standard = HCV RNA >LLOQ at EOT+12 weeks. LLOQ – lower limit of quantification; EOT12 – end of treatment +12 weeks; RTW0 – retreatment week 0. True positive indicates HCV RNA >LLOQ at EOT+12 weeks (treatment failure) and ΔALT >0IU/ml. False positive indicates HCV RNA <LLOQ at EOT+12 weeks (SVR12) but ΔALT >0IU/ml. True negative indicates HCV RNA <LLOQ at EOT+12 weeks (SVR12) and ΔALT <0IU/ml. False negative indicates HCV RNA >LLOQ at EOT+12 weeks (treatment failure) but ΔALT <0IU/ml.

Figure 5-7: ALT profiles for all 196 participants with available data in STOPHCV1 according to treatment duration.

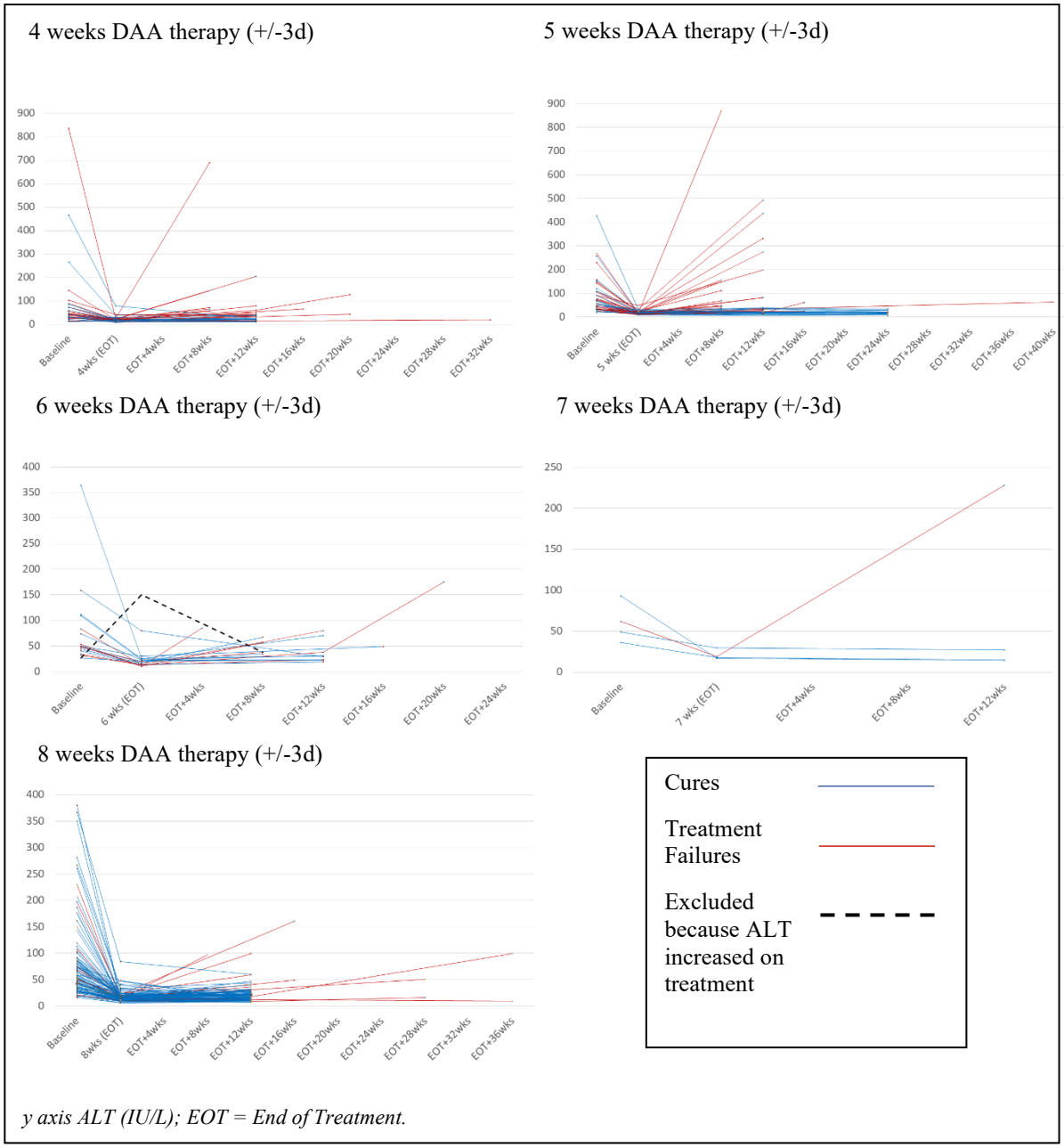
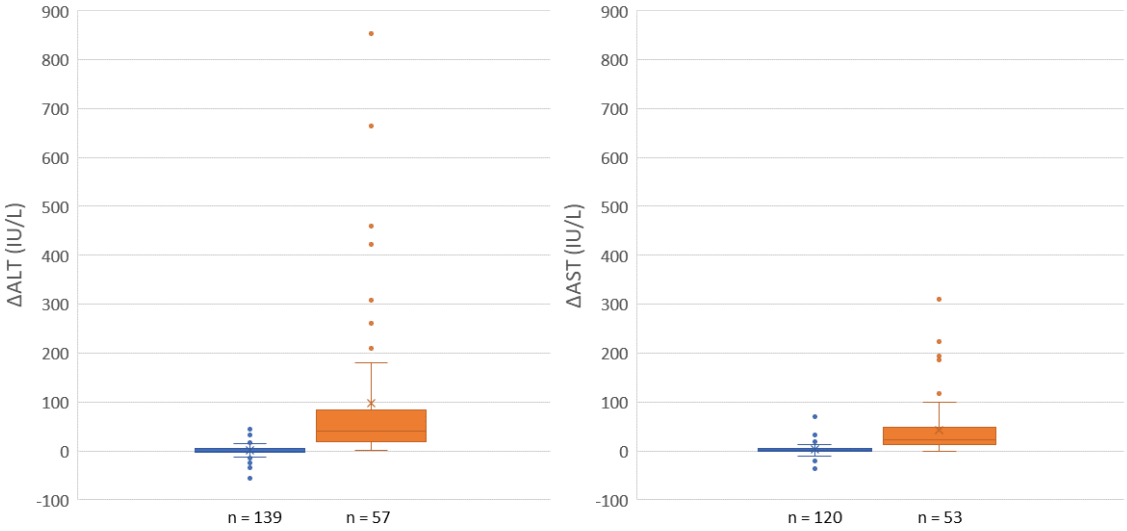


Table 18: Performance of ΔALT >0 and ΔAST >0 vs HCV RNA at EOT week 12 in STOPHCV1

STOPHCV1	HCV RNA >LLOQ (treatment failure)	HCV RNA negative (cure)	
Timing of ALT & AST in weeks since EOT (median [IQR])	12 (8, 13)	12 (12, 12)	
ALT (n=196)			
Median ΔALT (IQR) from EOT to EOT+12	+41 (+20, +85)	0 (-2, +5)	<0.001
Median ΔALT (IQR) genotype 1a	+42 (+20, +124)	+1 (-2, +5)	<0.001
Median ΔALT (IQR) genotype 1b	+41 (+19, +60)	-1 (-5, +3)	<0.001
Participants with ΔALT > 0	57	68	
Participants with ΔALT ≤ 0	0	69	
Sensitivity (95% C.I)	**100% (93.7, 100)**		
Specificity (95% C.I)	51.1% (42.4, 59.7)		
Positive predictive value (95% C.I) assuming 5% failure rate	46% (37 - 55)		
Negative predictive value (95% C.I) assuming 5% failure rate	100% (95 - 100)		
AUROC (95% C.I)	0.96 (0.94 - 0.99)		
Baseline ALT	+50 (+34, +90)	+55 (+31, +88)	0.885
EOT ALT	+17 (+14, +21.5)	+18 (+13, +23)	0.712
Decline in ALT from baseline to EOT	-32 (-58, -20)	-35 (-67, -18)	0.828
AST (n=173)			
Median ΔAST (IQR) from EOT to EOT+12	+23 (+13, +49)	+2 (-1, +5)	<0.001
Median ΔAST (IQR) genotype 1a	+23 (+10, +54)	+2 (-1, +6)	<0.001
Median ΔAST (IQR) genotype 1b	+22 (+14, +30)	+1 (0, +2.3)	<0.001
ΔAST > 0	52	77	
ΔAST ≤ 0	1	43	
Sensitivity (95% C.I)	**98.1% (89.9, 99.9)**		
Specificity (95% C.I)	35.8% (27.3, 45.1)		
Positive predictive value (95% C.I) with 5% failure rate	40% (32 - 49)		
Negative predictive value (95% C.I) with 5% failure rate	98% (88 - 100)		
AUROC (95% C.I)	0.92 (0.88 - 0.96)		
Baseline AST	39 (29 - 55)	39 (31 - 58)	0.674
EOT AST	20 (17 - 26)	20 (17 - 24)	0.794
Decline in AST from baseline to EOT	-19 (-31,-10)	-19 (-38.5, -9)	0.898

Figure 5-8: Box & Whisker plots of ΔALT (left, n = 194) and ΔAST (right, n= 174) in cures vs treatment failures in STOPHCV1



*Box plot showing interquartile range (IQR); bottom of box indicating Q1 [bottom 25%] and top of box indicating Q3 [top 75%]), range ('whiskers' indicating 'minimum' (Q1-1.5*IQR) and 'maximum'(Q3+1.5*IQR) values in data set), median (line within box) and outliers (observations that are numerically distant from rest of data shown as dots outside of whiskers). Maximum ΔALT and maximum ΔAST recorded in same participant. 6/7 participants with ΔALT outliers were ΔAST outliers.*

Figure 5-9: Receiver Operator Curve for ΔALT in SEARCH1 and STOPHCV1

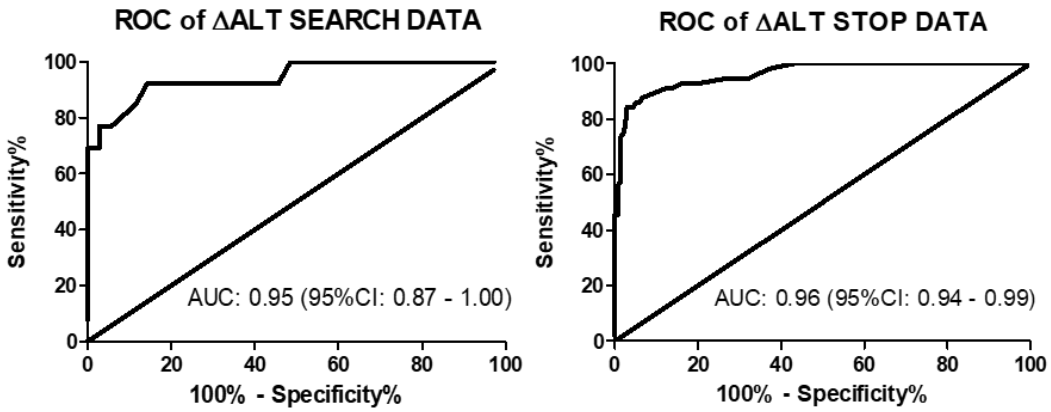
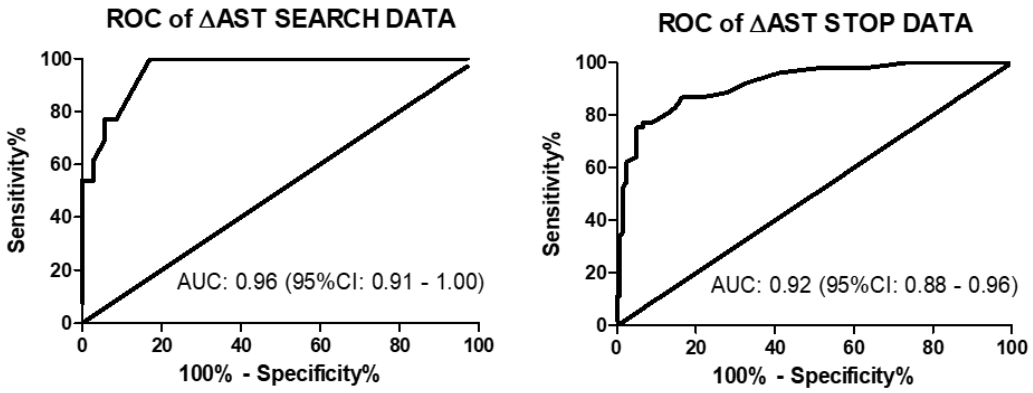


Figure 5-10: Receiver Operator Curve for ΔAST in SEARCH1 and STOPHCV1



Discussion

In this retrospective analysis of two independent HCV treatment-shortening studies I found that an increase in ALT or AST >0 IU/L within median 10 weeks after end of treatment is

highly sensitive for detecting treatment failure, compared with gold standard HCV RNA at EOT12.

In SEARCH-1 (n=48) an increase in ALT >0 IU/L after EOT picked up 12/13 individuals who experienced treatment failure by EOT12. This finding was replicated in STOPHCV1 (n=196), in which an increase in ALT after EOT was observed in 57/57 individuals who did not achieve SVR12 on shortened therapy. By comparison just 68/139 participants achieving SVR12 had an increase in ALT between EOT and EOT+12 weeks, making ΔALT >0 IU/L 100% (93.7 - 100) sensitive and 51.1% (42.4 - 59.7) specific in identifying treatment failure. ΔAST >0IU/L also performed well but appears to be less sensitive and specific than ALT in identifying treatment failure, with a lower median difference between cures and failures. AUROC for both liver enzymes demonstrated they are excellent markers for discriminating cures from treatment failures. Assuming a cure rate of 95%, typical of standard duration therapy, both tests had a negative predictive value exceeding 98% meaning the risk of a false negative result (missing a case of treatment failure) is exceptionally low.

ALT and AST are well-established markers of hepatocyte injury and ALT is known to be a more sensitive and specific compared with AST (which is found in skeletal and cardiac muscle as well as the kidneys). An association between pre-treatment liver enzyme levels and DAA treatment outcomes has been described previously[340], and greater declines in ALT and AST on treatment have been observed in individuals who cure, compared to those who do not achieve SVR, with standard duration therapy[343,346]. Likewise elevated levels of liver enzymes are associated with liver fibrosis and high HCV viral loads[347], which are both soft predictors of an inferior response to DAA therapy[156,348]. This study is, to my knowledge, the first evaluation of the performance of ΔALT and ΔAST after EOT as a surrogate for HCV RNA at EOT12 in detecting treatment failure and represents important proof of concept.

ALT monitoring after EOT could have a major cost-saving impact in resource limited settings, where nucleic acid testing is often expensive and restricted to few specialised, centralised laboratories. ALT testing is cheap ($2-5 in Vietnam) and can be performed from finger prick specimens in most laboratories. New point-of-care ALT testing technology has recently emerged which could negate the need for a lab altogether[349]. Furthermore ALT is measured routinely at EOT and EOT12 according to most HCV treatment guidelines[16,318], such that this strategy would not involve any additional clinic visits or blood testing. In the

long-term liver enzyme monitoring would be unlikely to replace PCR, and improved point-of-care HCV RNA testing will be preferable. However, this approach may enable more patients to access HCV treatment until that access to that technology becomes a reality.

Assuming a positive predictive value of 46% (figure 1), a negative predictive value of 100%, and a cure rate of 95%, this screening strategy would reduce viral load testing by 51%. In Vietnam this translates to a saving of US$18-46,000 per thousand patients treated (equivalent to approximately 36-90 courses of DAA therapy). In LMICs, where HCV treatment must be part- or fully funded by patients, this reduction in out-of-pocket costs could help prevent the kind of catastrophic health expenditures that have been associated with DAA therapy[350]. The 2022 updated WHO guidelines recommend the use of point-of-care (POC) HCV ribonucleic acid (RNA) assays as an additional approach alongside laboratory-based RNA assays to detect HCV viraemia. Pre-screening with ΔALT and ΔAST from EOT to EOT+12 weeks could further complement this approach in high-burden, resource-limited settings and remote regions of both high- and low-income countries. Work would be required to ensure ALT and AST are standardised across platforms.

Strengths & Limitations

The major strength of this study is in the replication of our finding from the SEARCH-1 pilot study in the larger independent STOPHCV1 study. The results were similar across divergent income settings with differing population demographics and infecting genotypes, using different antiviral treatments. Both trial populations had comprehensive clinical data, meaning we were able to include a high proportion of participants from each study (92% and 96% in SEARCH-1 and STOPHCV1 respectively).

The main limitation of this study is that both study populations were treated with short-duration DAA therapy, and it remains to be seen if this phenomenon persists with standard duration therapy. Similarly, both study populations had mild liver disease, with baseline ALT and AST levels within normal range. ALT dynamics are known to be altered in patients with cirrhosis[351], and a persistently elevated ALT after DAA therapy has been independently associated with high body mass index (BMI), diabetes, alcohol consumption and, opioid substitution therapy[344]. These conditions were poorly represented in the Vietnam study. While I found no evidence that genotype influenced change in ALT or AST after treatment, the

limited data with respect to non-1, non-6 genotypes, means my findings may not be applicable to all infecting genotypes.

Another limitation relates to timing of liver function testing, which was earlier than EOT12 in most patients who failed to achieve SVR12 (median 10 weeks after EOT, with a large range in STOPHCV1 from 5 to 42 weeks after EOT). Although ALT and AST timings were generally close to the EOT12 timepoint used to assess SVR (IQR = 8, 13), it would be preferable to test both cures and treatment failures at an identical timepoint. Furthermore, both trials had high rates of medication adherence, and it is not clear if an increase in ALT or AST after treatment would be reliable in those who haven't fully suppressed virus by EOT.

Despite these limitations, this study shows that an increase in ALT or AST >0 IU/L after end of treatment is a highly sensitive marker of virological rebound after shortened therapy, and this deserves further exploration in a prospective real-world study with standard duration therapy and in patients with cirrhosis. Given the large volume of real world data available a meta-analysis would also be helpful and may better define a cut off with improved specificity and positive predictive value. I am confident this approach has potential to reduce reliance on nucleic acid testing in resource-limited and remote settings, driving down costs and facilitating decentralisation of care.

Chapter 6

Discussion

The title of this thesis, *'Towards Elimination of Hepatitis C in Vietnam'* is intentionally broad and inclusive, to reflect the wide range of research questions that demand attention as Vietnam strives towards elimination of HCV. In chapters 2-5 I have described a meta-analysis of prevalence studies, a clinical trial evaluating novel HCV treatment strategies, and a diagnostic accuracy study. Each study makes a small yet important contribution to the HCV literature which I hope will stimulate further work. In this chapter I will contextualise my findings and discuss the studies' major limitations, describe what additional work could build on the results, and address what I perceive to be the major issues relating to the elimination of HCV in Vietnam.

Chapter 2 – seroprevalence of HBV, HCV and HDV in Vietnam

The systematic review and meta-analysis of prevalence studies described in chapter 2 provides useful detail about the viral hepatitis epidemic in Vietnam. Effective, real-time surveillance of new infections is essential for public health systems to target screening to at-risk groups, implement preventative measures and scale up treatment. A national online surveillance system was established in Vietnam in 2017 but has been hampered by technical shortcomings and a lack of motivation at the regional level, recording just 52,086 cases of HBV, and 6,792 cases of HCV by the end of 2019[193]. Systematic reviews and meta-analyses of published prevalence studies are not a substitute for real time surveillance. The data they include is generally a few years old and study heterogeneity diminishes accuracy. The Joanna Briggs Institute Systematic Review Index is imperfect and, as discussed in chapter 2, the DerSimonian–Laird random effects model has a tendency to generate implausibly narrow confidence bounds when the number of studies is small or when there are substantive

differences among study estimates[269]. These shortcomings notwithstanding, meta-analyses can still provide a useful snapshot of prevalence within different at-risk groups, particularly in the absence of a functioning surveillance system. In addition, they a cheap and efficient when compared with large scale serosurveys. Other high prevalence countries with inadequate hepatitis surveillance, such as Indonesia[352] and the Philippines[353], would benefit from a similar study in the short to medium term.

Concerning Hepatitis B, my major findings were a very high prevalence of HBsAg amongst antenatal populations (10.8% [10.1-11.6]) and adults in the general population (10.5% [10.0-11.0]) with similar prevalence in adults at high risk of exposure to blood-borne viruses. This suggests the epidemic is largely driven by chronic infections acquired in childhood (rather than by healthcare-related activities or intravenous drug use) and highlights that mother-to-child transmission is a major problem in Vietnam. It also predicts an extremely high burden of liver cirrhosis and HCC in years to come.

These HBV prevalence figures are also worth framing within the limitations of the study, however. Of the 44 HBV studies included, only 8 were from the last 5 years, and the data they reported was frequently older than that. Given that chronic HBV is mostly acquired at birth or in early childhood, HBsAg serosurveys of the adult population are like looking at light from a distant star: they show something that happened years ago. Many of the individuals surveyed would have been infected long before HBV vaccination was incorporated into the routine childhood immunisation schedule in 1997, and virtually all would have been born prior to introduction of universal birth dose vaccination in 2004. This means that some of the work to reduce future burden of HBV in Vietnam has already been done, and future serosurveys, that incorporate adults vaccinated at birth, will reflect this. HBsAg prevalence in children under 5 is used as a surrogate indicator of the cumulative incidence of chronic HBV and gives a better indication of the current situation. The Vietnam MoH estimates that HBsAg prevalence in under 5s remains greater than 1%, but robust data for this age group is lacking. Even if current rates of mother-to-child transmission are being over estimated, it is clear that birth dose vaccination remains well below WHO target levels. Vaccine hesitancy[354] and the COVID-19 pandemic[355] have both negatively impacted vaccine uptake in recent years. A renewed focus on maternal HBV screening and prophylaxis of MTCT is required, with effective surveillance of infections in children.

With regards to HCV, my finding that pooled prevalence of active infection in the general population is lower than historical estimates (0.26% [0.09-0.51]) is reassuring and may imply Vietnam will need to treat fewer infections that originally anticipated. However it is worth noting that current HCV antigen platforms are only 90-95% sensitive for detecting active infection compared to HCV RNA[226–229] and sensitivity of older assays is lower than this. I did not incorporate diagnostic platform into my analysis so my results may underestimate the true number of active infections in the population. In addition, 'adults in the general population' in my analysis were from community studies, outpatient studies and occupational surveys, and may have had fewer risk factors than might be expected in a true cross-sectional sample.

