

Difficult to treat populations with hepatitis C infection: Prisoners, people who inject drugs, and viral resistance

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Abstract

It is estimated that 71 million people are living with hepatitis C virus (HCV) infection globally. The availability of all-oral direct acting antiviral (DAA) medications for HCV infection has revolutionised treatment which is now well tolerated, short in duration and highly effective; cure rates exceed 95% in clinical trials. In this context, the World Health Organization (WHO) has proposed targets for the elimination of HCV as a public health threat by 2030. This includes an 80% reduction in HCV incidence and 65% reduction in HCV-related mortality. Achieving these targets will require sustained effort in diagnosing and treating HCV infection, as well as the development and implementation of models of care to reach populations both highly affected by HCV infection or those underserved by current models. Mathematical modelling data has demonstrated that in high and middle-income countries, HCV incidence will be most rapidly reduced if treatment is prioritised for people who inject drugs (PWID) who contribute most greatly to HCV transmission.

Prison populations globally are characterised by high HCV prevalence which is estimated to exceed 15%, and, in Australia, is as high as 50% amongst incarcerated PWIDs. PWIDs also experience high rates of incarceration on account of the criminalisation of injecting drug use (IDU). Despite this, HCV treatment rates within prisons have remained low due to multiple barriers to prison-based HCV care. As individuals re-enter the community from prison, they are also confronted by multiple competing priorities and linkage to HCV care was poor in the interferon era. How we can safely and effectively increase HCV treatment rates in the prison setting and beyond is not well defined.

While most people living with HCV are cured following first-line DAA therapy, there are a subset who relapse; those with advanced liver disease, including cirrhosis, are overrepresented in this group. It is therefore important to evaluate second-line salvage therapies in individuals with advanced disease to minimise HCV-mortality and mortality, in keeping with the WHO elimination goals.

This thesis includes a number of clinical studies to address the above issues. In the first study, I evaluated the safety and efficacy of HCV treatment delivered to prisoners by the Statewide Hepatitis Program which operates in all Victorian prisons. I identified that nurse-led HCV care can reach prisoners in large numbers, that it was safe, and that cure rates exceeded 95%. Importantly, over 80% of those treated had never engaged in specialist HCV care prior to incarceration, highlighting that prison-based treatment can reach a population that is poorly served by existing healthcare models. Given that approximately 70% of prisoners reported recent IDU prior to incarceration, prison-based HCV care can also potentially reduce HCV incidence and prevalence within the community more broadly via interrupting transmission.

In the second study, I investigated the likelihood of community DAA initiation amongst individuals released from prison with untreated HCV infection. I performed a randomised control trial evaluating a transitional intervention aimed at enhancing continuity of care following release. I identified that amongst unsupported individuals, DAA initiation rates were low. Our transitional model, however, was able to support a greater proportion of individuals to commence HCV treatment, whilst also significantly reducing the interval between community re-entry and DAA initiation, compared to those receiving standard of care. In the third study, I investigated the performance of non-invasive fibrosis algorithms amongst prisoners with HCV, including the AST-to-Platelet Index (APRI) and FIB-4 scores, to minimise the need for transient elastography and improve prison treatment efficiencies. I identified that these algorithms achieved high negative predictive values (NPV) at previously validated thresholds and, if incorporated into HCV assessment pathways, could minimise the need for further fibrosis determination. This is relevant for the dissemination of prison-based HCV programs domestically and internationally where access to transient elastography remains a barrier to care.

In the fourth study, I investigated the prevalence of HCV NS5A resistance associated substitutions (RAS) at treatment baseline in DAA-naïve participants with genotype (GT)1a, GT1b and GT3 infection. I demonstrated that the baseline NS5A RAS prevalence amongst Australians with GT1a infection was lower than other international regions, but comparable for GT1b and GT3 infection, and that next generation sequencing offered little improvement in diagnostic yield over population-based sequencing at baseline. These findings endorse Australia guidelines that HCV RAS testing is not required prior to first-line DAA therapy.

In the fifth study, I evaluated the safety and efficacy of sofosbuvir, elbasvir, grazoprevir $\pm$ ribavirin for relapsed HCV following treatment with an NS5A-inhibitor containing DAA regimen. I demonstrated that not only was this an effective salvage regimen for GT1/4, for which elbasvir and grazoprevir are licensed, but also for GT3 infection by using a multitargeted approach to suppress the emergence of resistant viral variants.

In the sixth study, I evaluated the safety and efficacy of sofosbuvir, velpatasvir and voxilaprevir for relapsed HCV in Australians with advanced liver disease who were treated via a pharmaceutical early access program. Despite a high prevalence of difficult to cure characteristics including GT3 infection, cirrhosis, portal hypertension and liver transplantation (LT), I identified high SVR12 rates. While treatment was generally well tolerated, there were three episodes of hepatic decompensation and protease inhibitor usage should be carefully monitored in those with advanced disease. This study provides reassuring evidence that this regimen is highly effective for relapsed HCV, irrespective of baseline characteristics.

Declaration

This is to certify that this thesis:

- i) contains no material that has been accepted for the award of any other degree or diploma at any university or other institution;
- ii) comprises only my original work except where indicated in the Preface;
- iii) includes due acknowledgement in the text to all other material used; and
- iv) is fewer than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices

Timothy Joseph Papaluca, November 2020

Preface

The work presented in this thesis was completed under the supervision of Professor Alexander Thompson, St Vincent's Hospital Melbourne and University of Melbourne, and co-supervised by A/Prof Joseph Doyle, Prof Mark Stooze and Prof Margaret Hellard, Burnet Institute and Monash University.

This thesis contains six results chapters, five of which have been published, or accepted for publication by peer reviewed journals. The remaining manuscript has been submitted for publication. In regard to chapter three, results have also been published as correspondence in a peer-reviewed journal and have been included in this thesis. Multiple co-authors have been involved, and where relevant, they have completed and signed a co-author authorisation form.

Chapter two describes the evaluation of the Statewide Hepatitis Program. Ms. Lucy McDonald, Ms. Anne Craigie, Ms. Annabelle Gibson, Dr David Iser and Prof Alexander Thompson contributed to the clinical management of the prisoners, forming the basis of this evaluation. Co-authors also contributed to data collection, assistance with data interpretation, study concept proposals, and/or critical review of the manuscript. This study was published in *The Journal of Hepatology* on 5th May 2019.

Chapter three describes the low likelihood of linkage to HCV care following release from prison and the positive impact of dedicated transitional support. Ms. Lucy McDonald, Ms. Anne Craigie and Ms. Amy Edwards were involved in identifying participants during prison-based HCV assessments. Annabelle Hoang and Alex Pappas played a key role in the dispensing and distribution of HCV DAAs. Other co-authors either contributed to study concept proposals, and/or critical review of the manuscript. The retrospective study was published as correspondence in *The Lancet Infectious Diseases* in November 2019 and the prospective study has been submitted for publication to a peer-reviewed journal.

Chapter four describes the use of non-invasive fibrosis algorithms for prisoners with HCV infection to optimise assessment efficiencies. Multiple co-authors were involved in data collection prior to undertaking the data analysis. The Statewide Hepatitis Program nurses, Ms. Anne Craigie, Ms. Lucy Donald and Ms Amy Edwards, as well as clinicians Prof Alexander Thompson, Dr David Iser, Dr Jacinta Holmes and Dr Michael MacIsaac were also involved in the clinical management of the prisoners, including the acquisition of transient elastography results. All co-authors contributed to study concept proposals, data collection and/or critical review of the manuscript. This study was accepted for publication in *PLoS One* on October 27th 2020.

Chapter five reports the baseline prevalence of NS5A RAS amongst a DAA-naïve Australians. All samples included were sequenced at The Victorian Infectious Disease Reference Laboratory by Ms. Jacinta O’Keefe under the supervision of Prof. Scott Bowden. All other authors assisted with the interpretation of the data, contribution to study concept proposals and/or critical review of the manuscript. This study was published in the Journal of Clinical Virology in November 2019.

Chapter six describes the efficacy and safety of sofosbuvir, elbasvir, grazoprevir and ribavirin for relapsed HCV infection. Many of the co-authors contributed to the clinical care of participants at the three alternate Melbourne-based hospitals involved in the study including Dr Marie Sinclair, A/Prof Paul Gow, Prof William Sievert, Dr Stephen Pianko, Dr NJ Arachchi and Dr Karla Cameron. Co-authors also contributed to the study concept proposals and/or critical review of the manuscript. This study was published in Liver International on 29th July 2019.

Chapter seven reports the efficacy and safety of sofosbuvir, velpatasvir and voxilaprevir for patients with relapsed HCV infection and advanced disease. Many of the co-authors contributed to the clinical care of the participants at 26 participating hospitals including Stuart Roberts, Simone Strasser, Katherine Stuart, Geoffrey Farrell, Gerry MacQuillan, Gregory Dore, Amanda Wade, Jacob George, Simon Hazeldine, James O’Beirne, Alan Wigg, Leslie Fisher, Bruce McGarity, Rohit Sawhney, Marie Sinclair, James Thomas, Ivan Valiozis, Martin Weltman, Mark Wilson, Aidan Woodward, Golo Ahlenstiel, Mazhar Haque, Miriam Levy, Emily Prewett, William Sievert, Siddharth Sood, Edmund Tse and Zina Valaydon. HCV NS5A RAS sequencing was performed at the Victorian Infectious Disease Reference Laboratory and Westmead Hospital by Prof Scott Bowden, Prof Mark Douglas and Ms Jacinta O’Keefe. Ms Kate New contributed to project administration. Co-authors were also involved in contribution to study concept proposals and/or critical review of the manuscript. This study was published online in Clinical Infectious Diseases on 5th September 2020.

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Thank you to my co-supervisors Associate Professor Joseph Doyle, Professor Mark Stoove and Professor Margaret Hellard. I have benefited greatly from your wealth of research experience, conceptualisation of the studies and dynamic approach to problem solving the challenges encountered during this thesis. Thank you to Professor Paul Desmond for your expertise in navigating the research process as chair of my Advisory Committee.

I would like to acknowledge the Statewide Hepatitis Program team for their significant contribution to this thesis. Whilst it has been my privilege to evaluate the clinical service, the thousands of prisoners treated by the service is testament to the hard work and determination of many healthcare professionals. Thank you in particular to Anne Craigie and Lucy McDonald who have been welcoming, supportive and adaptable since the thesis commenced. I am impressed by the kindness and compassion you show your patients every day and your strong commitment to the vulnerable prison communities. Thank you also for the laughs along the way. Thank you to Rebecca Winter for your contribution to C-LINK and your support in completing the thesis more broadly.

I also wish to acknowledge the contribution of Professor Scott Bowden and Ms. Jacinta O’Keefe who performed the HCV NS5A sequencing included in this thesis.

I would like to acknowledge the financial support for the C-LINK trial received from the St Vincent’s Hospital Research Endowment Fund. I also wish to acknowledge the financial support I received from the Department of Gastroenterology, St Vincent’s Hospital Melbourne and the Australian Government Postgraduate Research Scholarship.

I would also like to acknowledge my parents and my sisters for their love, support and kind encouragement. And finally thank you to my partner, Chris. Thank you for encouraging and supporting me to reach this point, carrying the work load when I have been snowed under and providing comfort when challenges have arisen. I am very excited about the next phase of life with you.

Publications and presentations arising from this thesis

Journal publications

Papaluca T, McDonald L, Craigie A, Gibson A, Desmond P, Wong D, Winter R, Scott N, Howell J, Doyle J, Pedrana A, Lloyd A, Stooove M, Hellard M, Iser D, Thompson A. Outcomes of treatment for hepatitis C in prisoners using a nurse-led, statewide model of care. *Journal of Hepatology*. 2019 May;70(5):839-846.

Papaluca T, Tambakis G, Iser D, Thompson A. Effective prison-based treatment and linkage to care after release. *The Lancet Infectious Diseases* 2019 Nov;19(11):1171.

Papaluca T, Craigie A, McDonald L, Edwards A, MacIsaac M, Holmes J, Jarman M, Lee T, Huang H, Chan A, Lai M, Sundararajan V, Doyle J, Hellard M, Stooove M, Howell J, Desmond P, Iser D, Thompson A. Non-invasive fibrosis algorithms are clinically useful for excluding cirrhosis in prisoners living with hepatitis C. Accepted for publication in *PLoS One* on 27th October 2020.

Papaluca T, O’Keefe J, Bowden S, Doyle J, Stooove M, Hellard M, Thompson A. Prevalence of baseline HCV NS5A resistance associated substitutions in genotype 1a, 1b and 3 infection in Australia. *Journal of Clinical Virology* 2019 Nov;120:84-87.

Papaluca T, Sinclair M, Gow P, Pianko S, Sievert W, Arachchi N, Cameron K, Bowden S, O’Keefe J, Doyle J, Stooove M, Hellard M, Iser S, Thompson A. Retreatment with elbasvir, grazoprevir, sofosbuvir +/- ribavirin is effective for GT3 and GT1/4/6 HCV infection after relapse. *Liver International* 2019 Dec;39(12):2285-2290.

Papaluca T, Roberts S, Strasser S, Stuart K, Farrell G, MacQuillan G, Dore G, Wade A, George J, Hazeldine S, O’Beirne J, Wigg A, Fisher L, McGarity B, Sawhney R, Sinclair M, Thomas J, Valiozis I, Weltman M, Wilson M, Woodward A, Ahlenstiel G, Haque M, Levy M, Prewett E, Sievert W, Sood S, Tse E, Valaydon Z, Bowden S, Douglas M, New K, O’Keefe J, Hellard M, Doyle J, Stooove M, Thompson A. Efficacy and safety of sofosbuvir/velpatasvir/voxilaprevir for HCV NS5A-inhibitor experienced patients with difficult to cure characteristics. *Clinical Infectious Diseases*, published online 5th September 2020.

Abstracts publications and oral presentations

Papaluca T, McDonald L, Craigie A, Gibson A, Desmond P, Wong D, Winter R, Scott N, Howell J, Doyle J, Pedrana A, Lloyd A, Stooze M, Hellard M, Iser D, Thompson A. Outcomes of treatment for hepatitis C in prisoners using a nurse-led, statewide model of care.

Oral presentation at Australian Gastroenterology Week (AGW) Gold Coast, Queensland, 2017

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Poster presentation at The Liver Meeting (AASLD) Washington DC, 2017

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1 Chapter 1: Introduction, literature review and aims of research

1.1 The Hepatitis C Virus

1.1.1 Introduction

The hepatitis C virus (HCV) is an Hepacivirus belonging to the Flaviviridae family (1). HCV infection, initially termed non-A, non-B viral hepatitis (NANBH), was suspected as a third viral entity accounting for the significant number of patients with transfusion-related hepatitis who were hepatitis A virus (HAV) and hepatitis B virus (HBV) seronegative (2, 3). Over the following 15 years, multiple attempts were made to identify this presumed viral entity and finally, via blind immunoscreening of cDNA expression libraries, clone 5-1-1 was identified leading to the first serological assays for the newly termed HCV infection (4, 5). This diagnostic assay was commercialised and implemented the following year (6, 7).

HCV infection is classified into genotypes and subtypes (8). There are six HCV genotypes, and their prevalence varies between geographical regions. Each genotype is further categorised into subtypes to account for the 20-25% variation in nucleotide sequences within a genotype. The NS5B RNA-dependant RNA polymerase (RdRp) is responsible for RNA replication, however the process is highly error prone resulting in significant variation in viral RNA sequences (9). This genetic variation between hepatitis C viruses within an individual are referred to as viral quasispecies (8).

The HCV virus consists of a 9.6kp positive sense RNA genome which includes non-translated 5' and 3' ends flanking the open reading frame. The 5' and 3' ends regulate ribosomal attachment and initiate translation and viral RNA synthesis, respectively (10, 11). The polyprotein, once translated, is approximately 3000 amino acids in length and is cleaved into viral structural and non-structural proteins. The structural proteins include the E1 and E2 which encapsulate the RNA viral genome, the core protein which forms the nucleocapsid and the p7 protein which regulates viral assembly (12, 13). The non-structural proteins are cleaved from the polyprotein to create NS2, NS3, NS4A, NS4B, NS5A and NS5B which each have specific functions. Novel HCV direct-acting antiviral (DAA) therapy inhibit the function of specific non-structural proteins and their roles are reviewed below.

1.1.1.1 Hepatitis C non-structural proteins

1.1.1.1.1 NS5B RdRp

The NS5B RdRp is the catalytic subunit responsible for replication of the HCV viral genome, by forming a negative RNA strand from template RNA, prior to transcription and translation (14). The polymerase functions rapidly but is also highly error prone, and as such, there is significant heterogeneity amongst

the virions leading the generation of HCV quasispecies, some which harbour drug resistance sequences (15, 16). The NS5B RdRp is approximately 590 amino acids in length and has a 'right hand' morphology, typical of many polymerases, with 'palm', 'finger' and 'thumb' subdomains (17, 18). The 'finger' subdomains are hypothesized to have an important role in the management of incoming nucleotide triphosphates and the template RNA strand, the 'palm' the catalytic subdomain and the 'thumbs' regulating the catalytic site. Inhibitors of the NS5B RdRp are either non-nucleotide or nucleotide inhibitors depending on their mechanism of action.

1.1.1.1.2 NS5A

The NS5A protein has an important role in viral replication and assembly, however its detailed biological role is yet to be completely defined (19). The NS5A protein is comprised of three structural domains. Domain I and II regulate viral replication and domain III, assembly. Unlike the NS5B RdRp and NS3 protease, the NS5A protein has no enzymatic activity but is postulated to assist in the formation of the virus-induced web like membranous structure required for viral replication. Inhibitors of NS5A were identified via the screening of compound libraries against cell-based replicon assays and are used clinically (20).

1.1.1.1.3 NS3/4A

Both the NS2 and NS3 proteases have well characterised enzymatic activity, however only NS3 protease inhibitors have advanced to clinical use. The NS3 protease is responsible for cleavage of the polypeptide chain to create discrete non-structural proteins. NS4A is a cofactor in this process which both optimises NS3 function and anchors the molecule to the endoplasmic reticulum (21). The initial clinical use of first-generation protease inhibitors (PI) including boceprevir, telaprevir, and later simeprevir and asunaprevir highlighted the utility of PIs in clinical use however they were limited by low barriers to resistance and lack of pan genotypic activity (22). PIs are also postulated to have additional antiviral effects by upregulating the activity of toll-like receptor (TLR) 3 which results in restored interferon (IFN)-B production (23).

1.1.2 HCV pathogenesis

HCV is a non-cytolytic virus and hepatocellular injury results from both immune- and metabolically-mediated mechanisms (24). Immune mediated cellular injury results from the activation of both innate and adaptive immune pathways.

HCV-infected hepatocytes upregulate production of type-1 IFN which induces cellular processes to resist infection and activates natural killer (NK) cells. NK cells are also recruited via cellular pathways initiated by pathogen-associated molecules (PAMPs) recognition and contribute to increased

production of INF-a/b (25). The NK cells are central to viral clearance which is achieved directly via inducing hepatocyte death (via cytolytic pathways) and indirectly by releasing inflammatory cytokines including INF- γ and Tumour Necrosis Factor alpha (TNFa) (26, 27).

The adaptive immune system response to HCV infection results in hepatitis and disease pathogenesis. Viral molecules are displayed via type I and II major histocompatibility complexes (MHC) which initiate a T helper (Th) immune response, upregulating CD40 ligand and TNFa production (28). Cytotoxic T lymphocytes are then activated resulting in cell-mediated indirect hepatocyte inflammation via the release of perforin, granzyme and TNFa or via direct hepatocyte death via Fas ligand (29, 30).

Those patients with a strong and sustained Th 1 initial immune response to HCV, driven by interleukin 12 and INF- γ , have a higher likelihood of HCV clearance during acute infection (31, 32). Most patients, however, do not achieve clearance, and the ensuing chronic infection results in persistent immune activation, hepatitis and progression to fibrosis and cirrhosis (33, 34).

In addition to these described immune mediated pathways, HCV infection induces metabolically mediated hepatocellular injury via increased insulin resistance, oxidative stress and hepatic steatosis, which may contribute to fibrosis progression. Insulin resistance, as determined by homeostasis model assessment (HOMA) scores, contributes significantly to hepatic steatosis and lipogenesis in chronic HCV infection which in turn is associated with fibrosis progression (35). There is also increased oxidative stress due to HCV mediated glutathione oxidation resulting in excessive reactive oxygen species (ROS) within hepatocellular mitochondria and the endoplasmic reticulum. NADPH pathways are also upregulated directly by the NS3 protease (36, 37). These changes are evidenced by increased serum oxidative markers in those with HCV infection (38). There is a tendency for hepatic steatosis in HCV infection (39) secondary to a direct effect from the HCV-core protein and indirectly, from the close association between HCV infection and alcohol misuse, diabetes and obesity (40). HCV GT3 infection, particularly, is associated with hepatic steatosis secondary to its postulated effect on lipid homeostasis including impaired secretion of lipids and the increased production and reduced degradation of de novo free fatty acids (41). These immune and metabolic processes contribute to sustained inflammation, fibrosis, proliferation, apoptosis and oxidative stress with an end result of hepatic fibrosis and cirrhosis (42).

1.1.3 Clinical manifestations and natural history of HCV infection

1.1.3.1 Acute HCV infection

HCV is transmitted via blood-blood contact. Following acute HCV infection, an individual may either achieve spontaneous clearance or develop chronic HCV infection, defined as detectable HCV RNA for

at least six months after exposure. The likelihood of spontaneous viral clearance is approximately 50% (43, 44). Whilst viral clearance occurs in most cases of acute HCV infections in less than 12 weeks, this has been documented to occur beyond one year (45-47).

Certain host and pathogen related factors predict the likelihood of developing chronic HCV after acute infection. Individuals who are icteric or symptomatic during acute infection, and those with either a low baseline viraemia or a rapid decline in HCV viral titres are more likely to achieve viral clearance and avoid chronic infection (48-50). Host factors associated with viral clearance (and avoidance of chronic infection) include particular interleukin-10 haplotypes, emergence of HCV specific CD4 cells, and high levels of inducible protein 10 (31, 51, 52). Viral factors include non GT1b infection and particular HCV hypervariable region sequences (50, 53).

1.1.3.2 Clinical manifestations of chronic HCV infection

Most individuals with chronic HCV infection are asymptomatic, and when symptoms are present they are often non-specific. The most frequently reported generalised symptoms include fatigue, nausea, anorexia, diarrhoea, abdominal pain, myalgia, arthralgia and weight loss (54). Compared with healthy controls, patients with HCV more commonly report abdominal pain, however, the remaining symptoms occur at similar frequencies (55). HCV-related symptoms, when present, are associated with reduced quality of life indices (56). Reductions in cognitive function and perceived cognitive impairment are also frequently reported (57, 58). The cognitive symptoms attributed to HCV infection do not correlated with the severity of underlying liver dysfunction, or encephalopathy or injecting drug use (IDU) status.

HCV infection may result in extrahepatic manifestations. Extrahepatic manifestations associated with HCV infection include but are not limited to porphyria, thyroid dysfunction, the development of non-organ specific autoantibodies, cryoglobulinemia, non-Hodgkin's lymphoma and membranoproliferative glomerulonephritis (59-64).

1.1.3.3 Progression to cirrhosis

HCV-related chronic hepatitis is associated with progression to fibrosis and cirrhosis. Multiple studies have investigated the likelihood of developing cirrhosis in the context of HCV infection, and estimates range from 7-55% (65-67). A meta-analysis including 111 studies determined the prevalence of cirrhosis was 16% 20 years post HCV infection, and the duration of infection was most strongly associated with cirrhosis (68). Once an individual develops advanced fibrosis (METAVIR F3), there is a high likelihood of progressing to cirrhosis, and incidence is approximately 10% per year (69). Particular demographic, biological and behavioural factors have been associated with a higher likelihood of

progression to cirrhosis. Demographic factors include male gender, non-black ethnicity and HCV infection at older ages (70-72). Medical comorbidities associated with progression include HBV and/or human immunodeficiency virus (HIV) coinfection and diabetes mellitus (73). Behavioural factors include excess alcohol consumption, marijuana and high dietary cholesterol intake (74, 75).

1.1.3.4 Hepatic decompensation

Hepatic decompensation events include ascites, encephalopathy, portal hypertensive bleeding or hepatocellular carcinoma (HCC). Jaundice also occurs frequently in advanced liver disease. Progression to decompensation amongst individuals with HCV related cirrhosis ranges between 4%-7.5% per annum in different series (69, 76). The most frequent initial decompensation events include progression from category A to B by Child Tourette Pugh (CTP) grading, development of HCC and de novo ascites. After progression to CTP category B, the likelihood of a liver related events is increased to 12.9% annually, and over 8 years follow up, 31% of patients with HCV-related decompensated cirrhosis progressed to transplantation (LT) or death in one series (63). Sustained virological response following HCV treatment is associated with an improvement in the frequency of these events and is discussed elsewhere (77, 78).

1.1.4 HCV testing

1.1.4.1 Antibody testing

HCV antibody testing can be performed using standard immunoassays processed in a laboratory or point of care (POC) rapid immunoassays. Standard immunoassay testing for anti-HCV using serum samples is performed using either enzymatic reactions (EIA) (which including enzyme-linked immunosorbent assay (ELISA)) or light emission testing including chemiluminescence immunoassay. The most recent 3rd generation EIA technologies detect antibodies to the HCV core protein as well as to the NS3, NS4 and NS5 antigens, and achieve high sensitivity and specificity of 92.2-98.9% and 100% respectively (79-81). In general, immunoassays can detect anti-HCV within 10-26 weeks of infection, however some patients will have detectable antibody within 8 weeks (82-84). Chemiluminescence immunoassays are increasingly available and have a similar sensitivity and specificity to EIA technologies (85, 86). Laboratory based EIA testing for anti-HCV can also be performed on dried blood spot (DBS) samples (87). In certain clinical settings, and when earlier generation EIA testing kits are used, anti-HCV can be undetectable in the setting of HCV viraemia. This includes those who are highly immunosuppressed (including transplantation recipients, advanced HIV infection and those receiving renal haemodialysis) or early in acute infection and in these settings, HCV RNA testing should be performed (88-90).

Rapid POC immunoassays are now approved by the US Food and Drug Administration (FDA). This includes the OraQuick HCV POC rapid immunoassay which is compatible with various patient samples including finger prick blood, venous sampling and saliva. When venous or finger prick samples are used, sensitivity and specificity are equivalent to laboratory-based EIA testing (99.7% and 99.9%, respectively), however they are reduced when saliva is used (sensitivity 98.1%) (91). A meta-analysis determined that the pooled sensitivity of POC technologies for the detection of anti-HCV was 98.9% with a specificity of 99.5% however, the OraQuick performed significantly better than other available assays (92).

1.1.4.2 HCV RNA testing

Where anti-HCV is detected, HCV RNA testing is performed to determine if an individual has active HCV infection (as opposed to prior spontaneous clearance or cure following treatment) (93). Nucleic acid testing is available in qualitative or quantitative assays and methods for RNA detection include polymerase chain reaction (PCR), transcription mediated amplification and branched RNA testing. The tests are performed using patient serum or plasma samples and have a threshold for detection of approximately 15 international units (IU)/mL. DBS samples can be used however the threshold for detection increases to 250-500 IU/mL (94, 95). A lower HCV viral load at baseline was associated with a higher likelihood of sustained virological response (SVR) following treatment with pegylated (PEG) IFN and ribavirin (RBV) (96, 97). Baseline HCV viral load does not predict SVR12 following DAA treatment. Some DAA regimens, however, can be described for a shorter duration where baseline HCV RNA viral load is low (98).

1.1.5 Cirrhosis determination

Cirrhosis determination is important to guide selection of an appropriate treatment regimen and to determine the need for ongoing follow-up and hepatoma surveillance post HCV cure. Different methods for cirrhosis assessment are discussed below.

1.1.5.1 Liver biopsy

Liver biopsy is regarded as the gold standard for cirrhosis determination (99). Liver biopsy involves sampling liver cells to histologically assess the inflammatory grade and fibrosis stage. Ideally, liver biopsies should be greater than 15mm in length and include 5-10 portal tracts (100, 101). The accuracy of liver biopsy for cirrhosis determination is affected by the number and size of biopsies collected, and the sensitivity for cirrhosis is increased when multiple samples are retrieved (100, 102). There can be variation in histological stage, however, between two biopsies collected at discrete locations within one liver. As liver biopsy samples only 1/50,000 of the liver by volume, a false negative result may

occur in up to 10-30% when investigating for cirrhosis (103-106). In one series which compared liver biopsies collected laparoscopically from both the left and right liver lobes simultaneously, there was variation in the reported grade and stage by 24.2% and 33.1% between samples, respectively (106).

Following collection, the liver tissue sample is assessed by a histopathologist. The introduction and use of standardised scores for liver fibrosis assessment, such as the METAVIR, Scheuer and Ishak scores have improved assessment reproducibility and reduced inter-observer discordance (107-109). The METAVIR score grades fibrosis stage from 0-4 depending on the detection of portal fibrosis, fibrosis septa and bridging fibrosis (108). Similarly, the Ishak score grades fibrosis from 0-6 depending on the presence and severity of the same key histological findings. Multiple data demonstrate that using these scoring systems improves concordance in pathologist assessments for fibrosis grade in particular (110, 111).

Liver biopsy is associated with procedural complications and approximately 1-5% will encounter serious adverse events requiring hospitalisation (112-115). Death is uncommon and is estimated to occur in 1/10,000 to 1/12,000 and more commonly in the setting of cirrhosis or malignant disease (113). In two large series, when complications had occurred, symptoms were present in 60% of participants within two hours of liver biopsy and 96% within 24 hours, and the other, severe pain or hypotension were manifest by three hours in all with adverse events (112, 113). The most significant procedural risk was biopsy related bleeding, which was as high as 4.5%, including subcapsular, intrahepatic and peritoneal bleeding (114). Other liver biopsy complications include transient bacteraemia, haemobilia, bilious ascites, pneumothorax, subphrenic abscess, sampling of incorrect organs, pain and hypotension (113, 116, 117). Many of these data originate prior to the routine use of ultrasound guidance for liver biopsy.