New data has emerged since I published my systematic review. An unpublished cross-sectional survey of 25,649 adults conducted in 32 provinces by the Vietnam MOH in 2018 and 2019, (which used Abbott Architect for HCV antibody and reflex antigen testing), found that 1.8% of individuals were antibody positive and 1.0% were HCV antigen positive[193]. In a 2022 study, among 14,675 adults surveyed in Ho Chi Minh City, 298 tested positive for HCV antibody (weighted prevalence 1·3% (95% CI, 1·1%-1·6%) and 50·6% (118 of 233) of those tested for HCV RNA had evidence of active infection[356]. These surveys, which include both high and low-risk groups, provide a more current estimate of pooled prevalence for the entire population. Nevertheless, my study shows that in the absence of obvious risk factors, prevalence of HCV antigen/RNA is low in Vietnam, such that universal screening of low-risk individuals is unlikely to be cost-effective.

Another important finding from the meta-analysis was that HCV prevalence in PWID is extremely high in Vietnam, far higher than the estimated global average (anti-HCV prevalence 72.5% [71.4 – 73.6] versus 52.3% [42·4–62·1])[265]. This estimate was based on data from 14 separate PWID populations which included 6666 individuals so should be robust. Interventions to prevent community transmission of HIV and HCV, such as needle and syringe programmes and opioid substitution therapy, were introduced from 2007. While these programmes have incorporated free antiretroviral therapy for people living with HIV, no such treatment has been available for those living with HCV. Intravenous drug use is officially labelled as a 'social vice' in Vietnam and forced incarceration for drug rehabilitation in facilities overseen by the Department of Social Vices Prevention (DSVP) remains common. As of April 2020, there were 97 public drug detoxification facilities, treating 34,982 patients, and only 16 organization and individual-based certified voluntary

drug detoxification facilities countrywide[357]. To achieve the 2030 WHO viral hepatitis elimination goals, EASL recommends that all barriers to the uptake of healthcare services by PWID be removed by changing policies and discrimination that hinder access. This includes the decriminalisation of minor, non-violent drug offences and the adoption of an approach based on public health promotion, respect for human rights and evidence[358].

Until PWID have equitable access to screening and treatment in Vietnam, HCV prevalence will remain high in this population. A major effectiveness-implementation trial evaluating an integrated model of HCV care for PWID has been underway in Hai Phong since 2020[191]. The model comprises large community-based mass screening, simplified treatment with SOF/DCV and major involvement of community-based organisations which reach out, link marginalised PWID to care, supervise treatment adherence and implement measures to prevent reinfection. Results are expected in 2023 and could have a major impact on policy.

My analysis of HCV genotype prevalence revealed a high prevalence of subtypes 1a, 1b, 6a and 6e but very few genotype 3 infections. Given the increasing evidence that genotype 6 treatment outcomes appear to be broadly similar to those of other non-3 genotypes, this finding supports my recommendation in chapter 4 that genotyping is not necessary in Vietnam, as it would seldom influence choice or duration of therapy.

With regards to Hepatitis D, my major finding was a lack of data. Given the high burden of HBV, there is almost certainly a high burden of HDV in Vietnam, though probably not on the scale seen in Mongolia[221], where HDV co-infection (as opposed to superinfection) is endemic. HDV is not screened for in public hospitals in Vietnam and remains absent from treatment guidelines and the MOH National Action Plan for viral hepatitis, published in 2021[193]. As new therapies (and therapeutic strategies) emerge, HDV screening should be incorporated into clinical practice. Well-designed studies (that are not restricted to individuals with liver disease) are warranted to clarify the burden of HDV. However, this will remain a low priority until affordable therapeutics become more widely available.

Chapter 3: SEARCH study stratum A (mild liver disease)

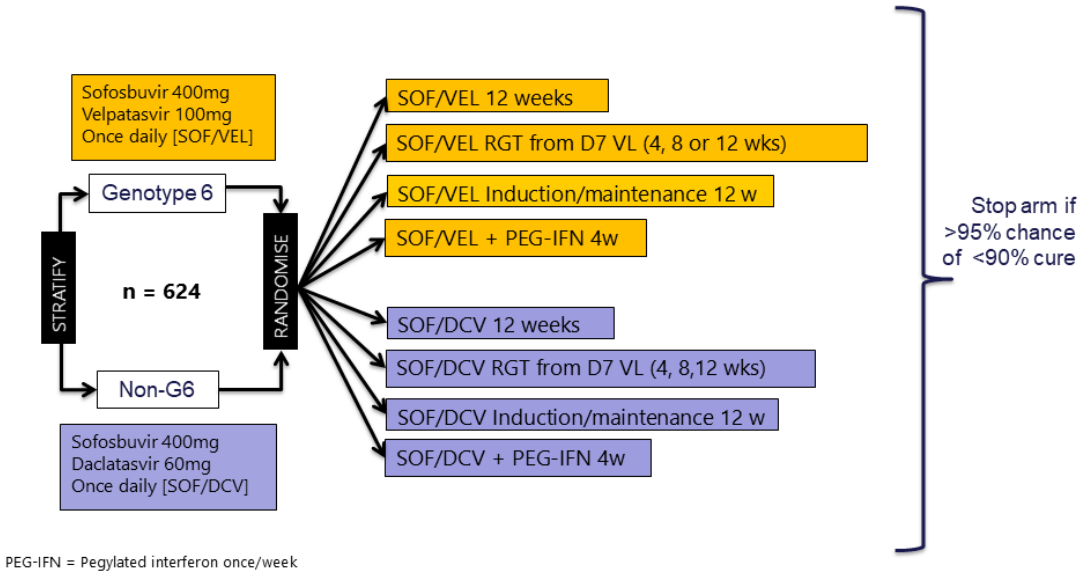
At a superficial level, the mechanistic pilot study described in chapter 3 provided a negative result: day 2 virological response does not adequately predict SVR12 with 4 weeks SOF/DCV treatment. We therefore could not accept the alternative hypothesis that high rates of cure are achieved with this approach. The main evidence on which this hypothesis was based came from a study in which 18 patients with genotype 1b infection were treated with ultra-short triple-class DAA therapy[173]. Given that HCV protease inhibitors are not widely available in most countries, and that the WHO now recommends forgoing genotyping before first-line treatment, it was rational to test the reliability of day 2 virological response with dual SOF/DCV therapy in patients infected with genotypes other than 1b.

Although day 2 virological response with SOF/DCV <500 IU/ml does not predict cure with 4 weeks treatment, early on-treatment virological response may still be a useful predictor of response to shortened therapy. In our study, participants who achieved SVR12 with 4 weeks treatment were more likely to have a day 7 HCV RNA < LLOQ than those who experienced treatment failure. However, this result did not reach statistical significance (p=0.054). A recent paper, published in May 2022, re-analysed data from a mathematical modelling-based response guided therapy study described in chapter 1[298]. The original study used HCV RNA trajectory from days 0, 2, 7, 14 and 28 to determine treatment duration. At reanalysis, they found day 7 HCV RNA was integral to predicting time-to-cure, whereas day 2 and day 28 were less helpful[359]. A possible explanation for the importance of day 7 viral load is that this constitutes the final phase of viral decline on DAA therapy (i.e. the second phase in the biphasic model), whereas day 2 could be part of a transient phase that precedes the final phase[360]. By day 28 most patients have HCV RNA <LLOQ and those that don't tend to have low level viraemia that likely represents low levels of non-infectious virions which are eventually cleared[302].

The optimal way to evaluate day 7 response guided therapy is in a randomised controlled trial (RCT). In 2020 we commenced recruitment for a multi-arm factorial RCT with adaptive design ('VIETNARMS'; trial registration: ISRCTN61522291) in HCMC and Hanoi. The trial is evaluating three treatment shortening strategies for HCV infection (versus standard care), one of which is day 7 RGT[303] (figure 6-1). In the RGT arms, participants with day 7 HCV RNA <LLOQ receive 4 weeks SOF/VEL or SOF/DCV, those with day 7 HCV RNA > LLOQ

but <250 IU/ml get 8 weeks and those >250 IU/ml receive 12 weeks. The study will complete recruitment in March 2023 and results will be shared in 2024.

Figure 6-1: VIETNARMS trial schema



SOF = sofosbuvir, DCV = daclatasvir, RGT = response guided therapy, VL = viral load, induction = 7days/week, maintenance = weekends off therapy

While results from the pilot study described in chapter 3 were disappointing in terms of the primary endpoint, it was encouraging that 100% (13/13) participants who did not achieve SVR12 were cured with 12 weeks retreatment (with the same DAA combination). It is also reassuring that the high number of putative NS5A mutations we identified were not clinically relevant. These findings support the notion that, unlike in other infections, there may be minimal risk in not eliminating HCV at the first attempt. Given that most patients with mild disease cure with treatment courses shorter than 12 weeks, there is a case for reducing standard duration of treatment to 8 weeks (irrespective of early virological response) and retreating the small increment in patients who fail to achieve SVR with the same DAAs. This approach would not entail additional monitoring and could dramatically reduce treatment costs, particularly in regions where DAA prices remain high. Testing the efficacy of 8 weeks SOF/DCV in patients with mild liver disease (with 12 weeks retreatment if required), with a minimal monitoring approach, would be straightforward and ethically sound and warrants

investigation. A health economics evaluation of potential cost savings, and how things might change as DAA prices decline, would also augment this research.

The SEARCH study was not powered to detect differences between cures and treatment failures with respect to host factors, viral factors, or drug levels. However, it is noteworthy that we found no evidence that any of the factors we evaluated were different in individuals who achieved SVR with 4 weeks SOF/DCV compared to those who did not. The fact that two individuals who cured with 4 weeks therapy still had HCV RNA > LLOQ at EOT implies that speed of elimination of HCV RNA from the blood is not the key determinant of outcome. If baseline virus levels, day 2 virological response, resistance mutations and drug levels are not key determinants of SVR after shortened therapy, it is worth considering what alternative mechanisms may be relevant.

As outlined in chapter 1, DAA's swiftly eliminate HCV antigen from blood, resulting in down-regulation of PD-1, and other inhibitory receptors in the liver. This leads to a rapid restoration of virus-specific CD8+ T cell function, which is known to be integral in spontaneous clearance of HCV. Romani et al evaluated T cell responses in 13 individuals who achieved SVR with 4 weeks triple therapy (NS5B-i/NS5A-i/PI) compared with 13 who did not achieve SVR[182]. They found that PD-1+ virus-specific CD8+ T cell subsets with cytotoxic capacity were present in a subset of chronic HCV-infected individuals at baseline and end of treatment, and this was associated with ability to achieve SVR with shortened treatment. The study involved flow cytometry analyses on thawed peripheral blood mononuclear cells and phenotyping was performed using monoclonal antibodies to assess the expression of multiple T cell lineage, activation and inhibitory receptors. They generated receiver operating characteristics curves for various CD4 and CD8 T cells with co-expressed inhibitory receptors, at both baseline and EOT. Some immune markers were promising for discriminating patients that would cure with 4 weeks treatment. Although this methodology is cumbersome and clearly not clinically practical for determining treatment duration, it indicates an important role of immunity in DAA mediated viral clearance after shortened therapy. One theory to have arisen from this is that immunomodulatory therapy alongside short-course DAA treatment might help achieve high rates of cure. In a small pilot study in Denmark, high rates of cure were achieved when PEG-IFN therapy was given alongside SOF/LDV and ribavirin for just 4 weeks[361]. In the VIETNARMS trial we are testing whether high rates of cure can be achieved with 4 weeks SOF/DCV or SOF/VEL when it is given

alongside 4 weeks of PEGIFN therapy. Should this ultrashort therapy with DAA + PEGIFN prove non-inferior to standard duration DAA therapy, questions will remain over whether this approach is practical, economical, or indeed ethical – given the side effects associated with interferon treatment. However, proof of concept would suggest that shorter treatment durations are achievable with combined DAA + immunomodulatory treatment which may be lead to investigation of more practical approaches that could be appropriate in some situations.

Rejuvenation of T cell responses following DAA-mediated elimination of viral antigen might also explain why retreatment after shortened therapy is highly effective[176,362]. It is plausible that patients recover some degree of immune function with unsuccessful first-line treatment, which helps permanently clear the virus when they are re-treated[181].

Chapter 4: SEARCH study stratum B (compensated cirrhosis)

In chapter 4 I showed that high rates of cure are achieved with 12 weeks SOF/DCV in genotype 6-infected individuals with compensated liver cirrhosis and advanced liver fibrosis. Since publication of our trial, and a separate large real-world study from Cambodia[317] (which also reported high rates of cure in genotype 6 infection with 12 weeks SOF/DCV), genotype 6 treatment guidelines have been updated. The Vietnam MoH stopped recommending addition of ribavirin to 12 weeks therapy and removed its recommendation for 24 weeks of therapy in individuals who cannot take ribavirin.

One of the major contributions this study made was to show that despite considerable genetic variation in genotype 6, with a high frequency of putative resistance-associated substitutions, outcomes with dual NS5B-i/NS5A-i therapy are very good and likely equivalent to those seen with other non-genotype 3 infections. There is therefore little reason to perform genotyping where it won't affect choice of therapy. Although this is consistent with WHO guidelines, when our data was presented to the Hospital for Tropical Disease they asked us to remove this recommendation from the report. Genotyping is no longer recommended as part of routine care by the Ministry of Health but remains part of the hospitals' standard of care and is a lucrative part of HCV treatment. I make this point to illustrate that policy change requires

engagement from multiple stakeholders in the public and private sector and potential cost-saving strategies should not be assumed to be welcomed by those who may stand to lose out financially in the short-term.

Chapter 5: ALT and AST analysis

My diagnostic accuracy study regarding changes in ALT levels after DAA therapy provides promising proof of concept for a strategy that could bring significant cost-saving and simply HCV care in remote regions. However as discussed in the limitations section of chapter 5, further evaluation is required in patients with cirrhosis and individuals treated with standard duration therapy, and to see if results are replicated with alternative ALT and AST platforms.

I am currently in discussions with the Infectious Disease Data Observatory (IDDO) regarding a potential systematic review of DAA studies with ALT data, and meta-analysis of ΔALT and ΔAST performance using the IDDO framework. This could provide more robust estimates of the accuracy of these biomarkers in detecting treatment failure, and might help better define a cut-off that preserves sensitivity but improves specificity.

Even if ΔALT proves a highly sensitive screen for treatment failure on alternative LFT plaforms and in patients with cirrhosis, ΔALT will not replace PCR testing, which ultimately defines SVR. In the long-term, we should still be striving for improved point-of-care HCV RNA testing in all settings. However, ALT monitoring may enable more patients to access HCV treatment until access to PCR testing is universally available.

The Big Picture

From 2014-2018 Hepatitis C treatment was arguably the most dynamic area of research in clinical medicine. When the SEARCH study was conceived, the exorbitant price of novel antiviral drugs meant that there was an urgent need to find ways to reduce costs and expand access to treatment with the cheapest and most widely available antivirals. There was legitimate concern regarding the potential for antiviral resistance, doubts over the efficacy of

pangenotypic DAAs in understudied genotypes, and uncertainty over drug tolerability and its impact on adherence.

Four years on, the scientific environment has changed: there is good evidence that DAAs are safe and well-tolerated, such that 8-12 weeks of treatment is acceptable for most individuals. Drug resistance has not presented a major problem, thanks to sofosbuvir's high barrier to resistance and the ability to administer fixed dose combination therapy. The advent of pangenotypic regimens has diminished the importance of viral genotypes and subtypes. Consequently, the personalised medicine that characterised the era of PEG-IFN therapy has been replaced by a drive for pragmatic and decentralised care. The most important studies in Hepatitis C since 2019 have been those that address these goals. The MINMON study, published in 2022 by Solomon et al, showed that a minimal monitoring approach was highly effective in both high- and low-income settings. Participants treated with 12 weeks SOF/VEL achieved excellent rates of cure without a need for pre-treatment genotyping, staggered drug dispensing, multiple scheduled visits, or laboratory monitoring. In the updated 2022 WHO guidelines, it is recommended that testing and treatment for HCV is delivered at peripheral health or community-based facilities, ideally at the same time. WHO advise that such services are integrated into existing care models (e.g. primary care, harm reduction facilities, and HIV services) and that testing, care, and treatment be delivered by trained non-specialists (e.g. primary care physicians or nurses). In this context, it is hard to foresee a lasting role of early on-treatment viral load testing which would increase both the cost and expertise required to treat HCV.

With respect to eliminating HCV in individuals under-served by existing models of care, long-acting antiviral injections could be crucial. Injectable drugs have proved highly effective for HIV[363] but there are currently no prospects of injectable antivirals for HCV. There has been one published study reporting efficacy for a retrievable coil-shaped long-acting DAA system in swine, compatible with nasogastric tube administration, with capacity to encapsulate and release gram levels of drugs while resident in the stomach[364]. However, cost and practicality of this approach are likely limited.

An HCV vaccine would also significantly reduce the global burden of HCV-associated disease, especially given that HCV cure by direct-acting antivirals does not lead to a complete reversion of T cell exhaustion and thus HCV reinfections still occur[97]. Barriers to HCV

vaccine development include virus diversity, limited models for testing vaccines, and an incomplete understanding of the protective immune responses[365]. Overcoming these challenges may be necessary for global control of HCV.

For Vietnam and other resource-constrained, high burden countries, the foremost barriers to HCV elimination in the short to medium term are financing and political leadership. Rwanda has shown how integrating HCV programs into existing services can help maximize effectiveness of public health spending. Rwanda was one of the first low-income countries to establish a national viral hepatitis control programme in 2012, and in 2018 launched a bold five-year HCV elimination plan with strong high-level governmental endorsement[366]. Its elimination strategy was carefully costed by integrating HCV testing and treatment services into the existing HIV response infrastructure, using its primary health care services to deliver hepatitis care (which was covered by its existing health insurance scheme), and procuring generic DAAs through voluntary licenses for as little as $60 per treatment course[188]. External funding from international donors also helped subsidise treatment for population categories not covered by insurance[9].

Vietnam is one of the top five fastest growing economies in the world and has potential to follow Rwanda's example. In recent years the government has channelled increasing capital into its viral hepatitis response: a new national action plan for 2021-2025 is funding improved surveillance and screening, promoting HBV vaccination, optimizing healthcare safety and implementing harm reduction services[193]. Vietnam's laudable response to the COVID-19 pandemic[367] - initially through successful surveillance and non-pharmaceutical interventions and latterly through comprehensive vaccine roll-out - has also demonstrated what is achievable with coordinated leadership and political will. Elimination of viral hepatitis remains some way off, but there are plenty of reasons for optimism.

Chapter 7

Dissemination of research

Chapter 2

My systematic review and meta-analysis of HBV, HCV and HDV seroprevalence studies in Vietnam was published in The Lancet Regional Health: Western Pacific in July 2022. https://www.thelancet.com/journals/lanwpc/article/PIIS2666-6065(22)00083-9/fulltext

I have presented the data at the annual SEARCH (South East Asia Research Collaborative for Hepatitis) meeting in Ho Chi Minh City. Professor Pham Minh Khue, who reviewed and contributed to the manuscript prior to publication, is a professor of public health at Haiphong University of Medicine and Pharmacy, and works closely with the Vietnam Ministry of Health. He has shared this data with health policy makers to maximise its impact.

Chapter 3

A manuscript titled *'Efficacy of ultra-short, response-guided sofosbuvir and daclatasvir therapy for HCV: a single arm mechanistic pilot study'* was submitted to eLife in July 2022 and simultaneously uploaded to the preprint server medRxiv. It underwent open peer review in October 2022 which is available to read here:

https://www.medrxiv.org/content/10.1101/2022.08.15.22278752v1

It has been provisionally accepted for publication and will be published in 2023.

The results of the study were shared with Vietnamese clinicians and scientists in an oral presentation at the Hospital for Tropical Disease National Hepatitis Conference, HCMC, July 2022. A poster describing the pharmacokinetic data was presented at the American Society of Tropical Medicine and Hygiene Conference in Seattle, USA, in October 2022.

Chapter 4

Stratum B of the SEARCH study was published in *Open Forum Infectious Disease* in July 2021.

https://academic.oup.com/ofid/article/8/7/ofab267/6295331

The results were shared with Vietnamese clinicians and scientists in an oral presentation at the Hospital for Tropical Disease National Hepatitis Conference, HCMC, July 2022.

Chapter 5

The transaminase study described in chapter 5 was submitted as an abstract for ID Week 2022 in Washington DC and awarded a Guerrant international travel grant. I presented the work as a poster at ID Week in October 2022. A copy of the poster is shown on the next page.

At ID Week I was invited by the editor of Clinical Liver Disease to submit a short report for a series on Innovations in Hepatitis Elimination. The manuscript has been accepted and is currently with editorial staff.

The work will be presented to Vietnamese clinicians at the SEARCH investigators annual meeting in December 2022.

Figure 7-1: Poster presented at ID Week, Washington DC, October 2022

Rise in ALT after HCV treatment is a highly sensitive screen for treatment failure: a diagnostic accuracy study

Barnaby Flower[1,2], Phuong Nguyen Thi Ngoc[1], Leanne McCabe[3], Chau Le Ngoc[1], Thu Vo Thi[1], Hang Vu Thi Kim[1], Thuan Dang Trong[1], Motiur Rahman[1,4], Guy Thwaites[1,4], Ann Sarah Walker[3,5,6], Le Manh Hung[7], Nguyen Van Vinh Chau[7], Graham S Cooke[Ψ2], Jeremy Day[Ψ1,4], on behalf of SEARCH & STOP-HCV investigators.

Ψ Contributed equally

Background

Nucleic acid testing to confirm cure after HCV therapy is expensive and requires technical expertise frequently unavailable in settings where disease prevalence is highest.

We investigated monitoring levels of ALT and AST in blood to identify treatment success in two independent treatment shortening trials reporting high rates of treatment failure.

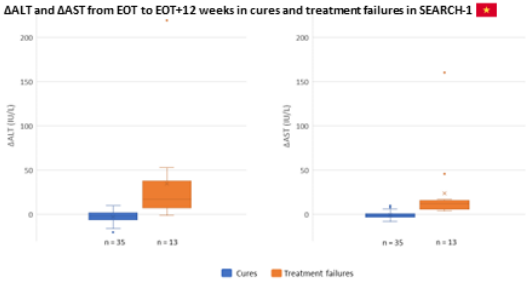
Methods

Using data from a short-course treatment trial in Vietnam (4-8 weeks treatment; n=52) which reported SVR rates of 75%, we describe how changes in ALT (ΔALT) and AST (ΔAST) from end of treatment (EOT) to EOT+12 weeks compared in individuals who cure (HCV RNA <LLOQ 12 weeks after EOT) with those that experience treatment failure.

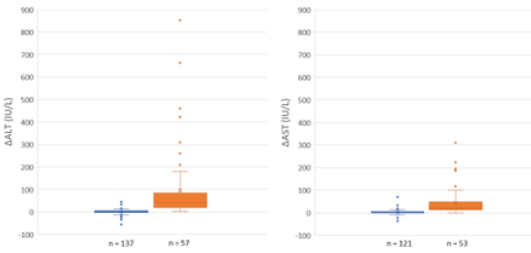
In a separate UK treatment shortening trial (n=202), which reported SVR rates of 71%, we tested the hypothesis that *any* elevation in ALT or AST between EOT and EOT12 is a sensitive marker of treatment failure.

Data

ΔALT and ΔAST from EOT to EOT+12 weeks in cures and treatment failures in SEARCH-1



ΔALT and ΔAST from EOT to EOT+12 weeks in cures and treatment failures in STOPHCV1



	SEARCH-1	STOPHCV1
N (available data)	48	196
Cures	35	139
Treatment failures	13	57
Median ΔALT (IQR) Cures	-2 IU/L (-5.5, +1.5)	0 IU/L (-2, +5)
Median ΔALT (IQR) Failures	+17 IU/L (+9, +38)	+41 IU/L (+20, +85)
Sensitivity (95% C.I.)	92% (64 – 99.8)	100% (93.7, 100)
Specificity (95% C.I.)	66% (48 - 81)	51.1% (42.4, 59.7)
AUROC (95% C.I.)	0.95 (0.87-1.00)	0.96 (0.94 -0.99)
Median ΔAST (IQR) Cures	-1 IU/L (-3, +1)	+2 IU/L (-1, +5)
Median ΔAST (IQR) Failures	+12 IU/L (+7, +15)	+23 IU/L (+13, +49)
Sensitivity (95% C.I.)	100% (75 - 100)	98.1% (89.9, 99.9)
Specificity (95% C.I.)	66% (48 - 81)	35.8% (27.3, 45.1)
AUROC (95% C.I.)	0.96 (0.91 - 1.00)	0.92 (0.88 - 0.96)

Results

In SEARCH-1 (Vietnam), among 48 individuals with data, 13 failed to achieve cure with 4-8 weeks sofosbuvir + daclatasvir.

ΔALT and ΔAST were higher in treatment failures than cures [median ΔALT (IQR):+17 IU/L (+7.5, +38) vs -2 IU/L (-6, +2)] (p< 0.001).

12/13 and 13/13 treatment failures had an increase in ALT and AST between EOT and EOT12, compared with 12/35 cures.

In STOPHCV1, 194/202 treated with 4-8wks ombitasvir, paritaprevir, ritonavir +/- dasabuvir had data, of which 57 failed to achieve cure.

ΔALT and ΔAST were again higher in treatment failures [median ΔALT (IQR):+41 IU/L (+20, +83) vs 0 IU/L (-2, +5)] (p<0.001)

Increase in ALT after EOT (ΔALT>0 IU/L) was
- **100% sensitive (95% C.I. [93.7 - 100])**
- **50% specific (95% C.I. [41.7 - 59.0])**

for detecting treatment failure.

ΔAST>0 IU/L performed similarly.

Interpretation

Rise in ALT after HCV therapy is a highly sensitive screen for treatment failure in patients with mild liver disease.

With rates of cure typical of standard duration therapy, this approach could half number of HCV RNA tests performed at EOT+12, reducing treatment costs and facilitating decentralisation of care in remote and resource-limited settings.

Chapter 8

COVID-19 impact statement

Four months after registering my PhD, the first cases of SARSCoV2 were reported in Wuhan, China. Ho Chi Minh City was one of the first places to report human-to-human transmission[368], and by March 2020, widespread emergency public health measures had been implemented to supress the outbreak. This included enforced quarantine of newly diagnosed cases (and their close contacts) in centralised facilities, a stay-at-home order, temporary suspension of outpatient services at the HTD, and closure of the international border. Meanwhile, as the scale of the outbreak in Europe became apparent, the UK government requested all doctors mobilise from extra-clinical commitments to assist the COVID-19 response. I returned to the UK on 23rd March 2020, as the country entered its first lockdown.

Impact of COVID-19 on the SEARCH study

Recruitment for SEARCH stratum A (chapter 3) and stratum B (chapter 4) was stopped early on account of the COVID-19 outbreak, with 52/60 (87%) and 41/43 (95%) of the target study populations enrolled. To navigate travel restrictions and the reduction in hospital outpatient services, we adapted trial follow up. This ensured that participants who were already enrolled attended the minimum number of visits required to establish primary endpoints, without compromising patient safety. Our strategy was broadly successful and, as discussed in chapters 3 and 4, early cessation of recruitment did not significantly compromise trial findings.

Impact of COVID-19 on thesis and other research

As well causing millions of deaths and wrecking most economies, COVID-19 has been a great disruptor of plans! International border closures, difficulties acquiring travel documents for ‘pandemic babies’, and lengthy military-led lockdowns meant this thesis was composed in various unanticipated locations, including Skien public library in Norway, my parents’ spare bedroom in Surrey and quarantine hotels in Oslo, HCMC and Cần Giờ. But while my family and I have had to relocate at short notice on a few occasions, we have been lucky to avoid serious ill health or family tragedy and

have benefited from the Wellcome Trust's financial support and colleague's adaptability and understanding of our changing circumstances.

The pandemic provided me an opportunity to be involved in vital clinical research which proceeded at unprecedented speed. At Imperial Hospitals I worked as a research fellow on the RECOVERY, COVACTA and REMAP-CAP clinical trials. I co-wrote a protocol for a SARSCoV2 diagnostics study and helped deliver REACT-2 (Real-time Assessment of Community Transmission-2), a series of studies funded by the Department of Health and Social Care assessing SARSCoV2 antibody tests to see how accurate they are and how easily people can use them at home. REACT-2 ultimately provided population level data regarding SARSCoV2 exposure and durability of antibody responses to infection and vaccination. Our data was used to guide government policy.

My involvement with REACT-2 led to requests to review other SARSCoV2 antibody seroprevalence studies and I was subsequently invited to write commentaries for The Lancet, The Lancet Regional Health Western Pacific and South East Asia Forum. The pandemic allowed me to collaborate with several talented clinical trialists, epidemiologists, statisticians, and basic scientists, from who I learned a huge amount. A large part of my development as a researcher is directly attributable to the dynamic scientific environment created by the emergence of COVID-19 in the first year of my PhD.

Summary of COVID-19 related work during my PhD

1. Flower B, Brown JC, Simmons B, et al. Clinical and laboratory evaluation of SARS-CoV-2 lateral flow assays for use in a national COVID-19 seroprevalence survey. Thorax. August 2020:thoraxjnl-2020-215732. doi:10.1136/thoraxjnl-2020-215732

2. Flower B, Atchison C. SARS-CoV-2 antibody seroprevalence in patients receiving dialysis in the USA. Lancet. 2020;396(10259):1308-1309. doi:10.1016/S0140-6736(20)32006-7

3. Flower B, Marks M. Did Laos really control the transmission of SARS-CoV-2 in 2020?. Lancet Reg Health West Pac. 2021;13:100202. doi:10.1016/j.lanwpc.2021.100202

4. Flower B, Delta variant sets off alarm bells in Vietnam, East Asia Forum, 01 August 2021, https://www.eastasiaforum.org/2021/08/01/delta-variant-sets-off-alarm-bells-in-vietnam/

5. Gibani MM, Toumazou C, Flower B, et al. Assessing a novel, lab-free, point-of-care test for SARS-CoV-2 (CovidNudge): a diagnostic accuracy study [published correction appears in Lancet Microbe. 2020 Nov;1(7):e280]. Lancet Microbe. 2020;1(7):e300-e307. doi:10.1016/S2666-5247(20)30121-X

6. Atchison C, Pristerà P, Flower B, et al. Usability and Acceptability of Home-based Self-testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibodies for Population Surveillance. Clin Infect Dis. 2021;72(9):e384-e393. doi:10.1093/cid/ciaa1178

7. Moshe M, Daunt A, Flower B, et al. SARS-CoV-2 lateral flow assays for possible use in national covid-19 seroprevalence surveys (React 2): diagnostic accuracy study. BMJ. 2021;372:n423. Published 2021 Mar 2. doi:10.1136/bmj.n423

8. Ward H, Cooke GS, Flower B, et al. Prevalence of antibody positivity to SARS-CoV-2 following the first peak of infection in England: Serial cross-sectional studies of 365,000 adults. Lancet Reg Health Eur. 2021;4:100098. doi:10.1016/j.lanepe.2021.100098

9. Ward H, Flower B, Garcia PJ, et al. Global surveillance, research, and collaboration needed to improve understanding and management of long COVID [published online ahead of print, 2021 Nov 10]. Lancet. 2021;S0140-6736(21)02444-2. doi:10.1016/S0140-6736(21)02444-2

10. Rosadas C, Khan M, Flower B et al Detection and quantification of antibody to SARS CoV 2 receptor binding domain provides enhanced sensitivity, specificity and utility. J Virol Methods. 2022 Apr;302:114475. doi: 10.1016/j.jviromet.2022.114475. Epub 2022 Jan 22. PMID: 35077719; PMCID: PMC8782753.

11. Ward H, Whitaker M, Flower B, et al. Population antibody responses following COVID-19 vaccination in 212,102 individuals. Nat Commun. 2022 Feb 16;13(1):907. doi: 10.1038/s41467-022-28527-x. PMID: 35173150; PMCID: PMC8850615.

12. Khan M, Rosadas C, Flower B, et al. Simple, sensitive, specific self-sampling assay secures SARS-CoV-2 antibody signals in sero-prevalence and post-vaccine studies. Sci Rep. 2022 Feb 3;12(1):1885. doi: 10.1038/s41598-022-05640-x. PMID: 35115570; PMCID: PMC8814240.

Bibliography

1. Rao VB, Johari N, du Cros P, Messina J, Ford N, Cooke GS. Hepatitis C seroprevalence and HIV co-infection in sub-Saharan Africa: A systematic review and meta-analysis. *Lancet Infect Dis*. 2015;15(7):819-824. doi:10.1016/S1473-3099(15)00006-7

2. WHO. *Hepatitis C Fact Sheet*.; 2021. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c. Accessed December 8, 2021.

3. World Health Organization (WHO). International Newsroom Meningitis Factsheet. https://www.who.int/news-room/fact-sheets/detail/meningitis. Published 2021.

4. Institute for Health Metrics and Evaluation (IHME). *Global Burden of Disease 2019 - Total Burden Related to Hepatitis C — Cause*.; 2019. https://www.healthdata.org/results/gbd_summaries/2019/total-burden-related-to-hepatitis-c-level-3-cause#:~:text=Summary Hepatitis C resulted in,due to hepatitis C%2C respectively.

5. Spearman CW, Dusheiko GM, Hellard M, Sonderup M. Hepatitis C. *Lancet*. 2019;394(10207):1451-1466. doi:10.1016/S0140-6736(19)32320-7

6. Grebely J, Page K, Sacks-Davis R, et al. The effects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. *Hepatology*. 2014;59(1):109-120. doi:10.1002/hep.26639

7. Foster GR. Quality of life considerations for patients with chronic hepatitis C. *J Viral Hepat*. 2009;16(9):605-611. doi:https://doi.org/10.1111/j.1365-2893.2009.01154.x

8. (WHO) WHO. *GLOBAL HEALTH SECTOR STRATEGY ON VIRAL HEPATITIS 2016–2021 TOWARDS ENDING VIRAL HEPATITIS*.; 2016.

9. DNDi (Drugs for Neglected Diseases Initiative). *The Opportunity to Eliminate Hepatitis c through Alternative Financing Mechanisms*.; 2022.

10. World Bank. *World Bank in Vietnam: Overview*.; 2021. https://www.worldbank.org/en/country/vietnam/overview#1.

11. Blach S, Zeuzem S, Manns M, et al. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol Hepatol*. 2017;2(3):161-176. doi:10.1016/S2468-1253(16)30181-9

12. Coalition for Global Hepatitis Elimination (A Programme of the Taskforce for Gloabl Health). Data dashboards - Viet Nam HBV & HCV. https://www.globalhep.org/country-progress/viet-nam. Published 2019.

13. Le Ngoc C, Tran Thi Thanh T, Tran Thi Lan P, et al. Differential prevalence and geographic distribution of hepatitis C virus genotypes in acute and chronic hepatitis C patients in Vietnam. *PLoS One*. 2019;14(3):e0212734. doi:10.1371/journal.pone.0212734

14. Pham VH, Nguyen HD, Ho PT, et al. Very high prevalence of hepatitis C virus genotype 6 variants in southern Vietnam: large-scale survey based on sequence determination. *Jpn J Infect Dis*. 2011;64(6):537-539.

15. Pham DA, Leuangwutiwong P, Jittmittraphap A, et al. High prevalence of Hepatitis C virus genotype 6 in Vietnam. *Asian Pac J Allergy Immunol*. 2009;27(2-3):153-160.

16. World Health Organization (WHO). *Guidelines for the Care and Treatment of Persons Diagnosed with Chronic Hepatitis C Virus Infection*.; 2018.

17. World Health Organization (WHO). *WHO Publishes Updated Guidance on Hepatitis C Infection – with New Recommendations on Treatment of Adolescents and Children, Simplified Service Delivery and Diagnostics*.; 2022. https://www.who.int/news/item/24-06-2022-WHO-publishes-updated-guidance-on-hepatitis-C-infection.

18. Forni D, Cagliani R, Pontremoli C, et al. Evolutionary Analysis Provides Insight Into the Origin and Adaptation of HCV . *Front Microbiol* . 2018;9. https://www.frontiersin.org/articles/10.3389/fmicb.2018.00854.

19. Quan P-L, Firth C, Conte JM, et al. Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proc Natl Acad Sci*. 2013;110(20):8194-8199. doi:10.1073/pnas.1303037110

20. Van Nguyen CuongAU - Bonsall, DavidAU - Ngo, Tue T.AU - Carrique-Mas, JuanAU - Pham, Anh H.AU - Bryant, Juliet E.AU - Thwaites, GuyAU - Baker, StephenAU - Woolhouse, MarkAU - Simmonds, PeterTI - Detection and Characterization of Homologues of Human Hepati D-VN. Detection and Characterization of Homologues of Human Hepatitis Viruses and Pegiviruses in Rodents and Bats in Vietnam. *Viruses*. 2018;10(3). doi:10.3390/v10030102

21. Pybus OG, Gray RR. The virus whose family expanded. *Nature*. 2013;498(7454):310-311. doi:10.1038/498310b

22. Pybus OG, Thézé J. Hepacivirus cross-species transmission and the origins of the hepatitis C virus. *Curr Opin Virol*. 2016;16:1-7. doi:https://doi.org/10.1016/j.coviro.2015.10.002

23. Simmonds P. The Origin of Hepatitis C Virus BT - Hepatitis C Virus: From Molecular Virology to Antiviral Therapy. In: Bartenschlager R, ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013:1-15. doi:10.1007/978-3-642-27340-7_1

24. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland P V. Transfusion-Associated Hepatitis Not Due to Viral Hepatitis Type A or B. *N Engl J Med*. 1975;292(15):767-770. doi:10.1056/NEJM197504102921502

25. Alter HJ, Prince AM. Workshop III: Transfusion-Associated Non-A, Non-B Hepatitis: An Assessment of the Causative Agent and Its Clinical Impact. *Transfus Med Rev*. 1988;2(4):288-293. doi:https://doi.org/10.1016/S0887-7963(88)70058-9

26. Alter H, Holland P, Purcell R, Popper H. TRANSMISSIBLE AGENT IN NON-A, NON-B HEPATITIS. *Lancet*. 1978;311(8062):459-463. doi:10.1016/S0140-6736(78)90131-9

27. Tabor E, Drucker J, Hoofnagle J, et al. TRANSMISSION OF NON-A, NON-B HEPATITIS FROM MAN TO CHIMPANZEE. *Lancet*. 1978;311(8062):463-466. doi:10.1016/S0140-6736(78)90132-0

28. Colombo M, Carnelli V, Gazengel C, Mannucci PM, Savidge GF, Schimpf K. TRANSMISSION OF NON-A, NON-B HEPATITIS BY HEAT-TREATED FACTOR VIII CONCENTRATE. *Lancet*. 1985;326(8445):1-4. doi:10.1016/S0140-6736(85)90055-8

29. Norkrans G, Frösner G, Hermodsson S, Iwarson S. Multiple Hepatitis Attacks in Drug Addicts. *JAMA*. 1980;243(10):1056-1058. doi:10.1001/jama.1980.03300360028019

30. Galbraith RM, Eddleston ALWF, Portmann B, Williams R, Gower PE. CHRONIC LIVER DISEASE DEVELOPING AFTER OUTBREAK OF HBsAg-NEGATIVE HEPATITIS IN HÆMODIALYSIS UNIT. *Lancet*. 1975;306(7941):886-890. doi:10.1016/S0140-6736(75)92126-1

31. Choo Q-L, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA cLone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome. *Science (80-)*.

1989;244(4902):359-362. doi:10.1126/science.2523562

32. Alter HJ, Purcell RH, Shih JW, et al. Detection of Antibody to Hepatitis C Virus in Prospectively Followed Transfusion Recipients with Acute and Chronic Non-A, Non-B Hepatitis. *N Engl J Med*. 1989;321(22):1494-1500. doi:10.1056/NEJM198911303212202

33. Simmonds P, Holmes EC, Cha T-A, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol*. 1993;74(11):2391-2399. doi:https://doi.org/10.1099/0022-1317-74-11-2391

34. Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. *Nature*. 2005;436(7053):933-938. doi:10.1038/nature04077

35. Lohmann V, Körner F, Koch J-O, Herian U, Theilmann L, Bartenschlager R. Replication of Subgenomic Hepatitis C Virus RNAs in a Hepatoma Cell Line. *Science (80-)*. 1999;285(5424):110-113. doi:10.1126/science.285.5424.110

36. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med*. 2005;11(7):791-796. doi:10.1038/nm1268

37. Hoofnagle JH, Feinstone SM. The Discovery of Hepatitis C — The 2020 Nobel Prize in Physiology or Medicine. *N Engl J Med*. 2020;383(24):2297-2299. doi:10.1056/NEJMp2031110

38. The Infected Blood Inquiry. Expert Report to the Infected Blood Inquiry : Hepatitis. 2020;(January).

39. Kuo G, Choo Q-L, Alter HJ, et al. An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-B Hepatitis. *Science (80-)*. 1989;244(4902):362-364. doi:10.1126/science.2496467

40. Japanese Red Cross Non-ANon-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. *Lancet*. 1991;338(8774):1040-1041. doi:10.1016/0140-6736(91)91901-6

41. Stramer SL, Glynn SA, Kleinman SH, et al. Detection of HIV-1 and HCV Infections among Antibody-Negative Blood Donors by Nucleic Acid–Amplification Testing. *N Engl J Med*. 2004;351(8):760-768. doi:10.1056/NEJMoa040085

42. Dunford L, Carr MJ, Dean J, et al. Hepatitis C virus in Vietnam: high prevalence of infection in dialysis and multi-transfused patients involving diverse and novel virus variants. *PLoS One*. 2012;7(8):e41266. doi:10.1371/journal.pone.0041266

43. Thursz M, Fontanet A. HCV transmission in industrialized countries and resource-constrained areas. *Nat Rev Gastroenterol Hepatol*. 2014;11(1):28-35. doi:10.1038/nrgastro.2013.179

44. Bruix J, Calvet X, Costa J, et al. PREVALENCE OF ANTIBODIES TO HEPATITIS C VIRUS IN SPANISH PATIENTS WITH HEPATOCELLULAR CARCINOMA AND HEPATIC CIRRHOSIS. *Lancet*. 1989;334(8670):1004-1006. doi:10.1016/S0140-6736(89)91015-5

45. Pereira BJG, Milford EL, Kirkman RL, Levey AS. Transmission of Hepatitis C Virus by Organ Transplantation. *N Engl J Med*. 1991;325(7):454-460. doi:10.1056/NEJM199108153250702

46. Esteban JI, Viladomiu L, Gonzalez A, et al. HEPATITIS C VIRUS ANTIBODIES AMONG RISK GROUPS IN SPAIN. *Lancet*. 1989;334(8658):294-297. doi:10.1016/S0140-6736(89)90485-6

47. Roggendorf M, Deinhardt F, Rasshofer R, et al. ANTIBODIES TO HEPATITIS C VIRUS. *Lancet*. 1989;334(8658):324-325. doi:10.1016/S0140-6736(89)90501-1

48. Platt L, Minozzi S, Reed J, et al. Needle and syringe programmes and opioid substitution therapy for preventing HCV transmission among people who inject drugs: findings from a Cochrane Review and meta-analysis. *Addiction*. 2018;113(3):545-563. doi:https://doi.org/10.1111/add.14012

49. O'Keefe D, Stoové M, Doyle J, Dietze P, Hellard M. Injecting drug use in low and middle-income countries: Opportunities to improve care and prevent harm. *J Viral Hepat*. 2017;24(9):714-724. doi:https://doi.org/10.1111/jvh.12741

50. Tohme RA, Holmberg SD. Is sexual contact a major mode of hepatitis C virus transmission? *Hepatology*. 2010;52(4):1497-1505. doi:https://doi.org/10.1002/hep.23808

51. Foster AL, Gaisa MM, Hijdra RM, et al. of HIV-infected Men Who Have Sex With Men. *Clin Infect Dis*. 2017;64(3):284-288. doi:10.1093/cid/ciw740

52. Witt MD, Seaberg EC, Darilay A, et al. Incident Hepatitis C Virus Infection in Men Who Have Sex With Men: A Prospective Cohort Analysis, 1984–2011. *Clin Infect Dis*. 2013;57(1):77-84. doi:10.1093/cid/cit197

53. Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical Transmission of Hepatitis C Virus: Systematic Review and Meta-analysis. *Clin Infect Dis*. 2014;59(6):765-773. doi:10.1093/cid/ciu447

54. Hauri AM, Armstrong GL HYJF. The global burden of disease attributable to contaminated injections given in health care settings. *Int J STD AIDS*. 2004;15(1):7-16. doi:10.1258/095646204322637182

55. Averbukh LD, Wu GY. Highlights for Dental Care as a Hepatitis C Risk Factor: A Review of Literature. *J Clin Transl Hepatol*. 2019;7(4):346-351. doi:10.14218/JCTH.2019.00023

56. Jafari S, Copes R, Baharlou S, Etminan M, Buxton J. Tattooing and the risk of transmission of hepatitis C: a systematic review and meta-analysis. *Int J Infect Dis*. 2010;14(11):e928-e940. doi:https://doi.org/10.1016/j.ijid.2010.03.019

57. Yang S, Wang D, Zhang Y, et al. Transmission of Hepatitis B and C Virus Infection Through Body Piercing: A Systematic Review and Meta-Analysis. *Medicine (Baltimore)*. 2015;94(47):e1893-e1893. doi:10.1097/MD.0000000000001893

58. El-Ghitany EM, Abdel Wahab MM, Abd El-Wahab EW, Hassouna S, Farghaly AG. A comprehensive hepatitis C virus risk factors meta-analysis (1989–2013); Do they differ in Egypt? *Liver Int*. 2015;35(2):489-501. doi:https://doi.org/10.1111/liv.12617

59. Frank C, Mohamed MK, Strickland GT, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet*. 2000;355(9207):887-891. doi:10.1016/S0140-6736(99)06527-7

60. Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology*. 2005;42(4):962-973. doi:https://doi.org/10.1002/hep.20819

61. Borgia SM, Hedskog C, Parhy B, et al. Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes. *J Infect Dis*. 2018;218(11):1722-1729. doi:10.1093/infdis/jiy401

62. Messina JP, Humphreys I, Flaxman A, et al. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*. 2015;61(1):77-87. doi:10.1002/hep.27259

63. Irekeola AA, Malek NA, Wada Y, Mustaffa N, Muhamad NI, Shueb RH. Prevalence of HCV genotypes and subtypes in Southeast Asia: A systematic review and meta-analysis. *PLoS One*. 2021;16(5):e0251673. https://doi.org/10.1371/journal.pone.0251673.

64. Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R TC. Hepatitis C virus genotype 7, a new genotype originating from central Africa. *J Clin Microbiol*. 2015;Mar;53(3):967-972. doi:10.1128/JCM.02831-14. Epub 2014 Dec 17.

65. Zhu YZ, Qian XJ, Zhao P QZ. How hepatitis C virus invades hepatocytes: the mystery of viral entry. *World J Gastroenterol*. 2014;Apr(7;20(13)):3457-3467. doi:10.3748/wjg.v20.i13.3457.

66. Abdel-Hakeem MS SN. Protective immunity against hepatitis C: many shades of gray. *Front Immunol*. 2014;Jun 16;(5:274). doi:10.3389/fimmu.2014.00274.

67. Sun J, Rajsbaum R YM. Immune and non-immune responses to hepatitis C virus infection. *World J Gastroenterol*. 2015;Oct 14(21(38)):10739-10748. doi:doi: 10.3748/wjg.v21.i38.10739

68. Seeff LB. Natural history of hepatitis C. *Hepatology*. 1997;26(S3):21S-28S. doi:https://doi.org/10.1002/hep.510260704

69. Loomba R, Rivera MM, McBurney R, et al. The natural history of acute hepatitis C: clinical presentation, laboratory findings and treatment outcomes. *Aliment Pharmacol Ther*. 2011;33(5):559-565. doi:https://doi.org/10.1111/j.1365-2036.2010.04549.x

70. Chu CM, Yeh CT, Liaw YF. Fulminant hepatic failure in acute hepatitis C: increased risk in chronic carriers of hepatitis B virus. *Gut*. 1999;45(4):613 LP - 617. doi:10.1136/gut.45.4.613

71. Lingala S, Ghany MG. Natural History of Hepatitis C. *Gastroenterol Clin North Am*. 2015;44(4):717-734. doi:https://doi.org/10.1016/j.gtc.2015.07.003

72. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature*. 2009;461(7265):798-801. doi:10.1038/nature08463

73. Le Ngoc C, Tran Thi Thanh T, Tran Thi Lan P, et al. Differential prevalence and geographic distribution of hepatitis C virus genotypes in acute and chronic hepatitis C patients in Vietnam. *PLoS One*. 2019;14(3):e0212734. doi:10.1371/journal.pone.0212734

74. Vogt M, Lang T, Frösner G, et al. Prevalence and Clinical Outcome of Hepatitis C Infection in Children Who Underwent Cardiac Surgery before the Implementation of Blood-Donor Screening. *N Engl J Med*. 1999;341(12):866-870. doi:10.1056/NEJM199909163411202

75. Gerlach JT, Diepolder HM, Zachoval R, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance[1] . *Gastroenterology*. 2003;125(1):80-88. doi:10.1016/S0016-5085(03)00668-1

76. Beinhardt S, Payer BA, Datz C, et al. A diagnostic score for the prediction of spontaneous resolution of acute hepatitis C virus infection. *J Hepatol*. 2013;59(5):972-977. doi:https://doi.org/10.1016/j.jhep.2013.06.028

77. Kaplan M, Gawrieh S, Cotler SJ, Jensen DM. Neutralizing antibodies in hepatitis c virus infection: a review of immunological and clinical characteristics. *Gastroenterology*. 2003;125(2):597-604. doi:10.1016/S0016-5085(03)00882-5

78. Haberstroh A, Schnober EK, Zeisel MB, et al. Neutralizing Host Responses in Hepatitis C Virus Infection Target Viral Entry at Postbinding Steps and Membrane Fusion. *Gastroenterology*. 2008;135(5):1719-1728.e1. doi:10.1053/j.gastro.2008.07.018

79. Grakoui A, Shoukry NH, Woollard DJ, et al. HCV Persistence and Immune Evasion in the

Absence of Memory T Cell Help. *Science (80-)*. 2003;302(5645):659-662. doi:10.1126/science.1088774

80. Thimme R, Bukh J, Spangenberg HC, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci*. 2002;99(24):15661-15668.

81. Thein H, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology*. 2008;48(2):418-431.

82. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical Outcomes after Transfusion-Associated Hepatitis C. *N Engl J Med*. 1995;332(22):1463-1466. doi:10.1056/NEJM199506013322202

83. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet*. 1997;349(9055):825-832. doi:10.1016/S0140-6736(96)07642-8

84. Dienstag JL, Ghany MG, Morgan TR, et al. A prospective study of the rate of progression in compensated, histologically advanced chronic hepatitis C. *Hepatology*. 2011;54(2):396-405. doi:https://doi.org/10.1002/hep.24370

85. Lee M-H, Hsiao TI, Subramaniam SR, et al. HCV Genotype 6 Increased the Risk for Hepatocellular Carcinoma Among Asian Patients With Liver Cirrhosis. *Off J Am Coll Gastroenterol | ACG*. 2017;112(7). https://journals.lww.com/ajg/Fulltext/2017/07000/HCV_Genotype_6_Increased_the_Risk_for.24.aspx.

86. Spiegel BMR, Younossi ZM, Hays RD, Revicki D, Robbins S, Kanwal F. Impact of hepatitis C on health related quality of life: A systematic review and quantitative assessment. *Hepatology*. 2005;41(4):790-800. doi:https://doi.org/10.1002/hep.20659

87. Evon DM, Stewart PW, Amador J, et al. A comprehensive assessment of patient reported symptom burden, medical comorbidities, and functional well being in patients initiating direct acting antiviral therapy for chronic hepatitis C: Results from a large US multi-center observational study. *PLoS One*. 2018;13(8):e0196908. https://doi.org/10.1371/journal.pone.0196908.

88. Forton DM, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. *Lancet*. 2001;358(9275):38-39. doi:10.1016/S0140-6736(00)05270-3

89. Lee KK, Stelzle D, Bing R, et al. Global burden of atherosclerotic cardiovascular disease in people with hepatitis C virus infection: a systematic review, meta-analysis, and modelling study. *Lancet Gastroenterol Hepatol*. 2019;4(10):794-804. doi:10.1016/S2468-1253(19)30227-4

90. Negro F, Forton D, Craxì A, Sulkowski MS, Feld JJ, Manns MP. Extrahepatic Morbidity and Mortality of Chronic Hepatitis C. *Gastroenterology*. 2015;149(6):1345-1360. doi:https://doi.org/10.1053/j.gastro.2015.08.035

91. Cacoub P, Saadoun D. Extrahepatic Manifestations of Chronic HCV Infection. *N Engl J Med*. 2021;384(11):1038-1052. doi:10.1056/NEJMra2033539

92. Hyeon CK, Chung MN, Sun HJ, Kwang HH, Kyu Oh D, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. *BMJ*. 2004;328(7446):983. doi:10.1136/bmj.38050.593634.63

93. McCormick SE, Goodman ZD, Maydonovitch CL SM. Evaluation of liver histology, ALT elevation, and HCV RNA titer in patients with chronic hepatitis C. *Am J Gastroenterol*.

91(8):1516-1522.

94. Anand BS VM. Assessment of correlation between serum titers of hepatitis c virus and severity of liver disease. *World J Gastroenterol*. 2004;10(16):2409-2411. doi:10.3748/wjg.v10.i16.2409] Anand BS, Velez M. Assessment of correlation between serum titers of hepatitis c virus and severity of liver disease. World J Gastroenterol 2004; 10(16): 2409-2411 [PMID: 15285030 DOI: 10.3748/wjg.v10.i16.2409]

95. Barreiro P, Labarga P, Fernández-Montero J V, et al. Longitudinal changes in viral RNA concentration in patients with chronic hepatitis C and/or HIV infection in the absence of antiviral therapy. *J Clin Virol*. 2013;58(2):391-395. doi:https://doi.org/10.1016/j.jcv.2013.06.031

96. Sacks-Davis R, group on behalf of the I study, Grebely J, et al. Hepatitis C Virus Reinfection and Spontaneous Clearance of Reinfection—the InC3 Study. *J Infect Dis*. 2015;212(9):1407-1419. doi:10.1093/infdis/jiv220

97. Thimme R. T cell immunity to hepatitis C virus: Lessons for a prophylactic vaccine. *J Hepatol*. 2021;74(1):220-229. doi:10.1016/j.jhep.2020.09.022

98. Alfei F, Kanev K, Hofmann M, et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature*. 2019;571(7764):265-269. doi:10.1038/s41586-019-1326-9

99. Rossi C, Butt ZA, Wong S, et al. Hepatitis C virus reinfection after successful treatment with direct-acting antiviral therapy in a large population-based cohort. *J Hepatol*. 2018;69(5):1007-1014.

100. Houglum J. Interferon: mechanisms of action and clinical value. *Clin Pharm*. 1983;Jan-Feb(2(1)):20-8.

101. Hoofnagle JH, Mullen KD, Jones DB, et al. Treatment of Chronic Non-A, Non-B Hepatitis with Recombinant Human Alpha Interferon. *N Engl J Med*. 1986;315(25):1575-1578. doi:10.1056/NEJM198612183152503

102. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon Alfa-2b Alone or in Combination with Ribavirin as Initial Treatment for Chronic Hepatitis C. *N Engl J Med*. 1998;339(21):1485-1492. doi:10.1056/NEJM199811193392101

103. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*. 2001;358(9286):958-965. doi:10.1016/S0140-6736(01)06102-5

104. Fattovich G, Giustina G, Favarato S, Ruol A. A survey of adverse events in 11 241 patients with chronic viral hepatitis treated with alfa interferon. *J Hepatol*. 1996;24(1):38-47. doi:10.1016/S0168-8278(96)80184-X

105. Marcellin P, Lau GKK, Zeuzem S, et al. Comparing the safety, tolerability and quality of life in patients with chronic hepatitis B vs chronic hepatitis C treated with peginterferon alpha-2a. *Liver Int*. 2008;28(4):477-485. doi:https://doi.org/10.1111/j.1478-3231.2008.01696.x

106. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009;461(7262):399-401. doi:10.1038/nature08309

107. Ghany MG, Strader DB, Thomas DL SL. American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology*. 2009;Apr;49((4)):1335-1374. doi:doi: 10.1002/hep.22759

108. Fried MW, Hadziyannis SJ, Shiffman ML, Messinger D, Zeuzem S. Rapid virological

response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *J Hepatol*. 2011;55(1):69-75. doi:10.1016/j.jhep.2010.10.032

109. Mangia A, Santoro R, Minerva N, et al. Peginterferon Alfa-2b and Ribavirin for 12 vs. 24 Weeks in HCV Genotype 2 or 3. *N Engl J Med*. 2005;352(25):2609-2617. doi:10.1056/NEJMoa042608

110. Berg T, von Wagner M, Nasser S, et al. Extended Treatment Duration for Hepatitis C Virus Type 1: Comparing 48 Versus 72 Weeks of Peginterferon-Alfa-2a Plus Ribavirin. *Gastroenterology*. 2006;130(4):1086-1097. doi:10.1053/j.gastro.2006.02.015

111. Bartenschlager R, Ahlborn-Laake L, Mous J JH. Nonstructural protein 3 of the hepatitis C virus encodes a serine-type proteinase required for cleavage at the NS3/4 and NS4/5 junctions. *J Virol*. 1993;Jul;67((7)):3835-3844. doi:10.1128/JVI.67.7.3835-3844.1993

112. Behrens SE, Tomei L, De Francesco R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J*. 1996;15(1):12-22. doi:https://doi.org/10.1002/j.1460-2075.1996.tb00329.x

113. Poordad F, McCone J, Bacon BR, et al. Boceprevir for Untreated Chronic HCV Genotype 1 Infection. *N Engl J Med*. 2011;364(13):1195-1206. doi:10.1056/NEJMoa1010494

114. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for Previously Untreated Chronic Hepatitis C Virus Infection. *N Engl J Med*. 2011;364(25):2405-2416. doi:10.1056/NEJMoa1012912

115. Sarrazin C, Kieffer TL, Bartels D, et al. Dynamic Hepatitis C Virus Genotypic and Phenotypic Changes in Patients Treated With the Protease Inhibitor Telaprevir. *Gastroenterology*. 2007;132(5):1767-1777. doi:10.1053/j.gastro.2007.02.037

116. Maasoumy B, Manns MP. Optimal treatment with boceprevir for chronic HCV infection. *Liver Int*. 2013;33(s1):14-22. doi:https://doi.org/10.1111/liv.12070

117. Jesudian AB, Jacobson IM. Optimal treatment with telaprevir for chronic HCV infection. *Liver Int*. 2013;33(s1):3-13. doi:https://doi.org/10.1111/liv.12079

118. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for Previously Treated Chronic HCV Genotype 1 Infection. *N Engl J Med*. 2011;364(13):1207-1217. doi:10.1056/NEJMoa1009482

119. Colombo M, Fernández I, Abdurakhmanov D, et al. Safety and on-treatment efficacy of telaprevir: the early access programme for patients with advanced hepatitis C. *Gut*. 2014;63(7):1150 LP - 1158. doi:10.1136/gutjnl-2013-305667

120. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary Study of Two Antiviral Agents for Hepatitis C Genotype 1. *N Engl J Med*. 2012;366(3):216-224. doi:10.1056/NEJMoa1104430

121. Gilead Sciences. Gilead Sciences to Acquire Pharmasset, Inc. for $11 Billion.https://www.gilead.com/news-and-press/press-room/press-releases/2011/11/gilead-sciences-to-acquire-pharmasset-inc-for-11-billion. Published 2011.

122. Abraham GM, Spooner LM. Sofosbuvir in the Treatment of Chronic Hepatitis C: New Dog, New Tricks. *Clin Infect Dis*. 2014;59(3):411-415. doi:10.1093/cid/ciu265

123. Afdhal N, Reddy KR, Nelson DR, et al. Ledipasvir and Sofosbuvir for Previously Treated HCV Genotype 1 Infection. *N Engl J Med*. 2014;370(16):1483-1493. doi:10.1056/NEJMoa1316366

124. Afdhal N, Zeuzem S, Kwo P, et al. Ledipasvir and Sofosbuvir for Untreated HCV Genotype 1

Infection. *N Engl J Med*. 2014;370(20):1889-1898. doi:10.1056/NEJMoa1402454

125. Kowdley K V, Gordon SC, Reddy KR, et al. Ledipasvir and Sofosbuvir for 8 or 12 Weeks for Chronic HCV without Cirrhosis. *N Engl J Med*. 2014;370(20):1879-1888. doi:10.1056/NEJMoa1402355

126. AASLD. Treatment-Naive Genotype 1a Without Cirrhosis: American Association for the Study of Liver Diseases. https://www.hcvguidelines.org/treatment-naive/gt1a/no-cirrhosis. Published 2021. Accessed October 5, 2022.

127. O'Brien TR, Lang Kuhs KA, Pfeiffer RM. Subgroup Differences in Response to 8 Weeks of Ledipasvir/Sofosbuvir for Chronic Hepatitis C. *Open Forum Infect Dis*. 2014;1(3):ofu110. doi:10.1093/ofid/ofu110

128. Ferenci P, Bernstein D, Lalezari J, et al. ABT-450/r–Ombitasvir and Dasabuvir with or without Ribavirin for HCV. *N Engl J Med*. 2014;370(21):1983-1992. doi:10.1056/NEJMoa1402338

129. Wang S-J, Huang C-F, Yu M-L. Elbasvir and grazoprevir for the treatment of hepatitis C. *Expert Rev Anti Infect Ther*. 2021;19(9):1071-1081. doi:10.1080/14787210.2021.1874351

130. Marshall AD, Cunningham EB, Nielsen S, et al. Restrictions for reimbursement of interferon-free direct-acting antiviral drugs for HCV infection in Europe. *Lancet Gastroenterol Hepatol*. 2018;3(2):125-133. doi:https://doi.org/10.1016/S2468-1253(17)30284-4

131. Fathi H, Clark A, Hill NR, Dusheiko G. Effectiveness of current and future regimens for treating genotype 3 hepatitis C virus infection: a large-scale systematic review. *BMC Infect Dis*. 2017;17(1):722. doi:10.1186/s12879-017-2820-z

132. Foster GR, Afdhal N, Roberts SK, et al. Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection. *N Engl J Med*. 2015;373(27):2608-2617. doi:10.1056/NEJMoa1512612

133. Zeuzem S, Foster GR, Wang S, et al. Glecaprevir–Pibrentasvir for 8 or 12 Weeks in HCV Genotype 1 or 3 Infection. *N Engl J Med*. 2018;378(4):354-369. doi:10.1056/NEJMoa1702417

134. Pawlotsky J-M, Negro F, Aghemo A, et al. EASL recommendations on treatment of hepatitis C: Final update of the series☆ *J Hepatol*. 2020;73(5):1170-1218. doi:10.1016/j.jhep.2020.08.018

135. Ghany MG, Morgan TR, Panel A-IHCG. Hepatitis C Guidance 2019 Update: American Association for the Study of Liver Diseases–Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Hepatology*. 2020;71(2):686-721. doi:https://doi.org/10.1002/hep.31060

136. Wyles DL, Ruane PJ, Sulkowski MS, et al. Daclatasvir plus Sofosbuvir for HCV in Patients Coinfected with HIV-1. *N Engl J Med*. 2015;373(8):714-725. doi:10.1056/NEJMoa1503153

137. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*. 2015;61(4):1127-1135. doi:https://doi.org/10.1002/hep.27726

138. Hézode C, Lebray P, De Ledinghen V, et al. Daclatasvir plus sofosbuvir, with or without ribavirin, for hepatitis C virus genotype 3 in a French early access programme. *Liver Int Off J Int Assoc Study Liver*. 2017;37(9):1314-1324. doi:10.1111/liv.13383

139. Poordad F, Schiff ER, Vierling JM, et al. Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. *Hepatology*. 2016;63(5):1493-1505. doi:https://doi.org/10.1002/hep.28446

140. Schwartländer B, Grubb I, Perriëns J. The 10-year struggle to provide antiretroviral treatment to people with HIV in the developing world. *Lancet*. 2006;368(9534):541-546. doi:10.1016/S0140-6736(06)69164-2

141. Clinton Health Access Initiative. *Hepatitis C Market Report*.; 2020.

142. Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical practice. *J Hepatol*. 2016;64(2):486-504. doi:10.1016/j.jhep.2015.09.011

143. Feld JJ, Jacobson IM, Hézode C, et al. Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. *N Engl J Med*. 2015;373(27):2599-2607. doi:10.1056/NEJMoa1512610

144. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, et al. Daclatasvir plus Sofosbuvir for Previously Treated or Untreated Chronic HCV Infection. *N Engl J Med*. 2014;370(3):211-221. doi:10.1056/NEJMoa1306218

145. AASLD-IDSA. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. 2021. https://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/AASLD-IDSA_HCVGuidance_October_05_2021.pdf.

146. Gupta N, Mbituyumuremyi A, Kabahizi J, et al. Treatment of chronic hepatitis C virus infection in Rwanda with ledipasvir–sofosbuvir (SHARED): a single-arm trial. *Lancet Gastroenterol Hepatol*. 2019;4(2):119-126. doi:10.1016/S2468-1253(18)30382-0

147. Dietz J, Kalinina O V, Vermehren J, et al. Resistance-associated substitutions in patients with chronic hepatitis C virus genotype 4 infection. *J Viral Hepat*. 2020;27(10):974-986. doi:https://doi.org/10.1111/jvh.13322

148. Murphy DG, Willems B, Deschênes M, Hilzenrat N, Mousseau R, Sabbah S. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5' untranslated region sequences. *J Clin Microbiol*. 2007;45(4):1102—1112. doi:10.1128/jcm.02366-06

149. Hedskog C, Parhy B, Chang S, et al. Identification of 19 novel hepatitis C virus subtypes-further expanding HCV classification. *Open Forum Infect Dis*. 2019;6(3):1-9. doi:10.1093/ofid/ofz076

150. Fiona McPhee, a* Joseph Ueland, a Vincent Vellucci, a Scott Bowden, b William Sievert c NZ. Impact of Preexisting Hepatitis C Virus Genotype 6 NS3, NS5A, and NS5B Polymorphisms on the In Vitro Potency of Direct- Acting Antiviral Agents. *Antimicrob Agents Chemother*. 2019;(November 2018):1-12.