Liver biopsy may be collected via a percutaneous or transjugular approach (99). A transjugular approach is preferred in the setting of ascites, where a diagnosis of peliosis hepatis or vascular tumour is suspected or when there is a need for ancillary vascular procedures to be performed concurrently (99).

1.1.5.2 Transient elastography

As liver biopsy is invasive, uncomfortable and associated with significant risk, alternate methods of cirrhosis determination have been developed. This includes transient elastography which records pulse-echo acquisitions following vibration transmission toward the liver, propagating an elastic sheer wave (118). The sheer wave is transmitted more rapidly with increasing hepatic “firmness”, which can be correlated to the severity of hepatic fibrosis or cirrhosis. Liver stiffness measurements are highly reducible and have low inter-observer variability (119). Initial studies investigating the accuracy of

transient elastography for cirrhosis (with liver biopsy as the reference standard) in participants with HCV infection demonstrated areas under receiver operating characteristic (AUROC) curve of 0.95 and 0.97 at cut off of 12.5kPa and 14.6kPa, respectively (120, 121). Negative predictive values of a liver stiffness measurement below these thresholds were 95-97%. The sensitivity of transient elastography to diagnose cirrhosis has subsequently be investigated in a large meta-analysis (122). The meta-analysis included 38 studies which reported AUROC values for the sensitivity and specificity of cirrhosis as compared to liver biopsy diagnosed METAVIR F4 disease. The mean AUROC was 0.94 and increased to 0.99 when adjusted for liver biopsy quality. Cut-off values for cirrhosis using transient elastography in these studies ranged from 10.2kPa to 17.6kPa. These data demonstrated, however, that transient elastography was less able to reliably differentiate severe fibrosis (F3/4 vs F2/1) and significant fibrosis (F2/3/4 vs F0/1), where the AUROC was reduced to <0.9.

Limitations of transient elastography include its applicability in obese patients, those with narrow intercostal spaces and or in the presence of ascites (118). Failure rates of transient elastography were 2.4-9.4% (118-120). 4.5% of transient elastography attempts were unsuccessful in one series and the sole predictor of failure on multivariate analysis was body mass index (BMI) >28 kg/m² (123) Elevations of alanine aminotransferase, hepatic congestion and recent oral intake have also been associated with falsely elevated liver stiffness measurements (124-126). The presence of steatosis can affect the diagnostic accuracy of transient elastography which lead to the development of the XL probe (127).

Liver stiffness measurements have been shown to reduce following successful HCV therapy, and in one series, there was a 32% decrease in liver stiffness measurements following DAA therapy (128). Other data demonstrate that amongst those individuals identified as cirrhotic prior to hepatitis C treatment, who then have transient elastography readings below the threshold for cirrhosis post SVR12, are still at risk of incident HCC, highlighting that transient elastography should be completed prior to initiation of hepatitis C therapy.

1.1.5.3 Non-invasive markers of fibrosis

1.1.5.3.1 *AST-to-platelet ratio index (APRI) score*

The APRI score was devised by Wai and colleagues as a non-invasive algorithm for predicting hepatic fibrosis and cirrhosis in patients with HCV infection (129). They identified variables most strongly associated with cirrhosis and fibrosis (with liver biopsy as the reference standard) in HCV infection. The APRI score is calculated as [(AST/upper limit normal AST)/platelets(10⁹/L)] x 100. APRI scores correlated well with fibrosis stage and the AUROC was 0.89 in the validation cohort. Cut-off APRI thresholds were determined for the exclusion and identification of cirrhosis which were <1.0 and >2.0, respectively. An APRI of <1.0 achieved a sensitivity of 89%, specificity of 75%, NPV of 98% and PPV of

38%, and only 11% of cirrhotic participants were misclassified as non-cirrhotic. An APRI of <2.0 was 57% sensitive, 93% specific and had PPV and NPV of 57% and 93% respectively. The NPV of APRI <1.0 for cirrhosis was 100% when applied to the validation cohort. These thresholds have been subsequently validated by several studies, and more recently included in a large systematic review. This identified that an APRI of 1.0 for the diagnosis of cirrhosis achieved an AUROC of 0.84 (130).

1.1.5.3.2 FIB-4 score

The FIB-4 score was developed to predict advanced fibrosis in patients with HCV and HIV coinfection. Developed by Sterling and colleagues, variables associated with fibrosis were identified and incorporated into the FIB-4 algorithm, which is calculated as $(\text{age}(\text{years}) \times \text{AST}) / (\text{platelets} \times \text{ALT}^{(1/2)})$ (131). The AUROC of FIB-4 to differentiate between Ishak grade 0-3 and 4-6 was 0.765 and a cut-off of 1.45 has a NPV of 90% for excluding advanced fibrosis and a sensitivity of 70%. At a higher cut-off of 3.25, the PPV was 65% and specificity 97%, however the sensitivity and NPV were reduced. Data validating the FIB-4 threshold of 1.45 for advanced fibrosis were included in a systematic review and the median AUROC was 0.87 (130). The pooled sensitivity and specificity were 90% and 58% respectively.

1.1.5.3.3 Hepascore

The Hepascore was developed to predict cirrhosis in patients with HCV infection (132). The score incorporated variables most strongly correlated with fibrosis stage on multivariate analysis and included age, gender, GGT, α 2-macroglobulin, hyaluronic acid and bilirubin. A threshold of 0.84 for the prediction of cirrhosis was associated with AUROC of 0.938. In the training cohort, this threshold achieved a sensitivity of 71% and specificity of 84%. A systematic review including multiple validation data identified a median AUROC of 0.79 for the detection of cirrhosis (130).

1.1.5.3.4 The enhanced liver fibrosis (ELF) score

The ELF score is an algorithm including several extracellular matrices which correlates well with fibrosis stage in chronic liver disease and includes tissue inhibitor of metalloproteinases 1, amino-terminal propeptide of type III procollagen and hyaluronic acid (133). Multiple validation data included in a systematic review determined a median AUROC of 0.88 for the diagnostic accuracy of ELF for cirrhosis (130).

1.1.5.4 Radiological investigations for the diagnosis of cirrhosis

There are multiple data exploring the accuracy of imaging modalities including ultrasound to diagnose or exclude hepatic cirrhosis (134-139). The imaging characteristics investigated in these data included surface nodularity, parenchymal heterogeneity, portal vein dilation, splenomegaly and ascites, and

the reference standard was histologically proven cirrhosis. There is however significant heterogeneity in reported sensitivity and specificity of these imaging characteristics between studies. For example, several series reported a high specificity for cirrhosis when liver surface nodularity or portal vein dilation were identified, which exceeded 90% (134, 135, 138). In these studies, however, the corresponding sensitivity of these key findings was low, reducing the overall diagnostic accuracy. In alternate data, the same imaging characteristics had both low specificity and sensitivity when compared with biopsy (136). Other studies reported no acceptable imaging characteristic for the diagnosis of cirrhosis, while another a high NPV for cirrhosis in the absence of splenomegaly or portal vein dilation (137, 139). As such, there is significant heterogeneity in the utility of these radiological characteristics.

1.1.5.5 Clinical examination findings for the diagnosis of cirrhosis

A meta-analysis determined that findings associated with decompensated cirrhosis including distended abdominal veins (likelihood ratio (LR) 11.0), encephalopathy (LR 10.0), and ascites (LR 7.2) were best able to predict cirrhosis (140). Lower LRs were identified for spider naevi (LR 4.3). An alternate systematic review identified that although clinical findings including ascites, collateral circulation, spider naevi, jaundice, firm liver edge and encephalopathy had high specificities between 75-98%, their sensitivities were less than 50% for most (141).

1.1.5.6 Benefits of identifying individuals with cirrhosis

Identification of individuals with hepatic cirrhosis is important to guide appropriate medical management to reduce liver related morbidity and mortality. Multiple data now demonstrate that enrolment in HCC surveillance programs for individuals with cirrhosis is associated with increased HCC survival (142). Identification of cirrhosis also allows appropriate screening for other complications of advanced liver disease, prior to clinical decompensation events, including variceal surveillance, with multiple therapeutics available for primary prophylaxis of variceal haemorrhage (143). Prognostically, diagnosing cirrhosis is important to predict the likelihood of future decompensation events and to tailor appropriate lifestyle advice for the patient. Patients with cirrhosis are also more vulnerable to infection with increase morbidity and mortality than non-cirrhotic individuals, and as such, a diagnosis of cirrhosis can inform appropriate vaccination recommendations (144).

1.1.6 HCV treatment

1.1.6.1 IFN-based HCV treatment

The initial studies investigating the efficacy and safety of IFN-alpha for HCV infection (then termed NANB hepatitis, prior to identification of HCV) were published in 1989 and identified that 24 weeks of

therapy achieved normalisation of ALT in a subset of patients, which was sustained in approximately 50% of responders (145). The addition of RBV to IFN was identified to improve efficacy without significant increased toxicity (146). In 2001 and 2002, respectively, two large randomised control trials were published demonstrating the superiority of combination PEG IFN alfa-2a/b and RBV for chronic HCV (147, 148). SVR rates were 42-46% for GT1 infection, and approximately 80% for HCV GT2 and GT3 infection after 48 weeks of therapy. PEG IFN and RBV thereafter remained first-line therapy for HCV infection for the following decade.

The use of first-generation PIs, including boceprevir and telaprevir, in combination with PEG IFN and RBV was later shown to improve efficacy in patients with HCV GT1 infection, and was incorporated into HCV treatment guidelines (149, 150). The initial pilot studies investigating IFN-free combinations of HCV DAA medications were published in 2012, which have now become first-line treatment (151).

1.1.6.2 DAA treatment of hepatitis C infection

The aim of hepatitis C DAA therapy is to achieve viral eradication. Novel hepatitis DAA therapies are associated with high rates of cure which exceeded 95% in clinical trials (152, 153). Cure from chronic hepatitis C infection is confirmed when no HCV virus is not detected in an individual's serum on PCR testing at least 12 weeks following completion of DAA treatment; this is termed SVR12. Non-SVR12 may be the result of virological breakthrough, relapse or reinfection. Virologic breakthrough is defined as detectable HCV RNA whilst on DAA therapy, suggestive of a high level resistant variant. HCV relapse is defined as detection of HCV RNA of the original viral lineage after completion of DAA therapy and reinfection identification of HCV RNA from an alternate viral lineage (154).

HCV DAAs have been available to Australian citizens via the Pharmaceutical Benefits Scheme since March 2016 (155). The initial DAA therapies reimbursed were sofosbuvir (SOF) + ledipasvir (LDV) for HCV GT1 infection and SOF + daclatasvir (DCV) for GT1 and GT3 infection. Combination ombitasvir + paritaprevir (ritonavir boosted) + dasabuvir (PrOD) was then listed in July 2016, and then elbasvir (ELB) + grazoprevir (GZR) in January 2017, both for the indication of HCV GT1 and GT4 infection. The first pan genotypic regimen available on the Australian Pharmaceutical Benefits Scheme was SOF + velpatasvir (VEL) which was made available from August 2017 and then subsequently, combination glecaprevir (GLE) + pibrentasvir (PIB), also for the indication of HCV GT1-6 infection, in August 2018. SOF and DCV were removed from the Pharmaceutical Benefits Scheme in 2020 due to the availability of combination pan genotypic regimens.

The first pan genotypic salvage regimen for NS5A inhibitor experienced patients with relapse was SOF + VEL + voxilaprevir (VOX) was made available to Australians via the Pharmaceutical Benefits Scheme in April 2019.

Recommendations for the treatment of HCV infection as guided by the 2020 Australian recommendations for the management of hepatitis C virus Infection and the 2018 EASL Treatment of Hepatitis C recommendations are presented below (154, 156).

1.1.6.2.1 Non-cirrhotic or compensated cirrhotic patients

Table 1.1: HCV DAA treatment for patients without cirrhosis or with compensated cirrhosis including recommendations from the 2020 Australian and the 2018 EASL HCV guidelines. HCV, hepatitis C virus; DAA, direct-acting antiviral.

Table 1.1: HCV DAA treatment for patients without cirrhosis or with compensated cirrhosis	Treatment naïve	Treatment experienced
Pan-genotypic regimens	Sofosbuvir / Velpatasvir [12 weeks] Glecaprevir / Pibrentasvir [8-12 weeks] ¹	Sofosbuvir / Velpatasvir [12 weeks] Glecaprevir / Pibrentasvir [8-16 weeks] ²
Genotype 1a	Sofosbuvir / Ledipasvir [8-12 weeks] ³ Elbasvir / Grazoprevir [12 weeks] Paritaprevir / Ombitasvir / Ritonovir / Dasabuvir [12 weeks]	Sofosbuvir / Ledipasvir [24 weeks] Elbasvir / Grazoprevir [12-16 weeks] ⁴ Paritaprevir / Ombitasvir / Ritonovir / Dasabuvir [12 weeks]
Genotype 1b	Sofosbuvir / Ledipasvir [8-12 weeks] ⁵ Elbasvir / Grazoprevir [8-12 weeks] ⁶ Paritaprevir / Ombitasvir / Ritonovir / Dasabuvir [8-12 weeks] ⁷	Sofosbuvir / Ledipasvir [12-24 weeks] ⁸ Elbasvir / Grazoprevir [12-16 weeks] ⁹ Paritaprevir / Ombitasvir / Ritonovir / Dasabuvir [12 weeks]
Genotype 2	No additional regimens recommended	No additional regimens recommended

Genotype 3	Sofosbuvir / Velpatasvir / Voxilaprevir [12 weeks] ¹⁰	Sofosbuvir / Velpatasvir / Voxilaprevir [12 weeks] ¹⁰
Genotype 4	Sofosbuvir / Ledipasvir [12 weeks] Elbasvir / Grazoprevir [12 weeks]	Sofosbuvir / Ledipasvir [12 weeks] Elbasvir / Grazoprevir [12 weeks]
Genotype 5 & 6	Sofosbuvir / Ledipasvir [12 weeks]	Sofosbuvir / Ledipasvir [12 weeks]

¹ Treatment-naïve patients with HCV genotype 1, 2, 4, 5, & 6 without cirrhosis should receive 8 weeks fixed-dose Glecaprevir / Pibrentasvir, and where cirrhosis is identified, treatment should be extended to 12 weeks. For HCV genotype 3 infection, patients with METAVIR F0-2 should be treated for 8 weeks, and for patients with METAVIR F3-4 liver disease, treatment should be extended to 12 weeks.

² Treatment-experienced patients with HCV genotype 1, 2, 4, 5, & 6 without cirrhosis should receive 8 weeks fixed-dose Glecaprevir / Pibrentasvir, and where cirrhosis is identified, treatment should be extended to 12 weeks. Treatment-experienced HCV genotype 3 patients without cirrhosis should receive 12 weeks fixed-dose Glecaprevir / Pibrentasvir, which should be extended to 16 weeks in those with cirrhosis.

³ EASL guidelines recommend 8 weeks sofosbuvir / ledipasvir for all treatment-naïve HCV genotype 1a patients without cirrhosis, however Australian guidelines only recommend this duration where baseline HCV viral load is <6 million IU/mL. Both guidelines recommend 12 weeks in the setting of cirrhosis.

⁴ Australian guidelines recommend 16 weeks Elbasvir / Grazoprevir +/- Ribavirin for treatment-experience genotype 1a patients irrespective of cirrhosis status, who have experienced on-treatment virologic failure to prior treatment. EASL guidelines do not recommend Elbasvir / Grazoprevir for treatment-experience patients, and only for treatment-naïve patients with baseline HCV viral load is <800,000 IU/mL.

⁵ EASL guidelines recommend 8 weeks fixed-dose sofosbuvir / ledipasvir in HCV GT1b patients without cirrhosis, where Australian guidelines suggest this duration is only considered where baseline HCV viral load is <6 million IU/mL. Treatment-naïve HCV genotype 1b patients should receive 12 weeks of therapy.

⁶ HCV genotype 1b infection in treatment-naïve patients with or without cirrhosis is treated with 8 weeks fixed dose elbasvir / grazoprevir. EASL guidelines including shortening duration to 8 weeks in the setting of METAVIR F0-2 disease.

⁷ EASL guidelines recommend that HCV genotype 1b infection is treated with 12 weeks fixed-dose paritaprevir / ombitasvir / ritonavir / dasabuvir, which can be reduced to 8 weeks in the setting of METAVIR F0-2 disease. Australian guidelines do not recommend the use of this regimen presently.

⁸ EASL guidelines do not recommend the use of sofosbuvir / ledipasvir for treatment-experienced HCV genotype 1b infection. Australian guidelines recommend that for treatment-experienced HCV genotype 1 patients without cirrhosis, sofosbuvir / ledipasvir is prescribed for 12 weeks, which is increased to 24 weeks in those with cirrhosis.

⁹ EASL guidelines do not recommend elbasvir / grazoprevir for treatment-experienced genotype 1b infection. Australian guidelines recommend 12 weeks of therapy for cirrhotic or non-cirrhotic patients with should be increased to 16 weeks for treatment-experienced patients with genotype 1 infection who have experienced on-treatment virologic failure to prior treatment.

¹⁰ Sofosbuvir / velpatasvir / voxilaprevir 12 weeks as first line therapy for HCV genotype 3 infection is recommended in EASL but not Australian HCV guidelines.

1.1.6.2.1.1 Genotype 1a infection

Four treatment options are recommended for GT1a infection including SOF + VEL, GLE + PIB, SOF + LDV, and GZR + ELB.

Fixed dose combination SOF + VEL is prescribed for GT1a infection for 12 weeks irrespective of cirrhosis status, PEG IFN RBV treatment experience or HIV coinfection. This recommendation is based on findings from ASTRAL-1 and ASTRAL-5 in which 95-98% of participants with GT1a infection achieved sustained virologic response at post-treatment week 12 (SVR12) (152, 157).

GLE + PIB achieved an SVR12 rate of 98% in patients without cirrhosis when treated for eight weeks in clinical trials (158). SVR12 rates were 98% in patients with cirrhosis when GLE + PIB was extended to 12 weeks (159).

SOF + LDV prescribed for 12 weeks achieves high rates of cure ($\geq 95\%$) for GT1a infection evidenced by ION-1, ION-3 and ION-4, irrespective of cirrhosis status or HIV co-infection (98, 160, 161). Duration of SOF + LDV may be reduced to eight weeks for non-cirrhotic, treatment naïve patients with GT1a infection (98) and is particularly efficacious when the baseline HCV viral load is < 6 million IU/mL (162). European guidelines allow for SOF + LDV eight weeks for all non-cirrhotic patients, irrespective of viral load (154). SOF + LDV is not recommended for treatment experienced patients as per the European Association for the Study of Liver (EASL) guidelines as therapy must either be extended to 24 weeks or include RBV (154). Australian guidelines recommend extension to 24 weeks when used in the setting of IFN RBV treatment experience, when this regimen is selected.

ELB + GZR 12 weeks achieved SVR12 rates of 92-97% for patients with GT1a infection in clinical trials, which was not affected by prior PEG IFN RBV exposure or cirrhosis status (163, 164). A post-hoc

analysis demonstrated that the SVR12 rate increased to 99% where no baseline NS5A resistance associated substitutions (RAS) were identified and baseline HCV RNA was <800,000 IU/mL, however efficacy was significantly reduced where NS5A RAS were detected. EASL guidelines therefore recommend that ELB + GZR only be prescribed for patients with a baseline HCV RNA <800,000 IU/mL due to the impracticalities of baseline NS5A RAS testing (154).

1.1.6.2.1.1 Genotype 1b infection

EASL guidelines recommend five regimens for the treatment of HCV GT1b infection (148). These include SOF + VEL, GLE + PIB, SOF + LDV, GZR + ELB and PrOD.

In ASTRAL-1, SOF + VEL was highly effective for GT1b infection and 99% of participants achieved SVR12 irrespective of cirrhosis status or prior treatment experience, including prior DAA exposure (152). In ASTRAL-5, SVR12 rates were 92% following 12 weeks SOF + VEL in HIV co-infected participants, however overall GT1b participant numbers were low and only one episode of relapse was recorded (92%, n=11/12) (157).

GLE + PIB prescribed for eight weeks for non-cirrhotic GT1a participants, irrespective of viral co-infection or prior treatment experience, achieved an SVR12 rate of 100% in clinical trials (158). For patients with compensated cirrhosis, treatment should be extended to 12 weeks on the basis of outcomes of the EXPEDITION-1 trial (SVR12 100%, n=39/39) (159).

The ION studies demonstrated SVR12 rates of 96-100% for GT1b participants following 12 weeks of SOF + LDV, irrespective of fibrosis stage or treatment experience (160, 165, 166). In a cohort of GT1b patients who were exclusively treatment-experienced (PEG IFN RBV +/- 1st generation PI), SVR12 rates reduced to 87% (n=20/23). SOF + LDV can be prescribed for eight weeks for non-cirrhotic, treatment naïve HCV GT1b patients supported by data from Kowdley et al in which SVR12 rates were 98% in participants with these characteristics (n=42/43) (98)

Twelve weeks GZR + ELB for GT1b infection in treatment-naïve or experienced patients, with or without cirrhosis or HIV co-infection, achieved SVR12 rates of 98-99% in several phase III clinical trials (163, 164, 167). ELB + GZR treatment can be shortened to eight weeks for GT1b infection in those without advanced fibrosis or cirrhosis (METAVIR F3/F4) supported by data from the STREAGER study (SVR12 97%, n=66/68) (168). This regimen is included in not included in the EASL guidelines for treatment experienced patients due to the impracticalities of resistance testing, where Australian guidelines recommend extension to 16 weeks for all treatment experienced individuals (154).

PrOD achieved excellent virological outcomes for GT1b infection in clinical trials. SVR12 rates were 98-100% for non-cirrhotic, treatment naïve participants including a subset with HIV-coinfection in three

clinical trials (169-171). In other studies which included treatment experienced participants, with or without compensated cirrhosis, SVR12 rates remained high (SVR12 99-100%) (172-174) (158-160). These data contribute to the recommendation that PrOD be prescribed for 12 weeks for GT1b infection, irrespective of these baseline characteristics (154). Treatment duration may be reduced to eight weeks for treatment naïve GT1b patients without advanced fibrosis or cirrhosis, supported by data from the GARNET study; this study included participants with METAVIR F0-F3 fibrosis and SVR12 rates were 97% (175). Episodes of virological relapse were concentrated in participants with F3 disease therefore therapy should be prescribed for 12 weeks in this setting. This regimen is included in the EASL guidelines only as it is no longer marketed in Australia (156).

1.1.6.2.1.2 Genotype 2 infection

Treatment guidelines recommend SOF + VEL or GLE + PIB for GT2 infection.

ASTRAL-II recruited non-cirrhotic, treatment naïve patients who were treated with SOF + VEL 12 weeks and identified an SVR12 rate of 99% (n=133/134) (176). SVR12 rates remained preserved (100%, n=104/104) in ASTRAL-I which included a larger proportional of treatment experienced GT2 participants including a proportion with cirrhosis, and in ASTRAL-5 (100%, n=11/11) which included participants with HIV coinfection (152, 157).

Eight weeks of combination GLE + PIB was efficacious for GT2 infection for non-cirrhotic patients, irrespective of prior treatment experience or HIV co-infection, evidenced by three clinical trials in which SVR12 rates were between 98-100% (153, 164, 177). For patients with HCV GT2 infection and compensated cirrhosis, SVR12 rates were 100% following 12 weeks GLE + PIB (159, 177).

1.1.6.2.1.3 Genotype 3

Three regimens are recommended for HCV GT3 treatment including SOF + VEL, GLE + PIB, and SOF + VEL + VOX.

The efficacy of fixed dose SOF + VEL for GT3 infection was investigated in the ASTRAL-3 trial. This demonstrated that overall, SVR12 outcomes were favourable and 95% of GT3 participants achieved cure (176). SOF + VEL was particularly efficacious for non-cirrhotic and treatment naïve participants, and SVR12 rates were 97% in both groups, respectively. SVR12 rates were diminished in those with compensated cirrhosis (91%), with prior treatment experience (90%), and when both characteristics were present (89%). These data have informed the EASL recommendation that SOF + VEL not be prescribed as first-line treatment for GT3 infection in individuals with compensated cirrhosis (154). ASTRAL-5 demonstrated an SVR12 rate of 92% for participants with HCV GT3 and HIV coinfection (157).

GLE + PIB is also efficacious for GT3 infection, and treatment duration is informed by an individual's baseline characteristics including cirrhosis status and prior treatment experience. In ENDURANCE-3, SVR12 rates were 95% amongst treatment naïve, non-cirrhotic participants treated with GLE + PIB for eight weeks, however only 17% of participants had advanced fibrosis (METAVIR F3) (178). The efficacy of eight weeks GLE + PIB for non-cirrhotic patients was endorsed by EXPEDITION-2 in which SVR12 was 100% amongst those with HCV GT3 and HIV co-infection (179). The SURVEYOR-2 trial investigated the impact of cirrhosis and prior treatment experience on GLE + PIB for HCV GT3 infection (180). SVR12 rates were 98% and 96% respectively in treatment naïve cirrhotic participants treated for 12 weeks and treatment experienced cirrhotic participants treated for 16 weeks. There are no high-quality data regarding the efficacy of 12 weeks GLE + PIB for cirrhotic GT3 patients who are treatment experienced. For treatment experienced, non-cirrhotic participants in SURVEYOR-2, SVR12 rates were 91% and 95% for those treated for 12 or 16 weeks, respectively. Similar outcomes have been identified in a pooled analysis of phase II and III GLE + PIB data with lower SVR rates amongst treatment experienced patients without cirrhosis, treated for 12 week (181).

EASL guidelines also include 12 weeks SOF + VEL + VOX as a first-line therapy for patients with cirrhosis and HCV GT3 infection, irrespective of treatment experience, due to the reduced efficacy of SOF + VEL in this subgroup (154). This is not recommended in Australian HCV guidelines (182). A reduced duration of eight weeks SOF + VEL + VOX for HCV GT3 participants was also associated with excellent outcomes (SVR12 96-99%) and included 25% who were treatment experienced and 20% with cirrhosis (183).

1.1.6.2.1.4 Genotype 4

Recommended regimens for HCV GT4 infection include SOF + VEL, GLE + PIB, SOF + LDV and GZR + ELB (154).

HCV GT4 infection responds favourably to 12 weeks SOF + VEL evidenced by ASTRAL-1 and ASTRAL-5 in which SVR12 rates were 100% which included patients with cirrhosis, prior treatment experience and HIV co-infection (152, 157).

GLE + PIB is efficacious for HCV GT4 infection, and duration is guided by prior treatment experience and cirrhosis status. When prescribed for non-cirrhotic patients, data demonstrate an SVR12 rate of 93% with eight weeks of therapy, irrespective of prior treatment experience (153). In cirrhotic patients, SVR12 rates were 99% and 100% in clinical trials when GLE + PIB was continued for 12 weeks. SVR12 rates were also high in HIV co-infected non-cirrhotic patients with HCV GT4 infection following eight weeks of GLE + PIB (159, 184).

SOF + LDV prescribed for 12 weeks for HCV GT4 infection was investigated in the SYNERGY trial, and the overall SVR12 rate was 95% with only one episode of relapse (185). An alternate phase II trial demonstrated excellent efficacy of SOF + LDV for GT4 cirrhotic participants (SVR12 100%, n=10/10), and whilst prior treatment experience resulted in numerically lower SVR12 rates, this did not reach statistical significance (91% vs 96%, p>0.05 (186).

GZR + ELB 12 weeks achieves excellent efficacy for HCV GT4 infection. This is supported by data from five clinical trials including participants with cirrhosis, HIV coinfection and those receiving opioid substitution therapy (OST) and SVR12 rates were 96-100%. Australian guidelines recommend that when GZR + ELB is prescribed for treatment experienced patients, duration should be extended to 16 weeks and RBV included due to the impracticalities of HCV NS5A RAS testing prior to treatment (182).

1.1.6.2.1.5 Genotype 5

Current treatment guidelines recommend SOF + VEL, GLE + PIB and SOF + LDV for HCV GT5 treatment. In ASTRAL-1, which included a subset of participants with cirrhosis (14%) and prior treatment experience (31%), SVR12 was achieved in 97% following 12 weeks of SOF + VEL (152).

Three registration studies have investigated the efficacy of GLE + PIB for HCV GT5 infection. All non-cirrhotic participants with GT5 infection achieved SVR12 when treated with eight – 12 weeks of GLE + PIB (153, 184). In EXPEDITION-1, all participants with HCV GT5 infection and cirrhosis were cured after 12 weeks GLE + PIB, however overall numbers were small (n=2) (159).

Treatment with SOF + LDV was associated with SVR12 rates of 95% in clinical trials when prescribed for 12 weeks for GT5 infection, including participants with compensated cirrhosis and prior treatment experience (187). There was one episode of relapse amongst both the cirrhotic and non-cirrhotic participants.

1.1.6.2.1.6 Genotype 6

Treatment regimens for HCV GT6 infection include SOF + VEL, GLE + PIB and SOF + LDV. ASTRAL-1 included HCV GT6 participants, of which 17% were treatment experienced and 15% had compensated cirrhosis and determined that 12 weeks SOF + VEL was associated with SVR12 in 100% (152). This informs the EASL recommendation for 12 weeks SOF + VEL for HCV GT6 irrespective of prior treatment experience or cirrhosis status (154).

Treatment with eight weeks GLE + PIB achieved SVR12 rates of 90% (one episode of relapse), which included a subset of participants who were treatment experienced (164). When treatment was extended to 12 weeks, SVR12 rates increased to 100% including for both cirrhotic and non-cirrhotic participants (159, 184). EASL guidelines recommend that non-cirrhotic patients are treated with GLE

+ PIB eight weeks, and cirrhotic patients for 12 weeks, irrespective of prior treatment experience (148).

1.1.6.2.2 Treatment of special populations

1.1.6.2.2.1 Treatment of HCV in patients with decompensated cirrhosis

Table 1.2: HCV DAA treatment regimens for patients with decompensated cirrhosis, including Australian and EASL HCV recommendations. HCV, hepatitis C virus; DAA, direct-acting antiviral.

Table 1.2 HCV DAA treatment for patients with decompensated cirrhosis	Treatment naïve	Treatment experienced
Pan-genotypic regimens	Sofosbuvir / Velpatasvir +/- weight-based ribavirin [12 weeks]	Sofosbuvir / Velpatasvir +/- weight-based ribavirin [12 weeks]
Genotype 1, 4, 5 or 6	Sofosbuvir / Ledipasvir +/- weight-based ribavirin [12 weeks]	Sofosbuvir / Ledipasvir +/- weight-based ribavirin [12 weeks]

Current HCV guidelines suggest that suitability for LT should be considered prior to HCV treatment in patient with decompensated liver disease and high baseline model for end stage liver disease (MELD) scores (154). If HCV treatment is commenced due to LT ineligibility or with a view to HCV cure / viral suppression to prevent post-LT HCV recurrence, the regimens approved for use in those with hepatic decompensation include SOF + VEL ± RBV, SOF + DCV ± RBV, SOF + LDV ± RBV and SOF + RBV.

SOF + VEL ± RBV is a pan-genotypic regimen licenced for use in decompensated cirrhosis. In ASTRAL-4, participants with HCV and CTP class B cirrhosis achieved SVR12 rates of 83%, 94% and 86%, respectively, when treated with SOF + VEL 12 weeks, SOF + VEL + RBV 12 weeks and SOF + VEL 24 weeks (188). SOF + DCV ± RBV is an alternate pan genotypic regimen for patients with decompensated cirrhosis. ALLY-1, a prospective, open-label phase III trial, recruited participants with hepatic decompensation who were randomised to 12 or 24 weeks of SOF + DCV + RBV (189, 190). SVR12 rates were 93% for CTP class A/B participants but reduced to 56% in those with CTP class C disease. This regimen was also evaluated in early access program studies which demonstrated SVR12 rates of 75% in an Australian series and 50-88% in the United Kingdom (191, 192).

Genotype specific regimens approved for HCV infection and decompensated cirrhosis include SOF + LDV for HCV GT1 and GT4 and SOF + RBV for GT2 infection. SOLAR-1 and SOLAR-2 investigated the efficacy of SOF + LDV + RBV prescribed for 12 or 24 weeks amongst participants waitlisted for LT with HCV GT1a, GT1b and GT4 (SOLAR-2 only) infection (193). Amongst CTP class B participants, SVR12

rates were 87% for 12 weeks and 89-96% for 24 weeks of therapy. SVR12 rates were lower for CTP class C patients and were 78-86% for 12 weeks and 85-87% for 24 weeks of therapy. Whilst SOF + RBV is approved for HCV infection in those with hepatic decompensation, this is not recommended in guidelines (154).

1.1.6.2.2.2 HBV-HCV co-infection

The selection of a DAA regimen for patients with HBV-HCV coinfection should be informed by data presented above, including consideration of prior treatment experience and cirrhosis status. HBV reactivation may occur in patients with HBV-HCV coinfection following DAA initiation (194). A Taiwanese series including 111 co-infected participants (defined as HBsAg positive and detectable HCV RNA) demonstrated that 53% who had a baseline HBV DNA of >20 IU/mL had an increase in their HBV viral load by at least one log₁₀ IU/mL prior to the SVR12 timepoint, and that in 5% this was associated an increased ALT >2x the upper limit of normal. HBV anti-viral therapy was commenced in three participants (195). EASL guidelines recommend initiating HBV anti-viral therapy prior to HCV DAA treatment for coinfecting individuals, and for patients who are HBsAg negative, HBcAb positive, serial monitoring of ALT and HBsAg be performed until the SVR12 timepoint (154).

1.1.6.2.2.3 Patients with significant renal impairment or receiving haemodialysis

Multiple DAA regimens can be prescribed for patients with stage 4 and 5 chronic kidney disease, including those receiving haemodialysis, including PrOD, GZR + ELB and GLE + PIB. SOF based regimens should be avoided where eGFR is <30ml/min/m² or for individuals with stage 4 and 5 chronic kidney disease due to the reduced renal clearance and accumulation of the SOF metabolite GS-331007 (196). RUBY-1 identified SVR12 rates of 100% amongst participants with HCV GT1 infection and stage 4 and 5 kidney disease following treatment with PrOD 12 weeks (197). GZR + ELB achieved an SVR12 rate of 94% for GT1 infection, including 6% who were cirrhotic and 75% prescribed haemodialysis (198). Similarly, GLE + PIB prescribed for 12 weeks for GT1-6 infection, including 19% of participants who were cirrhotic, achieved an SVR12 rate of 98% (199). There are no licenced treatment regimens for patients with both renal failure and hepatic decompensation, in whom PIs are contraindicated.

1.1.6.2.2.4 Treatment of HCV post-LT

The persistence of HCV infection post LT is universal and is associated with a high likelihood of recurrent cirrhosis, which occurs at a median of 10 years post LT (200, 201). HCV infection following LT may also result in fibrosing cholestatic hepatitis which is associated with high rates of graft failure and death (202). As such, HCV treatment should be pursued following LT to mitigate these risks. Phase III clinical trials in post-LT recipients provide reassuring data regarding the efficacy and safety of SOF + LDV + RBV, SOF + VEL and GLE + PIB for this population. In SOLAR-1 and SOLAR-2, SOF + LDV + RBV

was more efficacious when continued for 24 weeks (vs. 12 weeks) and achieved SVR12 rates of 98%-100% in non-cirrhotic, 95%-96% in compensated cirrhotic, 88%-100% in CTP class B and 75%-80% in CTP class C post-LT participants (190, 193). A study including 79 post LT participants with GT1-4 infection treated with 12 weeks SOF + VEL who were non-cirrhotic or had compensated cirrhosis achieved an SVR12 rate of 96% (203). Similarly, 98% of non-cirrhotic post-LT participants with HCV GT1-6 infection treated with 12 weeks GLE + PIB achieved SVR12, including a subset who were treatment experienced (204).

1.1.7 Improved clinical outcomes after successful HCV treatment

1.1.7.1 PEG IFN RBV era

Prior to the availability of all oral DAA therapy, PEG IFN RBV were prescribed for HCV treatment which were long duration and associated with significant toxicity (205). SVR rates were also significantly lower compared to contemporary DAA treatments (205). Data from this era however demonstrated improvements in clinical outcomes for patients with HCV infection who achieved SVR (compared with their non-SVR counterparts). These data demonstrate that SVR post PEG IFN RBV was associated with reduced all cause and liver-related mortality, progression to transplantation, and HCC incidence (77, 206). These improvements in morbidity and mortality are endorsed by multiple data including systematic reviews and metanalysis which determined a >50% reduction in all-cause mortality, liver related mortality and HCC, and that these benefits were enhanced in those with cirrhosis or HIV coinfection (78, 207, 208). SVR post successful HCV treatment has also been associated with a reduction in the hepatic portal venous gradient (HPVG) and a reduced likelihood of portal hypertensive complications, and similar improvements following DAA therapy are anticipated (209).

1.1.7.2 HCV DAA era

1.1.7.2.1 *Patients with compensated liver disease*

Data regarding improved liver and non-liver related morbidity and mortality following HCV DAA treatment is emerging. A United States Veterans Affairs Registry for Hepatitis C observational study identified an association between SVR12 and improved liver and non-liver related outcomes in Veterans with advanced fibrosis (FIB-4 score >3.25) after DAA therapy (210). These data indicate that achieving SVR12 was associated with a 79% reduction in all-cause mortality and 84% reduction in incident HCC, however the median follow-up period was limited to 1.6 years. Similarly, a French study determined that achieving SVR (PEG IFN RBV and DAA therapies) was associated with reduced all-cause mortality (HR 0.27 [0.18-0.42]), liver related mortality (HR 0.19 [0.10-0.36]), HCC (HR 0.26 [0.19-0.43]), and hepatic decompensation (HR 0.26 [0.17-0.39]), compared with their non-SVR counterparts

in those with compensated cirrhosis (211). Reduced all-cause mortality were also noted in the Veteran Affairs' cohort in those without advanced fibrosis (FIB-4 score <3.25) who achieved SVR12 post DAAs compared to non-SVR12 Veterans (59% reduction) or those never treated (69% reduction) (212).

1.1.7.2.2 Patients with decompensated liver disease

SVR12 following HCV DAA therapy is associated with improved liver-related outcomes in patients with decompensated cirrhosis. This includes a reduction in the HVPg which theoretically should confer a reduced likelihood of portal hypertensive complications including portal hypertensive bleeding, and ascites (213, 214). Studies which have investigated the safety and efficacy of DAA therapy in decompensated cirrhotic participants have shown improvements in CTP and MELD scores with when SVR12 is achieved (189, 192, 193). In ASTRAL-4, 47% of participants who achieved SVR12 improved at least one CTP class and over half had lower MELD scores (188). In SOLAR-2, 52% of CTP class B participants improved to class A with DAA treatment (193). Similar improvements in CTP class were observed in ALLY-1, a study of SOF + DCV +/- RBV in decompensated cirrhotic participants (189). HCV DAA treatment has also been associated with a reduced need for LT in patients who achieve SVR12. In Catalonia, Spain, following the introduction of DAAs, the frequency of LT waiting list admission reduced by 25% and 3-year survival rates post LT were also improved (215). The improvement in 3-year survival was driven by reduced mortality amongst HCV positive LT recipients who were treated with DAAs post-LT compared to those who underwent LT during the pre-DAA era (91% vs 76%, $p < 0.05$). Alternate data originating from Spain demonstrated that 25% of patients with HCV infection awaiting LT who achieved SVR12 could be delisted (216).

1.2 HCV risk factors for transmission and epidemiology

1.2.1 HCV transmission

Most incident HCV infections in developed countries are acquired through IDU or transfusion of contaminated bloods product, the latter of which has become infrequent since the introduction of routine blood product screening in the early 1990s. Other transmission sources include iatrogenic parental exposure, unsafe tattooing practices, and sexual and vertical transmission which are discussed below.

1.2.1.1 Injecting drug use

IDU remains the primary risk factor for HCV transmission in Australia and other high-income countries, and HCV prevalence amongst PWIDs is high. The odds ratio of prevalent HCV infection among PWIDs

compared with non-PWIDs exceeds 50 (217). A systematic review identified a wide midpoint prevalence of HCV infection amongst PWIDs of 9.8% to 97.4%, however, over 25 countries included in the analysis reported a prevalence of 60-80%, and in 12 countries this exceeded 80% (218).

HCV is transmitted amongst PWIDs via the sharing of needles, syringes and other injecting paraphernalia, and the virus can remain viable for up to 63 days on some injecting equipment (219). The ability for HCV to be transmitted through IDU is evidenced by the high likelihood of HCV seroconversion amongst PWIDs with short injecting histories. For example, 32% of PWIDs had HCV seroconversion within 12 months of IDU commencement, and this risk was enhanced in resource limited settings where it increased to 59% (220). HCV incidence, however, can be reduced by as much as 50% to 74% in settings where OST and needle and syringe exchange programs (NSPs) are readily available to PWIDs (221).

In Australia, mathematical modelling has demonstrated that IDU accounts for approximately 89% of incident HCV infection (222).

1.2.1.2 Blood and blood product transfusion

Blood product transfusions contributed significantly to HCV incidence and prevalence both in Australia and abroad prior to the introduction of routine HCV testing of blood donations. After the introduction of antibody testing in 1990, and subsequent HCV PCR testing in 1999, the risk of HCV infection has been significantly reduced to less than one per one million transfusions in developed countries (223, 224). Prior to 1990, it was determined that 190 of 100,000 blood product transfusions in Australia resulted in HCV infection (225). In similar series beyond Australia, as many 10% of blood transfusion recipients acquired HCV infection (226) and over 90% of patients with transfusion related hepatitis were retrospectively identified as HCV seropositive (227).

The populations most at risk of transfusion related HCV infection included those with haemophilia and thalassemia, who may have a reliance on blood products (228). Prior to 1990, 99% of haemophiliacs who received regular factor VIII concentrate were retrospectively identified as HCV seropositive (228).

In the era of risk-factor based screening and testing of blood donations in Australia, however, no cases of transfusion-related incident HCV infection were identified between 2007-2016 (229). Whilst this route of transmission has been largely eliminated in industrialised countries through these measures, blood transfusions, in addition to other iatrogenic HCV transmission, continues to contribute to incident infection in low- and middle-income countries (230).

1.2.1.3 Healthcare associated transmission

Healthcare associated HCV transmission includes incident infection from patient-to-patient, healthcare worker (HCW)-to-patient and patient-to-HCW exposure. This remains rare in most developed setting due to stringent infection control measures however deviations from aseptic technique, infection control or other procedural protocols can result in incident infection (231). In one region of low PWID prevalence, prior dental procedures, surgery and needle stick injuries were associated with an increased likelihood of HCV infection (232). Clusters of HCV infection have also been reported from unsafe injecting practices including shared use of syringes or drug vials between multiple patients, and cases of intentional diversion of a controlled substance by HCWs (233-235).

Specific healthcare scenarios associated with HCV transmission include organ transplantation and historical vaccination programs in resource limited settings using inappropriate infection control practices. There is a high likelihood of HCV infection when solid organs are received from an HCV seropositive donor. In one series, over 75% of recipients became HCV-seropositive or had detectable HCV RNA after solid organ transplantation from an HCV-seropositive donor (236). Subsequently, however, in the era of HCV RNA testing, HCV-antibody positive, RNA-negative organs are now utilised with negligible transmission risk (237). The role of HCV RNA positive donor organs for HCV seronegative recipients is also increasingly being explored and in Australia, HCV RNA positive donors are currently actively considered for transplantation, if a suitable recipient is identified (238, 239).

Healthcare associated HCV transmission has also been associated with large scale vaccination programs. The most significant of these was the Egyptian schistosomiasis vaccination program where the use of contaminated injecting equipment resulted in significant incident infection; HCV prevalence now exceeds 15% in some affected regions (240).

1.2.1.4 Sexual Transmission

The likelihood of sexual transmission of HCV is low (241, 242). The risk of HCV transmission between heterosexual monogamous couples has been investigated in two prospective studies (243, 244). The first recruited 500 monogamous couples who were followed for 15 years and the frequency of HCV seroconversion was determined. Overall, 4% of partners became HCV seropositive during follow up, however genotype discordance was noted in approximately half of cases suggesting an alternate source of HCV infection. Subsequent phylogenetic analysis demonstrated that true sexually acquired infection occurred in only 0.6%.

Risk factors for sexual transmission of HCV include heterosexuals with multiple sexual partners (OR 2.2-2.9), men who have sex with men (MSM) due to higher risk sexual practices and MSM with HIV coinfection (OR 4.1-5.7) (245). There has been an increase in HCV transmission particularly in HIV

positive MSM due to increased higher risk sexual practices since the introduction of antiretroviral therapy (ART), including in Australia and abroad (246, 247).

1.2.1.5 Vertical / perinatal transmission

The rate of HCV vertical transmission is low and is estimated to be between 5-6%, and the risk correlates with the magnitude of HCV RNA titres in pregnant woman (248, 249). The rate of vertical HCV transmission is increased approximately 2-fold, however, among HCV positive woman with HIV coinfection which is a significant risk factor for transmission (249). Vertical HCV transmission risk has not been associated with mode of delivery (vaginal birth compared with lower uterine caesarean section) or breastfeeding (250). There are no documented cases of HCV transmission from HCV seropositive, RNA negative women.

1.2.1.6 Tattoos and permanent make up procedures

Tattooing has been associated with the HCV transmission, particularly prior to 1990 (251). The transmission risk is enhanced when tattooing was performed in a non-professional setting (251, 252). Further studies including a large case-control trial have confirmed this association (OR 3.81) which was sustained even when other risk factors for transmission including IDU and prior blood transfusion were controlled (253). This risk has significantly declined since 1990, where tattooing is performed in professional settings.

1.2.2 HCV prevalence

1.2.2.1 Global HCV prevalence

A recent systematic review has estimated that 1.0% of the global population, or 71 million people, have HCV infection (254). This estimate was informed by prevalence data from 113 countries, which represent 92% of the global population. There was, however, significant heterogeneity in HCV prevalence between different geographical regions. Regions where HCV viraemia prevalence was high, exceeding 3%, included Central and South Asia, Eastern Europe and Africa. This included countries such as Gabon (7.0%), Mongolia (6.4%), Egypt (6.3%), Uzbekistan (4.3%) and Georgia (4.2%). Similarly, many regions had low HCV prevalence, and this was noted to be less than 0.1% in certain countries including the Netherlands, Samoa and Fiji. Six countries including China, Pakistan, India, Egypt, Russia and the USA accounted for over half of all HCV infections globally (254).

The prevalence of different HCV genotypes also varied between geographical regions. Genotype 1 was estimated to account for 44% of infections globally, genotype 3 for 25% and genotype 4 for 15%. Genotype 1 accounted for the majority (65%) of HCV infections in high- and middle-income countries,

where genotype 3 represented a higher proportion of HCV infections in middle- and lower-income countries compared with its global prevalence (36% vs 25%).

1.2.2.2 Australian HCV incidence and prevalence

HCV infection is a notifiable disease in Australia and data regarding HCV incidence and prevalence is publicly available through the Department of Health, Australian Government (255). In 2015, there were approximately 11,000 newly diagnosed HCV infections, reflecting an incidence of 46 per 100,000. In most cases the nature of these infection was unspecified (96%), however in 441 cases they were reported as newly acquired (4%). Most of these infections occurred in men (66%), and most were aged 30 years or older (77%). Notifications more commonly originated from metropolitan rather than regional locations (63%). Whilst Aboriginal and Torres Strait Islanders account for 3% of the Australian population, they accounted for 9% of newly diagnosed HCV infections.

Australia's HCV incidence was stable between 2011 to 2015 with 44.8-46.5 notifications received per 100,000 persons per year. Compared to the 10-year period preceding 2011, there had been a decline in HCV notifications by 12% (256).

In 2015, the number of Australians living with HCV infection was estimated to be 227,306 which included a proportion who remained undiagnosed (256). Since HCV direct-acting antiviral (DAA) treatments became available on the Australian Pharmaceutical Benefit Scheme in March 2016, it is estimated that 70,260 Australians have been treated, and that an additional 4,340 were treated prior to this via DAA early access programs and clinical trials (257). As such, an estimated 33% of Australians with chronic HCV infection have now been treated and contemporary prevalence data reflecting this treatment effect are anticipated. It is estimates that genotype 1 accounts for 55% and genotype 3 accounts for 38% of those chronically infected in Australia (258).

1.2.3 Epidemiology of HCV infection in prisons

1.2.3.1 Prevalence of HCV infection in prisons

Due to the close association between IDU and incarceration, there is a high prevalence of HCV infection amongst prison detainees globally. The prevalence of HCV infection in prisoners has recently been captured in two large systematic reviews (259, 260). One systematic review that included data from 46 countries identified a midpoint prevalence of HCV infection of 15.1%, however differences between geographical regions were noted (260). This included a high HCV prevalence of 20% in certain regions including Central Asia. The second systematic review investigated HCV seroprevalence (rather than viraemia) which was 26% based on multiple data sources (244). The highest prevalence was also noted in central Asia (30%).

Australian HCV prevalence data is informed by a National Prison Entrants' Blood Borne Virus survey which investigates HCV seroprevalence amongst persons entering each jurisdiction's prison systems. In 2016, 22% of the surveyed prisoners were HCV seropositive, which increased to 50% amongst prisoners who reported a history of IDU (261).

1.2.3.2 Incidence of HCV infection in prisons

Incidence of HCV infection amongst detainees has been estimated at 1.4 infections per 100-person years, with a range of 0.04-4.5 per 100-person years in one systematic review (259). The risk of incident infection was significantly greater amongst prisoners who reported a history of IDU (16.4 infections per 100-person years (range 5.5-34.2)). An alternate systematic review determined that HCV incidence ranged from 0.4-14.1% per annum, however due to the low availability of data only four countries were included in the analysis (260). Data from three of these countries reported HCV incidence of 0.4-1.7% however an Australian study demonstrated an incidence of 14.1% per annum amongst HCV seronegative prisoners incarcerated in one Australian jurisdiction who identified as PWID (262). Continued follow up of this cohort of incarcerated HCV seronegative PWIDs demonstrated that this incidence was sustained at 11.4 new HCV infections per 100-person years (263). Amongst individuals who identify as PWID who are incarcerated, there is a 62% increase in HCV incidence (264). There is no recent Australian data regarding HCV incidence amongst general detainees who do not report a history of IDU.

1.2.3.3 Prisons and PWID prevalence

There is a close association between PWIDs and incarceration due to the criminalisation of IDU and it is estimated that the lifetime risk of incarceration for PWIDs is 54-90%. Recidivist IDU following community re-entry is also strongly associated with reincarceration (265-267). Incarceration itself is also correlated with a likelihood of incident HCV infection (264). The prison setting provides an excellent setting for engaging PWIDs in HCV treatment as it is estimated that 50% of PWIDs incarcerated in Australia are HCV seropositive.

1.2.4 Prevalence of PWIDs globally and HCV infection amongst this group

The global prevalence of active PWIDs, defined as those who had injected drugs within 12 months, as well as the percentage who were anti-HCV positive was investigated in a recent systematic review (268). The analysis included data from 176 countries, representing over 99% of the global population aged 15 to 64 years old. The overall prevalence of current PWIDs was 0.33% and was greatest in eastern Europe (1.30%). In Australia, it is estimated that 0.59% of the population are current PWIDs.

Anti-HCV prevalence amongst PWIDs was also estimated in the systematic review (268). This determined an anti-HCV prevalence of 52.3% amongst PWIDs globally. This estimate was higher for the Australasian region (57.1%).

1.2.5 World Health Organisation (WHO) Viral Hepatitis Elimination Targets

Due to the significant global burden of HCV infection and the associated morbidity and mortality, the WHO developed an advocacy brief calling for the elimination of HCV and HBV infections as public health threats by 2030 (269). The agenda details the importance of increased testing, preventative health measures including vaccination and harm reduction, and reducing costs and improving availability of antiviral therapy. The stipulated targets for HCV infection, specifically, include an 80% reduction in HCV incidence and 65% reduction in HCV-related mortality.

1.2.5.1 Importance of HCV treatment for PWIDs and within prisons to achieve the WHO targets

Multiple mathematic modelling studies have identified that prioritising HCV treatment for PWIDs (rather than non- or ex-PWIDs) results in the most significant reduction of community HCV prevalence and incidence and is key to achieving the WHO Elimination Targets (270-273). Increasing treatment uptake amongst PWIDs has secondary benefits of treatment as prevention and interrupting onward transmission, in addition to the benefits for the individual. An Australian modelling study which considered HCV transmission, reinfection, treatment-related costs and interruption to liver disease progression determined that if 59/1,000 current Australian PWIDs were treated for HCV annually, the WHO elimination targets would be achieved in a cost-effective manner using a willingness-to-pay (WTP) threshold of \$50,000 AUD per quality adjusted life year (QALY) (274).

Modelling data also demonstrates that increasing HCV treatment within prisons can achieve significant reductions in community HCV incidence and prevalence, on account of the high HCV prevalence amongst prisoners. This data demonstrated that 28% of incident infections in Scotland were related to incarceration, and that HCV incidence was greatest in the immediate post-release period. Where prison-based HCV programs were able to treat 80% of all incarcerated PWIDs with HCV infection who had an anticipated incarceration duration of at least 16 weeks, the model anticipated a 45% reduction in Scotland's community HCV incidence and prevalence by 2030 through this intervention alone (275). Prison-based HCV treatment programs have also been modelled as cost effective, as evidence by English data using a WTP threshold of 13,000 pounds per QALY, where certain screening and treatment requirements were satisfied (276).

1.3 Prisons and HCV infection

1.3.1 Prison based HCV treatment

1.3.1.1 Barriers to prison-based HCV treatment

1.3.1.1.1 HCV Testing and assessment within the prison system

The WHO recommends that all prisoners are tested for blood borne viruses including HCV whilst incarcerated (277). Despite this, data demonstrate that only 34% of European countries have formal HCV testing protocols, and only 25% of USA prisons offer routine testing to their detainees (278-280). In some cases, where HCV testing is available, it is restricted to prisoners with risk factors for infection such as abnormal liver function tests or IDU status despite the high prevalence within prisons more broadly (281). Risk based testing can miss a significant number of prisoners with HCV infection, and is at odds with incarceration itself being significantly associated with HCV infection (217, 282). The likelihood of HCV testing within prisons in some developed countries remains low despite blood borne virus testing policies, and percentage of prisoners screened for HCV ranged from 3-30% in two studies from Canada and the United Kingdom (283, 284). Barriers to effective HCV testing include perceived stigma, low prioritisation of screening by prison health services operating within tight financial budgets, frequent prisoner transfer between discrete prison sites, lack of testing protocols and prisoner factors including lack of knowledge, fear of diagnosis or implications on IDU practices (285-287). Difficulties with venepuncture encountered by some PWIDs can also contribute to reduced testing rates and can be improved using novel testing modalities including dried-blood spot testing (288). The predominance of opt-in and risk-based HCV testing may be contributory and screening coverage could be improved by utilisation of opt-out testing protocols (285).

Once prisoners are diagnosed as HCV seropositive, further diagnostic testing including HCV RNA PCR are required for confirmation of current infection. In one series, however, only 62% of prisoners identified as HCV seropositive whilst incarcerated underwent PCR testing (289). A study from the US also demonstrated that only 18% of prisoners in one jurisdiction who screened positive for chronic HCV infection were referred for HCV assessment and treatment whilst incarcerated (289). Prisoners also require fibrosis assessment however data demonstrates the limited availability common modalities including transient elastography in prisons and reliance on external providers which results in significant assessment delays (290). These data demonstrate the ongoing challenges with HCV screening, diagnosis, referral, assessment and retention in care in the prison setting.

1.3.1.1.2 Treatment within the prison system

There are barriers within the prison to commencing and continuing HCV therapy. Many prison health services or government policies mandate physician review prior to commencing HCV therapy which results in significant treatment delays and transfer to external hospitals for assessment which is at odds with short average prison sentences (286, 290, 291).

Historical HCV treatments including PEG IFN RBV were also poorly suited to the prison setting on account of their long duration, high toxicity and high rates of discontinuation (291, 292). PEG IFN RBV was also contraindicated in those with severe or uncontrolled psychiatric illness and data demonstrate that up to 50% of prisoners report a mental health diagnosis which rendered many ineligible for treatment (293, 294). Prisoners also experience frequent interprison transfer across a jurisdiction which resulted in interruption and discontinuation of PEG IFN RBV treatment (287, 291).

DAA's have now replaced PEG IFN RBV as first-line HCV treatment. A barrier to their widespread use in the prison setting however has been the significant medication list price which ranges between \$54,000 and \$147,000 US dollars per treatment (295).

1.3.1.2 Efficacy of prison-based HCV treatment

1.3.1.2.1 PEG IFN RBV prison-based HCV treatment

There are seven studies which investigated the efficacy and safety of HCV treatment using PEG IFN RBV in the prison setting. In these studies, a minority of patients had cirrhosis which was no greater than 16% in any series (296-302). There was also a significant discrepancy between the number of prisoners with chronic HCV infection referred for treatment compared with those who initiated treatment, which was less than 50% in all studies, reflecting the significant barriers to HCV care as described. All models of care were resource intensive and reported a high reliance on specialist-led care and frequent hospital transfer for assessment and medical investigations. Rates of early discontinuation of PEG IFN RBV were 13-62% and overall SVR12 rates were 40-83% per protocol and 21-53% on intention-to-treat analysis. One study demonstrated that prison-based HCV treatment achieved similar outcomes to community-based care (302).

1.3.1.2 DAA based HCV treatment programs

At commencement of this thesis, there were no published data demonstrating the efficacy or safety of prison-based HCV treatment. At completion of this thesis, multiple data are now available which demonstrate that prison-based HCV treatment with DAAs is safe and achieves high rates of cure in large numbers of prisoners (303-310). Alternate models of HCV care are utilised in different regions. This includes telemedicine, where treatment is directed by a specialist who consults with prisoners

utilising video conferencing technology from a remote site. Other models including in-reach services where doctors and/or nurses with HCV expertise visit prison settings for assessments and initiation of HCV therapy and prison-based models where existing prison medical staff upskill in HCV management. In all studies, treatment discontinuation was uncommon.

1.3.2 Victorian prison system

The Victorian adult prison system is comprised of 15 adult prisons (13 male, two female), of which one is a step-down transition centre and three are privately operated. As of March 2020, there were 8,087 prisoners of whom 11% (n=864) identified as Aboriginal or Torres Strait Islander (311). Over 10,000 people are received into the Victorian prison system each year.

1.3.2.2 Victorian prison system, HCV and IDU

The National Prison Entrants' Bloodborne Virus Survey most recently determined a HCV seroprevalence of 24% amongst prisoners received into the Victoria prison system, and 48% amongst those who identified as PWIDs (261). Harm minimisation is available to Victorian prisoners and includes OST and bleach for the cleansing of syringes and other injecting paraphernalia. Approximately 11% of prisoners in Victoria are prescribed OST (312).

Prior to the introduction of DAA and the current nurse-led model of care, the existing model of HCV care used IFN-based treatment regimens, and included protocolised assessments and review by hepatologists, psychiatrists, prison medical officers, and hepatology clinical nurse consultants (unpublished data). Prison-based HCV treatment was only available in two prisons and PEG IFN RBV treatment was discontinued in the setting of interprison transfer. Approximately 5-10 prisoners annually were commenced on PEG IFN RBV.

1.3.3 The post prison release experience and linkage to HCV care

1.3.3.1 Competing priorities and challenges following community re-entry

Former prisoners encounter multiple challenges on community re-entry. Issues which require immediate attention include establishing stable accommodation, gainful employment, renegotiating old and new social networks and linking to healthcare services. Data regarding these key issues is discussed below, including how the continuum of HCV care amongst former prisoners performs amongst these competing priorities.