151. Hezode C, Reau N, Svarovskaia ES, et al. Resistance analysis in patients with genotype 1–6 HCV infection treated with sofosbuvir/velpatasvir in the phase III studies. *J Hepatol*. 2018;68(5):895-903. doi:10.1016/j.jhep.2017.11.032

152. Mettikanont P, Bunchorntavakul C, Reddy KR. Systematic review: epidemiology and response to direct-acting antiviral therapy in genotype 6 chronic hepatitis C virus infection. *Aliment Pharmacol Ther*. 2019;49(5):492-505. doi:10.1111/apt.15100

153. Lee SS, Abdo AA. Predicting antiviral treatment response in chronic hepatitis C: how accurate and how soon? *J Antimicrob Chemother*. 2003;51(3):487-491. doi:10.1093/jac/dkg135

154. Chung RT, Andersen J, Volberding P, et al. Peginterferon Alfa-2a plus Ribavirin versus Interferon Alfa-2a plus Ribavirin for Chronic Hepatitis C in HIV-Coinfected Persons. *N Engl J Med*. 2004;351(5):451-459. doi:10.1056/NEJMoa032653

155. Elgouhari HM, Zein CO, Hanouneh I, Feldstein AE, Zein NN. Diabetes Mellitus Is Associated

with Impaired Response to Antiviral Therapy in Chronic Hepatitis C Infection. *Dig Dis Sci*. 2009;54(12):2699-2705. doi:10.1007/s10620-008-0683-2

156. Al Marzooqi SH, Feld JJ. Sorting out cirrhosis: mechanisms of non-response to hepatitis C therapy. *Liver Int*. 2015;35(8):1923-1933. doi:https://doi.org/10.1111/liv.12861

157. Qureshi K, Petersen T, Andres J. Hepatitis C Treatment Differences in Elderly Patients: Single-Center Retrospective Study. *Ann Pharmacother*. 2019;54(2):113-121. doi:10.1177/1060028019871352

158. Maasoumy B, Buggisch P, Mauss S, et al. Clinical significance of detectable and quantifiable HCV RNA at the end of treatment with ledipasvir/sofosbuvir in GT1 patients. *Liver Int*. 2018;38(11):1906-1910. doi:https://doi.org/10.1111/liv.13932

159. Hezode C, Chevaliez S, Scoazec G, et al. P0843 : On-treatment viral kinetics do not predict SVR in patients with advanced liver disease receiving sofosbuvir in combination with daclatasvir or simeprevir for 12 weeks. *J Hepatol*. 2015;62:S654-S655. doi:10.1016/S0168-8278(15)31045-X

160. Kowdley K V, Nelson DR, Lalezari JP, et al. On-treatment HCV RNA as a predictor of sustained virological response in HCV genotype 3-infected patients treated with daclatasvir and sofosbuvir. *Liver Int Off J Int Assoc Study Liver*. 2016;36(11):1611-1618. doi:10.1111/liv.13165

161. Perelson AS, Guedj J. Modelling hepatitis C therapy-predicting effects of treatment. *Nat Rev Gastroenterol Hepatol*. 2015;12(8):437-445. doi:10.1038/nrgastro.2015.97

162. Lawitz EJ, Gruener D, Hill JM, et al. A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C. *J Hepatol*. 2012;57(1):24-31. doi:10.1016/j.jhep.2011.12.029

163. Guedj J, Perelson AS. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: Implications for treatment duration. *Hepatology*. 2011;53(6):1801-1808. doi:https://doi.org/10.1002/hep.24272

164. Sidharthan S, Kohli A, Sims Z, et al. Utility of Hepatitis C Viral Load Monitoring on Direct-Acting Antiviral Therapy. *Clin Infect Dis*. 2015;60(12):1743-1751. doi:10.1093/cid/civ170

165. Su F, Green PK, Berry K, Ioannou GN. The association between race/ethnicity and the effectiveness of direct antiviral agents for hepatitis C virus infection. *Hepatology*. 2017;65(2):426-438. doi:https://doi.org/10.1002/hep.28901

166. Kirby BJ, Symonds WT, Kearney BP, Mathias AA. Pharmacokinetic, Pharmacodynamic, and Drug-Interaction Profile of the Hepatitis C Virus NS5B Polymerase Inhibitor Sofosbuvir. *Clin Pharmacokinet*. 2015;54(7):677-690. doi:10.1007/s40262-015-0261-7

167. Rivero-Juarez A, Brieva T, Frias M, Rivero A. Pharmacodynamic and pharmacokinetic evaluation of the combination of daclatasvir/sofosbuvir/ribavirin in the treatment of chronic hepatitis C. *Expert Opin Drug Metab Toxicol*. 2018;14(9):901-910. doi:10.1080/17425255.2018.1506765

168. Squibb B-M. Daklinza (daclatasvir) tablets, for oral use. *Initial US Approv Bristol-Myers Squibb, New York, NY*. 2015.

169. EMA. Daklinza (SOF/DCV) Summary of Product Characteristics. *Eur Med Agency*. 2014.

170. Kracht PAM, de Gee EA, van der Poel A, et al. Introducing hepatitis C virus healthcare pathways in addiction care in the Netherlands with a Breakthrough project: a mixed method study. *Harm Reduct J*. 2019;16(1):45. doi:10.1186/s12954-019-0316-4

171. Petersen T, Townsend K, Gordon LA, et al. High adherence to all-oral directly acting antiviral HCV therapy among an inner-city patient population in a phase 2a study. *Hepatol Int*. 2016;10(2):310-319. doi:10.1007/s12072-015-9680-7

172. Jones CR, Flower BF, Barber E, Simmons B, Cooke GS. Treatment optimisation for hepatitis C in the era of combination direct-acting antiviral therapy: A systematic review and meta-analysis [version 1; peer review: 2 approved]. *Wellcome Open Res*. 2019;4. doi:10.12688/wellcomeopenres.15411.1

173. Lau G, Benhamou Y, Chen G, et al. Efficacy and safety of 3-week response-guided triple direct-acting antiviral therapy for chronic hepatitis C infection: a phase 2, open-label, proof-of-concept study. *lancet Gastroenterol Hepatol*. 2016;1(2):97-104. doi:10.1016/S2468-1253(16)30015-2

174. Kohli A, Kattakuzhy S, Sidharthan S, et al. Four-Week Direct-Acting Antiviral Regimens in Noncirrhotic Patients With Hepatitis C Virus Genotype 1 Infection. *Ann Intern Med*. 2015;163(12):899-907. doi:10.7326/M15-0642

175. Yakoot M, Abdo AM, Abdel-Rehim S, Helmy S. Response Tailored Protocol Versus the Fixed 12Weeks Course of Dual Sofosbuvir/Daclatasvir Treatment in Egyptian Patients With Chronic Hepatitis C Genotype-4 Infection: A Randomized, Open-label, Non-inferiority Trial. *EBioMedicine*. 2017;21:182-187. doi:10.1016/j.ebiom.2017.05.011

176. Cooke GS, Pett S ML et al. Strategic treatment optimization for HCV (STOPHCV1): a randomised controlled trial of ultrashort duration therapy for chronic hepatitis C [version 2; peer review: 2 approved]. *Wellcome Open Res*. 2021;6:93. https://doi.org/10.12688/wellcomeopenres.16594.2.

177. Sun J, Rajsbaum R YM. Immune and non-immune responses to hepatitis C virus infection. *World J Gastroenterol 2015; 21(38) 10739-10748 [PMID 26478666 DOI 103748/wjg.v21.i3810739] Sun J, Rajsbaum R, Yi M Immune non-immune responses to Hepat C virus Infect World J Gastroenterol*. 2015;21((38)):10739-10748. doi:10.3748/wjg.v21.i38.10739]

178. Simona U, Barbara A, Daniela T, et al. PD-1 Expression in Acute Hepatitis C Virus (HCV) Infection Is Associated with HCV-Specific CD8 Exhaustion. *J Virol*. 2006;80(22):11398-11403. doi:10.1128/JVI.01177-06

179. Wedemeyer H, Loos C, Hadem J, et al. Impaired cellular immune response after influenza, vaccination in patients with chronic hepatitis C infection. In: *Hepatology*. Vol 36. WB SAUNDERS CO INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA …; 2002:276A-276A.

180. Martin B, Hennecke N, Lohmann V, et al. Restoration of HCV-specific CD8+ T cell function by interferon-free therapy. *J Hepatol*. 2014;61(3):538-543. doi:10.1016/j.jhep.2014.05.043

181. Shrivastava S, Wilson E, Poonia B, et al. Augmentation of hepatitis C virus-specific immunity and sustained virologic response. *J Viral Hepat*. 2017;24(9):742-749. doi:https://doi.org/10.1111/jvh.12702

182. Romani S, Stafford K, Nelson A, Bagchi S, Kottilil S, Poonia B. Peripheral PD-1+ T Cells Co-expressing Inhibitory Receptors Predict SVR With Ultra Short Duration DAA Therapy in HCV Infection . *Front Immunol* . 2019;10. https://www.frontiersin.org/articles/10.3389/fimmu.2019.01470.

183. Li C, Pham VH, Abe K, Lu L. Nine additional complete genome sequences of HCV genotype 6 from Vietnam including new subtypes 6xb and 6xc. *Virology*. 2014;468-470:172-177. doi:10.1016/j.virol.2014.08.006

184. Li C, Yuan M, Lu L, et al. The genetic diversity and evolutionary history of hepatitis C virus in Vietnam. *Virology*. 2014;468-470:197-206. doi:10.1016/j.virol.2014.07.026

185. Boscarino JA, Sitarik A, Gordon SC, et al. Risk Factors for Hepatitis C Infection Among Vietnam Era Veterans Versus Nonveterans: Results from the Chronic Hepatitis Cohort Study (CHeCS). *J Community Health*. 2014;39(5):914-921. doi:10.1007/s10900-014-9863-5

186. Nguyen HA, Cooke GS, Day JN, Flower B, Phuong LT, Hung TM, Dung NT, Khoa DB, Hung LM, Kestelyn E, Thwaites GE, Chau NVV; SEARCH Investigators TH. The direct-medical costs associated with interferon-based treatment for Hepatitis C in Vietnam. *Wellcome Open Res*. 2020;Sep 11(4:129). doi:doi: 10.12688/wellcomeopenres.15408.2.

187. Nguyen Thanh D, Tran Thi Thanh T, Nghiem My N, et al. Baseline Characteristics and Treatment Cost of Hepatitis C atHospital for Tropical Diseases, Ho Chi Minh City, Vietnam inDirect-Acting Antiviral Treatment Era. *J Gastroenterol Hepatol Res*. 2019;8(5):2962-2971. doi:10.17554/j.issn.2224-3992.2019.08.857

188. Clinton Health Access Initiative Market Memo 2022. Hepatitis C Market Report. 2022;15(1):2020-2023.

189. The Global Fund. The Global Fund to fight AIDS, Tuberculosis and Malaria. https://www.theglobalfund.org/en/.

190. PEPFAR. The US President's Emergency Plan for AIDS Relief (PEPFAR). https://www.hiv.gov/federal-response/pepfar-global-aids/pepfar.

191. Rapoud D, Quillet C, Pham Minh K, et al. Towards HCV elimination among people who inject drugs in Hai Phong, Vietnam: study protocol for an effectiveness-implementation trial evaluating an integrated model of HCV care (DRIVE-C: DRug use & Infections in ViEtnam–hepatitis C). *BMJ Open*. 2020;10(11):e039234. doi:10.1136/bmjopen-2020-039234

192. Nguyen TTM, Nguyen LT, Pham MD, Vu HH, Mulvey KP. Methadone Maintenance Therapy in Vietnam: An Overview and Scaling-Up Plan. Bruce RD, ed. *Adv Prev Med*. 2012;2012:732484. doi:10.1155/2012/732484

193. Ministry of Health V. National Plan for Prevention and Control of Viral Hepatitis, Period 2021-2025. 2021. https://emoh.moh.gov.vn/publish/attach/getfile/408692.

194. Molès J-P, Vallo R, Khue PM, et al. HIV control programs reduce HIV incidence but not HCV incidence among people who inject drugs in HaiPhong, Vietnam. *Sci Rep*. 2020;10(1):6999. doi:10.1038/s41598-020-63990-w

195. Duong MC, Nguyen VTT, Otsu S, McLaws M-L. Prevalence of hepatitis B and C virus infections in hemodialysis patients in Vietnam: A systematic review and meta-analysis. *JGH Open*. 2020;4(1):29-38. doi:https://doi.org/10.1002/jgh3.12199

196. Sheena BS, Hiebert L, Han H, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol*. 2022;7(9):796-829. doi:10.1016/S2468-1253(22)00124-8

197. Dao D, Mize G, Pham LT, et al. EASL International Liver Fundation: The Vietnam Viral Hepatitis Alliance (V-VHA) dedicated to viral hepatitis initiatives in Vietnam. In: Vol 41. ; 2019:22-25.

198. McMahon BJ, Alward WLM, Hall DB, et al. Acute Hepatitis B Virus Infection: Relation of Age to the Clinical Expression of Disease and Subsequent Development of the Carrier State. *J Infect Dis*. 1985;151(4):599-603. doi:10.1093/infdis/151.4.599

199. Stevens CE, Beasley RP, Tsui J, Lee W-C. Vertical Transmission of Hepatitis B Antigen in

Taiwan. *N Engl J Med*. 1975;292(15):771-774. doi:10.1056/NEJM197504102921503

200. Tassopoulos NC, Papaevangelou GJ, Sjogren MH, Roumeliotou-Karayannis A, Gerin JL PR. Natural history of acute hepatitis B surface antigen-positive hepatitis in Greek adults. *Gastroenterology*. 1987;Jun(92(6)):1844-1850. doi:10.1016/0016-5085(87)90614-7

201. BEASLEY RP, TREPO C, STEVENS CE, SZMUNESS W. THE e ANTIGEN AND VERTICAL TRANSMISSION OF HEPATITIS B SURFACE ANTIGEN. *Am J Epidemiol*. 1977;105(2):94-98. doi:10.1093/oxfordjournals.aje.a112370

202. Okada K, Kamiyama I, Inomata M, Imai M, Miyakawa Y, Mayumi M. E Antigen and Anti-E in the Serum of Asymptomatic Carrier Mothers as Indicators of Positive and Negative Transmission of Hepatitis B Virus to Their Infants. *N Engl J Med*. 1976;294(14):746-749. doi:10.1056/NEJM197604012941402

203. Stevens CE, Toy PT, Tong MJ, et al. Perinatal Hepatitis B Virus Transmission in the United States: Prevention by Passive-Active Immunization. *JAMA*. 1985;253(12):1740-1745. doi:10.1001/jama.1985.03350360066020

204. McMahon BJ. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol Int*. 2009;3(2):334-342. doi:10.1007/s12072-008-9112-z

205. Nguyen LH, Ha NB, Vutien P, et al. Prevalence of hepatitis B virus genotype B in Vietnamese patients with chronic hepatitis B. *Hepatol Int*. 2009;3(3):461-467. doi:10.1007/s12072-009-9141-2

206. Bui TTT, Tran TT, Nghiem MN, et al. Molecular characterization of hepatitis B virus in Vietnam. *BMC Infect Dis*. 2017;17(1):601. doi:10.1186/s12879-017-2697-x

207. Chu C, Hussain M, Lok ASF. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology*. 2002;122(7):1756-1762. doi:10.1053/gast.2002.33588

208. Smedile A, Dentico P, Zanetti A, Sagnelli E, Nordenfelt E, Actis GC RM. Infection with the delta agent in chronic HBsAg carriers. *Gastroenterology*. 1981;Dec(81(6)):992-997.

209. Roulot D, Brichler S, Layese R, et al. Origin, HDV genotype and persistent viremia determine outcome and treatment response in patients with chronic hepatitis delta. *J Hepatol*. 2020;73(5):1046-1062. doi:10.1016/j.jhep.2020.06.038

210. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet*. 2011;378(9785):73-85. doi:10.1016/S0140-6736(10)61931-9

211. Miao Z, Zhang S, Ou X, et al. Estimating the Global Prevalence, Disease Progression, and Clinical Outcome of Hepatitis Delta Virus Infection. *J Infect Dis*. 2020;221(10):1677-1687. doi:10.1093/infdis/jiz633

212. Loureiro D, Castelnau C, Tout I, et al. New therapies for hepatitis delta virus infection. *Liver Int*. 2021;41(S1):30-37. doi:https://doi.org/10.1111/liv.14838

213. Cooke GS, Andrieux-Meyer I, Applegate TL, et al. Accelerating the elimination of viral hepatitis: a Lancet Gastroenterology & Hepatology Commission. *Lancet Gastroenterol Hepatol*. 2019;4(2):135-184. doi:10.1016/S2468-1253(18)30270-X

214. Palayew A, Razavi H, Hutchinson SJ, Cooke GS, Lazarus J V. Do the most heavily burdened countries have the right policies to eliminate viral hepatitis B and C? *Lancet Gastroenterol Hepatol*. 2020;5(10):948-953. doi:10.1016/S2468-1253(20)30011-X

215. Vietnam Ministry of Health. *The National Action Plan for Viral Hepatitis Prevention and Control 2015–2019, 2015*. https://thuvienphapluat.vn/van-ban/The-thao-Y-te/Quyet-dinh-739-QD-BYT-2015-phong-chong-benh-viem-gan-vi-rut-2015-2019-269703.aspx.

216. Berto A, Day J, Van Vinh Chau N, et al. Current challenges and possible solutions to improve access to care and treatment for hepatitis C infection in Vietnam: a systematic review. *BMC Infect Dis*. 2017;17(1):260. doi:10.1186/s12879-017-2360-6

217. Khue PM, Thuy Linh NT, Vinh VH, Dung LV, Nguyen Van B. Hepatitis B Infection and Mother-to-Child Transmission in Haiphong, Vietnam: A Cohort Study with Implications for Interventions. *Biomed Res Int*. 2020;2020:4747965. doi:10.1155/2020/4747965

218. Huy Do S. Epidemiology of Hepatitis B and C Virus Infections and Liver Cancer in Vietnam. *Euroasian J hepato-gastroenterology*. 2015;5(1):49-51. doi:10.5005/jp-journals-10018-1130

219. Nguyen VTT, Quang TD, Anh NT, et al. P1-011: Estimates and projection of disease burden and economic analysis for hepatitis B in Viet Nam. *J Viral Hepat*. 2018;25(June):38-38. doi:10.1111/jvh.07_12923

220. Global Health Data Exchange. Global Burden of Disease Study 2019 Data Input Sources Tool: Causes of death and disability in Vietnam. http://ghdx.healthdata.org/gbd-2019/data-input-sources?components=3&locations=20. Published 2019.

221. Stockdale AJ, Kreuels B, Henrion MYR, et al. The global prevalence of hepatitis D virus infection: Systematic review and meta-analysis. *J Hepatol*. 2020;73(3):523-532. doi:10.1016/j.jhep.2020.04.008

222. Abbas Z, Jafri W, Raza S. Hepatitis D: Scenario in the Asia-Pacific region. *World J Gastroenterol*. 2010;16(5):554-562. doi:10.3748/wjg.v16.i5.554

223. Pricewaterhouse Coopers. The long view: How will the global economic order change by 2050? *Pwc*. 2017;Februrary(February):1-72.

224. PRISMA. TRANSPARENT REPORTING of SYSTEMATIC REVIEWS and META-ANALYSES. https://prisma-statement.org/. Published 2021.

225. National Institute for Health Research. PROSPERO International prospective register of systematic reviews. https://www.crd.york.ac.uk/prospero/#aboutpage. Published 2022.

226. Freiman JM, Tran TM, Schumacher SG, et al. Hepatitis C Core Antigen Testing for Diagnosis of Hepatitis C Virus Infection: A Systematic Review and Meta-analysis. *Ann Intern Med*. 2016;165(5):345-355. doi:10.7326/M16-0065

227. Lemoine M, Mohamed Z, Chevaliez S, Shimakawa Y, Rwegasha J. Role of hepatitis C virus core antigen assay in hepatitis C care in Africa. *Lancet Gastroenterol Hepatol*. 2018;3(4):223-224. doi:10.1016/S2468-1253(18)30039-6

228. Wong XZ, Gan CC, Mohamed R, et al. Hepatitis C core antigen testing to diagnose active hepatitis C infection among haemodialysis patients. *BMC Nephrol*. 2020;21(1):480. doi:10.1186/s12882-020-02154-4

229. van Tilborg M, Al Marzooqi SH, Wong WWL, et al. HCV core antigen as an alternative to HCV RNA testing in the era of direct-acting antivirals: retrospective screening and diagnostic cohort studies. *Lancet Gastroenterol Hepatol*. 2018;3(12):856-864. doi:10.1016/S2468-1253(18)30271-1

230. JBI. *Critical Appraisal Checklist for Systematic Reviews and Research Syntheses*.; 2017. http://joannabriggs.org/research/critical-appraisal-tools.htmlwww.joannabriggs.org%0Awww.joannabriggs.org.

231. Borges Migliavaca C, Stein C, Colpani V, et al. How are systematic reviews of prevalence conducted? A methodological study. *BMC Med Res Methodol*. 2020;20(1):96. doi:10.1186/s12874-020-00975-3

232. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177-188. doi:https://doi.org/10.1016/0197-2456(86)90046-2

233. Freeman MF, Tukey JW. Transformations related to the angular and the square root. *Ann Math Stat*. 1950:607-611.

234. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Stat Med*. 1998;17(8):873-890. doi:https://doi.org/10.1002/(SICI)1097-0258(19980430)17:8<873::AID-SIM779>3.0.CO;2-I

235. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2021. https://www.r-project.org/.

236. Trung DT, Thao LTT, Hien TT, et al. Liver involvement associated with dengue infection in adults in Vietnam. *Am J Trop Med Hyg*. 2010;83(4):774-780. doi:10.4269/ajtmh.2010.10-0090

237. Barcus MJ, Hien TT, White NJ, et al. Short report: hepatitis b infection and severe Plasmodium falciparum malaria in Vietnamese adults. *Am J Trop Med Hyg*. 2002;66(2):140-142. doi:10.4269/ajtmh.2002.66.140

238. Chau TTH, Mai NTH, Phu NH, et al. Malaria in Injection Drug Abusers in Vietnam. *Clin Infect Dis*. 2002;34(10):1317-1322. doi:10.1086/340053

239. Nadol P, O'connor S, Duong H, et al. Findings from integrated behavioral and biologic survey among males who inject drugs (MWID) - Vietnam, 2009-2010: evidence of the need for an integrated response to HIV, hepatitis B virus, and hepatitis C virus. *PLoS One*. 2015;10(2):e0118304-e0118304. doi:10.1371/journal.pone.0118304

240. Hoang TV, Ha TTT, Hoang TM, et al. Impact of a methadone maintenance therapy pilot in Vietnam and its role in a scaled-up response. *Harm Reduct J*. 2015;12:39. doi:10.1186/s12954-015-0075-9

241. FollÃ©zou JY, Lan NY, Lien TX, et al. Clinical and biological characteristics of human immunodeficiency virus-infected and uninfected intravascular drug users in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg Am J Trop Med Hyg Am J Trop Med Hyg*. 1999;61(3):420-424. doi:10.4269/ajtmh.1999.61.420

242. NAKATA S, SONG P, DUC DAOD, et al. Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam. *J Gastroenterol Hepatol*. 1994;9(4):416-419. doi:https://doi.org/10.1111/j.1440-1746.1994.tb01265.x

243. Lien TTX, Trimoulet P, Yen VT, Lepers JP, Fleury HJA. Epidémiologie de l'infection par le virus de l'hépatite C au Vietnam. *Médecine Mal Infect*. 1997;27(11):893-897. doi:https://doi.org/10.1016/S0399-077X(97)80245-4

244. Tanimoto T, Nguyen HC, Ishizaki A, et al. Multiple routes of hepatitis C virus transmission among injection drug users in Hai Phong, Northern Vietnam. *J Med Virol*. 2010;82(8):1355-1363. doi:10.1002/jmv.21787

245. Kakumu S, Sato K, Morishita T, et al. Prevalence of hepatitis B, hepatitis C, and GB virus C/hepatitis G virus infections in liver disease patients and inhabitants in Ho Chi Minh, Vietnam. *J Med Virol*. 1998;54(4):243-248. doi:https://doi.org/10.1002/(SICI)1096-9071(199804)54:4<243::AID-JMV2>3.0.CO;2-4

246. Le L-VN, O'Connor S, Tran TH, et al. High hepatitis C virus infection among female sex

workers in Viet Nam: strong correlation with HIV and injection drug use. *West Pacific Surveill response J WPSAR*. 2019;10(3):9-18. doi:10.5365/wpsar.2019.10.1.002

247. Do SH, Yamada H, Fujimoto M, et al. High prevalences of hepatitis B and C virus infections among adults living in Binh Thuan province, Vietnam. *Hepatol Res*. 2015;45(3):259-268. doi:https://doi.org/10.1111/hepr.12350

248. Duong MC, McLaws ML. Screening haemodialysis patients for hepatitis C in Vietnam: The inconsistency between common hepatitis C virus serological and virological tests. *J Viral Hepat*. 2019;26(1):25-29. doi:10.1111/jvh.12994

249. Dang LVP, Nguyen QH, Ishizaki A, et al. Prevalence of Opportunistic Infections and Associated Factors in HIV-Infected Men Who Have Sex With Men on Antiretroviral Therapy in Bach Mai Hospital, Hanoi, Vietnam: A Case-Control Study. *Am J Mens Health*. 2020;14(3):1557988320926743-1557988320926743. doi:10.1177/1557988320926743

250. Dunford L, Carr MJ, Dean J, et al. A multicentre molecular analysis of hepatitis B and blood-borne virus coinfections in Viet Nam. *PLoS One*. 2012;7(6):e39027. doi:10.1371/journal.pone.0039027

251. Vietnam MOH. *Thông Tư 26/2013/TT-BYT Circular No. 26/2013/TT-BYT, Providing Guidance on the Blood Transfusion*. Hanoi; 2013. https://vanbanphapluat.co/circular-no-26-2013-tt-byt-guidance-on-the-blood-transfusion.