1.3.3.1.1 *Employment*

Former prisoners experience difficulties in establishing and maintaining gainful employment. American data demonstrates that 40% of former prisoners were not gainfully employed within 12

months of release (313). Similarly, only 30% of Norwegian prisoners achieved employment 3 years after re-entry (314). The challenge of maintaining employment was a key finding in a Finnish study which demonstrated that while gainful employment was achieved for a majority, this was sustained for only 10% (315). Factors associated with increased gainful employment following re-entry include pre-imprisonment work experience, connection with employers prior to release and returning to a conventional family setting, while drug and alcohol recidivism and chronic physical and mental health comorbidities were associated with unemployment (316). When gainful employment was achieved, multiple data indicated that this is associated with reduced recidivism and reincarceration (313, 314, 317, 318).

1.3.3.1.2 Housing

Australian data indicate that over 20% of Victorian prisoners were homeless or had unstable accommodation prior to incarceration (319). Stable accommodation remains a significant challenge following community re-entry; over 50% of Australian former prisoners will move residence two or more times within twelve months and 21% are immediately homeless post release (320). The challenges of house instability were endorsed by findings from a large American study which demonstrated an average of 2.59 residential moves per year amongst approximately 3000 recently released prisoners (321). This may be the result of individuals possessing little to no income, encountering discrimination from private landlords, having high reliance on others for housing security and being excluded from certain public housing programs (322). Despite these significant challenges, only 15% of Victorian prisoners were released with transitional support programs equipped to provide accommodation support (319). When the transition support for these 15% expired however, 44% of females and 22% of male former prisoners then became immediately homeless.

1.3.3.1.3 Recidivist illicit drug use

Australian data indicate that 55% of released prisoners resume illicit drug use within the first months following community re-entry, and 30% report IDU specifically (323). Of those with recidivist IDU, 50% reported the shared use of injecting paraphernalia. More contemporary Australian data identified that 41% of 533 ex-prisoners with an IDU history reported recidivist IDU within 6 months of prison release, most of whom relapsed within the first month (324). Other Australian and international data identified likelihoods of drug and alcohol recidivism between 8-68%. A Russian study evaluating time to recidivist drug use identified a median period of 30 days, and the likelihood of relapse was higher amongst those who were unemployed (325).

The high rates of recidivist IDU and low opiate tolerance following incarceration contribute to the increased likelihood of death, largely secondary to non-intentional opiate overdose, in the immediate post release period. Of 30,237 prisoners followed for a mean period of 1.9 years, 443 died resulting in an overall mortality rate of 777 per 100,000 person years (326). The risk of death was 12.7 times higher than their community counterparts in the immediate 2 weeks following prison release with a 129-fold relative risk of death from overdose during this time-period. This risk is enhanced in the immediate two weeks following release and is 3-8 times higher during this time than compared with 3-12 weeks post release (327). There is also a high incidence of non-fatal overdose in the first 2 weeks following prison release which was 27-29 per 100-person years in alternate data (328, 329).

1.3.3.1.4 Health and linkage to care

Incarceration may confer a period of stability for individuals and health conditions can be optimised (330). Up to 90% of prisoners, however, report no regular primary care physician prior to incarceration and as such, the stability of any chronic disease management achieved in prison is interrupted (331). Additionally, 75-90% of recently released prisoners in the USA do not have public or private health insurance within 3 months of community re-entry which adds to the complexity of accessing medical care (332, 333). In this context, there are multiple health-related challenges encountered by prisoners during community re-entry. Poor healthcare utilisation has been attributed to competing priorities including homelessness and unstable housing, poor resources required to make and attend appointments, drug and alcohol recidivism, reincarceration and poverty (334).

There is a high prevalence of HIV infection amongst prisoners, particularly in the USA, and it is anticipated that one in seven of USA citizens with HIV infection will be incarcerated each year (335). As such, prisons provide an ideal setting to engage people living with HIV infection in care (336). There are however barriers to HIV care continuity on community re-entry. For example, only 30% of prisoners living with HIV infection engaged in prison-based care had their anti-retroviral medication prescription dispensed within two months of re-entry, which was provided prior to release (337). Furthermore, only 50% had linked to care within 3 months, evidenced by a HIV viral load test result (338). Moreover, 30% of those former prisoners had detectable HIV viraemia suggestive of anti-retroviral medication interruption. The median time to linkage to care was significantly longer for black Americans. Recidivist drug and alcohol use in HIV positive ex-prisoners is associated with a lower likelihood of linkage to care, poor adherence to anti-retroviral treatments and increased high risk behaviours (339). Other factors negatively associated with HIV linkage included conditional release and reincarceration, whilst the presence of multiple medical comorbidities, and longer duration of incarceration had positive associations (340). There is also a high reliance on emergency services amongst former prisoner with HIV infection with 56% presenting to emergency departments within

12 months of release, most commonly for febrile syndromes, substance misuse related presentations, trauma and mental health diagnoses (341). Many prisoners with HIV released from prisons in the USA reported that linking with HIV care post release was significantly more challenging than anticipated (342). A recent systematic review which investigated facilitators and barriers to HIV care following incarceration to inform HCV linkage to care strategies identified that social support, linkage to an existing primary care provider, and receipt of OST facilitated linkage to HCV care (343). Barriers to HCV care included perceived lack of healthcare information and lack of specialised linkage to care programs whilst unstable housing, age less than 30 years, HIV related stigma, poor providers' attitudes and lack of post release integration services were barriers to success linkage to HIV transitional care (Yanes-Lane).

In the absence of a regular healthcare provider, the emergency department is frequently used by former prisoners for urgent care. One study identified that recent incarceration was significantly associated with emergency department presentation, and also a history IDU, opiate use, sexually transmitted infection and survival sex trading (344). Furthermore, in one Australian jurisdiction, 20% of those released from prison were admitted to hospital within 12 months, more commonly Aboriginal or Torres Strait Islanders, females and in people with mental health diagnoses (345).

Former prisoners with mental health diagnoses are at particular risk of deterioration during re-entry. This includes 50% of former prisoners with substance use disorder reporting recidivist drug use shortly after release (346). Those prisoners re-entering the community with mental health diagnoses also reported poorer health, had more frequent hospitalisations, more suicidal ideation, greater difficulties securing housing and employment and less financial support from family than those without these diagnoses (347). There is also a higher rate of reincarceration amongst this group (348).

There is also a disproportionally high prevalence of non-communicable diseases including hypertension and type II diabetes amongst prisoners, and less than half these individuals were successfully linked to care post release (349, 350).

1.3.3.1.5 Social Engagement

An important matter regarding community re-entry is re-establishing social connections with family and friends. Re-engaging previously meaningful relationships can however be a source of anxiety for released prisoners and many report insecurities regarding how they will be received (351). Certainly, some prisoners and their family identify challenges during reconnection, should that desire to reconnect be present at all (352). Data demonstrate that where successful social reconnections are established, there may be resultant benefits for the individual including a lower likelihood of recidivism, a higher likelihood of employment and improved overall sense of wellbeing (353). Re-

establishing social relationships also tend to result in increased housing, financial and emotional support from family members (354). Nonetheless, the success of social engagement is affected by the complex interplay of issues facing re-entering individuals and is less likely to be successful or sustained in the setting of substance misuse, mental health diagnoses, weakened family ties, unstable housing and lower levels of employment (351).

1.3.3.1.6 Likelihood of HCV linkage to care in this context

There is limited data exploring the likelihood of former detainees with HCV infection linking to care post community re-entry. One retrospective study investigated the likelihood of individuals released from prison with untreated HCV linking with HCV care following community re-entry (as evidenced by a HCV RNA blood test result). Of the 1605 individuals followed for 6 months, only 138 (9%) engaged with care within this time frame.

1.3.3.2 Initiatives to improve linkage to care amongst recently release prisoners

Due to the poor continuity in healthcare amongst former detainees, and the high burden of communicable and non-communicable diseases amongst this group, multiple interventions have been developed and evaluated to improve healthcare continuation. Such interventions are described below, including a small study describing HCV linkage to care initiatives.

1.3.3.2.1 Case management

The efficacy of intensive case management during community re-entry, and its impact on healthcare utilisation, has previously been described. This includes the 'Project Bridge' intervention which was initially described in 2001 and provides case management, mental health triage, referral to substance misuse services, community program engagement and referral to HIV management services, where appropriate, for recently released prisoners (355). This intervention achieved linkage to medical care for 75% of participants (where a need was identified), and 67% of those reporting substance misuse were also linked to appropriate drug and alcohol services. These data highlight that transition support can significantly improve continuation of medical care during community re-entry.

Multiple subsequent studies have highlighted the benefit of intensive case management for released prisoners, particularly for those with HIV infection (356-360). Interventions evaluated in these studies included video conference introductions with community case managers prior to release, scheduled review meetings in the community, transportation to medical appointments, brief educational interventions, assistance with social security and housing, referral to mental health and drug recidivism services, and connection with employment agencies. One such program delivered by peer navigators was associated with a higher likelihood of linkage to HIV care within 30 days (OR 2.15),

which was sustained at 12 months post release (359). Similarly, 79% of prisoners enrolled in another case management re-entry program were engaged with appropriate medical care and 74% also received additional community services (357, 358). This benefit was most pronounced where successful contact with participants was achieved both within the prison (prior to release) and community, and then the prison alone, and then community alone, suggesting the importance of initiating contact whilst incarcerated to build rapport and to facilitate appropriate discharge planning.

Qualitative data also supports the acceptability of case management interventions provided during re-entry (356). Case management for HIV positive individuals following release is associated with reduced internalised or perceived stigma which in turn minimised apprehension in accessing community HIV services (360).

Case management of released prisoners with significant mental health diagnosis resulted in a higher likelihood of linkage to primary health care (87% vs 38% amongst controls) and continuation or commencement of psychotropic medications (80% vs 38% amongst controls) (361). Similarly, amongst individuals with mental health diagnoses engaged with transition services, median time to linkage was two days compared with 185 days in a historical matched cohort (361).

1.3.3.2.2 Discharge planning / educational interventions during incarceration

Prison-based discharge planning and educational interventions have been associated with improved healthcare utilisation following release from prison. Regarding HIV care, two studies have demonstrated that prisoners who received comprehensive discharge planning including referral to community HIV providers were 10 times more likely to achieve healthcare continuity (358, 362). Similarly, 75% of HIV positive prisoners who had video-conferencing arranged with their community HIV provider during discharge planning were retained in care in the community (363). HIV disease management education programs delivered prior to release also resulted in a higher likelihood of linkage to care and also sustained linkage at 12 months post release (364).

Brief educational interventions regarding sexually transmitted infections and their transmission were associated with lower likelihoods of high-risk sexual behaviours following release (365, 366). This effect was enhanced when education was provided over multiple sessions. Similarly, prisoners with latent tuberculosis who were released with partial treatment were more likely to complete therapy in the community (65% vs 48%, $p > 0.05$) where prison-based tuberculosis group education was provided (367). Educational programs for prisoners with mental health diagnoses designed to foster health-related knowledge and other important life skills have also been associated with enhanced rates of healthcare engagement (361).

1.3.3.2.3 Transportation provision

Assistance with transportation to and from medical appointments following community re-entry has been positively associated with linkage to HIV care (aOR 2.04 [CI 1.49-2.79]) highlighting that these practicalities remain a significant barrier to care (364). Another study demonstrated a numerically higher likelihood of linkage to care amongst former prisoners provided with transportation support (368). Qualitative data also describes the importance of social reconnections to facilitate transportation assistance from family and peers (342).

1.3.3.2.4 Monetary incentives

One randomised control trial investigated whether a \$5 USD incentive could increase continuation of isoniazid for latent tuberculosis amongst individuals released on treatment (367). The researchers noted that while 65% of prisoners with latent tuberculosis were released during their isoniazid treatment course, only 24% of matched historical controls continued therapy after release. They therefore randomised prisoners to tuberculosis education and \$5 USD payment versus education alone. 37% of former prisoners in both arms attended transition clinics following release, and although the effect of the cash payment wasn't additive, the rate of linkage to care achieved through education alone was significantly higher than in the matched historical cohort.

1.3.3.2.5 Transition clinics and linkage with primary care physicians

The impact of transition clinics on healthcare continuity has been investigated in multiple clinical trials. One study identified a high engagement in transitional care, with a median period of 10 days between re-entry and review (350). Prisoners with HIV were retained in care for at least six months in 86% of cases, however this was lower for those whose primary diagnosis was hypertension (45%), diabetes (43%) or opioid dependence (33%).

Another transition intervention study determined whether medical, psychosocial and chronic disease self-management would result in higher primary health care utilisation and minimise emergency department presentations (369). Prisoners were randomised to either the transition clinic or to standard of care (appointment scheduled with primary care physician on a prisoner's behalf immediately post release). Whilst the rates of retention in primary health care was not statically different between groups (37.7% vs 47.1%), those engaged in transition clinic care were significantly less likely to presented to the emergency department for urgent care during follow up. The cohort was significant for high rates of homelessness (38%), ethnic minorities (86%) and those without a primary healthcare provider prior to incarceration (331).

Furthermore, an Australian study identified that former prisoners who had engaged with primary health care within one month of community re-entry were more likely to utilise mental health (aHR 1.65), alcohol and other drug services (aHR 1.48) and subsequent primary care services over the following 6 months (370). Prisoners with mental health diagnoses who were managed by transition clinics on community re-entry were more likely to be engaged with appropriate psychiatric services following release (361).

1.3.3.3 Transition interventions specific to HCV infection

Only one study has investigated HCV transitional care during prisoner re-entry. This was a single arm clinical trial in which prisoners with HCV who were released without treatment were recruited (371). Following community re-entry, participants were supported to attend medical appointments by providing phone call reminders, appointment rescheduling, accompaniment to appointments, and reimbursement for public transportation. They were also referred to their prior health provider to facilitate HCV treatment, or to another healthcare provider affiliated with the study prior to release. Overall 31% were linked to HCV care, defined as attending a least one appointment with an HCV provider and 20% commenced HCV treatment.

It is anticipated that linkage to ongoing specialist care for cirrhotic individuals who are successfully treated for hepatitis C infection whilst incarcerated is also low, however there is limited data to support this at present.

1.4 HCV viral resistance and impact on salvage retreatment amongst non-SVR12 patients

1.4.1 HCV RAS

Multiple viral quasispecies are identified within an individual due to the error prone NS5B RdRp polymerase, as previously discussed. As such, a population of genetically distinct, though closely related HCV quasispecies exist within an individual. Some HCV quasispecies will harbour naturally occurring amino acid substitutions which reduce their susceptibility to approved NS5B, NS5A and NS3/4A protease inhibitors. Those which confer a >2-fold reduced sensitivity to particular DAAs are termed HCV RAS.

In general, HCV RAS have reduced viral fitness compared with wild-type virus and therefore represent a minority of quasispecies in most DAA naïve individuals. These resistance quasispecies are selected, however, with the introduction of DAA therapy as wild-type virus is rapidly eliminated and can therefore be implicated in non-SVR12.

While HCV RAS are identified in the sequences coding for the NS5B, NS5A and NS3/4A proteins, this thesis investigated HCV NS5A RAS only at treatment baseline and their implication on HCV treatment. As such, the baseline prevalence and significance of NS5A RAS only, and their implications on first line and second-line salvage treatment are discussed below.

1.4.2 Prevalence of NS5A RAS at baseline

The prevalence of baseline HCV RAS in DAA naïve populations vary between different geographical regions. At commencement of this thesis, there was no Australia-specific NS5A RAS prevalence data available.

1.4.2.1 GT1a infection

Multiple studies have investigated the prevalence of GT1a NS5A RAS in DAA naïve patients. A metanalysis which included NS5A RAS data from 35 phase I, II and III trials determined a mean prevalence of 13.0% (372). NS5A RAS prevalence was highest in the Oceanic region, in which 15.6% samples harboured resistant quasispecies, and was lowest in Europe (12.1%). The most prevalent GT1a NS5A RAS was M28V in all regions. Other NS5A-inhibitor registration trials have demonstrated a GT1a NS5A RAS prevalence of 11-23% at baseline (153, 373, 374), and amongst country specific series, the prevalence was 11-23% (375-377). Australian data was published in 2019 and identified a comparable baseline frequency of HCV GT1a NS5A RAS (12%), and M28V remained the most commonly identified resistance quasispecies (378).

1.4.2.2 GT1b infection

The prevalence of GT1b NS5A RAS was identified as 17.6% in a large metanalysis, which was highest in Europe (16.8%) and lowest in the Asian Pacific region (15.6%), and the Y93H was the most prevalent NS5A RAS in all regions (372). Other international data including large registration trials and other country specific series have demonstrated varying baseline prevalence between 16-33% (153, 373-376, 379, 380).

1.4.2.3 GT3 infection

Fewer data are available regarding baseline GT3 NS5A RAS prevalence. Large registration trials and smaller country specific series identified frequencies of 12-19%, and Y93H was most commonly identified (153, 374, 376). The prevalence of GT3 NS5A RAS was significantly higher in China, where it exceeded 50% due to the high baseline prevalence of dual A30K and L31M NS5A RAS in combination.

1.4.2.4 Non-GT1 and -GT3 infection

There is limited data regarding non-GT1 and -GT3 infection. These are restricted to registration and country specific series which report a wide GT2 NS5A prevalence range of 28.4-98%, which varied by subtype (181, 374, 376, 379). The most prevalent NS5A RAS in GT2 infection was L31M. The range of NS5A RAS in GT4 infection detected at baseline was 45.0% to 60.2% and amino-acid substitutions at position 58 were most commonly identified. In registration trials, NS5A RAS were identified in GT5 and GT6 infection in 8.8-26.8%, and 50%, respectively. Amino acid substitutions were most commonly at position 28 in GT6 infection.

1.4.3 Effect of NS5A RAS at baseline by treatment regimen

1.4.3.1 SOF + LDV

The presence of LDV specific RAS at baseline was associated with reduced SVR12 rates in treatment-experienced GT1a patients treated for 12 weeks (SVR2 67-72%) (372, 381). Therefore, guidelines recommend that treatment duration is extended to 24 weeks in the setting of prior PEG IFN RBV exposure. SVR12 rates were also lower in treatment naïve participants treated for eight weeks who had baseline NS5A RAS, however efficacy was restored when treatment was extended to 12 weeks (381). NS5A RAS identified at baseline in treatment-naïve cirrhotic patients with GT1a infection was also associated with lower SVR12 rates (86%), however due to the small numbers included in this study, no alteration to treatment duration is recommended. There was no association between presence of baseline LDV specific RAS and reduced efficacy in HCV GT1b patients (372). There is insufficient data to determine the impact of baseline NS5A RAS on the efficacy of SOF + LDV in GT4, GT5 and GT6 infection.

1.4.3.2 SOF + DCV

In both GT1 and GT3 infection, prior treatment experience did not affect the likelihood of cure following 12 week SOF + DCV in non-cirrhotic participants, irrespective of baseline NS5A RAS (382, 383). SVR12 rates were lower however in cirrhotic (vs non-cirrhotic) participants which reflects under treatment rather than the effect of baseline NS5A RAS. Therefore, recommendations suggest 24 weeks SOF + DCV for cirrhotic patients with HCV infection (154).

1.4.3.3 PrOD

There was no significant reduction in treatment efficacy where baseline NS5A RAS were identified in participants with GT1a or GT1b HCV infection who were treated with PrOD (373).

1.4.3.4 GZR + ELB

Baseline NS5A RAS can significantly reduce the efficacy of 12 weeks GZR + ELB in those with HCV GT1a infection. The C-EDGE trial identified that SVR12 rates were 58% and 99% amongst GT1a participants with and without baseline NS5A RAS, respectively (163). A pooled analysis also demonstrated that baseline NS5A RAS at positions 28, 30, 31, 58 and 93 significantly reduced the efficacy of 12 weeks GZR + ELB (384). SVR12 rates reduced to 64% in participants who were treatment experienced and had baseline NS5A RAS. This effect could be mitigated by extension of therapy to 16 weeks and the addition of RBV, which achieved SVR12 rates of 100%. Baseline NS5A RAS did not affect GZR + ELB efficacy in HCV GT1b infection.

1.4.3.5 SOF + VEL

The impact of baseline NS5A RAS for GT1-6 HCV infection was investigated in a pooled analysis study including all phase III SOF + VEL clinical trial data (participants with decompensated cirrhosis excluded) (374). For non-GT3 infection, the presence of baseline NS5A RAS did not reduce SOF + VEL efficacy. In GT3 infection however, SVR12 rates were numerically lower in participants with baseline HCV NS5A RAS, however this did not reach statistical significance. The efficacy of SOF + VEL was significantly lower however in those with GT3 infection who harboured the Y93H NS5A RAS at baseline (86% vs 98%, $p < 0.05$). The impact of baseline NS5A RAS in individuals with hepatic decompensation treated with SOF + VEL has also been investigated. In participants with GT1 infection, hepatic decompensation and baseline NS5A RAS, treatment with SOF + VEL for 12 and 24 weeks achieved SVR12 rates of 80% and 90%, respectively (188). The impact of baseline NS5A RAS, however, was overcome when SOF + VEL was prescribed with RBV for 12 weeks (SVR12 98-100%). There is insufficient data to determine the effect of NS5A RAS at baseline in decompensated GT3 patients.

1.4.3.6 GLE + PIB

The effect of baseline NS5A RAS amongst GT1-6 participants included in phase II and III GLE + PIB trials was analysed in a pooled analysis study (181). There was minimal effect on treatment outcomes of GLE + PIB eight-12 weeks in compensated cirrhotic and non-cirrhotic participants with two notable exceptions. This included reduced efficacy (SVR12 90%) in treatment-experienced GT3 participants, irrespective of cirrhosis status, treated with GLE + PIB for 12 weeks. This could be overcome by extension of treatment duration to 16 weeks (SVR12 94-96%). Similarly, efficacy was reduced in GT3 participants treated for eight weeks who harboured the A30K variant at baseline (SVR12 78%), which could be overcome by extending therapy to 12 weeks (SVR12 93%). No other significant impacts of baseline NS5A RAS were identified.

1.4.4 Prevalence of NS5A RAS at treatment failure and evolution after treatment

NS5A RAS may be selected during DAA treatment and are implicated in virological relapse and breakthrough, accounting for their high prevalence at treatment failure (21). NS5A RAS were detected in >95% of patients who failed SOF + LDV with HCV GT1 infection (385). More than one NS5A RAS were detected in 35%. In a pooled analysis of all SOF + VEL phase II/III trials, NS5A RAS were detected in 85% of non-SVR12 participants and similarly, in 92% of non-SVR12 patients who failed GLE + PIB, with the Y93H RAS most frequent in both series (374, 386). NS5A RAS were detectable in 72% and 29% of GT1a and GT1b patients, respectively, who had failed PrOD (373).

The prevalence of treatment-emergent NS5A RAS has also been investigated in several real-world studies. A German series which included 626 non-SVR12 patients demonstrated that 77-81% with GT1a, 83-94% with GT1b and 84% of those with GT3 infection had detectable NS5A RAS at non-SVR12 (387). Substitutions conferring resistance to NS5A inhibitors were most commonly detected at position 30 for GT1a and position 93 for GT1b and GT3 infection. A French study determined that 85% of non-SVR12 patients had NS5A RAS at treatment-failure and in almost all, the detected RAS conferred at least >10-fold increase in resistance to NS5A inhibitors available at that time (388).

NS5A RAS demonstrate excellent viral fitness and therefore can persist as a dominant quasispecies for many years after relapse (373, 389). Amongst LDV exposed patients with non-SVR12, NS5A RAS were detected in 86% of patients 96 weeks' post treatment (373). Similarly, amongst Ombitasvir exposed non-SVR12 patients, NS5A RAS were detected and remained the dominant quasispecies in 96% of patients 48 weeks after treatment (373).

1.4.5 Retreatment strategies and effect of treatment-emergent RAS

Table 1.3: Recommendations for HCV retreatment for NS5A-inhibitor experienced patients and impact of baseline NS5A RAS. SVR12, sustained virologic response 12 weeks after therapy; HCV, hepatitis C virus; RAS, resistance-associated substitution.

	Regimen	SVR12 rate	Impact of baseline HCV NS5A RAS
Pan-genotypic	Sofosbuvir / Velpatasvir / Voxilaprevir [12 weeks]	96%	No
	Sofosbuvir / Glecaprevir / Pibrentasvir [12-16 weeks]	96%	No
	Sofosbuvir / Velpatasvir / Ribavirin [12-24 weeks]	91% ¹	Yes ²
Genotype 1 & 4	Glecaprevir / Pibrentasvir	89-91% ³	Yes

	[12-16 weeks]		
	Sofosbuvir / Elbasvir / Grazoprevir [16-24 weeks]	96%	No

¹ SVR12 rates varied by genotype and were 96% for HCV GT1 & GT2 infection and 78% for GT3 infection

² SVR12 rate was 100% for GT3 participants without HCV NS5A RAS and 77% in those with NS5A RAS at baseline

³ SVR12 rates were 89% for those treated with GLE / PIB for 12 weeks and 91% for those treated for 16 weeks

1.4.5.1 SOF + VEL + VOX

The efficacy of SOF + VEL + VOX for relapsed HCV amongst DAA-experienced participants was investigated in two large registration trials, POLARIS-1 and POLARIS-4 (390). In POLARIS-1, non-SVR12 NS5A inhibitor experienced participants, 46% of whom had compensated cirrhosis, were treated with 12 weeks SOF + VEL + VOX. The overall SVR12 rate was 96% and was not affected by genotype or the presence of baseline NS5A or NS3 RAS. A numerically lower SVR12 was noted in those with cirrhosis (cirrhotic 93% vs non-cirrhotic 99%), however this did not reach statistical significance. POLARIS-4 included DAA (but not NS5A-inhibitor) experienced participants with relapse. Forty-six percent of participants were cirrhotic and 98% achieved SVR12 after 12 weeks SOF + VEL + VOX. Treatment was generally well tolerated in both series.

Several real-world studies have also investigated the efficacy of SOF + VEL + VOX amongst non-SVR12 populations, most of whom NS5A-inhibitor experienced. This includes large series from the USA, Italy and Spain (391-393). All three studies demonstrated SVR12 rates of 95-96% per protocol. The USA real-world study, however, suggested that efficacy is reduced in SOF + VEL experienced participants, and in the Spanish study amongst participants with GT3 infection and cirrhosis. There is no Australian data regarding the efficacy or safety of this regimen in those with advanced disease.

The impact of NS5A and NS3 RAS at baseline in DAA-experienced patients undergoing salvage treatment with SOF + VEL + VOX was investigated in a pooled analysis of phase III registration trials (394). 82.7% of participants had NS3 and/or NS5A RAS, and 79% had NS5A RAS specifically, at baseline. The presence of NS3 and/or NS5A RAS did not affect treatment outcomes and SVR12 rates were comparable irrespective of the number of RAS or type of RAS identified, including at position 93.

1.4.5.2 GLE + PIB

The MAGELLAN-1 Part 2 trial investigated the efficacy of 12- or 16- weeks of GLE + PIB for the management of DAA-naïve and -experienced GT1 and GT4 participants, including a subset (34%) who had compensated cirrhosis (395). Overall, SVR12 rates were suboptimal and were 89% for 12 weeks

and 91% for 16 weeks of treatment. SVR12 rates were particularly low amongst participants who were both NS5A- and NS3/4A PI-experienced treated for 12 or 16 weeks (79% and 81%, respectively). For patients who were only NS5A-experienced treated for 16 weeks, however, SVR12 outcomes were acceptable (94%) and were excellent for those with prior NS3/4A PI experience only (100%) for both 12- and 16-weeks of treatment (379). In participants with no HCV RAS, SVR12 rates were 100%. Amongst those with NS5A RAS, 83% in participants treated for 12 weeks and 94% treated for 16 weeks achieved SVR12. In patients with baseline NS5A and NS3 RAS, there was a high rate of on treatment virologic breakthrough (33%).

MAGELLAN-1 Part 1 similarly identified reduced efficacy in participants without cirrhosis and relapsed HCV infection who were NS5A-inhibitor +/- NS3/4A PI experienced (91-93%) compared with those NS3/4A PI experienced alone (100%) after treatment for 12 weeks (396). SVR12 rates for participants with baseline NS3 RAS only, NS5A RAS only and both NS3 and NS5A RAS were 100%, 91% and 93%, respectively.

1.4.5.3 SOF + GLE + PIB

There is emerging data regarding the efficacy of SOF in combination with fixed dose GLE + PIB and RBV as a salvage regimen for NS5A inhibitor experienced patients with relapsed HCV. This was investigated in MAGELLAN-3 in which participants, including those with cirrhosis, who had failed 8, 12 or 16 weeks of GLE + PIB were retreated with SOF + GLE + PIB + RBV for either 12- or 16- weeks (397). All GT3 participants, and those NS3/4A PI or NS5A-experienced prior to GLE + PIB failure were treated for 16 weeks. The SVR12 rate was 96% overall, irrespective of the presence of baseline NS5A and NS3 RAS, and the regimen was well tolerated.

1.4.5.4 SOF + ELB + GZR

SOF in combination was GZR + ELB with RBV is an effective retreatment regimen for NS3- or NS5-experienced participants with HCV relapse and GT1 and GT4 infection. This was investigated in the REVENGE study in which patients with prior DAA failure, most of who had advanced fibrosis or cirrhosis, were treated for 16-24 weeks. SVR12 rates were 96% and the sole treatment failure occurred in a patient who died between EOT and SVR12 timepoints. No effect of baseline HCV NS5A or NS3 RAS were identified. There is no data regarding the efficacy of this regimen for non-GT 1 & 4 infection.