252. Vietnam Plus. Voluntary blood donation a popular movement in Vietnam.https://en.vietnamplus.vn/voluntary-blood-donation-a-popular-movement-in-vietnam/224689.vnp. Published April 12, 2022.

253. Hyams KC. Risks of Chronicity Following Acute Hepatitis B Virus Infection: A Review. *Clin Infect Dis*. 1995;20(4):992-1000. doi:10.1093/clinids/20.4.992

254. Katelaris PH, Robertson G, Bradbury R, Tippett G, Hoa DQ, Ngu MC. Seroprevalence of hepatitis viruses in children in rural Viet Nam. *Trans R Soc Trop Med Hyg*. 1995;89(5):487. doi:10.1016/0035-9203(95)90077-2

255. Hipgrave DB, Nguyen T V, Vu MH, et al. Hepatitis B infection in rural Vietnam and the implications for a national program of infant immunization. *Am J Trop Med Hyg*. 2003;69(3):288-294.

256. Nguyen TH, Vu MH, Nguyen VC, et al. A reduction in chronic hepatitis B virus infection prevalence among children in Vietnam demonstrates the importance of vaccination. *Vaccine*. 2014;32(2):217-222. doi:10.1016/j.vaccine.2013.11.004

257. Miyakawa M, Yoshida L-M, Nguyen H-AT, et al. Hepatitis B virus infection among pregnant mothers and children after the introduction of the universal vaccination program in Central Vietnam. *Sci Rep*. 2021;11(1):8676. doi:10.1038/s41598-021-87860-1

258. de Villiers MJ, Nayagam S, Hallett TB. The impact of the timely birth dose vaccine on the global elimination of hepatitis B. *Nat Commun*. 2021;12(1):6223. doi:10.1038/s41467-021-26475-6

259. Nguyen Si Anh H, Vo H-L, Hoang Bao L, Tran Minh H, Tran Thi Thu H, Kien VD. Hepatitis B Birth Dose Vaccination among Vietnamese Children: Implications for the Expanded Program on Immunization. Liu Y, ed. *Biomed Res Int*. 2019;2019:3453105. doi:10.1155/2019/3453105

260. Tran TK, Gottvall K, Nguyen HD, Ascher H, Petzold M. Factors associated with antenatal care adequacy in rural and urban contexts-results from two health and demographic

surveillance sites in Vietnam. *BMC Health Serv Res*. 2012;12(1):40. doi:10.1186/1472-6963-12-40

261. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive–active immunoprophylaxis in infants born to HBsAg-positive mothers. *J Viral Hepat*. 2012;19(2):e18-e25. doi:https://doi.org/10.1111/j.1365-2893.2011.01492.x

262. Wen W-H, Chang M-H, Zhao L-L, et al. Mother-to-infant transmission of hepatitis B virus infection: Significance of maternal viral load and strategies for intervention. *J Hepatol*. 2013;59(1):24-30. doi:10.1016/j.jhep.2013.02.015

263. Chen H, Lin L, Hu F, et al. Effects of Maternal Screening and Universal Immunization to Prevent Mother-to-Infant Transmission of HBV. *Gastroenterology*. 2012;142(4):773-781.e2. doi:10.1053/j.gastro.2011.12.035

264. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol*. 2014;61(1, Supplement):S45-S57. doi:https://doi.org/10.1016/j.jhep.2014.07.027

265. Degenhardt L, Peacock A, Colledge S, et al. Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. *Lancet Glob Heal*. 2017;5(12):e1192-e1207. doi:10.1016/S2214-109X(17)30375-3

266. Grebely J, Dalgard O, Conway B, et al. Sofosbuvir and velpatasvir for hepatitis C virus infection in people with recent injection drug use (SIMPLIFY): an open-label, single-arm, phase 4, multicentre trial. *Lancet Gastroenterol Hepatol*. 2018;3(3):153-161. doi:10.1016/S2468-1253(17)30404-1

267. Vietnam Ministry of Health. *Increased Access to Treatment for HIV/HCV Co-Infected Patients in Vietnam*.; 2021. https://vaac.gov.vn/tang-cuong-tiep-can-dieu-tri-viem-gan-c-doi-voi-nguoi-benh-dong-nhiem-hiv-viem-gan-c-tai-viet-nam.html.

268. Blake A, Smith JE. Modeling Hepatitis C Elimination Among People Who Inject Drugs in New Hampshire. *JAMA Netw Open*. 2021;4(8):e2119092-e2119092. doi:10.1001/jamanetworkopen.2021.19092

269. Cornell JE, Mulrow CD, Localio R, et al. Random-Effects Meta-analysis of Inconsistent Effects: A Time for Change. *Ann Intern Med*. 2014;160(4):267-270. doi:10.7326/M13-2886

270. da Silva Filipe A, Sreenu V, Hughes J, et al. Response to DAA therapy in the NHS England Early Access Programme for rare HCV subtypes from low and middle income countries. *J Hepatol*. 2017;67(6):1348-1350. doi:10.1016/j.jhep.2017.06.035

271. Gupta N, Mbituyumuremyi A, Kabahizi J, et al. Treatment of chronic hepatitis C virus infection in Rwanda with ledipasvir–sofosbuvir (SHARED): a single-arm trial. *Lancet Gastroenterol Hepatol*. 2019;4(2):119-126. doi:10.1016/S2468-1253(18)30382-0

272. Prokunina-Olsson L, Muchmore B, Tang W, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet*. 2013;45(2):164-171. doi:10.1038/ng.2521

273. Franco S, Aparicio E, Parera M, Clotet B, Tural C, Martinez MA. IFNL4 ss469415590 variant is a better predictor than rs12979860 of pegylated interferon-alpha/ribavirin therapy failure in hepatitis C virus/HIV-1 coinfected patients. *AIDS*. 2014;28(1). https://journals.lww.com/aidsonline/Fulltext/2014/01020/IFNL4_ss469415590_variant_is_a_better_predictor.17.aspx.

274. Ansari MA, Pedergnana V, L C Ip C, et al. Genome-to-genome analysis highlights the effect of the human innate and adaptive immune systems on the hepatitis C virus. *Nat Genet*. 2017;49(5):666-673. doi:10.1038/ng.3835

275. Ansari MA, Aranday-Cortes E, Ip CL, et al. Interferon lambda 4 impacts the genetic diversity of hepatitis C virus. *Elife*. 2019;8:e42463. doi:10.7554/eLife.42463

276. Nitta Y, Kawabe N, Hashimoto S, et al. Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. *Hepatol Res*. 2009;39(7):675-684. doi:https://doi.org/10.1111/j.1872-034X.2009.00500.x

277. National Institutes of Health. *Common Terminology Criteria for Adverse Events (CTCAE)*.; 2017. https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf.

278. Thomson E, Ip CLC, Badhan A, et al. Comparison of Next-Generation Sequencing Technologies for Comprehensive Assessment of Full-Length Hepatitis C Viral Genomes. Loeffelholz MJ, ed. *J Clin Microbiol*. 2016;54(10):2470 LP - 2484. doi:10.1128/JCM.00330-16

279. Smith DA, Fernandez-Antunez C, Magri A, et al. Viral genome wide association study identifies novel hepatitis C virus polymorphisms associated with sofosbuvir treatment failure. *Nat Commun*. 2021;12(1):6105. doi:10.1038/s41467-021-25649-6

280. Smith DA, Bradshaw D, Mbisa JL, et al. Real world SOF/VEL/VOX retreatment outcomes and viral resistance analysis for HCV patients with prior failure to DAA therapy. *J Viral Hepat*. 2021;28(9):1256-1264. doi:https://doi.org/10.1111/jvh.13549

281. Manso CF, Bibby DF, Lythgow K, et al. Technical Validation of a Hepatitis C Virus Whole Genome Sequencing Assay for Detection of Genotype and Antiviral Resistance in the Clinical Pathway . *Front Microbiol* . 2020;11. https://www.frontiersin.org/articles/10.3389/fmicb.2020.576572.

282. Bradshaw D, Mbisa JL, Geretti AM, et al. Consensus recommendations for resistance testing in the management of chronic hepatitis C virus infection: Public Health England HCV Resistance Group. *J Infect*. 2019;79(6):503-512. doi:10.1016/j.jinf.2019.10.007

283. StataCorp. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC. 2019.

284. SAS L. PKanalix version 2020R1. Antony, France. 2022.

285. de Kanter CTMM, Drenth JPH, Arends JE, et al. Viral Hepatitis C Therapy: Pharmacokinetic and Pharmacodynamic Considerations. *Clin Pharmacokinet*. 2014;53(5):409-427. doi:10.1007/s40262-014-0142-5

286. Hospital for Tropical Diseases Ethics Commitee V. HTD EC (764 Vo Van Kiet, ward 1, district 5, HCMC, Viet Nam; (+84) 28 3923 8704; bv.bnhietdoi@tphcm.gov.vn), ref: CS/BND/18/25 Approved 07/06/2018. Presented at the:

287. Vietnam Ministry of Health. Ethical Evaluation Commitee in Biomedical Research of Ministry of Health, Vietnam (138A Giang Vo, Ba Dinh, Ha Noi, Viet Nam; (+84) 0462732156; admin@iecmoh.vn), ref: 6172/QĐ-BYTtnam MoH Ethics. Presented at the:

288. ICREC. Imperial College Research Ethics Committee, UK (Level 2, Medical School Building, St Marys Campus, London W2 1PG; +44 (0)207 594 9484; researchethicscommittee@imperial.ac.uk), ref: 17IC4238. Approved 18/12/2018. Presented at the:

289. OXTREC. Oxford Tropical Research Ethics Committee, UK (University of Oxford Research Services, University Offices, Wellington Square, Oxford OX1 2JD; +44 (0) 1865 (2)82106; (oxtrec@admin.ox.ac.uk); ref: 43-17 Approved 04/10/2018. Presented at the:

290. World Medical Association. *WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects*. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/.

291. Cooke GS. ISRCTN registry. ISRCTN17100273. https://doi.org/10.1186/ISRCTN17100273. Published 2018.

292. Flower B, McCabe L, Le Ngoc C, et al. High cure rates for HCV genotype 6 in advanced liver fibrosis with 12 weeks sofosbuvir and daclatasvir: The Vietnam SEARCH Study. *Open Forum Infect Dis*. June 2021. doi:10.1093/ofid/ofab267

293. Maasoumy B, Vermehren J, Welker M-W, et al. Clinical value of on-treatment HCV RNA levels during different sofosbuvir-based antiviral regimens. *J Hepatol*. 2016;65(3):473-482. doi:10.1016/j.jhep.2016.04.006

294. Emmanuel B, Wilson EM, O'Brien TR, Kottilil S, Lau G. Shortening the duration of therapy for chronic hepatitis C infection. *lancet Gastroenterol Hepatol*. 2017;2(11):832-836. doi:10.1016/S2468-1253(17)30053-5

295. Cooke GS, Pett S ML et al. Strategic treatment optimization for HCV (STOPHCV1): a randomised controlled trial of ultrashort duration therapy for chronic hepatitis C. *[version 1; peer Rev 1 Approv 1 Approv with Reserv Wellcome Open Res /wellcomeopenres165941)*. 2021;6:93. doi:https://doi.org/10.12688

296. Sulkowski MS, Flamm S, Kayali Z, et al. Short-duration treatment for chronic hepatitis C virus with daclatasvir, asunaprevir, beclabuvir and sofosbuvir (FOURward study). *Liver Int*. 2017;37(6):836-842. doi:https://doi.org/10.1111/liv.13335

297. El-Shabrawi MH, Abdo AM, El-Khayat HR, Yakoot M. Shortened 8 Weeks Course of Dual Sofosbuvir/Daclatasvir Therapy in Adolescent Patients, With Chronic Hepatitis C Infection. *J Pediatr Gastroenterol Nutr*. 2018;66(3). https://journals.lww.com/jpgn/Fulltext/2018/03000/Shortened_8_Weeks_Course_of_Dual.15.aspx.

298. Etzion O, Dahari H, Yardeni D, et al. Response guided therapy for reducing duration of direct acting antivirals in chronic hepatitis C infected patients: a Pilot study. *Sci Rep*. 2020;10(1):17820. doi:10.1038/s41598-020-74568-x

299. Perelson AS, Guedj J. Modelling hepatitis C therapy--predicting effects of treatment. *Nat Rev Gastroenterol Hepatol*. 2015;12(8):437-445. doi:10.1038/nrgastro.2015.97

300. Kohli A, Osinusi A, Sims Z, et al. Virological response after 6 week triple-drug regimens for hepatitis C: a proof-of-concept phase 2A cohort study. *Lancet (London, England)*. 2015;385(9973):1107-1113. doi:10.1016/S0140-6736(14)61228-9

301. Charatcharoenwitthaya P, Wongpaitoon V, Komolmit P, et al. Real-world effectiveness and safety of sofosbuvir and nonstructural protein 5A inhibitors for chronic hepatitis C genotype 1, 2, 3, 4, or 6: a multicentre cohort study. *BMC Gastroenterol*. 2020;20(1):47. doi:10.1186/s12876-020-01196-0

302. Pal V, Ancha N, Mann J, Modi AA. Effect of Low Positive End of Treatment Viral Load with Direct-Acting Antiviral Therapy on Sustained Virologic Response. Peltekian KM, ed. *Can J Gastroenterol Hepatol*. 2020;2020:8815829. doi:10.1155/2020/8815829

303. McCabe L, White IR, Chau NVV, et al. The design and statistical aspects of VIETNARMS: a strategic post-licensing trial of multiple oral direct-acting antiviral hepatitis C treatment strategies in Vietnam. *Trials*. 2020;21(1):413. doi:10.1186/s13063-020-04350-x

304. Boyle A, Marra F, Peters E, et al. Eight weeks of sofosbuvir/velpatasvir for genotype 3 hepatitis C in previously untreated patients with significant (F2/3) fibrosis. *J Viral Hepat*. 2020;27(4):371-375. doi:10.1111/jvh.13239

305. Clinton Health Access Initiative. HCV Market Intelligence Report 2021 and Preliminary HBV Market Insights. 2021:23-34.

306. Smolders EJ, Jansen AME, ter Horst PGJ, Rockstroh J, Back DJ, Burger DM. Viral Hepatitis C Therapy: Pharmacokinetic and Pharmacodynamic Considerations: A 2019 Update. *Clin Pharmacokinet*. 2019;58(10):1237-1263. doi:10.1007/s40262-019-00774-0

307. Ahmed B, Munir B, Ghaffar A, et al. Pharmacokinetics profile of serum and cellular Sofosbuvir along with its concentration effect analysis in HCV patients receiving Sofosbuvir and Ribavirin. *Pak J Pharm Sci*. 2019;32.

308. Dahari H, Halfon P, Cotler SJ. Resurrection of response-guided therapy for sofosbuvir combination therapies. *J Hepatol*. 2016;65(3):462-464. doi:10.1016/j.jhep.2016.05.028

309. Clinton Health Access Initiative. *Hepatitis C Market Report*. Vol 15.; 2020.

310. Antonini TM, Coilly A, Rossignol E, et al. Sofosbuvir-Based Regimens in HIV/HCV Coinfected Patients After Liver Transplantation: Results From the ANRS CO23 CUPILT Study. *Transplantation*. 2017;102(1):119-126. https://www.unboundmedicine.com/medline/citation/28846559/Sofosbuvir_Based_Regimens_in_HIV/HCV_Coinfected_Patients_After_Liver_Transplantation:_Results_From_the_ANRS_CO23_CUPILT_Study_.

311. Lionetti R, Calvaruso V, Piccolo P, et al. Sofosbuvir plus daclatasvir with or without ribavirin is safe and effective for post-transplant hepatitis C recurrence and severe fibrosis and cirrhosis: A prospective study. *Clin Transplant*. 2018;32(2):e13165. doi:10.1111/ctr.13165

312. Rockstroh JK, Ingiliz P, Petersen J, Peck-Radosavljevic M, Welzel TM, Van der Valk M, Zhao Y, Jimenez-Exposito MJ ZS. Daclatasvir plus sofosbuvir, with or without ribavirin, in real-world patients with HIV-HCV coinfection and advanced liver disease. Antivir Ther. *Epub 2016 Nov 15 PMID 27845298*. 2017;22(3):225-. doi:doi: 10.3851/IMP3108.

313. Lacombe K, Fontaine H, Dhiver C, et al. Real-World Efficacy of Daclatasvir and Sofosbuvir, With and Without Ribavirin, in HIV/HCV Coinfected Patients With Advanced Liver Disease in a French Early Access Cohort. *J Acquir Immune Defic Syndr*. 2017;75(1):97—107. doi:10.1097/qai.0000000000001342

314. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*. 2015;61(4):1127-1135. doi:10.1002/hep.27726

315. Mettikanont P, Bunchorntavakul C, Reddy KR. Systematic review: epidemiology and response to direct-acting antiviral therapy in genotype 6 chronic hepatitis C virus infection. *Aliment Pharmacol Ther*. 2019;49(5):492-505. doi:10.1111/apt.15100

316. Mei Y-Y, Chen Y-M, Wu Y-K, Zhang X-H, Xu W-X. Efficacy and Safety of Sofosbuvir-Based Direct-Acting Antiviral Agents Treatment for Patients with Genotype 3/6 Hepatitis C Virus Infection. *Can J Gastroenterol Hepatol*. 2020;2020:8872120. doi:10.1155/2020/8872120

317. Zhang M, O'Keefe D, Iwamoto M, et al. High sustained viral response rate in patients with hepatitis C using generic sofosbuvir and daclatasvir in Phnom Penh, Cambodia. *J Viral Hepat*. 2020;27(9):886-895. https://www.unboundmedicine.com/medline/citation/32358826/High_sustained_viral_response_rate_in_patients_with_hepatitis_C_using_generic_sofosbuvir_and_daclatasvir_in_Phnom_Penh,_Cambodia.

318. Nguyen DT, Tran TTT, Nghiem NM, et al. Effectiveness of sofosbuvir based direct-acting antiviral regimens for chronic hepatitis C virus genotype 6 patients: Real-world experience in Vietnam. *PLoS One*. 2020;15(5):e0233446. https://doi.org/10.1371/journal.pone.0233446.