1.4.5.5 SOF + VEL +/- RBV

The use of NS3/4A PIs are not recommended for patients with CTP class B/C cirrhosis due to the risk of hepatotoxicity and hepatic decompensation. As such, SOF + VEL +/- RBV is the preferred regimen for DAA-experienced patients with HCV relapse and hepatic decompensation. The efficacy of 24 weeks

of SOF + VEL + RBV was investigated in a phase II trial in participants with GT1-3 infection, some of whom had compensated cirrhosis, who had previously failed an NS5A-containing regimen (398). Overall, the SVR12 rate was 91%, and was 96% in HCV GT1 and GT2 infection, with no impact of baseline NS5A RAS on outcomes. In GT3 infection however, SVR12 was 78% overall, and decreased to 77% where baseline NS5A RAS were detected (compared to an SVR12 rate of 100% in those without baseline NS5A RAS). As such, SOF + VEL + RBV should be used for retreatment following non-SVR12 where PIs are contraindicated.

1.5 Conclusions

The above literature review serves as a background to this thesis and highlights the tremendous progress that has been made in the management of HCV infection. In particular, the introduction of DAA therapy has revolutionised HCV treatment and high rates of cure are anticipated after first line-therapy. In this setting, the WHO has proposed elimination targets including an 80% reduction in HCV incidence and 65% in HCV-related mortality by 2030.

Despite this, there remain certain groups who are difficult to treat. This includes PWIDs who are underserved by existing models of care, and prisoners for whom there was a dearth of clinical services to provide prison-based treatment in large numbers at commencement of this thesis. Finally, DAA-experienced individuals with relapse and advanced disease also present unique challenges in determining a retreatment strategy that is safe and effective.

Each of these groups, however, are also important for achieving the WHO targets. PWIDs and prisoners are high transmitting populations and HCV treatment can not only have significant benefits for the individual, but also for the community more broadly. Therefore, models of care appropriate for the prison system require development and evaluation. Dedicated HCV transitional care is also needed to improve engagement amongst those individuals who are released from prison untreated. Those with advanced liver disease contribute most greatly to HCV-related morbidity and mortality and therefore it is also important to continue to evaluate optimal management strategies for those with HCV-relapse and difficult to cure characteristics to mitigate these risks.

1.6 Hypotheses and Aims of this research

In this context, the hypothesis' and aims of this research include:

Hypothesis' of this research:

- 1) That nurse-led prison based hepatitis C treatment can be delivered safely and effectively to individuals in prison in large numbers
- 2) That transitional healthcare for individuals released from the prison who are living with hepatitis C will increase the likelihood of initiation of antiviral therapy in the community
- 3) That simple blood-based fibrosis algorithms could be used with high confidence to exclude cirrhosis in a prison setting to improve hepatitis C treatment efficiencies
- 4) That combination SOF, ELB, GZR and RBV will achieve high rates of hepatitis C cure in NS5A inhibitor experienced patients, irrespective of hepatitis C genotype
- 5) Certain NS5A inhibitor experienced patient subgroups with relapsed hepatitis C infection may respond less favourably to second line salvage therapy

Aims of research:

- 1) To evaluate the efficacy and safety of prison-based HCV treatment delivered by the Statewide Hepatitis Program across the jurisdiction of Victoria, Australia
- 2) To design and evaluate a transitional healthcare model which supports untreated individuals with HCV infection released from the prison system to commence DAA treatment
- 3) To evaluate the performance of non-invasive fibrosis algorithms for prisoners with HCV infection and determine their impact on program efficiencies within the prison sector
- 4) To determine the prevalence of baseline HCV NS5A RAS amongst DAA-naïve Australians
- 5) To evaluate the safety and efficacy of SOF, ELB, GZR and RBV for NS5A-inhibitor experienced participants with HCV relapse and non-genotype 1 & 4 infection
- 6) To determine the safety and efficacy of SOF, VEL and VOX for NS5A-inhibitor experienced Australians with HCV relapse and difficult to cure characteristics

2 Chapter 2: Outcomes of treatment for hepatitis C in prisoners using a nurse-led, statewide model of care

2.1 Introduction and Summary

Approximately 71 million people are living with HCV globally (254). The introduction of DAAs means that HCV treatment is now all-oral, well tolerated and short in duration and cure rates exceed 95% (152, 153). In order to meet the WHO viral hepatitis elimination targets, HCV DAA treatments must be prioritised for PWIDs who most greatly contribute to incident infection in Western countries (274). As PWIDs have a high lifetime risk of incarceration, the prevalence of HCV in prisons globally is also high (259). Therefore, the prison setting provides an opportunity to engage and treat PWIDs living with HCV in large numbers. Despite this, multiple barriers to prison-based HCV care have historically resulted in low prisoner treatment rates both in Australia and internationally (399). This includes limited local access to clinicians, the requirement for hospital-based specialist review, budgetary constraints and IFN-based therapies which had high toxicity yet low efficacy.

In this context, the Statewide Hepatitis Program, a nurse-led in-reach model-of-care was developed to provide viral hepatitis care for all prisoners in Victoria, Australia. This program operates across all 14 adult prisons in Victoria and provides decentralised HCV care, therefore minimising the need for prisoner transfer and specialist review. This study evaluated the safety and efficacy of HCV treatment provided by the Statewide Hepatitis Program. We demonstrated that prison-based HCV treatment using DAA therapies was highly effective and cure rates exceeded 95% per protocol. Treatment was also well tolerated, and adverse events were uncommon. Importantly, less than 20% of those treated had previously participated in specialist care prior to incarceration, highlighting the important contribution prisons can provide in engaging this marginalised population.

This study was published in *The Journal of Hepatology*.

Reference:

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The text in its entirety is included in this chapter. The prison version from the journal is included as an appendix.

2.2 Original Article

Outcomes of treatment for hepatitis C in prisoners using a nurse-led, state-wide model of care

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2.2.1 Abstract

Background: Treatment programs for people who inject drugs (PWID), including prisoners, are important for achieving hepatitis C elimination targets. There are multiple barriers to treatment of hepatitis C in prisons, including access to specialist physicians, testing and antiviral therapy, short prison sentences, and frequent inter-prison transfer. A state-wide program for assessment and management of hepatitis C was developed in Victoria, Australia to improve access to care for prisoners. This nurse-led model of care is supported by telemedicine to provide decentralised care within all prisons in the state.

Methods: We prospectively evaluated the feasibility and efficacy of the nurse-led model of care for hepatitis C within the 14 adult prisons over a 13-month period. The primary end point was sustained virological response at week 12 post-treatment (SVR12) using per protocol analysis.

Results: There were 416 prisoners included in the analysis. The median age was 41 years, 90% were male, 50% had genotype 3 and 44% genotype 1 hepatitis C and 21% had cirrhosis. Injecting drug use was reported by 68% in the month prior to prison entry, 54% were receiving opioid substitution therapy, and 86% reported never previously engaging with specialist HCV care. Treatment duration was eight weeks in 24%, 12 weeks in 59%, and 24 weeks in 16% of treatment courses. The SVR12 rate was 96% (301/313) per-protocol. Inter-prison transfer occurred during 26% courses of treatment but was not associated with lower SVR12 rates. No treatment-related serious adverse events occurred.

Conclusion: Hepatitis C treatment using a decentralised, nurse-led model of care is highly effective and can reach large numbers of prisoners. Large scale prison treatment programs should be considered to support hepatitis C elimination efforts.

2.2.2 Introduction

The World Health Organization (WHO) has set global targets for the elimination of viral hepatitis as public health threat¹. For hepatitis C, the goals are to reduce incidence by 80% and mortality by 65% by 2030. In Western countries where injecting drug use is the dominant risk factor for hepatitis C transmission², eliminating incident infection will require coordinated efforts using harm reduction strategies as well as “treatment as prevention” to interrupt transmission among people who inject drugs (PWID). This population has not typically been well engaged with specialist care, highlighting the need to develop new models of care for hepatitis C among marginalised, high transmitting populations.

Prison systems provide a unique opportunity to scale up hepatitis C treatment³. The prevalence of hepatitis C is higher in prisoners than in the general population, reflecting the criminalisation of drug use and the frequent detention of PWIDs.^{4,5} In Australia, 46% of prisoners self-report a lifetime history of injecting drug use, and the hepatitis C seroprevalence among incarcerated PWID is greater than 50%⁶. Harm reduction strategies currently available in Australian prisons include bleach for cleansing of injection devices and opioid substitution therapy (OST). Despite this, ongoing incident hepatitis C infection has been reported among PWID in Australian prisons⁷. The median sentence length in Australia is less than six months and there are high rates of recidivism, meaning that prisoners frequently cycle between incarceration and freedom, creating new networks for hepatitis C transmission.⁸ Mathematical modelling studies have demonstrated that the global elimination of HCV will require widespread treatment of key risk populations, in particular PWID.^{9,10} Treatment programs in prisons, where a large numbers of PWID can be screened and treated, could therefore significantly contribute to the HCV elimination agenda⁹.

Despite WHO recommending universal screening of all prisoners for hepatitis C³, rates remain low in many regions worldwide and treatment programs have been limited by multiple barriers¹¹⁻¹⁴. Historical barriers have included the nature of interferon-based therapy (parenteral, long duration, significant toxicity and poor efficacy) as well as systemic barriers (limited local access to clinicians, need for hospital-based specialist review, frequent transfer between prisons interrupting care, and budgetary policy requiring that funding for testing and treatment come from local prison budgets rather than national health schemes). The recent introduction of direct acting antivirals (DAAs) for the treatment of hepatitis C has provided highly effective therapy that is simple, safe, and short duration – ideal for the prison environment. However, DAAs are expensive, which has limited the implementation of prison treatment programs worldwide¹⁵; less than 20% of surveyed European countries provided access to hepatitis C treatment in all prisons.¹⁴ Where treatment is available to

people in custody, it has often been restricted to prisoners with advanced liver disease.^{16, 17} Such a policy will withhold effective treatment from most people living with hepatitis C in the prison, including those at risk of transmission.

In March 2016, the Australian Government approved universal access to DAAs for all Australians living with chronic hepatitis C, including prisoners. A decentralised nurse-led model of care was therefore developed to make viral hepatitis assessment and treatment available to all prisoners across all 14 prisons in the state of Victoria, Australia. We present the first evaluation of the feasibility and efficacy of this novel model of care for hepatitis C.

2.2.3 Methods

Model of care

Australia has a policy of state-sponsored universal health care for all members of the general community, managed by the federal government. However, prisoners are not eligible for this scheme, and medical care during the period of incarceration becomes the budgetary responsibility of the local correctional service under the state government. Such a funding silo challenges the feasibility of DAA therapy given the high list price of these medications. The Australian Government, recognising the importance of providing DAAs to prisoners if hepatitis C elimination is to be achieved, classified DAA treatment for hepatitis C under both the general drug schedule for the community, as well as a highly-specialised schedule, which allows drugs to be provided to prisoners with the requirement that they are prescribed by a specialist and dispensed to prisons from a hospital pharmacy.

In this context, the Victorian Statewide Hepatitis Program was developed by St Vincent's Hospital, Melbourne under contract to the Department of Justice and Regulation, State Government of Victoria, Australia. At the time of evaluation, there were 14 prisons for adults (12 male, two female) in the state of Victoria, with capacity for 7441 prisoners (Figure 1). In the 12 month period from November 2015, more than 10,500 prisoners were received into the Victorian prison system.¹⁸ Each of the 14 Victorian prison sites was serviced by the program, as described below. The program staff included two full-time hepatitis program nurses, three part-time hepatologists (0.25 equivalent fulltime (EFT) staffing in total), and a central pharmacist and pharmacy technician (time commitment increased from 0.2 to 0.6 EFT during the period of evaluation). A centralised electronic medical record was accessible at each prison as well as from St Vincent's Hospital. The medical record could be accessed by nursing, medical, and pharmacy staff to review and coordinate relevant investigations and track prisoner movements across the system to minimise interruptions of care from referral to the sustained virological response (SVR12) time-point.

Figure 2.1: Distribution of Victorian prisons, Australia.

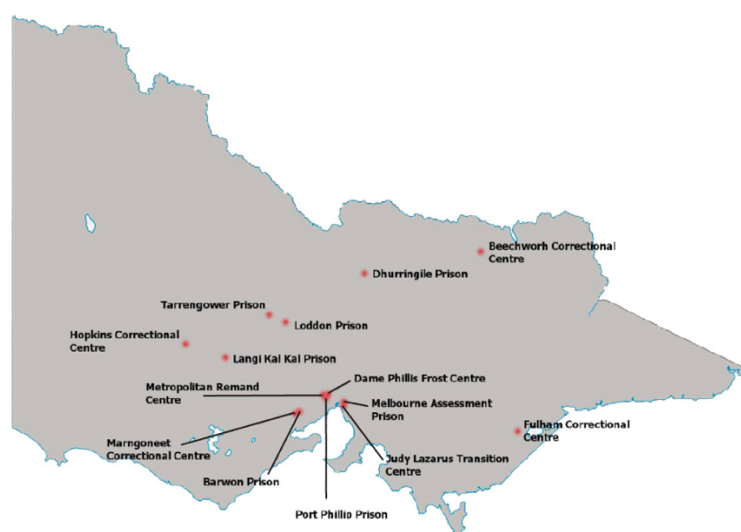


Fig. 1. Distribution of Victoria Prisons, Australia.

Victorian prison healthcare policy is that all prisoners are offered opt-in screening for viral hepatitis at first prison reception and at each transfer between prison sites. This is the responsibility of the primary healthcare service at each prison. If a prisoner was seropositive for hepatitis C, or self-reported a previous diagnosis of hepatitis C, they were referred to the Statewide Hepatitis Program for protocol-driven face-to-face assessment by a program nurse at their residing prison. A program nurse visited each prison on a weekly, fortnightly or monthly basis. Assessment involved a detailed clinical questionnaire including evaluation of risk behaviour, liver stiffness measurement using transient elastography (portable FibroScanTM), and blood-based investigations, including general biochemistry, hepatitis C viral load and genotype, hepatitis B (HBV) and HIV serology. The presence of comorbid medical conditions was assessed, including psychiatric illness (self-reported). The evaluation of risk behaviour included assessment of active injecting drug use, defined as injecting in the month prior to incarceration. Injecting drug use in prison was defined as the using drugs requiring needles and/or other injecting apparatus in either the current or a previous incarceration. Significant alcohol intake included pre-imprisonment or historical significant daily drinking (more than two standard drinks, most days, for both men and women)¹⁹ or binge drinking (heavy intermittent alcohol intake).

Prisoners identified as having chronic hepatitis C infection were then triaged as either 'low risk' or 'higher risk'. 'Low risk' prisoners were either non-cirrhotic or had compensated cirrhosis without significant comorbidities, for whom the hepatologist prescribed DAA therapy after a paper-based consult only with a program nurse, without the physician having direct interaction with the prisoner. 'Higher risk' prisoners had evidence of cirrhosis (clinical/FibroScan), including all prisoners who had decompensated cirrhosis and/or significant comorbid conditions potentially complicating hepatitis C

treatment such as renal failure or HBV coinfection. 'Higher risk' prisoners were referred for either a tele-medicine consult (conducted from their local prison) or face-to-face assessment by a hepatologist. Face-to-face consultation for prisoners located at one of the 13 peripheral prisons required prisoner transfer to the central prison. At clinical review, it was decided whether 'higher risk' prisoners were to commence DAA therapy immediately or required further assessment prior to treatment initiation.

Prisoners with hepatitis C and cirrhosis were enrolled in surveillance programs for hepatocellular carcinoma (HCC) and oesophageal varices as recommended by consensus guidelines^{20, 21}. A viral hepatitis education program for prisoners and prison staff, including primary care staff, drug and alcohol service providers and correctional officers, was provided at all 14 correctional facilities to support the clinical service.

Harm reduction

An opioid substitution therapy (OST) program is available in Victorian prisons, which 11% of prisoners utilised in 2016²². Bleach is available which can be used to clean injecting equipment. There is no provision of clean needles or syringes.

Treatment eligibility

To be eligible for treatment prisoners had to be 18 years of age or older, have evidence of chronic hepatitis C with detectable serum HCV RNA, and have an adequate sentence duration to facilitate complete hepatitis C treatment while incarcerated (between eight to 24 weeks depending on the selected treatment regimen). It was not a requirement however, that prisoners remained in prison to complete blood tests at week 12 post-treatment. Prisoners commenced on DAA therapy who were released early (early parole) were provided with their remaining medication to complete treatment in the community. Prisoners for whom a short sentence duration made them ineligible for treatment whilst incarcerated were referred to a healthcare service for treatment upon release to the community.

Standard correctional pharmacy procedure required that medications be repackaged into blister packs at the central hospital pharmacy before being couriered to the prisons. Initial packs were prepared to include 28 days of therapy, and subsequent packs were distributed to each prison monthly thereafter, until therapy was completed. Two of the 14 prisons provided medications to prisoners in weekly packets while the remaining 12 prisons dispensed medications to the prisoners daily. Medications moved with patients at time of inter-prison transfer to minimise treatment interruption and prisoner location was confirmed in real time using the electronic medical record. When DAAs first became available on March 1st 2016, the program had capacity to initiate six prisoners per week. This

subsequently increased to 16 prisoners per week in June 2016 with increased pharmacy staff resources.

Analysis

This analysis evaluated hepatitis C treatment outcomes among all prisoners who commenced therapy over the 13-month period between 1st November 2015, when the program started, and 1st December 2016.

All prisoners had serum HCV RNA tested at baseline, at the end-of-treatment (EOT), and at week 12 post-treatment. The pre-specified period of follow-up was to week 12 post treatment. The primary endpoint was SVR12, which was defined as undetectable serum HCV RNA 12 weeks after the EOT. Secondary end points included EOT response (defined as undetectable serum HCV RNA at EOT), confirmed HCV reinfection (defined as detectable serum HCV RNA with HCV genotype switch between EOT and SVR12) and treatment-associated adverse events. Relapse was defined as prisoners who achieved an EOT response, but in whom serum HCV RNA was detectable at week 12 post-treatment, without HCV genotype switch.

We considered two analyses for virological outcome – the primary outcome was rate of SVR12 per protocol, including those prisoners with a serum HCV RNA result at 12 weeks post-treatment (complete follow-up). This analysis was performed to account for the high number of prisoners ‘lost to freedom’; that is prisoners released to freedom after the EOT but prior to SVR12, as well as a small number of prisoners who were granted parole during their treatment course. We also considered overall SVR12 rate by intention to treat.

Data were described using median and interquartile ranges (IQR). Categorical data were described as number and percentage. Comparisons between groups were made using appropriate statistical tests after considering distribution of data.

Ethical considerations

This study was approved by the St Vincent’s Hospital Melbourne Human Research Ethics Committee.

2.2.4 Results

Prisoner characteristics

During the 13-month period of evaluation, 949 prisoners were assessed by the Statewide Hepatitis Program, of whom 562 (59%) prisoners were eligible for treatment, and 416 (44%) were commenced on therapy during the period of evaluation. Reasons for treatment ineligibility are detailed in supplementary table 2.2. Baseline characteristics are described in detail in Table 2.1. In brief, the

cohort was predominately male (90%), the median age was 41 [34-46] years, 80% were Caucasian, 12% were Indigenous, and 4% were Asian. Cirrhosis was present in 21%. HIV and HBV co-infection were uncommon, but past HBV infection (anti-HBc positivity) was present in 24%. The majority of prisoners responded to detailed questions about their past and/or current injecting practices. Injecting drug use was reported by 68% (n=262/386) in the month prior to incarceration and the most common drug of choice was heroin (60%, n=120/200). Mental health comorbidities were reported by 70%. Only 14% of prisoners had previously seen a specialist to discuss their hepatitis C; 94% were treatment naïve (Table 2.1).

Table 2.1: Prisoner characteristics.

Characteristics	N = 416
Age, yr, median [IQR]	41 [34-46]
Male sex, n (%)	376 (90)
Ethnicity, n (%)	
Caucasian	332 (80)
Indigenous	51 (12)
Asian	15 (4)
Other	18 (4)
Body mass index, kg/m ² , mean [IQR]	31 [27-34]
HCV genotype, n (%)	
Genotype 1a	184 (44)
Genotype 1b	14 (3)
Genotype 2	8 (2)
Genotype 3	208 (50)
Genotype 6	2 (1)
HCV viral load, IU/ml, median [IQR]	685,000 [192,000-2,630,000]
ALT, U/ml, median [IQR]	88 [55-145]
Platelet count, median [IQR]	218 [176-256]
HBV serology, n (%)	
HBsAg ⁺	6 (2)
Anti-HBs ⁺	310 (75)
Anti-HBc ⁺	98 (24)
HIV serology, n (%)	8 (2)
Liver stiffness measurement [~] , n (%)	
<9.5 kPa	278 (71)
9.5-12.5 kPa	40 (10)
>12.5 kPa	71 (19)
Cirrhosis, n (%)	86 (21)
Compensated	77 (19)
Decompensated	9 (2)
Opiate substitution therapy	228 (54)
Comorbid psychiatric illness [*]	291 (70)
Psychotropic medication	208 (50)
Significant alcohol history [#] , n (%)	243 (58)
Injecting drug use, n (%)	
PWID, current/previous	389 (94)
Injecting prior to incarceration ⁺	262/386 (68)
Injecting in prison, current/previous [^]	130/228 (57)
Previous specialist care for HCV, n (%)	59 (14)
Treatment experienced, n (%)	25 (6)

~ Valid liver stiffness measurements using transient elastography were obtained in 94% (n=398). The remaining 6% (n=27) of prisoners were either cirrhotic (n=10) evidenced by clinical decompensation and/or radiological evidence of portal hypertension or had unsuccessful elastography due to body habitus (n=17), ^{*} Self-reported or

recorded from medical record at initial review, # Recent (pre-imprisonment or historically significant daily drinking (more than 2 standard drinks, most days, for men and women) or binge drinking (significant intermittent alcohol intake), + injecting drugs use within 1 month of incarceration, ^ injecting drug use in the current or previous incarceration. Legend: ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; PWID, people who inject drugs.

HCV DAA therapy and virological response

Table 2.2: Treatment regimens and durations.

Treatment regimen	(N = 416)
Sofosbuvir 400 mg/ledipasvir 90 mg, n (%)	
8 weeks	100 (24)
12 weeks	77 (18)
24 weeks	4 (1)
Sofosbuvir 400 mg/daclatasvir 90 mg ± RBV, n (%)	
12 weeks	147 (35)
24 weeks	65 (16)
Sofosbuvir 400 mg/RBV 1,000–1,200 mg, n (%)	5 (1)
Paritaprevir 150 mg/ritonavir 100 mg/ombitasvir 25 mg/dasabuvir 250 mg, n (%)	16 (4)
Sofosbuvir 400 mg/PegIFN ± RBV, n (%)	2 (1)

Legend: RBV, ribavirin.

The DAA regimens prescribed are listed in Table 2.2. The rate of SVR12 was 96% (n=301/313) per protocol (Table 2.3). SVR12 rates exceeded 95% for all genotypes (Gt) including Gt1a (96%, n=137/143), Gt1b (100%, n=11/11), Gt2 (100%, n=5/5), Gt3a (96%, n=147/153) and Gt6 (100%, n=1/1). There was no difference in SVR12 between cirrhotic and non-cirrhotic prisoners (97% vs 94%, p=0.51). SVR12 rates by ITT analysis were lower (SVR12 72%, n=301/416, Table 2.3); the most common reason for not achieving SVR12 was loss to follow-up due to being released to freedom (90%, n=103/115).

Table 2.3: Virological response.

Virological response EOT, n (%)	Overall (N = 416)	Per protocol (n = 364)
HCV PCR negative	364 (88)	364 (100)
Lost to freedom*	52 (12)	–
Virological breakthrough	0 (0)	0 (0)
Treatment-related death	0 (0)	–
SVR12, n (%)	(N = 416)	(n = 313)
HCV PCR negative	301 (72)	301 (96)
Genotype 1a	137/184 (74)	137/143 (96)
Genotype 1b	11/14 (79)	11/11 (100)
Genotype 2	5/8 (63)	5/5 (100)
Genotype 3	147/208 (71)	147/153 (96)
Genotype 4/5	–	–
Genotype 6	1/2 (50)	1/1 (100)
Cirrhosis		
Yes	59/86 (69)	59/63 (94)
No	242/330 (73)	242/250 (97)
Lost to freedom	103 (25)	–
Virological relapse	11 (2.8)	11 (3.5)
Reinfection	1 (0.2)	1 (0.5)
Treatment-related death	0 (0)	–

*Lost to freedom; prisoners either released on-treatment or after end-of-treatment but prior to week 12 post-treatment. Legend: HCV, hepatitis C virus; EOT, end-of-treatment; SVR12, sustained virological response at post-treatment week 12.

Treatment failure was due to virological relapse in 11 prisoners (Supplementary Table 2.1), and confirmed hepatitis C reinfection in one prisoner. Three of the 11 prisoners who relapsed had treatment interruptions of greater than one-week duration, including two prisoners prescribed sofosbuvir plus daclatasvir for 24 weeks who received only six and seven weeks respectively due to being released on parole; both were HCV PCR positive on re-incarceration within the follow-up period. The one prisoner with confirmed reinfection was diagnosed by recurrent HCV viraemia between EOT and SVR12, with a switch from Gt1a to 3 between EOT and SVR12 time points, and who had risk factors for reinfection.

Treatment-related adverse events were infrequent (7.9%, 41 events in 33 participants, Table 2.4). One prisoner (0.2%) discontinued treatment at week eight of 12 due to insomnia (SVR12 achieved). There were no treatment related deaths. Nine prisoners had decompensated liver disease at treatment initiation (Child Pugh B, n=4 and Child Pugh C, n=5). The SVR12 rate was 71% (n=5/7) amongst this group. Two prisoners experienced virological relapse and two prisoners were released to freedom prior to SVR12. One Child Pugh C prisoner achieved SVR12 and was referred to a liver transplantation service and waitlisted for transplantation while incarcerated. This patient underwent successful orthotopic liver transplantation following release from prison. No *de novo* HCCs were diagnosed during the period of evaluation.

Table 2.4: Adverse events.

Adverse events, n (%)	N = 416
Treatment-related	
Headache	12 (2.9)
Gastrointestinal symptoms	9 (2.2)
Lethargy	8 (1.9)
Rash	4 (1.0)
Myalgia	3 (0.7)
Serious adverse events	0 (0)
Treatment-related deaths	0 (0)
Leading to discontinuation of treatment	
Treatment-related	
Insomnia	1 (0.2)

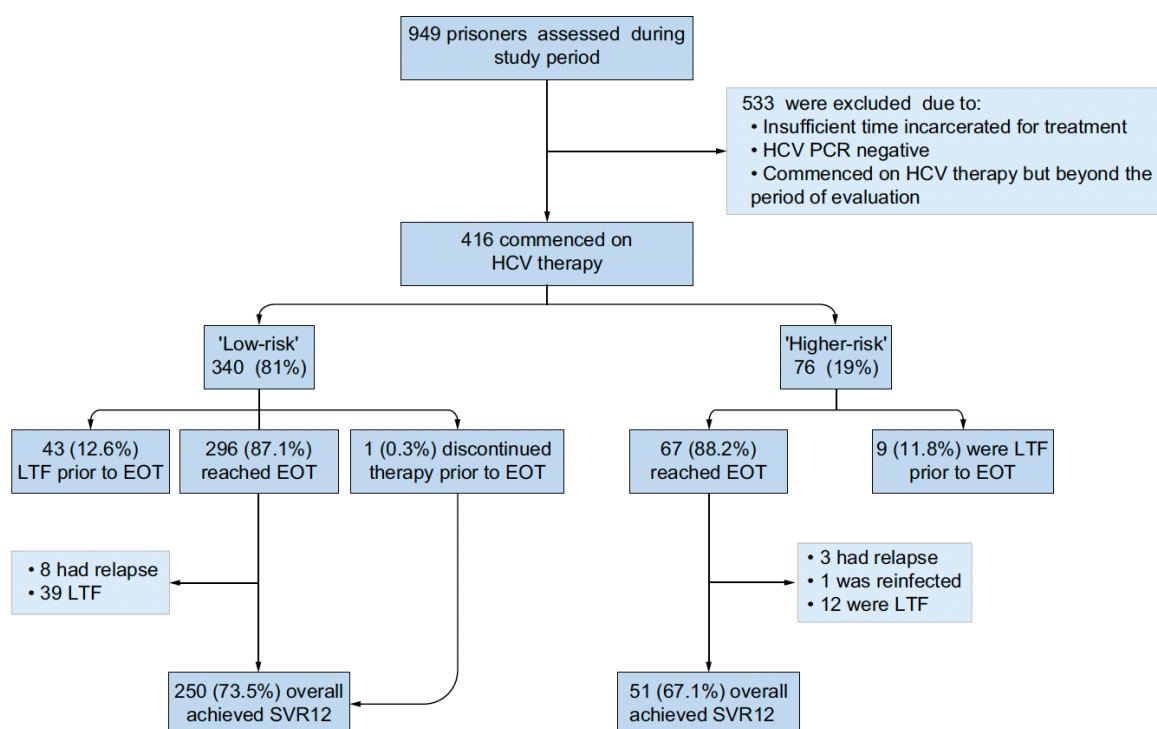
There were six prisoners who were HCV-HBV co-infected. All were non-cirrhotic. One prisoner who was HBeAg-positive with a high serum HBV DNA level was initially commenced on antiviral therapy for hepatitis B (tenofovir 300mg daily), and then subsequently hepatitis C, four weeks later. Five prisoners were HBeAg-negative and had serum HBV DNA levels <2000 IU/mL at baseline. These prisoners were monitored with LFTs and serial HBV DNA levels. Two prisoners experienced on-

treatment increase in serum HBV DNA level without biochemical flare and antiviral therapy for HBV was not commenced. Past HBV infection was present in 24% of the cohort. There were no cases of on-treatment or post-treatment hepatitis flare to suggest HBV reactivation.