319. Hu C, Yuan G, Liu J, et al. Sofosbuvir-Based Therapies for Patients with Hepatitis C Virus Infection: Real-World Experience in China. Peltekian KM, ed. *Can J Gastroenterol Hepatol*. 2018;2018:3908767. doi:10.1155/2018/3908767

320. Wu D-B, Jiang W, Wang Y-H, et al. Safety and efficacy of sofosbuvir-based direct-acting antiviral regimens for hepatitis C virus genotype 6 in Southwest China: Real-world experience of a retrospective study. *J Viral Hepat*. 2019;26(3):316-322. doi:https://doi.org/10.1111/jvh.13033

321. Omata M, Kanda T, Wei L, et al. APASL consensus statements and recommendation on treatment of hepatitis C. *Hepatol Int*. 2016;10(5):702-726. doi:10.1007/s12072-016-9717-6

322. Pol S, Bourliere M, Lucier S, et al. Safety and efficacy of daclatasvir-sofosbuvir in HCV genotype 1-mono-infected patients. *J Hepatol*. 2017;66(1):39-47. doi:10.1016/j.jhep.2016.08.021

323. Welzel TM, Petersen J, Herzer K, et al. Daclatasvir plus sofosbuvir, with or without ribavirin, achieved high sustained virological response rates in patients with HCV infection and advanced liver disease in a real-world cohort. *Gut*. 2016;65(11):1861 LP - 1870. doi:10.1136/gutjnl-2016-312444

324. El-Hariri M, Abd El Megid AG, Taha Ali TF, Hassany M. Diagnostic value of Transient Elastography (Fibroscan) in the evaluation of liver fibrosis in chronic viral hepatitis C: Comparison to liver biopsy. *Egypt J Radiol Nucl Med*. 2017;48(2):329-337. doi:https://doi.org/10.1016/j.ejrnm.2017.03.026

325. Pawlotsky J-M, Negro F, Aghemo A, et al. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol*. 2018;69(2):461-511. doi:10.1016/j.jhep.2018.03.026

326. Gottwein JM, Pham L V, Mikkelsen LS, et al. Efficacy of NS5A Inhibitors Against Hepatitis C Virus Genotypes 1–7 and Escape Variants. *Gastroenterology*. 2018;154(5):1435-1448. doi:10.1053/j.gastro.2017.12.015

327. Bonsall D, Ansari MA, Ip C, et al. ve-SEQ: Robust, unbiased enrichment for streamlined detection and whole-genome sequencing of HCV and other highly diverse pathogens. *F1000Research*. 2015;4:1062. doi:10.12688/f1000research.7111.1

328. Singer JB, Thomson EC, McLauchlan J, Hughes J, Gifford RJ. GLUE: a flexible software system for virus sequence data. *BMC Bioinformatics*. 2018;19(1):532. doi:10.1186/s12859-018-2459-9

329. Singer JB, Thomson EC, Hughes J, et al. Interpreting viral deep sequencing data with glue. *Viruses*. 2019;11(4):1-12. doi:10.3390/v11040323

330. De Luca A, Di Giambenedetto S, Lo Presti A, et al. Two Distinct Hepatitis C Virus Genotype 1a Clades Have Different Geographical Distribution and Association With Natural Resistance to NS3 Protease Inhibitors. *Open forum Infect Dis*. 2015;2(2):ofv043-ofv043.

doi:10.1093/ofid/ofv043

331. Dahari H, Canini L, Graw F, et al. HCV kinetic and modeling analyses indicate similar time to cure among sofosbuvir combination regimens with daclatasvir, simeprevir or ledipasvir. *J Hepatol*. 2016;64(6):1232-1239. doi:10.1016/j.jhep.2016.02.022

332. Gambato M, Canini L, Lens S, et al. Early HCV viral kinetics under DAAs may optimize duration of therapy in patients with compensated cirrhosis. *Liver Int*. 2019;39(5):826-834. doi:https://doi.org/10.1111/liv.14014

333. Dahari H, Guedj J, Perelson AS, Layden TJ. Hepatitis C Viral Kinetics in the Era of Direct Acting Antiviral Agents and IL28B. *Curr Hepat Rep*. 2011;10(3):214-227. doi:10.1007/s11901-011-0101-7

334. Sakai H, Miwa T, Ikoma Y, et al. Development of diffuse large b-cell lymphoma after sofosbuvir-ledipasvir treatment for chronihepatitis c: A case report and literature review. *Mol Clin Oncol*. 2020;13(3):1-6. doi:10.3892/mco.2020.2071

335. Simmons B, Saleem J, Hill A, Riley RD, Cooke GS. Risk of Late Relapse or Reinfection With Hepatitis C Virus After Achieving a Sustained Virological Response: A Systematic Review and Meta-analysis. *Clin Infect Dis*. 2016;62(6):683-694. doi:10.1093/cid/civ948

336. Lee Y Bin, Nam JY, Lee J-H, et al. Differential Effect of HCV Eradication and Fibrosis Grade on Hepatocellular Carcinoma and All-cause Mortality. *Sci Rep*. 2018;8(1):13651. doi:10.1038/s41598-018-31839-y

337. Backus LI, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A Sustained Virologic Response Reduces Risk of All-Cause Mortality in Patients With Hepatitis C. *Clin Gastroenterol Hepatol*. 2011;9(6):509-516.e1. doi:10.1016/j.cgh.2011.03.004

338. Tada T, Kumada T, Toyoda H, et al. Viral eradication reduces all-cause mortality, including non–liver-related disease, in patients with progressive hepatitis C virus-related fibrosis. *J Gastroenterol Hepatol*. 2017;32(3):687-694. doi:https://doi.org/10.1111/jgh.13589

339. Graham CS. The Current Status of US and Global Access to Direct-Acting Antiviral Regimens for Hepatitis C Virus Infection. *Clin liver Dis*. 2020;16(1):16-19. doi:10.1002/cld.925

340. Xie Z, Deng K, Xia Y, et al. Efficacy and safety of direct-acting antiviral therapies and baseline predictors for treatment outcomes in hepatitis C patients: A multicenter, real-world study in Guangdong, China. *J Med Virol*. 2022;94(9):4459-4469. doi:https://doi.org/10.1002/jmv.27851

341. Huynh T, Zhang J, Hu K-Q. Hepatitis C Virus Clearance by Direct-acting Antiviral Results in Rapid Resolution of Hepatocytic Injury as Indicated by Both Alanine Aminotransferase and Aspartate Aminotransferase Normalization. *J Clin Transl Hepatol*. 2018;6(3):258-263. doi:10.14218/JCTH.2018.00014

342. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet*. 1991;337(8749):1058-1061. doi:10.1016/0140-6736(91)91707-2

343. Naravadi VVR, Gonzalez B, Cotler S, Loy V. Normalization of Alanine Aminotransferase Levels at the End of Treatment with Direct-Acting Antiviral Therapy for Chronic Hepatitis C Infection Is Associated with Sustained Virological Response: 803. *Off J Am Coll Gastroenterol | ACG*. 2016;111. https://journals.lww.com/ajg/Fulltext/2016/10001/Normalization_of_Alanine_Aminotransferase_Levels.803.aspx.

344. Tacke F, Boeker KHW, Klinker H, et al. Baseline risk factors determine lack of biochemical

response after SVR in chronic hepatitis C patients treated with DAAs. *Liver Int*. 2020;40(3):539-548. doi:https://doi.org/10.1111/liv.14186

345. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig L, LijmerJG Moher D, Rennie D, de Vet HCW, Kressel HY, Rifai N, Golub RM, Altman DG, Hooft L, Korevaar DA, Cohen JF F the SG. *STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies.*; 2015.

346. El Kassas M, Alboraie M, Mostafa A, et al. After successful hepatitis C virus antiviral therapy: It looks that normal alanine aminotransferase level is not the normal. *J Clin Lab Anal*. 2018;32(3):e22296. doi:https://doi.org/10.1002/jcla.22296

347. Akkaya O, Kiyici M, Yilmaz Y, Ulukaya E YO. Clinical significance of activity of ALT enzyme in patients with hepatitis C virus. *World J Gastroenterol*. 2007;13:(41):5481-5485. doi:10.3748/wjg.v13.i41.5481]

348. Zeuzem S, Rockstroh JK, Kwo PY, et al. Predictors of response to grazoprevir/elbasvir among HCV genotype 1 (GT1)–infected patients: integrated analysis of phase 2-3 trials [abstract 700]. In: *Liver Meeting 2015*. San Francisco, CA; 2015.

349. Howell J, Van H, Pham M, et al. Validation of novel point-of-care test for alanine aminotransferase measurement: A pilot cohort study. *Clin Liver Dis*. 2022;20(4):136-139. doi:https://doi.org/10.1002/cld.1261

350. Sui B, Chang E, Zhao K, Qiu Y, Hou W, Chan P. Cost estimate and budget impact analysis of implementing the new guidelines for management of hepatitis C virus in China: a micro-costing study. *Lancet*. 2017;390:S102. doi:10.1016/S0140-6736(17)33240-3

351. Huynh T, Zhang J HK. Hepatitis C Virus Clearance by Direct-acting Antiviral Results in Rapid Resolution of Hepatocytic Injury as Indicated by Both Alanine Aminotransferase and Aspartate Aminotransferase Normalization. *J Clin Transl Hepatol*. 2018;6(3):258-263. doi:10.14218/JCTH.2018.00014.

352. Muljono D. Epidemiology of Hepatitis B and C in Republic of Indonesia. *Euroasian J Hepato-Gastroenterol*. 2017;7((1)):55-59.

353. Minta AA, Silva MWT, Shrestha A, et al. Hepatitis B surface antigen seroprevalence among children in the Philippines, 2018. *Vaccine*. 2021;39(14):1982-1989. doi:https://doi.org/10.1016/j.vaccine.2021.02.042

354. Thanh Thi Le X, Ishizumi A, Thi Thu Nguyen H, et al. Social and behavioral determinants of attitudes towards and practices of hepatitis B vaccine birth dose in Vietnam. *Vaccine*. 2020;38(52):8343-8350. doi:https://doi.org/10.1016/j.vaccine.2020.11.009

355. VietnamPlus. UNICEF Representative commends Vietnam's immunisation system amid COVID-19.https://en.vietnamplus.vn/unicef-representative-commends-vietnams-immunisation-system-amid-covid19/234060.vnp.

356. Kim T V, Le DH, Dao DVB, et al. Demonstration of a population-based HCV serosurvey in Ho Chi Minh City, Viet Nam: Establishing baseline prevalence of and continuum of care for HCV micro-elimination by 2030. *Lancet Reg Heal – West Pacific*. 2022;27. doi:10.1016/j.lanwpc.2022.100524

357. Hieu DM, Gray B, Tuan DM, Colman BP. Facing drug addiction: Vietnam's struggle with opioids. *Drug Sci Policy Law*. 2021;7:20503245211034936. doi:10.1177/20503245211034934

358. EASL. *EASL Policy Statement : Drug Use and the Global Hepatitis C Elimination Goal*.; 2020. https://easl.eu/wp-content/uploads/2020/08/full-version-easl-policy-statement-on-drug-

use-and-the-global-hepatitis-c-elimination-goal.pdf.

359. Goyal A, Churkin A, Barash D, et al. Modeling-Based Response-Guided DAA Therapy for Chronic Hepatitis C to Identify Individuals for Shortening Treatment Duration. *Open Forum Infect Dis*. 2022;9(5):ofac157. doi:10.1093/ofid/ofac157

360. Guedj J, Dahari H, Rong L, et al. Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. *Proc Natl Acad Sci*. 2013;110(10):3991-3996. doi:10.1073/pnas.1203110110

361. Øvrehus ALH, Krarup H, Birkemose I, et al. Four weeks of ledipasvir/sofosbuvir and ribavirin with or without pegylated interferon for chronic hepatitis C in non-cirrhotic people who inject drugs. A randomized trial. *J Hepatol*. 2018;68(4):840-842. doi:10.1016/j.jhep.2017.11.031

362. Flower B, Hung LM, Mccabe L, et al. Efficacy of ultrashort, response guided sofosbuvir and daclatasvir therapy for Hepatitis C: a single arm, mechanistic, pilot study. *medRxiv*. January 2022:2022.08.15.22278752. doi:10.1101/2022.08.15.22278752

363. Orkin C, Arasteh K, Górgolas Hernández-Mora M, et al. Long-Acting Cabotegravir and Rilpivirine after Oral Induction for HIV-1 Infection. *N Engl J Med*. 2020;382(12):1124-1135. doi:10.1056/NEJMoa1909512

364. Verma M, Chu JN, Salama JAF, et al. Development of a long-acting direct-acting antiviral system for hepatitis C virus treatment in swine. *Proc Natl Acad Sci*. 2020;117(22):11987-11994. doi:10.1073/pnas.2004746117

365. Bailey JR, Barnes E, Cox AL. Approaches, Progress, and Challenges to Hepatitis C Vaccine Development. *Gastroenterology*. 2019;156(2):418-430. doi:10.1053/j.gastro.2018.08.060

366. Umutesi G, Shumbusho F, Kateera F, et al. Rwanda launches a 5-year national hepatitis C elimination plan: A landmark in sub-Saharan Africa. *J Hepatol*. 2019;70(6):1043-1045. doi:10.1016/j.jhep.2019.03.011

367. Nguyen TA, Nguyen BTC, Duong DT, Marks GB, Fox GJ. Experience in responding to COVID-19 outbreaks from Vietnam. *Lancet Reg Heal – West Pacific*. 2021;7. doi:10.1016/j.lanwpc.2020.100077

368. Phan LT, Nguyen T V, Luong QC, et al. Importation and Human-to-Human Transmission of a Novel Coronavirus in Vietnam. *N Engl J Med*. 2020;382(9):872-874. doi:10.1056/NEJMc2001272

369. Metcalfe C. Biostatistics: A Foundation for Analysis in the Health Sciences. 7th edn. Wayne W. Daniel, Wiley, 1999. No. of. pages: xiv+755+appendices. Price: £28.95. ISBN 0-471-16386-4. *Stat Med*. 2001;20(2):324-326. doi:https://doi.org/10.1002/1097-0258(20010130)20:2<324::AID-SIM635>3.0.CO;2-O

370. G. PO, Eleanor B, Rachel T, et al. Genetic History of Hepatitis C Virus in East Asia . *J Virol*. 2009;83(2):1071-1082. doi:10.1128/JVI.01501-08

371. Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. *Hepatology*. 2014;59(1):318-327. doi:https://doi.org/10.1002/hep.26744

372. Posada D. jModelTest: Phylogenetic Model Averaging. *Mol Biol Evol*. 2008;25(7):1253-1256. doi:10.1093/molbev/msn083

1
2
3
4
5
6
7
8
9
10

Appendix A

Supplementary information for chapter 2

Full titles of included studies

Appendix A Table i: Hepatitis B studies (n=44)

Title	Author
Short report: hepatitis B infection and severe Plasmodium falciparum malaria in Vietnamese adults	Barcus et al 2002
Prevalence of hepatitis A, B, C and E virus markers among patients with elevated levels of Alanine aminotransferase and Aspartate aminotransferase in Phnom Penh (Cambodia) and Nha Trang (Central Vietnam) [French]	Buchy et al 2004
Malaria in injection drug abusers in Vietnam	Chau et al 2002
Sexual practices, partner concurrency and high rates of sexually transmissible infections among male sex workers in three cities in Vietnam	Clatts et al 2015
Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam	Cordier et al 1993
Acute viral hepatitis in Hanoi, Viet Nam	Corwin et al 1996
High prevalences of hepatitis B and C virus infections among adults living in Binh Thuan province, Vietnam	Do et al 2015
A multicentre molecular analysis of hepatitis B and blood-borne virus coinfections in Viet Nam	Dunford et al 2012
Risk factors for hepatitis B infection in rural Vietnam	Duong et al 2009
Hepatitis B and C virus infections among patients with end stage renal disease in a low-resourced hemodialysis center in Vietnam: a cross-sectional study	Duong et al 2015 i
Challenges of hemodialysis in Vietnam: experience from the first standardized district dialysis unit in Ho Chi Minh City	Duong et al 2015 ii
Shewhart Charts and Two-Monthly Screening Interval to Monitor Hepatitis C and Hepatitis B Virus Infections in Two-Year Prospective Cohort Study of Hemodialysis Patients in Vietnam	Duong et al 2016
Clinical and biological characteristics of HIV infected and uninfected IDUs in HCMC Vietnam	Follezou et al 1999
Prevalence of and Factors Associated with Reproductive Tract Infections among Pregnant Women in Ten Communes in Nghe An Province, Vietnam	Goto et al 2005
Hepatitis B infection in rural Vietnam and the implications for a national program of infant immunization	Hipgrave et al 2003
Impact of a methadone maintenance therapy pilot in Vietnam and its role in a scaled-up response	Hoang et al 2015
Discrepancies in prevalence trends for HIV, hepatitis B virus, and hepatitis C virus in Haiphong, Vietnam from 2007 to 2012	Ishizaki et al 2017
Prevalence of hepatitis B, hepatitis C and GB virus C /hepatitis G virus infections in liver disease patients and habitants in HCM vietnam	Kakumu et al 1998

Seroprevalence of hepatitis viruses in children in rural Viet Nam	Katelaris et al 1995
Impact of Nucleic Acid Testing (NAT) on screening blood donors at a tertiary center in Vietnam	Kha To et al 2020
Reproductive tract infections including sexually transmitted infections: a population-based study of women of reproductive age in a rural district of Vietnam	Lan et al 2008
Epidemiology of hepatitis C virus infection in Vietnam. [French]	Lien et al 1997
High hepatitis C virus infection among female sex workers in Viet Nam: strong correlation with HIV and injection drug use	Linh-Vi et al 2019
The Prevalence and Components of Metabolic Syndrome in Men from Infertile Couples and Its Relation on Semen Analysis	Minh et al 2021
Hepatitis B virus infection among pregnant mothers and children after the introduction of the universal vaccination program in Central Vietnam	Miyakawa et al 2021
Findings from Integrated Behavioral and Biologic Survey among males who inject drugs (MWID)-Vietnam, 2009-2010: Evidence of the need for an integrated response to HIV, hepatitis B virus, and hepatitis C virus	Nadol et al 2015
High hepatitis C virus (HCV) prevalence among men who have sex with men (MSM) in Vietnam and associated risk factors: 2010 Vietnam Integrated Behavioural and Biologic Cross-Sectional Survey	Nadol et al 2016
Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam	Nakata et al 1994
High rates of positive viral hepatitis serologic in patients attending a city hospital challenge healthcare providers	Ngo et al 2009
The impact of dengue haemorrhagic fever on liver function	Nguyen et al 1997
Highly endemic hepatitis B infection in rural Vietnam	Nguyen et al 2006
Prevalence of HBV infection among different HIV-risk groups in Hai Phong, Vietnam	Nguyen et al 2011
A reduction in chronic hepatitis B virus infection prevalence among children in Vietnam demonstrates the importance of vaccination	Nguyen et al 2014
High burden of hepatocellular carcinoma and viral hepatitis in Southern and Central Vietnam: Experience of a large tertiary referral center, 2010 to 2016	Nguyen-Dinh et al 2018
Hepatitis B Infection and Mother-to-Child Transmission in Haiphong, Vietnam: A Cohort Study with Implications for Interventions	Pham et al 2020
Prevalence and factors associated with chronic Hepatitis B infection among adults in the Central Highland, Vietnam	Pham et al 2020 ii
Risks for HIV, HBV, and HCV infections among male injection drug users in northern Vietnam: A case-control study	Quan et al 2009
Markers of hepatitis C and B virus infections among blood donors in Ho Chi Minh City and Hanoi, Vietnam.	Song et al 1994
Prevalence of hepatitis B and hepatitis C in healthy adults in Ho Chi Minh City	Terakawa et al 2011
Baseline Characteristics and Treatment Cost of Hepatitis C at Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam in Direct-Acting Antiviral Treatment Era	Thanh et al 2020
Liver involvement associated with dengue infection in adults in Vietnam	Trung et al 2010
Hepatitis B in Ho Chi Minh City, Viet Nam	Van Be et al 1992
Epidemiological Characteristics of Advanced Hepatocellular Carcinoma in	Van Quang Le et al 2019

the Northern Region of Vietnam	
Prevalence of hepatitis B & hepatitis C virus infections in potential blood donors in rural Vietnam.	Viet et al 2012

1

2

3 **Appendix A Table ii: Hepatitis C studies (n=44)**

Title	Author
Prevalence of hepatitis A, B, C and E virus markers among patients with elevated levels of Alanine aminotransferase and Aspartate aminotransferase in Phnom Penh (Cambodia) and Nha Trang (Central Vietnam) [French]	Buchy et al 2004
Malaria in injection drug abusers in Vietnam	Chau et al 2002
Prevalence and incidence of HCV infection among Vietnam heroin users with recent onset of injection	Clatts et al 2009
HIV, Hepatitis C, and Other Sexually Transmitted Infections Among Male Sex Workers in Ho Chi Minh City, Vietnam	Colby et al 2016
Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam	Cordier et al 1993
Acute viral hepatitis in Hanoi, Viet Nam	Corwin et al 1996
High prevalences of hepatitis B and C virus infections among adults living in Binh Thuan province, Vietnam	Do et al 2015
Hepatitis C virus in Vietnam: high prevalence of infection in dialysis and multi-transfused patients involving diverse and novel virus variants	Dunford et al 2012
Hepatitis B and C virus infections among patients with end stage renal disease in a low-resourced hemodialysis center in Vietnam: a cross-sectional study	Duong et al 2015 i
Challenges of hemodialysis in Vietnam: experience from the first standardized district dialysis unit in Ho Chi Minh City	Duong et al 2015 ii
Shewhart Charts and Two-Monthly Screening Interval to Monitor Hepatitis C and Hepatitis B Virus Infections in Two-Year Prospective Cohort Study of Hemodialysis Patients in Vietnam	Duong et al 2016
Risk Behaviors for HIV and HCV Infection Among People Who Inject Drugs in Hai Phong, Viet Nam, 2014	Duong et al 2018
Screening haemodialysis patients for hepatitis C in Vietnam: The inconsistency between common hepatitis C virus serological and virological tests	Duong et al 2019
Clinical and biological characteristics of HIV infected and uninfected IDUs in HCMC Vietnam	Follezou et al 1999
Impact of a methadone maintenance therapy pilot in Vietnam and its role in a scaled-up response	Hoang et al 2015
Discrepancies in prevalence trends for HIV, hepatitis B virus, and hepatitis C virus in Haiphong, Vietnam from 2007 to 2012	Ishizaki et al 2017
Prevalence of hepatitis B, hepatitis C and GB virus C /hepatitis G virus infections in liver disease patients and habitants in HCM Vietnam	Kakumu et al 1998
Seroprevalence of hepatitis viruses in children in rural Viet Nam	Katelaris et al 1995

Impact of Nucleic Acid Testing (NAT) on screening blood donors at a tertiary center in Vietnam	Kha To et al
Epidemiology of hepatitis C virus infection in Vietnam. [French]	Lien et al 1997
High hepatitis C virus infection among female sex workers in Viet Nam: strong correlation with HIV and injection drug use	Linh-Vi et al 2019
HIV control programs reduce HIV incidence but not HCV incidence among people who inject drugs in HaiPhong, Vietnam	Molès et al 2020
Findings from Integrated Behavioral and Biologic Survey among males who inject drugs (MWID)-Vietnam, 2009-2010: Evidence of the need for an integrated response to HIV, hepatitis B virus, and hepatitis C virus	Nadol et al 2015
High hepatitis C virus (HCV) prevalence among men who have sex with men (MSM) in Vietnam and associated risk factors: 2010 Vietnam Integrated Behavioural and Biologic Cross-Sectional Survey	Nadol et al 2016
Findings from Integrated Behavioral and Biologic Survey among males who inject drugs (MWID)-Vietnam, 2009-2010: Evidence of the need for an integrated response to HIV, hepatitis B virus, and hepatitis C virus	Nadol et al 2016
Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam	Nakata et al 1994
Lack of association between acquisition of TT virus and risk behavior for HIV and HCV infection in Vietnam	Nerurkar et al 1999
High rates of positive viral hepatitis serologic in patients attending a city hospital challenge healthcare providers	Ngo et al 2009
The impact of dengue haemorrhagic fever on liver function	Nguyen et al 1997
Prevalence and risk factors for hepatitis C infection in rural north Vietnam	Nguyen et al 2007
Acceptability and Usability of HCV Self-Testing in High Risk Populations in Vietnam	Nguyen et al 2021
High burden of hepatocellular carcinoma and viral hepatitis in Southern and Central Vietnam: Experience of a large tertiary referral center, 2010 to 2016	Nguyen-Dinh et al 2018
Risks for HIV, HBV, and HCV infections among male injection drug users in northern Vietnam: A case-control study	Quan et al 2009
Hepatitis C virus seroprevalence in the general female population from 8 countries	Quesada et al 2015
Towards Targeted Interventions in Low- and Middle-Income Countries: Risk Profiles of People Who Inject Drugs in Haiphong (Vietnam)	Riondel et al 2020
The evolution of hepatitis C in the kidney transplant recipient at CHO ray hospital	Sinh et al 2012
Markers of hepatitis C and B virus infections among blood donors in Ho Chi Minh City and Hanoi, Vietnam.	Song et al 1994
Multiple routes of hepatitis C virus transmission among injection drug users in Hai Phong, Northern Vietnam	Tanimoto et al 2010
Prevalence of hepatitis B and hepatitis C in healthy adults in Ho Chi Minh City	Terakawa et al 2011
Prevalence of hepatitis virus types B through E and genotypic distribution of HBV and HCV in Ho Chi Minh City, Vietnam	Tran et al 2003
Liver involvement associated with dengue infection in adults in Vietnam	Trung et al 2010
Epidemiological Characteristics of Advanced Hepatocellular Carcinoma in the Northern Region of Vietnam	Van Quang et al 2019

Prevalence of hepatitis B & hepatitis C virus infections in potential blood donors in rural Vietnam.	Viet et al 2012
Prevalence and correlates of HCV monoinfection and HIV and HCV coinfection among persons who inject drugs in Vietnam	Zhang et al 2015

1

2

3 **Appendix A Table iii: HIV studies with HBV or HCV co-infection data (n=10)**

Title	Author
TAHOD-LITE: Antiretroviral Treatment for Adult HIV Infection in Asia, 1998 to 2013	Boettiger et al 2015
HBV and HCV Coinfection among HIV/AIDS Patients in the National Hospital of Tropical Diseases, Vietnam	Bùi et al 2014
Prevalence of Opportunistic Infections and Associated Factors in HIV-Infected Men Who Have Sex With Men on Antiretroviral Therapy in Bach Mai Hospital, Hanoi, Vietnam: A Case-Control Study	Dang et al 2020
Epidemiology of hepatitis C virus infection in Vietnam. [French]	Lien et al 1997
Viral hepatitis among HIV+ patients in northern Vietnam	Mohan et al 2017
High Proportion of HIV-HCV Coinfected Patients with Advanced Liver Fibrosis Requiring Hepatitis C Treatment in Haiphong, Northern Vietnam (ANRS 12262)	Nguyen et al 2016
Factors associated with HIV RNA viral loads in ART-naive patients: implications for treatment as prevention in concentrated epidemics	Rangarajan et al 2016
Penicilliosis and AIDS in Haiphong, Vietnam: evolution and predictive factors of death	Son et al 2014
Long-term viral suppression and immune recovery during first-line antiretroviral therapy: a study of an HIV-infected adult cohort in Hanoi, Vietnam	Tanuma et al 2017

4

5 **Appendix A Table iv: HDV studies (n=7)**

Title	Author
HDV infection rates in northern Vietnam	Binh et al 2018
A multicentre molecular analysis of hepatitis B and blood-borne virus coinfections in Viet Nam	Dunford et al 2012
High prevalence of hepatitis delta virus among persons who inject drugs, Vietnam	Hall et al 2015
Predominance of HBV Genotype B and HDV Genotype 1 in Vietnamese Patients with Chronic Hepatitis	Nghiem et al 2021
Prevalence and genotype distribution of hepatitis delta virus among chronic hepatitis B carriers in Central Vietnam	Nguyen et al 2017
High prevalence and significance of hepatitis D virus infection among treatment-naive HBsAg-positive patients in Northern Vietnam	Sy et al 2013
Prevalence of hepatitis virus types B through E and genotypic distribution of HBV and HCV in Ho Chi Minh City, Vietnam	Tran et al 2003

Appendix A Table v: Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence data for all 72 included studies

Critical appraisal of study quality was performed by BF (first author) and HVTK (third author). Discrepancies regarding study eligibility were resolved through discussion between investigators (HVTK, BF).