Nurse-led care was delivered effectively across the state-wide network

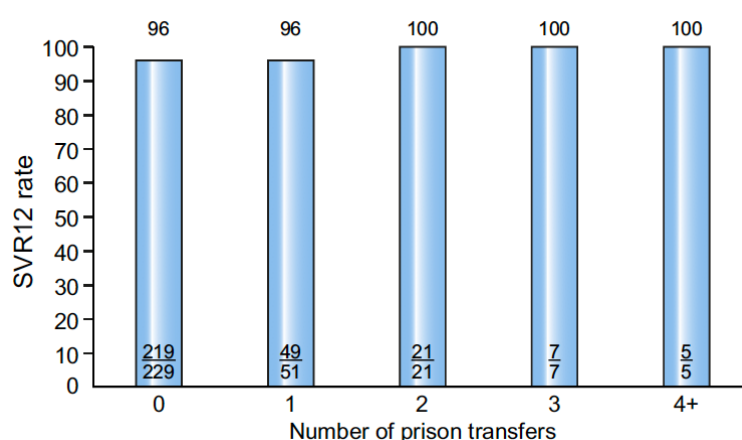
Most prisoners (82%, n=340) were suitable for treatment based on nurse-led evaluation only and did not require a formal hepatologist assessment ('low risk'). The remaining 76 prisoners (18%) were categorised as 'higher risk' and required either face-to-face (13%, n=55), tele-medicine hepatologist consultation (3%, n=13), or both (2%, n=8) (Figure 2). Rates of SVR12 were comparable in 'low risk' and 'higher risk' prisoners, both per protocol (96% vs 93%, p=0.27), and by ITT (74% vs 67%, p=0.26) analysis (Figure 2).

Figure 2.2: Decentralised, nurse-led model of care. HCV, hepatitis C virus; EOT, end-of-treatment; LTF, loss to freedom; SVR12, sustained virological response at post-treatment week 12.



The decentralised, nurse-led model of care removed the requirement for most prisoners to attend a central prison or hospital for hepatitis C assessment and treatment. However, 26% of prisoners still had at least one prison transfer while receiving DAA therapy (n=110, range 0-6). The frequency of prison transfers while receiving hepatitis C treatment was not associated with diminished SVR12 outcomes (SVR12 96-100%, Fisher's exact test p=0.89) (Figure 3).

Figure 2.3: Treatment outcomes (SVR12 rate) by number of inter-prison transfers while receiving HCV DAA therapy.



Legend: DAA, direct-acting antiviral; SVR12, sustained virological response at post-treatment week 12.

The cascade of care from referral is presented in detail for each prison in Supplementary Table 2.2. Assessing the cascade of care following referral to the program, 562 (59%) were eligible for treatment and 416 (44%) were commenced on therapy. The majority of those ineligible had an insufficient remaining period of incarceration to complete treatment (61%, n=235/387). At the time of evaluation, the average time from assessment to treatment was 4.6 months, reflecting in large part the fact that assessments commenced in November 2015, 4 months prior to reimbursement of DAAs in March 2016. The period between assessment and initiating treatment has since fallen to an average of 3 weeks.

2.2.5 Discussion

This is the first large scale hepatitis C prison management program to be implemented across an entire jurisdiction. The decentralised, nurse-led model of care implemented across 14 prisons has overcome many of the traditional systemic barriers to providing effective and efficient hepatitis C treatment to large numbers of prisoners, including the need for specialist consultation and hospital transfer, and treatment interruption from prisoner transfer between correctional facilities with partitioned clinical services. Importantly hepatitis C cure rates achieved by this new model were high.

Although many countries continue to mandate specialist physician management for hepatitis C therapy initiation²³, our data demonstrate that nurse-led treatment is safe and effective in the correctional setting, with only 18% of assessed prisoners requiring specialist consultation. Whilst inter-prison transfer was frequent (driven by movement in the justice system rather than related to prisoners need to access hepatitis C services), it did not lead to treatment interruption or diminished efficacy. The model of care allows high treatment numbers with existing resources now permitting

16 new treatment starts per week. The key features of the program that likely support its success include the nurse-led model delivering DAA therapy locally, the state-wide coverage across all correctional facilities, the use of information technology including telemedicine and a central electronic medical record, a centralised pharmacy distribution with real-time prisoner tracking, and federal government policy supporting prisoner access to DAAs.

An important benefit of the program is that it engages a marginalised population in hepatitis C treatment and primary healthcare more broadly. Many prisoners had a history of current or recent injecting prior to incarceration, and most had psychiatric comorbidities. As expected, a high proportion (86%) had never previously consulted a clinician about hepatitis C treatment. PWID are the group at greatest risk of hepatitis C infection in many developed countries and are traditionally difficult to engage in health care. Prison-based hepatitis C treatment programs can therefore play a vital role in increasing care and treatment in this group.

There is now a global focus on how to best utilise public health platforms to eliminate hepatitis C as a public health threat. Mathematical modelling has estimated that for Victoria to achieve the WHO elimination targets by 2030¹, treatment rates among current PWID will need to be scaled up to approximately 59/1000, or around 1,300 active PWID per year^{9, 24}. Sustaining this level of treatment uptake in the community will require increased testing and retention of PWID in care⁹, including the treatment of prisoners, many of whom have a history of current or recent injecting.

The success of our program has been recognised by a recent increase in resourcing to increment treatment start numbers to 20 per week (1040/year). This treatment rate means the program would potentially contribute >50% of the overall yearly elimination target for treatment scale-up among PWID in Victoria, based on our data that 68% of prisoners with hepatitis C were considered current PWID. This highlights the vital contribution that prison treatment programs can make as part of a broader elimination agenda.

Limitations of this study include the large proportion of prisoners 'lost to freedom', most of whom were released from prison between EOT and SVR12. The study, however, evaluates a proposed model of care to increase treatment scale-up amongst a priority population, rather than to assess DAA effectiveness, which is already established^{25, 26}. Despite a high proportion of prisoners who were 'lost to freedom', approximately 90% did complete DAA therapy while incarcerated and most are anticipated to have achieved cure. Previous Australian studies have demonstrated a hepatitis C incidence of >10% annually amongst incarcerated PWID⁷. In our study there was only one confirmed case of hepatitis C reinfection identified, although the duration of follow-up was only 12 weeks post treatment; with a longer duration of follow-up a higher number of reinfections is anticipated. It is

possible that a number of the cases of relapse were in fact reinfections with the same hepatitis C genotype; regardless, the number of relapses was also low. The program supports retreatment of those reinfected to engage prisoners at highest risk for transmission, key for reducing prison hepatitis C prevalence and achieving community elimination targets. Finally, detailed data concerning the rate of opt-in screening at reception, overseen by the prison medical staff, was not available, this step in the cascade of care preceding referral to the program. The estimate of the percentage of viraemic prisoners treated was informed by Australian data estimating hepatitis C seroprevalence within prisons, the proportion therefore anticipated to be hepatitis C RNA positive and the short average duration of imprisonment (Supplementary Table 2.2). Although the average time between assessment and treatment was 4.6 months at the time of evaluation, there was a 4 month disparity between commencement of clinical assessments in November 2015 and hepatitis C treatment availability in March 2016. The period between assessment and initiating treatment has since fallen to an average of 3 weeks, with 75% of prisoners assessed now starting DAA therapy.

Future work will explore refinements to prison-based models of care. This will include evaluating the role for peer workers to increase prisoner engagement and the role for point-of-care testing to increase screening uptake and reduce time from assessment to treatment start. This will also examine the success of linkage to community care for prisoners released to freedom, including treatment completion rates for those who are released on-treatment, as well as treatment uptake in those referred to community treatment centres for DAA therapy post-release. In addition, long-term follow-up studies to monitor for HCV reinfection are underway.

In conclusion, DAA therapy for hepatitis C using a decentralised, nurse-led model of care was highly effective in increasing hepatitis C treatment access to a large population of prisoners, with high rates of hepatitis C cure. Given the high prevalence of hepatitis C among prisoners, with many reporting current or recent injecting drug use, large scale prison treatment programs should be promoted to benefit both the individual prisoner and also hepatitis C elimination efforts in the broader community.

2.2.6 Further discussion

A high prevalence of cirrhosis was noted in the study cohort, which was multifactorial. Duration of hepatitis C infection was identified as a contributing factor, evidenced by the higher median age of cirrhotic versus non cirrhotic participants included in the cohort. Whilst the overall median age of the cohort was 41 years, the median age of those with cirrhosis was 49 years [IQR 44-55]. Secondly, whilst rates of HBV and HIV co-infection were low, 58% of participants identified prior alcohol misuse which was assessed a contributory for the higher levels of cirrhosis than anticipated. Finally, as weekly hepatitis C treatment initiations were capped throughout the time period evaluated in this study,

individuals with cirrhosis were prioritised for treatment above those without cirrhosis, contributing to the high prevalence of those with advanced liver disease included in this group.

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3 Chapter 3: The CLINK trial – a prospective randomised controlled trial investigating linkage to HCV care following release from prison

3.1 Introduction and Summary

Prison-based HCV treatment for people living with HCV infection has now been shown to be safe and effective and can reach individuals in large numbers (310, 400). However, on account of short average prison sentences and strict treatment eligibility criteria in some countries, many prisoners are released untreated. Following release from prison, individuals encounter a number of immediate competing priorities relating to housing, employment, and renegotiating social networks, and it is unclear how HCV treatment is prioritised in this setting (313, 319). At commencement of this thesis, there was no data regarding the likelihood of HCV DAA initiation, or the efficacy of dedicated transitional interventions, amongst those individuals released from prison with untreated HCV infection. Multiple interventions to enhance linkage to care on community re-entry, however, have been evaluated for other medical conditions including HIV infection which demonstrate favourable outcomes regarding community healthcare engagement and treatment continuity (359, 362). The aims of this chapter were therefore to determine the likelihood of prisoners with HCV infection linking to care following release, and whether a dedicated HCV transitional intervention could enhance DAA initiation rates.

Included in this chapter are two publications exploring these aims. The first was published as correspondence and demonstrates that the likelihood of community HCV treatment initiation amongst individuals released from prison after comprehensive HCV assessment was low. Overall, 25% initiated HCV treatment within 6 months, however the only variable associated with commencement was reincarceration and treatment within the prison. In this context, we developed an intervention to support individuals to commence treatment after community re-entry. This model of care streamlined the care cascade between community re-entry and treatment initiation, and also removed financial barriers to treatment including fees for medical appointments and medications. This intervention increased the likelihood of commencing HCV treatment during community re-entry and has important implications for how we best manage individuals during transitions moving forward.

The retrospective data has been published as correspondence in *The Lancet Infectious Diseases*. The prospective randomised controlled trial manuscript has been submitted to a peer-reviewed journal for consideration of publication.

3.2 Original correspondence – retrospective study

Prison based treatment of communicable disease can be safe and effective, however post release linkage to care remains suboptimal

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3.2.1 Correspondence

We read with interest the article 'Challenges and opportunities for the management of infectious diseases in Europe's prisons: evidence-based guidance' by Tavošchi et al.¹ The authors describe the high burden of communicable diseases including hepatitis C (HCV) in European prisons and how prioritising healthcare in this sector has benefits for both prisoners and the broader community. Similarly, in Australia there is a disproportionately high HCV seroprevalence amongst prisoners, as high as 50% amongst people who inject drugs (PWID).²

The World Health Organisation has prioritised the elimination of HCV as a public health threat by 2030³, and the prison setting must be used to engage prisoners living with HCV in care to achieve these targets. The high prevalence of both HCV infection and PWIDs, and the short average length of incarceration makes the prison ideal for diagnosing infection and providing short duration, highly effective direct-acting antiviral therapy (DAAs). In this setting we recently described a nurse-led model-of-care for HCV treatment in prison which is safe and achieves cure rates greater than 95% amongst prisoners.⁴ These data endorse the author's recommendations and demonstrate that system-wide prison programs are feasible and effective. Importantly, over 80% of these prisoners had never engaged in specialist HCV care in the community and 68% reported injecting within one month of incarceration. Prison-based healthcare can engage people who do not traditionally participate in HCV care and interrupt onward transmission networks.

Another important healthcare gap is linkage to care post-release from prison. In a pilot study, we evaluated the rate of successful linkage to HCV care among recently released prisoners. All prisoners had a comprehensive HCV assessment whilst incarcerated and were planned for treatment before release. On release, prisoners were referred to a local primary care practice with a detailed summary of their clinical work-up, to streamline DAA prescription. There were no cost barriers to treatment uptake. The rate of treatment uptake was determined by registration with the national drug authorisation registry, required before DAA treatment can be dispensed. Among 75 former prisoners, only 19 (25%) were prescribed DAAs within 6 months of release. Of these, 7/19 (37%) commenced

treatment in prison when re-incarcerated during this period. Re-incarceration was the only predictor significantly associated with DAA initiation.

This period of time immediately post release from prison can be a challenging, characterised by competing priorities including renegotiating social networks, finding stable accommodation and managing recidivism.⁵ There is a need for new models of care to optimize linkage to care post release from prison for this vulnerable population.

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3.3 Original manuscript – prospective study

C-LINK: a prospective randomised controlled trial demonstrating that care navigation post release from prisons increases initiation of hepatitis C treatment

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3.3.1 Abstract

Background:

Hepatitis C treatment within prisons is safe and effective and can reach individuals in large numbers. Some prisoners, however, are released untreated. On community re-entry, individuals are faced with a number of immediate competing priorities, and in this context, linkage to hepatitis C care is low. Interventions targeted at improving healthcare continuity following re-entry have yielded positive outcomes for other health diagnoses however data regarding hepatitis C transitional care is limited.

Methods:

We conducted a prospective randomised controlled trial comparing a hepatitis C care navigator intervention to standard of care for individuals released from Victorian prisons with untreated hepatitis C infection. The primary outcome was prescription of hepatitis C direct-acting antivirals (DAA) within six months of community re-entry.

Results:

46 participants were randomised. The median age was 36 and 59% were male. Ninety percent (n=36/40) had injected drugs within six months of incarceration, 50% self-reported a psychiatric diagnosis and 28% were prescribed opioid substitution therapy. Twenty-two were randomised to care navigation and 24 to standard of care. Individuals randomised to the intervention were more likely to commence hepatitis C DAAs within six months of re-entry (73%, n=16/22 vs 33% n=8/24, p<0.01), and the median time between re-entry and DAA prescription was also significantly shorter (21 days [11-42] vs 82 days [44-99], p=0.049). The ability to successfully contact participants who were randomised

to care navigation was the sole factor predicting treatment initiation (93%, n=14/15 vs 29%, n=2/7, $p<0.01$).

Conclusions:

Hepatitis C transitional care for individuals re-entering the community is associated with a higher likelihood of DAA initiation and reduced time to treatment. Treatment rates amongst unsupported individuals are otherwise low. Similar programs should be implemented to contribute towards hepatitis C elimination.

3.3.2 Introduction

It is estimated that 71 million people are living with hepatitis C virus infection globally (1). The availability of direct acting antiviral medications (DAA) has revolutionised hepatitis C treatment and cure rates exceed 95% in clinical trials (2, 3). In this context, the World Health Organisation (WHO) has proposed elimination targets for hepatitis C infection including an 80% reduction in incidence by 2030 (4). To achieve these targets, treatment should be prioritised to high transmitting populations including people who inject drugs (PWID) (5). As PWIDs experience a high lifetime risk of incarceration, prisons are an important setting to engage and treat people living with hepatitis C (6). Prison-based hepatitis C treatment has now been shown to be safe and effective, able to reach prisoners in large numbers, and also reduce incident infection by preventing transmission (7-10). However, even where prison treatment programs are available, short average sentence durations mean many prisoners are released untreated.

Following release from prison, individuals are faced with multiple immediate competing priorities including the need to find secure housing and employment, the renegotiation of social networks, and managing the risk of return to substance use (11-14). This period is also defined by high rates of morbidity and mortality, most commonly from accidental overdose or injury (15, 16). Despite the high burden of medical and psychiatric illness amongst prisoners, in the context of these challenges, linkage to healthcare following re-entry is low (17-19). This interruption of the care cascade includes poor linkage to hepatitis C care for those individuals who are released untreated. We have previously shown that only 25% of prisoners living with hepatitis C had commenced treatment within 6 months of release, despite forwarding their community healthcare provider a comprehensive summary of their prison-based assessment (20). The only factor positively associated with DAA commencement was reincarceration with subsequent treatment by prison-based hepatitis C services. Data from North America similarly demonstrates a low likelihood of linkage to hepatitis C care following community re-entry, which ranges between 26-37% (21, 22). This vulnerable group is therefore inadequately served by existing healthcare models.

Multiple interventions designed to improve healthcare continuity amongst people leaving prison have been evaluated including case-management, educational interventions, transportation assistance and dedicated transition clinics, particularly amongst individuals living with HIV (23-27). Such interventions have been associated with favourable outcomes including continuity of HIV anti-retroviral medications, engagement in mental health and drug and alcohol services, and sustained involvement with primary healthcare. Interventions tailored to support individuals during this critical period can also reduce emergency department presentations and hospitalisations (28). A recent prospective, single arm study demonstrated that 31% of individuals with HCV infection who received transitional care co-ordination and patient navigation were linked to HCV care within 180 days of release (29). There is, however, no randomised controlled data investigating the effect of transitional interventions on linkage to hepatitis C care following community re-entry.

Whilst there are substantial challenges with linkage to viral hepatitis care during community re-entry, achieving cure for these individuals is important to mitigate the long-term risks of untreated hepatitis C infection including the development of cirrhosis and hepatocellular carcinoma, and more broadly to contribute to viral elimination by interrupting onward transmission. We therefore investigated the feasibility and efficacy of a post-release care navigation to improve the likelihood of recently released prisoners with untreated hepatitis C infection commencing HCV DAAs.

3.3.3 Methods

We performed a randomised controlled trial to identify the likelihood of commencing HCV DAA treatment amongst individuals who received hepatitis C case-management using a care navigator, within six months of prison release, compared to those receiving standard of care.

Study design and setting

We evaluated a care navigator-led linkage to care intervention to support hepatitis C treatment uptake following incarceration. This study was performed in the community setting; however, participants were identified during incarceration at prison-based hepatitis C assessments. We have previously established a successful nurse-led state-wide prison in-reach program which provides comprehensive hepatitis C management for all prisoners in Victoria, Australia (7). Prisoners who were assessed by the Program between 1st October 2018 and 15th March 2020 but whom had an anticipated release date within four weeks of their initial assessment were eligible for inclusion. The aims and nature of the study were discussed with potential participants who then verbally consented to participate and provided community contact details for either themselves and/or an associate. They also provided written consent for the release of pharmaceutical claims information for the six months following community re-entry to determine their hepatitis C treatment status. Associates included

family members, community healthcare providers and peers. All participants were provided with a toll-free number at the time of their prison-based assessment and were encouraged to contact the study co-ordinator following community re-entry to discuss treatment. Once prison release was confirmed, participants were randomised electronically using a randomisation key generated by a third party to either care navigation or standard of care on a 1:1 basis, and were stratified by opioid substitution therapy (OST) prescription which was anticipated to be associated with higher rates of linkage to hepatitis C care.

Participants

Participants were identified during prison-based assessments. Inclusion criteria for participation included i) active hepatitis C infection, defined as a positive hepatitis C RNA PCR; ii) ability to provide at least one telephone number for community contact post release; iii) age 18 to 65 years; and iv) ineligibility for prison-based hepatitis C treatment (anticipated release within 4 weeks of initial assessment). Prisoners were excluded if i) they had hepatic cirrhosis (liver stiffness measurement of ≥ 12.5 kPa); ii) they were prescribed medications contraindicated to co-administration with hepatitis C DAA; iii) they remained under the authority of the Department of Justice including those serving a community-based correction order following incarceration and/or iv) hepatitis C treatment was commenced in prison.

Study Intervention

Participants randomised to care navigation received a transitional intervention including telephone-based consultations and support, prescription of hepatitis C DAA medications, and reimbursements for i) DAA medication co-payments (\$16.80-\$116.40 AUDs), ii) study participation time (vouchers at baseline, end-of-treatment, and SVR12 timepoints (\$20, \$50, \$100 AUD, respectively)) and OST, if applicable. OST, including methadone and buprenorphine, are reimbursed for Australian citizens, however a weekly dispensing fee of \$35.00 AUD is charged by community pharmacies.

Following re-entry, community pharmacies are reimbursed by the Department of Justice, Victorian Government to dispense an individual's OST for four weeks, when prescribed during incarceration. OST dispensing fees were continued to be reimbursed for care navigation recipients for the duration of hepatitis C treatment (\$140-280 AUD), which was paid directly to the dispensing pharmacy.

Medications were prescribed via the Australian Pharmaceutical Benefits Scheme, which reimburses DAA therapy for all Australians with chronic hepatitis C infection. The prescription was dispensed at St Vincent's Hospital Melbourne and then medications were packaged into monthly blister packs and distributed to a participants' accommodation or their community pharmacy for collection via registered post. The initial four weeks of therapy was sent to the participant, and then the remaining

four to eight weeks were sent once treatment initiation was confirmed by the participant or community pharmacy.

Reimbursement with vouchers for a supermarket chain were provided for study participation time at treatment baseline, end-of-treatment and after completion of SVR12 blood tests, where they remained engaged in care. A toll-free number was provided to study participants and telephone contact could be initiated by either the study coordinators or the participant themselves. The prison database was screened intermittently to determine if participants had been reincarcerated, and if so, a Statewide Hepatitis Program nurse was informed so that treatment could be commenced or continued within the prison. Where there were concerns regarding the mental or physical wellbeing of a participant or treatment-related adverse events, a face-to-face review at St Vincent's Hospital Melbourne or a regional hospital were arranged.

Participants randomised to the case management intervention were contacted via telephone within two weeks of prison release. Where an associate was nominated, they were contacted by the study team to ascertain contact details for the study participant. If contact was not established within six weeks of community re-entry, no further attempts were made. If contact was successful, the care navigation model was described to the participant who re-consented for ongoing participation.

Standard of care

In Victoria, standard discharge protocols for re-entering individuals include a non-compulsory medical appointment to create a discharge summary of diagnoses and medications for their community healthcare provider. If an individual is prescribed OST, they are provided with a community prescriber and dispensing pharmacy, and therapy is subsidised for four weeks.

For participants randomised to standard of care, an additional comprehensive summary of information from their prison-based HCV assessment was shared with a nominated community healthcare provider, where identified. This included results of blood-based investigations including HCV RNA PCR and genotype, hepatitis B virus (HBV) serology, liver and renal biochemistry and transient elastography results, where available. The referral also included information about DAA prescribing and identifying potential drug-drug interactions. An alert was created for participants prison-based electronic medical record to indicate that they remained untreated and should be referred to the Statewide Hepatitis Program in the event of reincarceration. Standard of care participants were also provided with a toll-free number on which they could contact the study coordinators following release. When contact was initiated by the participant, they were directed to their local healthcare provider to commence HCV treatment.

Participants randomised to standard of care were contacted by the study team six months post release to determine hepatitis C treatment status. All hepatitis C treatments in Australia are reimbursed through a national scheme that registers prescriptions written for an individual. All participants consented to investigators contacting the national register at the end of follow-up to confirm whether any prescription for hepatitis C treatment had been generated for an individual. At six months post-release, participants who remained hepatitis C treatment naïve in the standard-of-care arm were eligible to receive care navigation equivalent to participants in the intervention arm.

Data collection

Baseline characteristics were collected during participants' Statewide Hepatitis Program prison-based assessment. This included demographics, a detailed clinical questionnaire regarding HCV diagnosis and prior management, evaluation of risk behaviours, liver stiffness measurements (using transient elastography) for fibrosis determination and blood-based investigations including full blood count, liver and renal biochemistry, HCV RNA and genotype, and HIV / HBV serology. Blood based investigations results were transcribed from the Statewide Hepatitis Program operational database. Regarding injecting drug use (IDU) practices, a history of IDU was defined as having ever injected an illicit substance. Recent IDU was defined as the injecting of an illicit substance within the six months prior to incarceration. Self-reported medical and psychiatric comorbidities, and medications as documented in their prison health record were also recorded. It was noted whether a participant was receiving daily OST prior to release, all of whom were referred to a community OST prescriber prior to discharge by the prison healthcare provider.

Following release, the time interval between community re-entry and i) successful contact as well as ii) DAA commencement were noted. The receipt of DAA medications, as well as completion of all tablets (self-reported), defined as end-of-treatment (EOT), were recorded. Pathology request forms were mailed to participants prior to the SVR12 timepoint, enabling them to access their local pathology provider. Participants in the intervention group continued to be followed until a HCV PCR test 12 weeks after completion of therapy was performed. HCV cure was defined as an absence of detectable HCV RNA on PCR testing at least 12 weeks after completion of therapy. Instances of reincarceration were recorded during follow-up. The occurrence and frequency of adverse events was recorded. Data from the national drug reimbursement schedule was the final source-of-truth for DAA prescription at 6 months in those participants who were uncontactable.

Outcomes for analysis

The primary endpoint was the frequency of participants who were prescribed HCV DAAs within six months of prison release. Secondary endpoints included time to DAA prescription, frequency of community- and prison-based treatment initiations, completion of treatment and SVR12 outcomes.

Data analysis

Power calculations were conducted prior to study commencement anticipating 25% of participants in the standard of care and 55% in the case-management groups would commence HCV DAAs within 6 months of community re-entry. These thresholds were informed by our respective data where 25% of unsupported individuals had commenced DAAs within 6 months of community re-entry following incarceration. It was anticipated that the transitional hepatitis C care would improve initiation rates by greater than two fold, and therefore 55% was selected. A target of 96 participants was identified to achieve a power of 80% and alpha value of 0.05. However, recruitment was terminated in March 2020 due to the SAR-CoV-2 global pandemic which resulted in the suspension of face-to-face prison-based HCV assessments.

We considered the primary outcome of DAA initiation using intention-to-treat analysis. Categorical variables were applied to chi-squared tests (or Fisher's Exact tests for samples less than five) while continuous variables were applied to (parametric) t-tests and (non-parametric) Mann-Whitney/Kruskal-Wallis tests for symmetrically and asymmetrically distributed data, respectively. Statistical analysis was performed using STATA 12.0 (StataCorp LP, TX, USA). Statistical significance was defined at the 5% level.

Ethics

This research protocol was approved by St Vincent's Hospital Human Research Ethics Committee (REF HREC/17/SVHM/282).

3.3.4 Results

Participant characteristics

In total, 46 participants were randomised over 16 months. Participants were predominantly male (n=28, 61%), the median age was 36 years and 20% identified as Indigenous Australian (Table 1). Infection with hepatitis C genotype 1 was most commonly detected (n=19, 41%), followed by genotype 3 (n=14, 30%). One participant was HIV co-infected. No participant had active HBV co-infection, however eight participants were HBsAg-negative and anti-HBc positive consistent with prior exposure. Most participants had no or mild fibrosis (LSM $\leq$ 9kPa, n=31/35, 38%), and a minority had advanced fibrosis (LSM 9-12.4kPa, n=4/35, 11%). None were cirrhotic. All participants reported a history of IDU and most reported IDU within the six months prior to incarceration (n=36/40, 90%).

There was a high prevalence of self-reported psychiatric comorbidities (n=23, 50%) and approximately a third were prescribed OST at the time of prison release (n=13, 28%). Twenty (43%) participants were reincarcerated during the 6-month follow-up. Baseline characteristics were well matched between the case management and standard of care groups (Table 3.1) with the exception of the prevalence of self-reported psychiatric diagnoses: most commonly anxiety, depression and drug induced psychosis (case-management n=16/22, 73% vs standard of care n=9/24, 38%, p=0.02). There was, however, no significant difference in the number of participants prescribed psychotropic medications.

Table 3.1: Baseline participants characteristics.

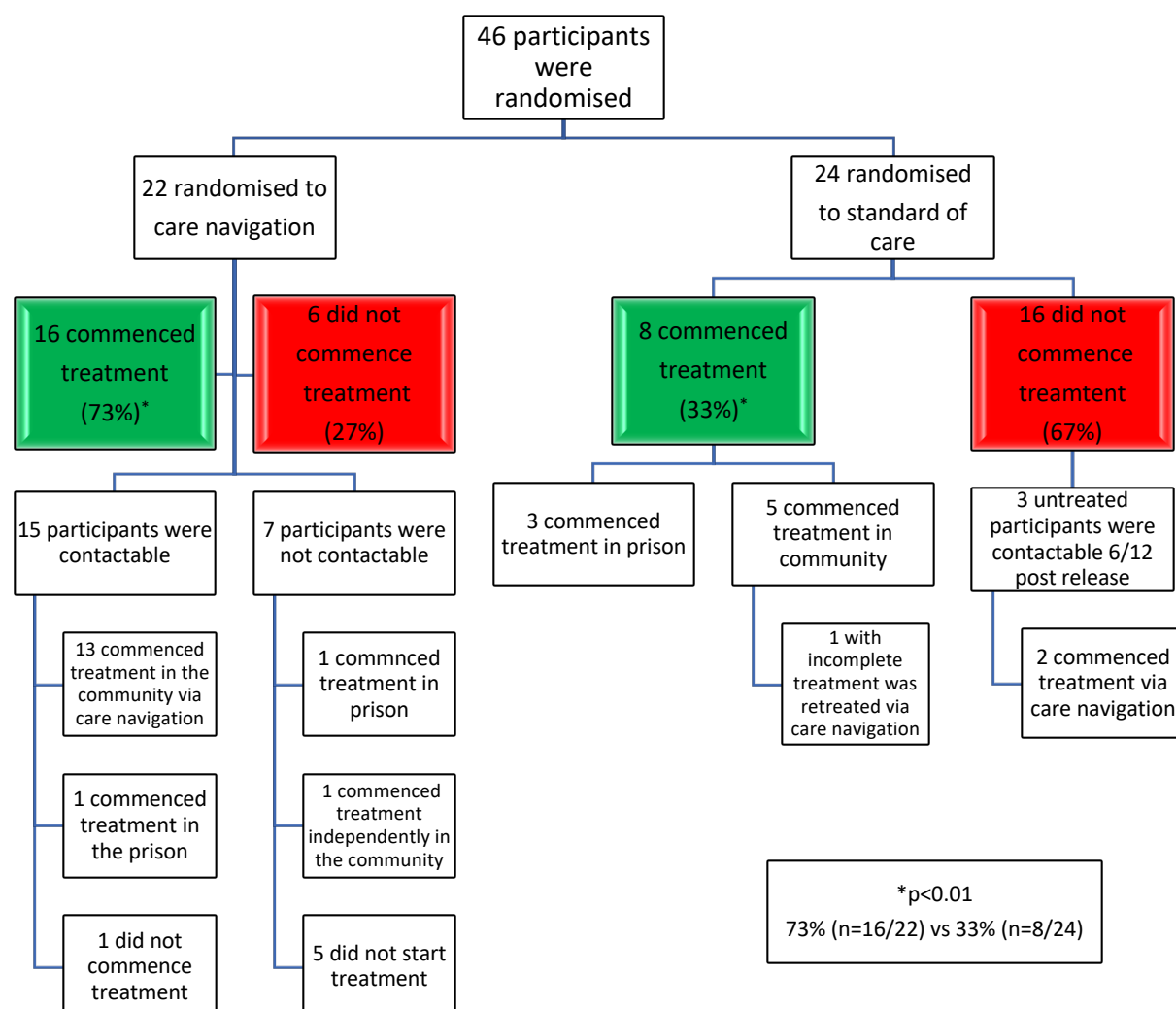
Table 3.1: Baseline characteristics	Total (n=46)	Care navigation (n=22)	Standard of care (n=24)	P value
Age, years, median [IQR]	36 [32-41]	35 [31-41]	36 [32-41]	NS
Male sex, n (%)	27 (59)	13 (59)	14 (58)	NS
Body mass index, kg/m ² , median [IQR]	26.3 [21.5-29.1]	26.6 [22.1-29.2]	25.4 [21.4-29.8]	NS
Indigenous Australian, n (%)	9 (20)	5 (23)	4 (17)	NS
HCV genotype, n (%)				NS
- 1	19 (41)	9 (41)	10 (42)	
- 2	2 (5)	2 (9)	0 (0)	
- 3	14 (30)	7 (32)	7 (29)	
- 4	1 (2)	1 (4)	0 (0)	
- NA	10 (22)	3 (14)	7 (29)	
ALT, U/mL, median [IQR]	63 [37-102]	62 [35-142]	63 [37-87]	NS
Platelet count, median [IQR]	270 [213-326]	269 [214-323]	278 [199-353]	NS
HBV serology, n (%)				NS
- HBsAg	0 (0)	0 (0)	0 (0)	
- Anti-HBs	33 (72)	16 (72)	17 (71)	
- Anti-HBc	8 (17)	3 (18)	5 (21)	
HIV coinfection, n (%)	1 (2)	1 (5)	0 (0)	NS
Liver Stiffness, n (%)				NS
- < 6 kPa	17/35 (49)	8/15 (53)	9/20 (45)	
- 6 – 9 kPa	14/35 (40)	7/15 (47)	7/20 (35)	
- 9 – 12.5 kPa	4/35 (11)	0 (0)	4/20 (20)	
- > 12.5 kPa	0/35 (0)	0 (0)	0/20 (0)	
Cirrhosis, n (%)	0 (0)	0 (0)	0 (0)	NS
Injecting drug use, n (%)				NS
- PWID, ever	46 (100)	22 (100)	24 (100)	
- PWID, within 6 months of incarceration	36/40 (90)	18/21 (86)	18/19 (95)	NS
Comorbid psychiatric illness, n (%)	23 (50)	16 (73)	9 (38)	0.02
Psychotropic medication, n (%)	20 (43)	13 (59)	7 (29)	NS
OST at prison release	13 (28)	7 (32)	6 (25)	NS
Reincarceration*, n (%)	20 (43)	11 (50)	9 (38)	NS

*Reincarceration within the 6-month period of follow up. Legend: HCV, hepatitis C; ALT, alanine aminotransferase, ALT; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; PWID, person who injects drugs; OST, opioid substitution therapy.

Likelihood of hepatitis C treatment initiation, *Primary outcome: hepatitis C treatment initiation*

Care navigation was associated with a higher rate of linkage to care and hepatitis C treatment initiation post-release from prison. Sixteen of the 22 participants (73%) randomised to care navigation commenced hepatitis C treatment within six months following release from prison, compared to 8 of the 24 participants (33%) in the standard of care arm ($p=0.01$) (figure 1). The median time between community re-entry and prescription of hepatitis C DAAs was 21 days [IQR 11-42 days] for individuals randomised to care navigation and 82 days [IQR 44-99 days] for standard of care participants ($p=0.049$).

Figure 3.1: Participant consort diagram



Cascade of care, care navigator intervention arm

Overall, 16 participants randomised to the care navigation commenced treatment within six months of community re-entry, 15 (94%) of whom did so via the intervention. This included 13 treated directly in the community by the care navigator and two who were fast tracked for prison-based treatment by care navigator referral following reincarceration. One additional participant, who was unable to be contacted by the care navigator, was prescribed treatment in the community via an alternate community healthcare provider. The proportion of participants randomised to care navigation retained in hepatitis C care at each treatment milestone is displayed in figure 2.

In total, 38 points of contact were recorded for the 22 participants (supplemental table 4.1). While a personal telephone number was most frequently provided (n=12), successful participant contact was more commonly achieved via a family member (n=10 [parent n=7, alternate family member n=3]) than with the participant directly (n=4) (Supplemental table 4.1). Overall, 20 participants or their associates were contactable. In five cases however, the associate was either unable (n=4) or unwilling (n=1) to facilitate onward contact with the participant, and 15 participants themselves were ultimately contactable. A median of 4 contact attempts were made to engage the participants (range 1-17).

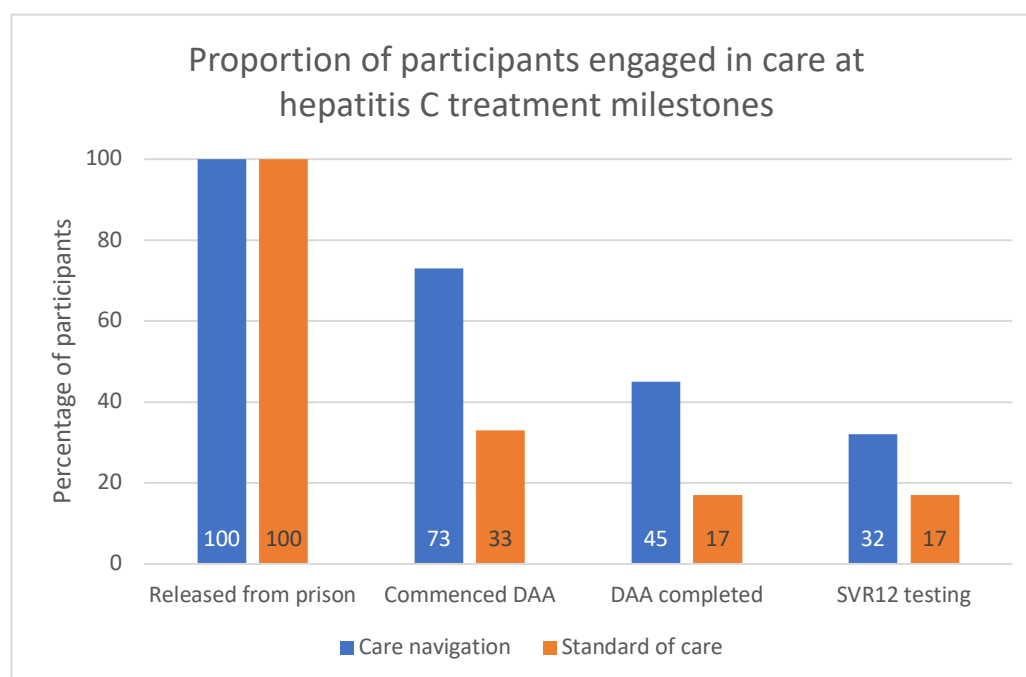
Of the 15 participants who were directly contactable, 13 (87%) were successfully commenced on HCV DAA therapy by the care navigator. The median times between prison release and i) successful contact with participant or associate, ii) successful contact with participant themselves and iii) commencement of HCV DAAs was 9.5 days [IQR 5-20], 21 days [IQR 5-36] and 38 days [IQR 24-48], respectively. The period between DAA receipt and initiation was less than four weeks for 11 participants and greater than four weeks for two. Ten participants were treated with eight weeks glecaprevir + pibrentasvir and three were prescribed 12 weeks sofosbuvir + velpatasvir. Medications were either mailed to the participants' community pharmacy for collection (n=7), to their home (n=4) or were provided to the participant in person (n=2). Nine participants completed treatment and four had partial treatment between 28 and 56 days. Seven had SVR12 bloods collected; six achieved SVR12 and one participant who had incomplete treatment, relapsed, and is undergoing retreatment. A participant with HIV co-infection who discontinued anti-retroviral therapy following community re-entry was also referred for specialist HIV care by the care navigator and hepatitis C and HIV treatments were initiated simultaneously. Of the 13 who commenced treatment, seven (54%) changed telephone number during follow-up after initial contact (range 0-2).

Two participants in the intervention arm commenced hepatitis C treatment in prison following reincarceration, with the reincarceration episode identified by the care navigator. Following identification of reincarceration, the care navigator informed the prison hepatitis treatment service

who expedited prison-based DAA initiation. Both were treated with glecaprevir + pibrentasvir. One completed treatment and one was released at treatment week six with their remaining medication.

Treatment was overall well tolerated. One participant reported nausea and one developed Bell's Palsy at treatment week four, which was not thought to be treatment related following clinical review.

Figure 3.2: Proportion of participants engaged in care at hepatitis C treatment milestones.



Legend: DAA, direct-acting antivirals; SVR12, sustained virologic response at post-treatment week 12.

Standard of care group

As per the study protocol, participants in the control arm were not directly contacted until six months post-release from prison. Six of 24 participants were able to be contacted at six months, which was achieved through an associate (n=5) or directly (n=1). A further three associates were contactable but were unable to facilitate onward contact with the participant themselves. Prescription of hepatitis C treatment during this period was documented from the national drug prescription database which approves and registers all hepatitis treatments in Australia in real time. In total, eight of 24 commenced treatment within six months of prison release, including five treatments prescribed in the community and three prescribed in the prison following reincarceration (figure 1). Of the five community prescriptions, two participants self-reported completion, two discontinued treatment between day 7-14, and the outcome for one is unknown. Two participants in the prison achieved SVR12, and one was released prior to EOT. Only one participant in the standard of care arm initiated contact with the study team using the toll-free number provided during the period of follow up.

All participants randomised to standard of care were eligible to switch to case management at six months post release, if they remained untreated. Of the sixteen participants who remained untreated, only three were contactable, either directly or via their nominated associate. Two subsequently commenced treatment via the intervention. The third was being evaluated for treatment at a tertiary hospital outpatient clinic and elected to continue with this health service. Another participant in the standard of care arm who had incomplete community-initiated treatment (7 days sofosbuvir + velpatasvir) was contacted through their nominated community healthcare provider, enrolled in care navigation and retreated.

Community- vs prison-based hepatitis C treatment initiations according to treatment arm

Comparing the frequency of community-based DAA initiations during follow-up, 14 (64%) participants randomised to care navigation and five (21%) receiving standard of care commenced treatment in the community. This difference was significant ($n=14/22$, 64% vs $n=5/24$, 21% $p=0.01$).

Of the 14 receiving care navigation who initiated DAAs in the community, 13 did so via the intervention. Of the five participants treated in the standard of care arm, two were treated by their primary physician, one via a supervised injecting facility outreach program, and one through a hospital-based outpatient clinic. One participant had treatment registered by the national database, but the details of the clinical setting were not available.

Overall, 20 (43%) participants were reincarcerated at least once during follow-up. The rate of incarceration was similar between groups (care navigation $n=11/22$, 50% vs standard of care $n=9/24$, 38%, $p=0.55$). The proportion of reincarcerated participants treated in prison was lower amongst those randomised to care navigation ($n=2/11$, 18% vs standard of care $n=3/9$, 33%, $p=0.61$). However, when participants who had already commenced hepatitis C DAA in the community prior to reincarceration were excluded, enrolment in care navigation was associated with a numerically higher likelihood of hepatitis C treatment initiation during reincarceration compared to standard of care ($n=2/5$, 40% vs $n=3/9$, 33%, $p=1.0$).

Predictors of treatment initiation

Predictors of treatment initiation were considered on univariate analysis for both the care navigation and control groups (Table 3.2). The only factor associated with a higher likelihood of DAA commencement amongst those randomised to care navigation was achieving successful participant contact following release ($n=14/15$, 93% vs $n=2/7$, 29%, $p=0.01$). No baseline factors predicted treatment initiation amongst those randomised to standard of care (table 3.2).

Table 3.2: Predictors of treatment initiation in participants randomised to care navigation and standard of care.

Variable	Care navigation (n=22)		P value	Standard of care (n=24)		P value
	Treated (n=16)	Not treated (n=6)		Treated (n=8)	Not treated (n=16)	
Male sex	56% (n=9)	67% (n=4)	1.0	75% (n=6)	50% (n=8)	0.39
Age	35 [31-41]	38 [34-43]	0.36	33 [31-40]	38 [32-42]	0.48
Indigenous	12.5% (n=2)	50% (n=3)	0.10	13% (n=1)	19% (n=3)	1.0
Reincarceration	50% (n=8)	50% (n=3)	1.0	38% (n=3)	38% (n=6)	1.0
Opioid substitution therapy	38% (n=6)	17% (n=1)	0.62	13% (n=1)	31% (n=5)	0.62
Psychiatric comorbidity	81% (n=13)	50% (n=3)	0.28	25% (n=2)	44% (n=7)	0.66
IDU within 6/12 of incarceration (n=36/40)	94% (n=15/16)	60% (n=3/5)	0.13	86% (n=6/7)	100% (n=12/12)	0.37
Successful participant contact following incarceration	88% (n=14)	17% (n=1)	0.01	-	-	-

Legend: IDU, injecting drug use.

3.3.5 Discussion

This is the first randomised controlled trial to demonstrate that care navigator-led hepatitis C case management is associated with significant improvement in DAA initiation rates amongst individuals following release from prison. Overall, 73% of participants enrolled in care navigation commenced HCV DAAs within six months of community re-entry, 94% of whom had done so via the intervention. The time to treatment in the care navigator arm was also significantly shorter, an important benefit of streamlined care. The model can minimise the need for repeat diagnostic tests, medical appointments, and specialist referral prior to treatment. The model also promotes hepatitis C care as a priority, important in a time of competing priorities, including securing stable housing and income following community re-entry, ministering personal relationships, and managing recidivist behaviours. It is therefore not surprising that only 33% of participants randomised to standard-of-care commenced treatment during follow-up. Of these, almost 40% did so within prison following reincarceration.

These results are in keeping with our previous findings in a retrospective audit in which only 25% of individuals commenced DAAs within 6 months of incarceration and are similar to other international data (19-21) - without dedicated transition support, linkage to care is low.

We observed a higher likelihood of DAA initiation than Akiyama and colleagues, who performed a single arm study in the USA evaluating hepatitis C care coordination and patient navigation for recently released individuals. The higher commencement rate amongst our participants is likely multifactorial. Firstly, we were able to successfully contact a greater number of participants following re-entry, which our data identifies is key for transitional care engagement. Secondly, we created a model of care which enhanced immediate treatment initiation without significant time or fiscal expenditure, rather than supporting an individual to navigate existing protracted healthcare pathways. Finally, our model incorporated a pathway for accelerated prison-based treatment initiation, anticipating a high rate of reincarceration amongst this group.

The ability to contact participants following re-entry was key to engaging individuals in care, and was the sole predictor of treatment initiation amongst those randomised to care navigation. Overall, 14 of the 15 participants who were directly contactable commenced hepatitis C treatment, 13 of whom did so in the community via the intervention. This suggests that those re-entering the community prioritise their hepatitis C management highly, and when supported to treatment initiation, high engagement is achieved. Although only a minority of untreated participants randomised to standard of care were contactable at six months post release, again, two of three were commenced on hepatitis C treatment via the intervention. This further endorses the model's ability to overcome inherent barriers to care on re-entry and engage those poorly served by existing healthcare pathways.

We did, however, encounter difficulties in maintaining contact with some participants whilst on treatment and to the SVR12 timepoint. This was most frequently due to a change in contact details; in fact, most participants who were treated via care navigation changed primary telephone numbers at least once. The difference between an individual's anticipated and actual contact details, accommodation and social networks before and after prison release is also evidenced by approximately 80% of control participants being uncontactable at six months post release. Successful engagement in hepatitis C transitional care is therefore dependent on early and active contact immediately following release from prison when there appears to be some certainty about an individual's contact information. Furthermore, as only one participant-initiated contact following prison release via the toll-free number provided, the responsibility of establishing contact must lie with transitional care coordinators in order to achieve a level of engagement adequate to justify resourcing.

The time to hepatitis C treatment was also significantly shorter for those randomised to care navigation. Prior Australian data has identified that almost half of individuals with an IDU history resumed IDU within six months of community re-entry (13). Given the high likelihood of recidivist IDU amongst this group and the potential for onward transmission, reducing time between re-entry and DAA initiation may also have important implications in reducing incident infection in the community.

Overall, 43% of the participants were reincarcerated during follow up. The success of our model was in part due to accelerated treatment initiation for individuals noted to be reincarcerated during surveillance of the prison database. Prison reception staff address several healthcare issues for an incoming prisoner and in this context, referral for hepatitis C treatment may be missed or delayed. Our data demonstrate that active monitoring for reincarceration is associated with a numerically higher rate of prison-based hepatitis C treatment. More broadly however, these data emphasize the close association between PWIDs and detention, and the cyclical movements between community and prison. In this context, enhanced collaboration between prison and community healthcare providers is important to manage the complex needs of this population across siloed medical services. In Australia there is an existing workforce of integrated hepatitis nurses who assist in coordinating hepatitis C care, including for individuals re-entering the community from prisons. These data support the formalisation of this role and referral pathways between Justice healthcare departments and this sector. Given the frequent interaction between PWID and the prison sector, we must also continue to advocate for prison-based hepatitis C treatment internationally where not currently standard practice, and continue to evaluate systems to improve hepatitis C treatment throughput where in place. This may include strategies such as point-of-care diagnostics at prison entry, or initiation of hepatitis C DAA prior to re-entry with linkage to a transitional healthcare service on release.

This study has several limitations. Firstly, due to the SARS-CoV-2 global pandemic and the suspension of prison-based hepatitis C assessments in our jurisdiction, recruitment was terminated early. As such, although the differences in the rates of DAA initiation between groups were significant, we acknowledge that the overall sample size is small. Secondly, this model relies on a policy of universal access to DAA for all persons living with hepatitis C infection irrespective of fibrosis or PWID status, as is the current practice in Australia, which may affect its applicability in other settings globally when treatment is restricted. The importance of treating PWIDs however is increasingly being recognised by policy makers globally and access to DAAs is similar in a large number of high-income countries. Some individuals with whom the study was discussed were subsequently sentenced and treated whilst incarcerated and did not necessarily come to the attention of the study team. Whilst no individual refused participation in the study during prison-based assessments, this limits our ability to

confidently determine the number of individuals who were randomised compared to the number for which study participation was explored.

Future studies will explore broader psychosocial determinants of hepatitis C transitional care engagement to refine the future models. A cost-effectiveness analysis is also planned to determine whether assertive hepatitis C transitional care for individuals leaving the prison system is a cost effective method of contributing to hepatitis C elimination which should be adopted more broadly.

In summary, transitional care navigation increases the likelihood that individuals living with hepatitis C are successfully engaged in care following release from prison, and also reduces time to treatment. Treatment rates amongst unsupported individuals are otherwise low, likely due to low prioritisation of hepatitis C care in the setting of multiple re-entry challenges. Similar programs should be developed and implemented to make a meaningful contribution to improving health outcomes amongst this marginalised population.

3.3.6 References

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4 Chapter 4: The use of non-invasive fibrosis algorithms for excluding cirrhosis in prisoners

4.1 Introduction and Summary

Whilst prison-based HCV treatment using DAAs is safe and effective, there remain a number of barriers preventing the widespread implementation of prison-based HCV programs internationally (295, 383). Furthermore, where such programs are operational, treatment throughput can also be impeded by a range of factors. These barriers may be system, prisoner, economic or treatment-related and each requires further evaluation to both understand, and mitigate, their impact. One important barrier is the need for cirrhosis determination prior to DAA initiation (275). Cirrhosis determination prior to hepatitis C treatment is important for the selection of an appropriate DAA regimen and to determine those who should be enrolled in HCC surveillance following successful treatment (156). TE is the most reliable non-invasive method for fibrosis assessment, however it is also poorly suited to the prison setting being costly, difficult to transport across an entire jurisdiction and presents challenges relating to staff credentialing (401). Blood-based fibrosis algorithms, including the APRI and FIB-4, have been shown to achieve excellent NPVs for the exclusion of cirrhosis and advanced fibrosis, respectively, which could streamline HCV assessment in prisons (129, 131, 402). These algorithms, however, have not been evaluated amongst prison-based cohorts.

The aim of this study was to evaluate the ability of blood-based algorithms to exclude cirrhosis in prisoners, and to determine the impact on reducing the need for TE if incorporated into HCV assessment protocols in the prison setting. We also investigated the utility of including age thresholds in fibrosis determination pathways. We identified that both APRI and FIB-4 at thresholds of 1.0 and 1.45, respectively, achieved NPVs for cirrhosis of >95% and if used as a trigger for TE, would reduce referrals for this investigation by over 70%. We also developed and validated a novel fibrosis assessment pathway for use in prisoners which includes both categorical age thresholds and the APRI. This evaluation is included in the manuscript below.

This study has been accepted for publication in *PLoS One*.

Reference:

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4.2 Original manuscript

Non-invasive fibrosis algorithms are clinically useful for excluding cirrhosis in prisoners living with hepatitis C

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4.2.1 Abstract

Background and Aims:

Prison-based HCV treatment rates remain low due to multiple barriers, including accessing transient elastography for cirrhosis determination. The AST-to-platelet ratio index (APRI) and FIB-4 scores have excellent negative predictive value (NPV) in hospital cohorts to exclude cirrhosis. We investigated their performance in a large cohort of prisoners with HCV infection.

Methods:

This was a retrospective cohort study of participants assessed by a prison-based hepatitis program. The sensitivity, specificity, NPV and positive predictive value (PPV) of APRI and FIB-4 for cirrhosis were then analysed, with transient elastography as the reference standard. The utility of age thresholds as a trigger for transient elastography was also explored.

Results:

Data from 1007 prisoners were included. The median age was 41, 89% were male, and 12% had cirrhosis. An APRI cut-off of 1.0 and FIB-4 cut-off of 1.45 had NPVs for cirrhosis of 96.1% and 96.6%, respectively, and if used to triage prisoners for transient elastography, could reduce the need for this investigation by 71%. The PPVs of APRI and FIB-4 for cirrhosis at these cut-offs were low. Age ≤ 35 years alone had a NPV for cirrhosis of 96.5%. In those >35 years, the APRI cut-off of 1.0 alone had a high NPV $>95\%$.

Conclusion:

APRI and FIB-4 scores can reliably exclude cirrhosis in prisoners and reduce requirement for transient elastography. This finding will simplify the cascade of care for prisoners living with hepatitis C.

4.2.2 Introduction

The advent of direct acting antivirals (DAAs) has revolutionised the treatment of chronic hepatitis C virus (HCV) infection. People living with HCV can now be treated with all oral, short duration, and well tolerated DAA regimens which achieve cure rates in excess of 95% [1,2]. In this context, the World Health Organization (WHO) has established targets for HCV elimination, including an 80% reduction in incident infections and a 65% reduction in HCV related mortality by 2030 [3]. Mathematical modelling has demonstrated that to achieve these targets, concerted efforts must be made to treat key populations with high risk for transmission including people who inject drugs (PWID) [4].

Prisons provide an excellent opportunity to identify and treat people living with HCV given the over-representation of people with injecting drug use histories and the associated high prevalence of chronic HCV [5]; HCV seroprevalence exceeds 15% in prisons globally, and in Australia is as high as 50% amongst incarcerated PWIDs [6,7]. Multiple data have now demonstrated that prison-based HCV treatment is safe and effective and can reach prisoners in large numbers [8-10]. Despite this, rates of HCV treatment in prisons worldwide remain low due to multiple barriers [11]. These include frequent prisoner transfer interrupting treatment, short sentence durations, the need for hospital transfer for specialist review and limited resourcing for HCV testing and treatment [12,13]. One important barrier is access to TE (e.g. FibroScan®) for cirrhosis determination in the correctional setting [14].

Cirrhosis determination prior to HCV DAA initiation is important for decision-making regarding treatment regimen and duration, and to identify those who require ongoing specialist referral for cirrhosis management and hepatocellular cancer (HCC) surveillance [15]. TE is the most accurate non-invasive tool for cirrhosis determination and has largely replaced liver biopsy in the community [16]. Access to TE within the correctional sector however is challenging and remains a significant barrier to care due to its high cost, limited availability of equipment within prison health services, frequent need for prisoner transfer and issues with security [10,17].

Based on routine blood tests, the AST-to-platelet ratio index (APRI) and the FIB-4 score were developed to predict advanced fibrosis and cirrhosis, using community recruited samples [18-21]. Both scores have subsequently been validated and demonstrate excellent negative predictive value for excluding advanced fibrosis or cirrhosis. Indeed, the WHO recommends both APRI and FIB-4 as cost-effective tools for the assessment of hepatic fibrosis in resource-limited settings [22]. Prisoner populations, however, are characterised by male gender, younger age, as well as multiple high-risk behaviours that differ considerably from the hospital populations used to validate these algorithms. Validation of simple serum-based fibrosis markers for use in the correctional setting to triage prisoners

at risk of cirrhosis would be clinically useful and help improve the cascade of care by reducing treatment costs and the time delay and logistical challenges associated with TE.

We have therefore evaluated the accuracy of non-invasive fibrosis algorithm scores including APRI and FIB-4 as a practical triage tool for excluding cirrhosis in the correctional setting. This analysis was conducted using a large cohort of prisoners with HCV infection with a view to simplifying clinical pathways by reducing the number of prisoners requiring TE, therefore improving the HCV cascade of care.

4.2.3 Materials and methods

Data availability statement

The data included in this analysis includes sensitive information regarding prisoners under the care of the Department of Justice and Regulation, Victorian Government. As such, in keeping with Department of Justice regulations, this data cannot be made publicly available. Data queries regarding the hepatitis C non-invasive fibrosis algorithm dataset can be forwarded to Bernadette De Graff, Clinical Research Compliance Manager, Department of Gastroenterology, St Vincent's Hospital Melbourne, Bernadette.degraff@svha.org.au.

Participants

This was a retrospective cohort study investigating the performance of the APRI and FIB-4 in a prison setting. The cohort provided prospectively collected data from all prisoners with HCV infection evaluated through the Statewide Hepatitis Program in Victoria, Australia. Prisoners evaluated for HCV infection were consecutively recruited from November 2015. This model of care has been described in detail previously [8]. Prisoners who self-reported a prior HCV diagnosis or who were identified as HCV seropositive whilst incarcerated were referred to the Statewide Hepatitis Program for face-to-face protocol-driven assessments with a program nurse. Clinical assessments were conducted at all 15 adult prisons throughout Victoria, which are serviced by the program. Pathology testing was performed by the diagnostic laboratory affiliated with each correctional facility. A database was created containing participants' baseline clinical characteristics, blood test results and liver stiffness measurement (LSM) median scores, interquartile ranges (IQR) and IQR:median ratios. Blood-based investigations that were recorded included haemoglobin, white blood cell count, platelet count, creatinine, urea, sodium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, albumin, international normalised ratio (INR), hepatitis B/C serology, HIV serology, and HCV genotype and viral load.

Prisoners were excluded if they had insufficient blood test results to calculate the APRI or FIB-4 scores within 6 months of TE, if TE had not been performed, if IQR:median ratio exceeded 0.3 or the absence of current HCV infection, defined as detectable HCV RNA (figure 1).

Liver cirrhosis determination

TE was used for cirrhosis determination at each prisoner's initial clinical assessment to guide appropriate management. Cirrhosis was defined as a median LSM of ≥ 12.5 kPa, in keeping with Australian HCV consensus guidelines [23, 24]. Blood-based and radiologically investigations were also arranged at this review, where appropriate. TE was performed using FibroScan® (Echosens) with the 'M' or 'XL' probe, as determined by the participant's body mass index (BMI) and body habitus. Measurements were collected in the mid-axillary line until ten valid readings were recorded. The LSM results was deemed valid only when the IQR/median ratio was less than 0.3 and when performed prior to HCV DAA initiation. All transient elastography was performed by three hepatology clinical nurse consultants who had performed at least 100 supervised examinations for credentialing. The APRI and FIB-4 scores were then determined for each prisoner. The APRI was calculated as $[(AST / \text{Upper limit of normal of AST}) \times 100] / [\text{Platelets}(10^9/L)]$ [18]. The FIB-4 score was calculated as $[(\text{age} \times AST) / (\text{platelets}(10^9/L) \times ALT^{1/2})]$ [19].

Statistical methodology and analysis

Statistical analyses were performed using STATA 12.0 (StataCorp LP, College Station, TX, USA). Categorical data were described as number and percentage and interval data as median and IQR.

Data were used to determine the sensitivity, specificity, PPV and NPV of the APRI and FIB-4 scores to diagnose or exclude cirrhosis using standard analytical approaches. Performance of APRI and FIB-4 were assessed against gold-standard TE assessments. Cut-offs of 1.0 and 2.0 for APRI, and 1.45 and 3.25 for FIB-4 were selected on the basis of having been previously validated in other data for the diagnosis of cirrhosis and advanced fibrosis (METAVIR F3/4), respectively [18-20]. Two analyses for FIB-4 at these thresholds were considered, including for advanced fibrosis (LSM ≥ 9.5 kPa) (for which this algorithm was originally validated) and for cirrhosis (≥ 12.5 kPa). The reduction in requirement for TE was also determined should prisoners be triaged for TE using these algorithms cut-offs.