1. Was the sample frame appropriate to address the target population?
2. Were study participants recruited in an appropriate way?
3. Was the sample size adequate?
4. Were the study subjects and setting described in detail?
5. Was data analysis conducted with sufficient coverage of the identified sample?
6. Were valid methods used for the identification of the condition?
7. Was the condition measured in a standard, reliable way for all participants?
8. Was there appropriate statistical analysis?
9. Was the response rate adequate, and if not, was the low response rate managed appropriately?

Study	JBI checklist indicators									Score	Comment
	1	2	3	4	5	6	7	8	9		
Barcus et al 2002	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sampling, non-representative sample (severe malaria)
Binh et al 2018	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sampling, non-representative sample (85% male)
Boettiger et al 2015	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Buchy et al 2004	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sampling, underpowered
Bùi et al 2014	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random; entire centre's population
Chau et al 2002	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Clatts et al 2009	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random sampling; non-representative sample (male IVDU only)
Clatts et al 2015	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random; non-representative (sex workers)
Colby et al 2016	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random sampling; non-representative (sex workers)
Cordier et al 1993	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random, non-representative, male HCC only
Corwin et al 1996	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Dang et al 2020	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Do et al 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Multi-stage cross-sectional
Dunford et	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive

al 2012											sampling
Dunford et al 2012	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Duong et al 2009	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Cross sectional but non-representative rural sample
Duong et al 2015 i	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Duong et al 2015 ii	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Duong et al 2016	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random; entire centre population
Duong et al 2018	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Duong et al 2019	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random; entire centre population
Follezou et al 1999	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	6	Non-representative sample (very high rates HIV)
Goto et al 2005	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Hall et al 2015	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sampling, lacks baseline characteristics
Hipgrave et al 2003	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Hoang et al 2015	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Ishizaki et al 2017	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Kakumu et al 1998	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling of hepatitis patients. Details of sampling strategy for general population lacking
Katelaris et al 1995	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8	Under powered for HCV prevalence
Kha To et al 2020	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Lan et al 2008	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-representative sample (married women age 18-49)
Lien et al 1997	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-cross sectional sampling
Linh-Vi et al 2019	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Minh et al 2021	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random, non-representative sample (males from infertile couples)
Miyakawa et al 2021	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	7	Non-random sample, high drop out >30%
Mohan et al 2017	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random retrospective chart review

Molès et al 2020	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random response-driven sampling
Nadol et al 2015	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random response-driven sampling
Nadol et al 2016	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random response-driven sampling
Nakata et al 1994	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive and retrospective sampling
Nerurkar et al 1999	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	7	Non-random sampling, diagnostics were combo of sera or filter paper-blotted whole blood
Nghiem et al 2021	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random, consecutive sampling
Ngo et al 2009	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	6	Non-random, consecutive sampling, non-representative sample (inpatients and outpatients), minimal baseline characteristics
Nguyen et al 1997	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	6	Non-random consecutive sampling, non-representative sample (patients with severe Dengue), under-powered for HCV
Nguyen et al 2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Nguyen et al 2007	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Nguyen et al 2011	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Nguyen et al 2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Nguyen et al 2017	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sampling, non-representative
Nguyen-Dinh et al 2018	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random sampling, Oraquick diagnostics
Pham et al 2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	Non-random retrospective sample
Pham et al 2020 ii	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9	
Quan et al 2009	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	
Quesada et al 2015	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random snowball sampling using peer recruiters
Rangarajan et al 2016	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-representative sample (females only)
Riondel et al 2020	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling

Sinh et al 2012	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	7	Non-random response-driven sampling
Son et al 2014	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	6	Non-random consecutive sample, lacking baseline characteristics
Song et al 1994	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling, non-representative (inpatients with penicilliosis), under powered for HBV/HCV prevalence
Sy et al 2013	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random sampling
Tam et al 2016	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling, non-representative (HCV and HIV positive patients excluded)
Tanimoto et al 2010	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random response driven sampling
Tanuma et al 2017	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Terakawa et al 2011	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random sampling, non-representative (healthy workers at major companies)
Thanh et al 2020	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sample, non-representative (HCV-infected outpatients)
Tran et al 2003	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random sampling, non-representative (healthy outpatients)
Truong et al 2016	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random consecutive sample
Trung et al 2010	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random consecutive sampling
Van Be et al 1992	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	6	Non-random sampling, unclearly defined study populations, no baseline characteristics
Van Quang et al 2019	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random, retrospective sample
Viet et al 2012	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7	Non-random sampling; non-representative (*potential* blood donors, HBV-vaccinated individuals excluded)
Zhang et al 2015	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8	Non-random sampling

Sample size adequacy was assessed using the formula $n = Z^2P(1-P)/d^2$*, where n is the sample size, Z is the statistic corresponding to level of confidence (95%), P is expected prevalence (10% HBV, 1-10% HCV depending on risk-group) and d is precision (corresponding to effect size)*[369].

Appendix B

Supplementary information for chapters 3 & 4

Lab genotyping methodology SEARCH stratum A and B (chapters 3 & 4)

Viral RNA was extracted from 140 µl of plasma using MagNa pure 96 DNA and viral NA small volume kit (Roche Diagnostics, Basel). Viral RNA was reverse transcribed using superscript III reverse transcriptase protocol as described earlier[370]. The resulting cDNA was used for amplification of 236 bp 5'UTR, 377 bp NSB5 and 464 bp core region using primers through nested PCR as described earlier[370]. Amplicons were run on 1.5% agarose gel (Merck, Germany) and purified using the Agincourt AMPure XP (Beckman Coulter, Inc) according to the manufacturer's protocol. Purified amplicons were sequenced both forward and reverse direction using Big Dye Terminator Cycle Sequencing Ready Reaction Kit and Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems).

The nucleotide sequences were aligned and the consensus sequence was generated. This includes 236 bp 5'UTR (position 78 to 314), 464 bp core sequence (position 288 to 752) and 377 bp NS5B (position 8259 to 8636) of GenBank sequence AY051292. Forty seven well characterized HCV whole genome sequences representing all genotypes and subtypes were downloaded from GenBank and used as reference sequences[371]. The reference sequences and the sequences from the current study were subjected to phylogenetic analysis using Genious 8.0.5 software package (http://www.geneious.com). NSB5 sequences (if NS5B sequence is not available, core or 5'UTR sequences respectively) from the present study and the corresponding region of reference sequences were aligned with MUSCLE alignment program available within the Genious package. The sequence alignments were then subjected to Jmodel test to identify the best model for phylogenetic analysis[372]. The suggested nucleotide substitution model (GTR+G+I) was subsequently used in phylogenetic analysis using Neighbor-Joining method (available in Genious package). To confirm the reliability of phylogenetic tree analysis, bootstrap resampling and reconstruction were carried out 1000 times. All sequences from this study was submitted to GenBank (accession numbers for 5'UTR MH191406—MH191721; core region MH191722—MH192024 and, NS5B region MH192025—MH192337).

Methodological detail of PK/PD analysis in stratum A (chapter 3)

Sofosbuvir and GS-331007 were extracted from 100 µL of plasma using phospholipid removal in the 96-well plate format (Phree, 8E-S133-TGB, Phenomenex), followed by separation on a Gemini, 50 mm × 2.0 mm I.D. 5 µm, column (00B-4435-B0, Phenomenex). Quantification was performed using

selected reaction monitoring for the transitions m/z 530.2–>243.2 (sofosbuvir), 536.2–>243.1 (isotope-labelled internal standard for sofosbuvir), 261.3–>113.1 (GS-331007), and 265.3–>113.1 (isotope-labelled internal standard for GS-331007).

The lower limit of quantification (LLOQ) was set to 1.95 ng/mL for sofosbuvir and 20.5 ng/mL for GS-331007. A total of 9 quality control samples (3×low, 3×mid and 3×high concentration) were analysed for each analyte within each batch of clinical samples (96-well plate), resulting in an accuracy of 2.81-2.93% RSE for sofosbuvir and 2.19-3.50% RSE for GS-331007.

DCV was extracted from 100 µL of plasma using supported liquid extraction in the 96-well plate format (ISOLUTE® SLE+ 96-well plate, 820-0200-P01, Biotage), followed by separation on a Gemini, 50 mm × 2.0 mm I.D. 5 µm, column (00B-4435-B0, Phenomenex). Quantification was performed using selected reaction monitoring for the transitions m/z 739.5–>339.3 (DCV) and 747.5–>339.3 (isotope-labelled internal standard for DCV). The LLOQ was set to 1.64 ng/mL for DCV. A total of 9 quality control samples (3×low, 3×mid and 3×high concentration) were analysed within each batch of clinical samples (96-well plate), resulting in an accuracy of 2.46-2.62% RSE for DCV.

PK/PD analysis

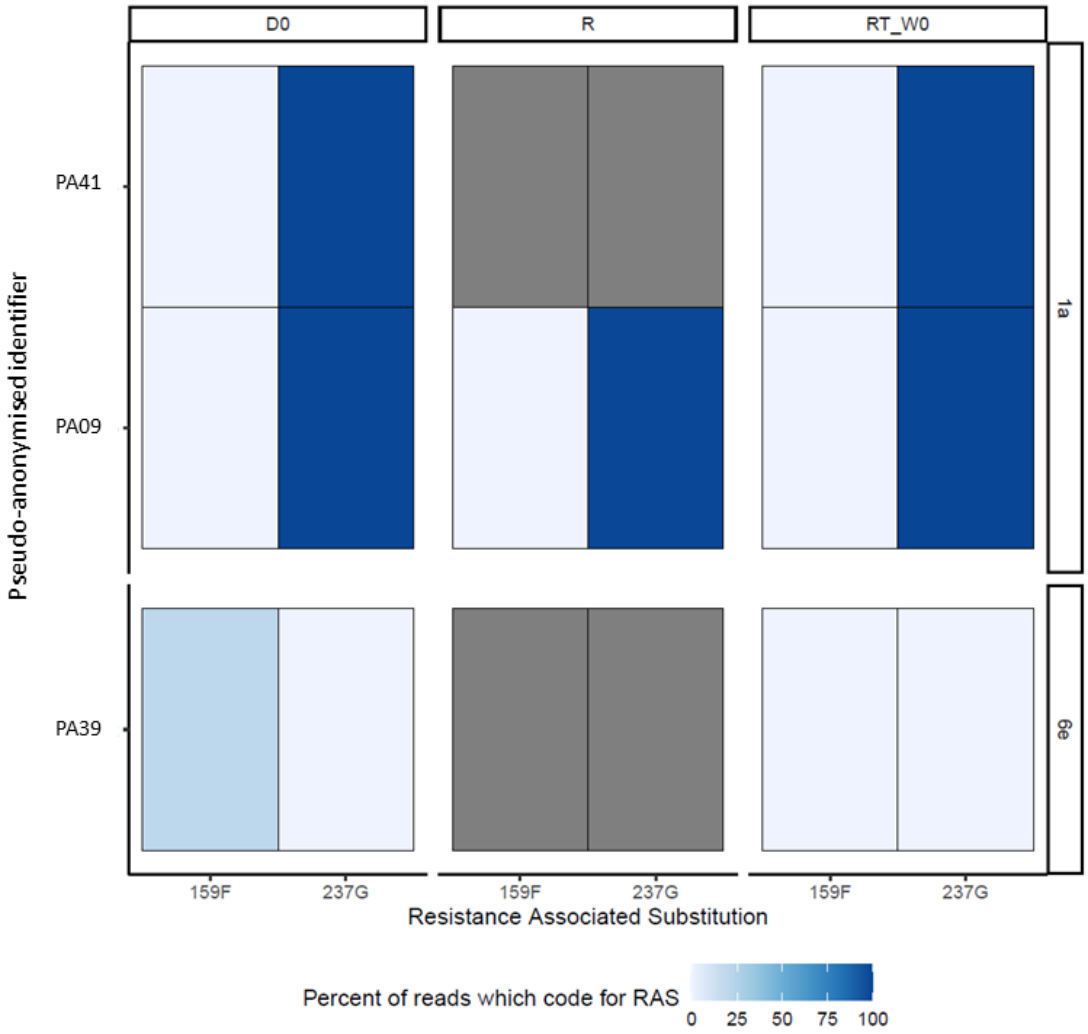
Maximum concentration (C_{max}) and time to reach maximum concentration (t_{max}) were derived directly from the observed drug concentrations. The software's automatic slope calculator was used to derive the elimination rate (λ) (adjusted R^2 value with uniform weighting) and terminal elimination half-life ($t_{1/2}$). The drug exposure measured as area under the concentration-time curve (AUC) was calculated for each drug using the trapezoidal method. Linear interpolation was used for acceding concentrations and log-linear interpolation for descending concentrations. For the individual analysis, the linear method was used for all measurements due to accumulation between the day 0 and day 28 measurements. Both the AUC to the last time point (AUC_{last}, 8 hours for SOF and 24 hours for GS-331007 and DCV) and AUC to infinity (AUC_{inf}) were calculated.

A non-compartmental pharmacodynamic analysis were conducted using viral load data from enrolment to day 14 to calculate area under the viral load – time curve and terminal elimination half-life of the viral clearance curve, using the same methodology as explained above. In addition, the relative reduction in viral load between enrolment and day 1, and between enrolment and day 7 were calculated. Ordinary linear regression of drug exposure (AUC_{last}) from the individual pharmacokinetic analysis and the outcome measurements were performed in GraphPad Prism 9.3.1 (GraphPad Software, San Diego, California USA).

Limitations of PK/PD analysis

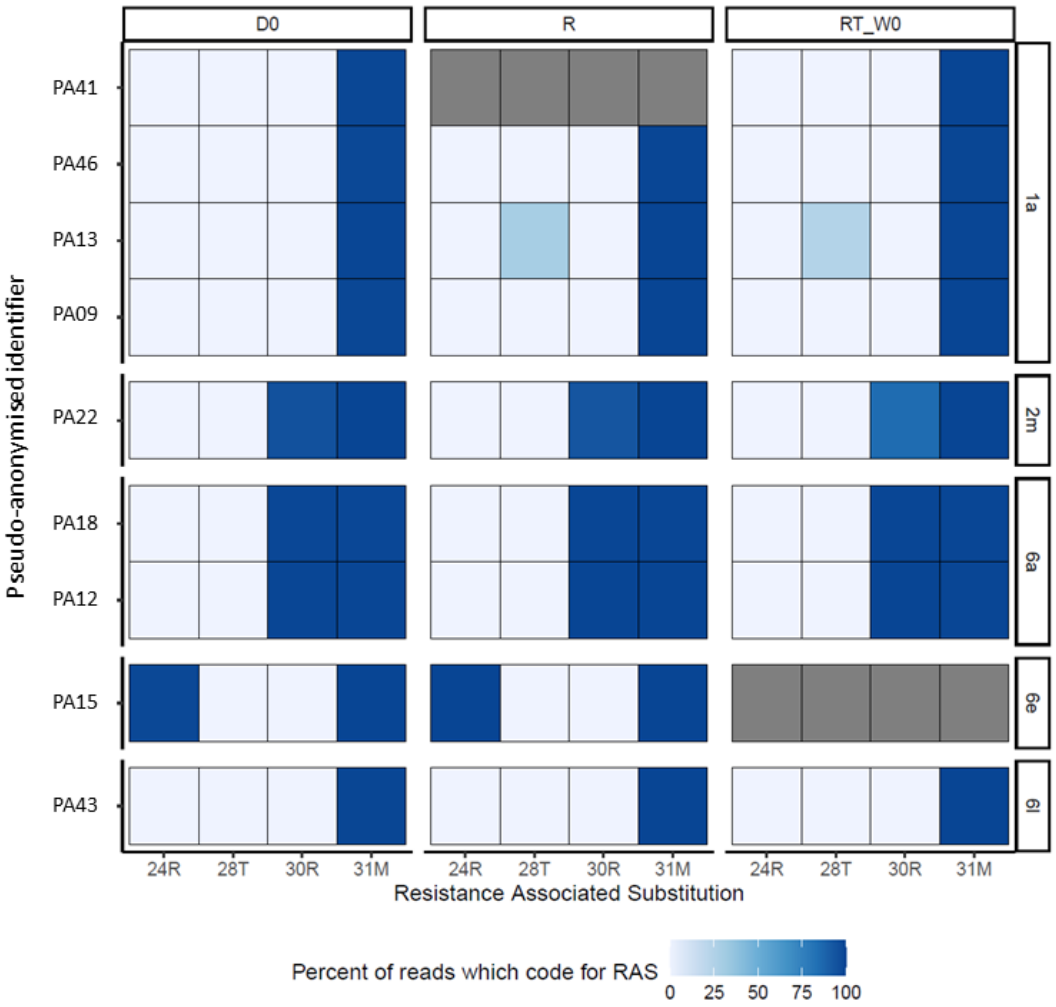
Two different sampling schedules were used to collect pharmacokinetic samples on day 0 and day 28, resulting in an overlapping sampling profile overall. Therefore, data collected within an individual on a specific day was not dense enough to justify a non-compartment analysis. However, if the data on day 0 and 28 were combined. it resulted in a complete pharmacokinetic profile for the individual. Two separate pharmacokinetic analyses were carried out. A naïve-pooled analysis was performed separately conducted on day 0 and 28 data. This resulted in a summary of exposure (AUC and Cmax) and half-life of the drugs, but it would not be possible to link these measurements to treatment outcome due to the different sampling strategies. Therefore, a second analysis was performed in which the data from day 0 and 28 were pooled for each individual. This resulted in complete pharmacokinetic profiles for each patient, and the pharmacokinetic-pharmacodynamic analysed demonstrated no significant relationship between drug exposure and treatment outcome. Drug accumulation was observed for the sofosbuvir metabolite GS-331007 and DCV between day 0 and 28. However, the individual analysis should still generate median exposure values for each patient, which can be linked with treatment outcome. Another drawback is that no pre-dose samples were collected. Therefore, the non-compartmental analysis will assume no concentration at day 0 on day 28 for GS-331007 and DCV, even though drug accumulation was observed.

Appendix B - Figure i: Original R plot of SOF-RAS at baseline (D0), time of virological rebound (R) and start of retreatment (RT_W0) from which Figure 3-14 was derived.



Grey boxes represent missing data

Appendix B - Figure ii: Original R plot of DCV-RAS at baseline (D0), time of virological rebound (R) and start of retreatment (RT_W0) from which Figure 3-14 was derived.



Grey boxes represent missing data

Appendix C

Supplementary Information for Chapter 5

Appendix C Table i: STARD Checklist for reporting diagnostic accuracy studies

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	Title. page 1
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	Abstract, page 2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	Introduction, p3
	4	Study objectives and hypotheses	
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Methods, p3-4
Participants	**6**	Eligibility criteria	Methods, p3-4
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Methods, p3-4
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Methods, p3-4, published trial papers
	9	Whether participants formed a consecutive, random or convenience series	Methods, p4, published trial papers
Test methods	**10a**	Index test, in sufficient detail to allow replication	Methods, p4, published trial papers
	10b	Reference standard, in sufficient detail to allow replication	Methods, p4, published trial papers
	11	Rationale for choosing the reference standard (if alternatives exist)	Methods p4
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Methods p4
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Methods, p4
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	Methods, Appendix figures 1 & 2
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	Methods, p4, Appendix figures 3 & 4
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	Methods, p4
	15	How indeterminate index test or reference standard results were handled	N/A
	16	How missing data on the index test and reference standard were handled	Methods, Appendix

			figures 1 & 2
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Methods, Appendix figures 1 & 2
	18	Intended sample size and how it was determined	Published trial papers
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Appendix figures 3 & 4
	20	Baseline demographic and clinical characteristics of participants	Appendix table 1
	21a	Distribution of severity of disease in those with the target condition	Appendix table 1
	21b	Distribution of alternative diagnoses in those without the target condition	Appendix table 1
	22	Time interval and any clinical interventions between index test and reference standard	Appendix figures 1 & 2
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Appendix figures 3 & 4
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Figure 1
	25	Any adverse events from performing the index test or the reference standard	N/A
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	Discussion p6
	27	Implications for practice, including the intended use and clinical role of the index test	Discussion, p6
OTHER INFORMATION			
	28	Registration number and name of registry	In original papers
	29	Where the full study protocol can be accessed	Both study protocols available with published papers
	30	Sources of funding and other support; role of funders	Acknowledgements