In addition to APRI and FIB-4, given the young average age of prisoner populations globally and the slow natural history of HCV infection [24], the sensitivity, specificity, NPV and PPV of binary age thresholds alone to predict or exclude cirrhosis were also analysed, to determine if stratifying fibrosis assessment by age could minimise the need for TE while maintaining high NPV. The age thresholds were determined by the data and the quartile values (25th, 50th and 75th) were selected for analysis. The cohort was then separated by these age values and we determined the predictive value of APRI

at a cut off of 1.0 in these groups separately to contribute to new clinical pathways for fibrosis assessment for the entire prison cohort incorporating age categories. This novel fibrosis assessment pathway was then validated using data from an additional cohort of prisoners.

Ethics Statement

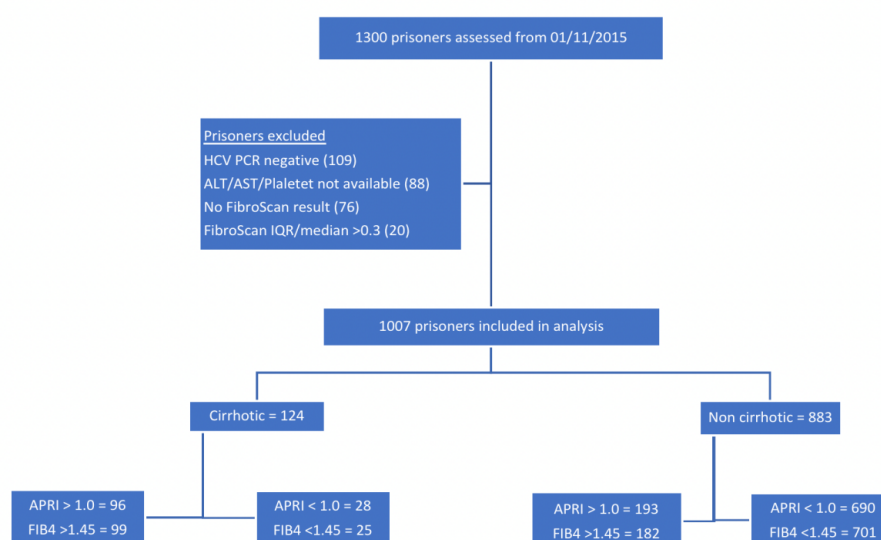
This study was approved by the St Vincent's Hospital Human Research Ethics Committee. The study was granted a waiver of consent as all data were analysed anonymously. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines.

4.2.4 Results

Prisoner characteristics

In total, data from 1300 was assessed. Two hundred and ninety three prisoners were excluded including 109 who were HCV PCR negative, 88 who had insufficient blood test results to calculate APRI and/or FIB-4 (n=88), 76 who did not undergo TE (n=76) and 20 who had LSM IQR:median >0.3 (n=20) (Figure 1). As such, 1007 were included in the analysis. Baseline characteristics are described in Table 4.1. In short, prisoners were prominently male (89%), the median age was 41 [35-47] years, HCV GT3 infection was the most common genotype (46%), and there were low rates of HBV and HIV coinfection (1%). One hundred and twenty-four individuals (12%) were cirrhotic, as defined by TE (LSM $\geq$ 12.5kPa, n=124/1007). The median APRI score was 0.66 [0.41-1.09] and median FIB-4 score was 1.02 [0.69-1.54] (Table 4.2). The median time between the collection of blood-based investigations and transient elastography was 34 days [IQR 13-86 days].

Figure 4.1: Consort diagram.



Legend: HCV, hepatitis C; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range; APRI, AST-to-platelet ratio.

Table 4.1: Baseline characteristics of prisoners at initial assessment.

Baseline characteristics	n=1007
Age, median [IQR]	41 [35-47]
BMI, median [IQR]	28.4 [25.3-32.2]
Male sex, n (%)	899 (89)
Indigenous Australian, n (%)	133 (13)
HCV viral load IU/mL, median [IQR]	757,000 [193,250-3,130,750]
HCV Genotype, n, (%)	
- GT1a	435 (43)
- GT1b	33 (3)
- GT2	26 (3)
- GT3	466 (46)
- GT4	2 (0.5)
- GT6	8 (0.5)
- NA	37 (4)
HBsAg positive, n (%)	14 (1)
HIV Ab positive, n (%)	12 (1)
ALT, median [IQR]	81 [53-132]
AST, median [IQR]	52 [36-79]
Platelets, median [IQR]	234 [192-276]
Cirrhotic, n (%)	124 (12)

Legend: BMI, body mass index; GT, genotype; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range.

Table 4.2: Results of cirrhosis determination using APRI, FIB-4 and elastography.

Cirrhosis determination	n=1007
APRI score, n (%)	
- < 1.0	718 (71)
- 1.01 – 2.0	196 (19)
- > 2.0	93 (9)
FIB-4 score, n (%)	
- < 1.45	726 (72)
- 1.45 – 3.25	236 (23)
- >3.25	45 (45)
Liver Stiffness Measurements, n (%)	
- < 6 kPa	356 (36)
- 6 – 9.4 kPa	435 (43)
- 9.5 – 12.4 kPa	92 (9)
- ≥ 12.5 kPa	124 (12)

Legend: APRI, AST-to-platelet ratio.

Performance of APRI or FIB-4 scores for cirrhosis in prisoners

The performance of the APRI and FIB-4 scores for cirrhosis (LSM $\geq$ 12.5kPa) at previously validated thresholds are presented in Table 4.3. An APRI score cut-off of 1.0 had a sensitivity and specificity for cirrhosis of 77.4% and 78.1%, respectively. The NPV of APRI $<$ 1.0 to exclude cirrhosis was 96.1%. If this cut-off was incorporated into prison fibrosis assessment protocols where only prisoners with an APRI $\geq$ 1.0 were referred for TE, the need for FibroScan[®] would reduce by 71% (n=718/1007). The PPV at this threshold was low (33.2%), meaning that these prisoners would still require TE for cirrhosis confirmation. At the higher APRI cut-off of 2.0, the PPV was higher (48.4%), but resulted in lower NPV (91.4%) and sensitivity (36.3%) – using an APRI cut-off of 2.0 alone to exclude cirrhosis would have meant that 64% (n=79/124) cases of cirrhosis were missed (Supplementary table 4.1).

Table 4.3: Sensitivity, specificity, NPV and PPV of the APRI and FIB-4 scores to diagnose or exclude cirrhosis (LSM $\geq$ 12.5kPa) represented as percentages.

		Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		< 12.5kPa (n=883) n (%)	$\geq$ 12.5kPa (n=124) n (%)				
For prediction of cirrhosis	All prisoners (n=1007) n (%)						
APRI							
$\leq$ 1.0	718 (71)	690 (78)	28 (23)	77	78	33	96
$>$ 1.0	289 (29)	193 (22)	96 (77)				
$\leq$ 2.0	914 (91)	835 (95)	79 (64)	36	95	48	91
$>$ 2.0	93 (9)	48 (5)	45 (36)				
FIB-4							
$\leq$ 1.45	713 (71)	688 (78)	25 (20)	80	78	34	97
$>$ 1.45	294 (29)	195 (22)	99 (80)				
$\leq$ 3.25	960 (95)	871 (99)	89 (72)	28	99	75	91
$>$ 3.25	47 (5)	12 (1)	35 (28)				

Legend: APRI, AST-to-platelet ratio; NPV, negative predictive value; PPV, positive predictive value.

Similar patterns were observed using different FIB-4 cut-offs, with the lower cut-off score of 1.45 associated with a high NPV for the exclusion of cirrhosis, and a low PPV (Table 4.3). At a higher cut-off score of 3.25, the specificity of the score was enhanced, however the sensitivity and NPV were significantly reduced (Table 4.3).

The performance of FIB-4 for diagnosing or excluding advanced fibrosis (LSM ≥ 9.5 kPa) at previously validated threshold was also analysed (Supplemental table 4.1).

Age thresholds for the exclusion of cirrhosis

The sensitivity, specificity, NPV and PPV of age thresholds alone for cirrhosis were determined. The binary age thresholds selected for analysis were 35, 41 and 47 years, representing the quartile values for the cohort. The binary variable of prisoner age ≤ 35 achieved a NPV for cirrhosis of 96.5%, due to the low prevalence in this group (3.5%, $n=9/258$) (table 4.4). If no formal fibrosis assessment was performed for prisoners aged ≤ 35 , nine cases of cirrhosis would be missed, representing 1% of the total cohort. The NPV of the age thresholds 41 and 47 years alone to exclude cirrhosis decreased to 94.7% and 91.6%, respectively, due to the increasing prevalence of cirrhosis in these age groups (Table 4.4). Furthermore, as the age threshold increased, the number of prisoner with cirrhosis who were missed, increased. We also calculated the NPV using the combination of age and APRI (APRI cut-off of 1.0 for prisoners aged ≤ 35 and >35 to exclude cirrhosis (Table 4.4). Amongst the age ≤ 35 years subgroup, an APRI cut-off of 1.0 was associated with a NPV of 99.0%, however would result in 26% of this age group requiring TE who had an APRI >1.0 . In those >35 years old, the NPV of APRI 1.0 remained satisfactory and was $>95\%$, which could reduce the need for TE by 70% in this age group (table 4.5).

Table 4.4: Ability of age alone to diagnose or exclude cirrhosis, with TE as the reference standard.

	All prisoners ($n=1007$) n (%)	Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		< 12.5 kPa ($n=883$) n (%)	≥ 12.5 kPa ($n=124$) n (%)				
For prediction of cirrhosis							
Age							
≤ 35	258 (26)	249 (28)	9 (7)	92	28	26	97
> 35	749 (74)	634 (72)	115 (93)				
≤ 41	528 (52)	500 (57)	28 (23)	77	57	20	95
> 41	479 (48)	383 (43)	96 (77)				
≤ 47	752 (75)	689 (78)	63 (51)	49	78	24	92
> 47	255 (25)	194 (22)	61 (49)				

Legend: NPV, negative predictive value; PPV, positive predictive value.

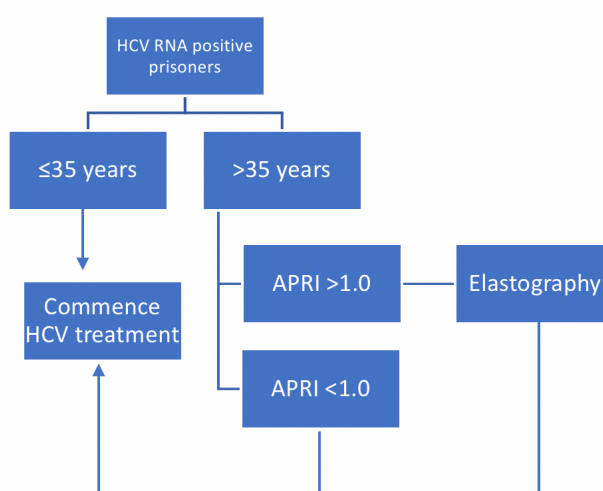
Table 4.5: The performance of APRI in age categories ≤ 35 and >35 to diagnose or exclude cirrhosis.

	All prisoners (n=1007) n (%)	Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		$< 12.5\text{kPa}$ (n=883) n (%)	$\geq 12.5\text{kPa}$ (n=124) n (%)				
For prediction of cirrhosis							
Age + APRI							
≤ 35							
APRI ≤ 1.0	192 (19)	190 (21)	2 (2)	78	76	11	99
APRI > 1.0	66 (7)	59 (7)	7 (6)				
> 35							
APRI ≤ 1.0	526 (52)	500 (57)	26 (21)	78	79	40	95
APRI > 1.0	223 (22)	134 (15)	89 (71)				

Legend: APRI, AST-to-platelet ratio; NPV, negative predictive value; PPV, positive predictive value.

Finally, a novel fibrosis assessment algorithm of performing TE only in prisoners >35 years and only when $\text{APRI} \geq 1.0$ would achieve a NPV of $>95\%$ for cirrhosis and reduce the need for TE by 78% of the entire cohort (Figure 2). The NPV for cirrhosis was sustained at 97% when this fibrosis assessment pathway was applied to a validation cohort of 189 prisoners, 11% ($n=20/189$) of whom were cirrhotic, and referral for TE would be reduced by 83% ($n=156/189$) (Supplemental table 4.2).

Figure 4.2: A novel clinical pathway for fibrosis assessment prior to HCV treatment in prisons. This pathway achieves a NPV for cirrhosis of $>95\%$ in all prisoners and minimises the need for FibroScan by 78%.



Legend: HCV, hepatitis C virus; APRI, AST-to-platelet ratio.

4.2.5 Conclusions

HCV treatment within prisons is important for global viral elimination, however the requirement for TE for fibrosis assessment presents barriers to efficient HCV care in custodial settings. Our data demonstrate that the APRI and FIB-4 serum scores can be useful to stratify risk of cirrhosis in a prison population, using validated thresholds from hospital-based cohorts. When applied to our cohort of prisoners with HCV infection, APRI and FIB-4 thresholds of 1.0 and 1.45, respectively, were associated with NPVs for cirrhosis in excess of 96%. The sensitivity and PPV for both algorithms, however, was low. The data, therefore, demonstrate that the strength of these algorithms lies in the exclusion, rather than confirmation, of cirrhosis. The NPV of binary age thresholds for excluding cirrhosis were also investigated. The NPV of age ≤ 35 alone exceeded 96%, and could be incorporated into fibrosis assessment protocols to improve HCV treatment throughput. The use of APRI and FIB-4 have been prioritised by the WHO for cirrhosis determination in low- and middle-income countries due to their availability and low cost [18]. Similarly, prison health services provide complex care to prisoners in large numbers, including for HCV infection, yet operate under significant fiscal restrictions. Therefore, the use of these indices to minimise cost and streamline care should be expanded.

Both APRI (threshold of 1.0) and FIB-4 (threshold 1.45) had NPVs of $>96\%$ for cirrhosis. We believe that this NPV is such that a second test for fibrosis determination is not required. The use of these thresholds to determine need for TE would result in a 70% reduction in the number of prisoners being referred for TE. As such, these indices could help simplify pathways of care for HCV in prisons and promote increased treatment capacity. While mobile FibroScan[®] units are available, high equipment costs and mandatory training requirements mean that it is not feasible to have them routinely available in many prisons. As there are significant costs and logistics associated with transporting prisoners to tertiary hospitals or a central prison where a FibroScan[®] might be available, the reliance on TE for fibrosis assessment represents a significant challenge for expanding HCV treatment coverage in prisons. Using these algorithms to reduce the need for TE in the majority while maintaining a high NPV is significant, as delays in facilitating this investigation are at odds with the short average duration of incarceration, meaning some prisoners would be released without treatment. The NPV of APRI and FIB-4 are influenced by cirrhosis prevalence, and our cohort of predominately young male prisoners is well suited for their use.

Our data also demonstrate that age ≤ 35 years has a very high NPV for cirrhosis (96.5%), and that there is little clinical utility in applying blood-based algorithms or TE to this population. Evaluation of liver fibrosis in those ≤ 35 years might be restricted to people with clinical risk factors for cirrhosis, such as multiple spider naevi. In prisoners >35 years there is a higher prevalence of cirrhosis, and in this group

formal fibrosis evaluation is important. APRI <1.0 had a NPV for cirrhosis of $>95\%$ in prisoners >35 years, reducing the need for TE by 70% in this age group. Therefore, LSM could be reserved for those prisoners >35 years who have APRI >1.0 . Combined, this approach achieves a NPV of $>95\%$ across the entire cohort and minimised the need for TE by 78% (Figure 2). When applied to a validation cohort, the NPV of this approach was sustained and $<20\%$ would require referral for TE.

Despite the high NPV of the FIB-4, APRI and age ≤ 35 to exclude cirrhosis, a small number of cirrhotic prisoners were miscategorised. Using a FIB-4 cut off of 1.45 for TE, 2% ($n=25/1007$) of the overall cohort and 20% ($n=25/124$) of cirrhotics were incorrectly identified as non-cirrhotic. This was similar for an APRI threshold of 1.0 (3% miscategorised, $n=28/1007$). All miscategorised prisoners were Child-Turcotte-Pugh class A and had preserved platelet counts. As such, the main implication for those miscategorised is exclusion from HCC surveillance. Achieving cure for these prisoners however will lead to significant reductions in their HCC risk and more broadly, non-HCC liver related morbidity and mortality [26]. Therefore, the reduction in prison-based HCV treatment throughput if TE is required for all prisoners must be considered. By stratifying the need for TE using fibrosis algorithms, it is anticipated that program efficiencies would be streamlined and that a greater number of prisoners would be treated, preventing the progression to cirrhosis and HCC in many.

The data demonstrate that the strength of APRI and FIB-4 are for excluding, rather than diagnosing cirrhosis. The higher APRI and FIB-4 scores at thresholds of 2.0 and 3.25, which are recommended in WHO guidelines, were analysed for their ability to diagnose cirrhosis. Whilst achieving high specificity at these thresholds ($>95\%$ for both APRI 2.0 and FIB-4 3.25), their sensitivity was low and the majority of prisoners with cirrhosis were not diagnosed. As such, these algorithms perform poorly when used to diagnose cirrhosis within custodial settings and offer little clinical utility in prison HCV assessment pathways.

There is now increased focus on how best to utilise public health platforms to achieve the WHO elimination goals by 2030. Mathematical modelling has demonstrated to achieve this agenda, PWIDs must be prioritised as they contribute most to incident HCV infection [4]. The effectiveness of prison-based HCV care has now been widely described [8-10]. However, the propagation of similar programs worldwide requires simplification of HCV assessment algorithms to reduce cost, particularly for other countries operating within tight fiscal budgets. Our analysis demonstrates that APRI and FIB-4 maintain a high NPV for cirrhosis when used at their validated thresholds in the prison setting. Our new algorithm incorporating age as a categorical variable and only performing non-invasive blood test algorithms to exclude cirrhosis in those >35 years exclusively is a practical way to more aggressively reduce the number requiring LSM while maintaining a high NPV. Such strategies will expedite HCV

assessment and minimise costs and therefore have an important role in the prison settings in the era of viral hepatitis elimination.

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5 Chapter 5: Prevalence of baseline HCV NS5A resistance associated substitutions in genotype 1a, 1b and 3 infection in Australia

5.1 Introduction and Summary

It is estimated that 71 million people are living with HCV globally (240). Within a chronically infected individual, there are a range of genetically distinct viral quasispecies on account of the poor fidelity of the HCV RNA polymerase. Some quasispecies harbour genetic sequences, which when translated, confer resistance to HCV DAAs including NS5A, NS5B and NS3/4A inhibitors. These are termed HCV RAS. The prevalence of HCV NS5A are relevant as they may impact treatment outcomes following DAA therapy when detected at baseline. Furthermore, Australian HCV guidelines do not currently recommend HCV RAS testing at baseline and it is therefore important to understand their prevalence to confirm the appropriateness of this recommendation (403).

The Victorian Infectious Disease Reference Laboratory (VIDRL) received funding from the National Health and Medical Research Council of Australia to provide HCV NS5A sequencing for Australians living with HCV infection, both at baseline and amongst those who had relapsed. All sequencing was performed by Jacinta O’Keefe and Scott Bowden at VIDRL. This study analysed a subset of these sequencing results and specifically included those who were verified as HCV DAA naïve. We identified that the prevalence of NS5A RAS at baseline was lower in Australia for GT1a infection and similar for GT1b and GT3 infection when compared to international data, supporting Australia guidelines regarding HCV RAS testing. We also compared the diagnostic yield of population-based vs next generation sequencing and did not identify any significant benefit to utilising this technology at baseline.

This study was published in *The Journal of Clinical Virology*.

Reference:

Papaluca T, O’Keefe J, Bowden S, Doyle J, Stooze M, Hellard M, Iser D, Thompson AJ. Prevalence of baseline HCV NS5A resistance associated substitutions in genotype 1a, 1b and 3 infection in Australia. *Journal of Clinical Virology*. 2019 Nov 1;120:84-7

The text in its entirety is included in this chapter. The prison version from the journal is included as an appendix.

5.2 Original article

Prevalence of baseline HCV NS5A resistance associated substitutions in genotype 1a, 1b and 3 infection in Australia

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5.2.1 Abstract

Background:

Direct-acting antivirals (DAA) have revolutionised hepatitis C virus (HCV) treatment, and most regimens include an NS5A inhibitor. Certain amino-acid substitutions confer resistance to NS5A inhibitors, termed resistance-associated substitutions (RAS). If present at baseline, they can reduce virological response rates. Population-based sequencing (PBS) is generally used for baseline sequencing, however next generation sequencing (NGS) reduces the threshold for detection of sequences encoding RAS from 20% to 5%. We determined the prevalence of NS5A RAS at baseline amongst Australian chronically infected with genotype (GT)1a, GT1b and GT3 HCV, using both PBS and NGS.

Methods:

Samples from DAA-naïve individuals were received at the Victorian Infectious Disease Reference Laboratory between June 2016 and December 2018. All samples were analysed for NS5A RAS using PBS. A subset of GT1 HCV samples were processed using NGS technology (Vela Diagnostics, Singapore) to determine the improvement in sensitivity.

Results:

In total, 672 samples were analysed using PBS. The baseline prevalence of NS5A RAS was 7.6% for GT1a (n=25/329), 15.7% for GT1b (n=8/51) and 15.1% for GT3 (n=44/292). NGS only marginally increased sensitivity for NS5A RAS at baseline in GT1a (16% vs 17%) and GT1b (29% vs 36%).

Conclusion:

The prevalence of NS5A RAS in GT1a HCV in Australia was low compared with international data, and was similar to other reported international prevalence for GT1b and GT3 infection. NGS at baseline only marginally increased sensitivity for the detection of NS5A RAS in patients with GT1 HCV and cannot be recommended for routine use at baseline in clinical practice.

5.2.2 Introduction

It is estimated that hepatitis C virus (HCV) infection affects 71 million people globally (1). Direct-acting antiviral (DAA) therapy targeting the NS3, NS5A and the NS5B proteins has revolutionised HCV treatment, and cure rates exceed 90% in real world settings. Amongst those infected, the poor fidelity of the HCV viral polymerase generates a range of genetic variants, some which result in amino-acid polymorphisms. These can reduce viral susceptibility to DAAs and are known as resistance-associated substitutions (RAS); they have been detected at baseline and virological relapse.

The presence of HCV NS5A RAS at baseline can affect treatment outcomes and certain NS5A RAS have been implicated in diminished sustained virological response rates (SVR) (2-4). As such, guidelines recommend HCV NS5A RAS testing at treatment baseline for particular regimens, including elbasvir/grazoprevir; for this regimen, the detection of specific NS5A RAS requires extension of treatment duration and addition of ribavirin (5). The prevalence of baseline HCV RAS varies among geographical regions. In Australia there are limited data regarding the prevalence of baseline genotype (GT)1a and GT1b NS5A RAS (6). Recent data has demonstrated a baseline GT1a NS5A RAS prevalence of 11.9% in one Australian region (7), however there are no Australian prevalence data regarding baseline HCV GT3 or GT1b NS5A RAS, nor data evaluating the utility of next generation sequencing (NGS) at baseline.

Population-based sequencing (PBS) for detection of NS5A RAS has a threshold limit of approximately 20%. The added yield of NGS, as well as the clinical relevance of low frequency variants remains unclear (8). In this context, we determined the frequency of NS5A RAS in DAA-naïve HCV GT1a, 1b and 3 in Australia, using both PBS and NGS.

5.2.3 Objectives

We tested for NS5A RAS in a large population of DAA-naïve individuals who had a baseline serum sample sent for sequencing studies prior to DAA treatment in Victoria, Australia. We restricted analysis to individuals infected with HCV GT1a, GT1b or GT3 infection. All subjects were tested for NS5A RAS using PBS. A subset of participants with GT1a and GT1b HCV were also tested for NS5A RAS using NGS.

5.2.4 Study design

Clinical samples

All serum samples received at the Victorian Infectious Disease Reference Laboratory between June 2016 and December 2018 were investigated. HCV genotype was previously determined with the Abbott RealTime genotyping II assay or the Versant HCV Genotype 2.0 assay (Siemens Healthcare Diagnostics, Surrey, UK). All participants were verified as DAA-naïve from their medical record. RNA

was extracted from 200 µL of patient serum and eluted to 100 µL by an automated method, using the Abbott m2000 instrument (Abbott Molecular Abbott Park, IL) and the Sample Preparation System_{RNA} kit (AbbottMolecular).

Population sequencing approach

The NS5A region (639nt) was amplified in a nested PCR using primers with Superscript III one-step RT-PCR system with Platinum Taq (Invitrogen, Waltham, MA) and Taq DNA Polymerase (Qiagen, Hilden, Germany). Purified PCR product was sequenced using ABI-Prism 3730 Genetic Analyser (Applied Biosystems, Life Technologies, Ltd, Paisley, UK). Using the ABI sequence analysis program SeqScape version 2.1.1 (Applied Biosystems), a consensus sequence was generated and aligned to a stored GenBank HCV reference sequence (Subtype 1a H77, subtype 1b Con 1, and subtype 3a HPCK3A).

Next Generation Sequencing approach

A subset of HCV GT1 samples were also tested using the NGS platform *Sentosa*[®] SQ HCV Genotyping assay (Vela Diagnostics, Singapore). With an initial input of 530 µL of serum, RNA was isolated from serum samples by an automated method using the Vela Diagnostics *Sentosa*[®] SX101 instrument. The PCR amplification step was performed off-board and the plate returned to the *Sentosa*[®] SX101 instrument for normalisation, enzymatic shearing, purification, adapter ligation and library pooling. After emulsion PCR and enrichment of the ion sphere particles, the amplicons were then subjected to NGS on the *Sentosa*[®] SQ301 Sequencer, with the data analysed by the *Sentosa*[®] SQ Suite software. The threshold for detection of this platform is 5%.

Definition of NS5A resistance associated substitutions

HCV NS5A RAS were defined as polymorphisms encoded by the NS5A gene associated with at least two-fold reduced susceptibility to available NS5A inhibitors in *in-vitro* cell-based replicon assays (9-11). All changes at positions 24, 28, 30, 31, 58, 92 and 93 were investigated.

Ethical considerations

This study was approved by the St Vincent's Hospital Melbourne Human Research Ethics committee (HREC/17/SVHM/143).

5.2.5 Results

Prevalence of NS5A RAS using PBS

In total, 672 samples were analysed. There were 329 HCV GT1a samples, and 26 NS5A RAS were identified in 25 patient samples (7.6%, n=25/329) (Table 5.1). The most frequent GT1a NS5A RAS were

M28V (2.7%, n=9/329) and L31M (1.9%, n=6/329). Dual NS5A RAS were identified in one sample (Table 5.1).

There were 51 HCV GT1b samples. NS5A RAS were identified in 15.7% (8/51) (Table 1). Y93H was most frequently identified (7.8%, n=4/51), then L31M (5.9%, n=3/51) and A92T (2.0%, n=1/51).

Table 5.1: Prevalence of NS5A RAS detected via population sequencing at baseline, presented by genotype, as frequencies and (percentages).

Frequency of baseline HCV RAS, n (%)	NS5A RAS	GT1a	GT1b	GT3
Samples with NS5A RAS identified		25/329 (7.6)	8/51 (15.7)	44/292 (15.1)
Single RASs at NS5A position:				
28	M28L	1 (0.3)		
	M28T	2 (0.6)		
	M28V	9 (2.7)		
30	A30K			15 (5.1)
	A30S			4 (1.4)
	A30V			1 (0.3)
	Q30H	1 (0.3)		
	Q30R	1 (0.3)		
31	L31M	6 (1.8)	3 (5.9)	
	L31V	1 (0.3)		
92	A92T		1 (2.0)	
93	Y93H	1 (0.3)	4 (7.8)	18 (6.2)
	Y93N	1 (0.3)		
	Y93S	1 (0.3)		
Dual RAS at NS5A position				
28, 58	M28V, H58Q	1 (0.3)		
30, 31	A30K, L31M			2 (0.7)
93, 30	Y93H, A30K			2 (0.7)
	Y93H, A30T			2 (0.7)

Legend: GT, genotype; RAS, resistance-associated substitution; HCV, hepatitis C virus.

There were 292 GT3 HCV samples (subtype 3a accounts for almost all GT3 infection in Australia(12)) and 47 NS5A RAS were identified in 44 patient samples (15.1%, n=44/292)(Table 5.1). The most

prevalent single RAS included Y93H (6.2%, n=18/292) and A30K (5.1%, n=15/292). Dual RAS were identified in six samples (Table 5.1).

Benefit of NGS at baseline in GT1a and 1b samples

Eighty-nine GT1a and 14 GT1b samples were also analysed using NGS, of which 75 and 10 respectively had no NS5A RAS detected by PBS. NGS confirmed that the RAS detected by PBS were the dominant quasiespecies (Table 2). NGS detected additional low frequency variants in 2/89 GT1a samples (in one of which no RAS was detected by PBS) and 1/14 GT1b samples, Table 2).

Table 5.2: Comparison between the frequency of detection of NS5A RAS using PBS and NGS methodologies. GT1a samples 45 and 86 and GT1b sample 9 (highlighted in blue) represent serum samples where a single or dual NS5A RAS were identified by NGS (including the percentage of the prevalent virus) which was not detected by PBS. GT, genotype.

Sample code	Population based sequencing	Next Generation sequencing
GT1a		
8	M28V	M28V (52.3%)
11	M28V	M28V (91.1%)
15	L31M	L31M (99.65%)
21	Y93H	Y93H (16.4%)
36	L31M	L31M (28.3%)
37	L31M	L31M (99.54%)
39	M28T	M28T (99.78%)
45	M28V	M28V (96.7%) Q30R (5.6%)
50	Q30H	Q30H (99.7%)
53	M28T	M28T (17.0%)
62	M28V	M28V (99.1%)
83	L31M	L31M (45.2%)
86	No RAS detected	L31M (5.4%)
87	L31M	L31M (98.9%)
88	M28V	M28V (66.4%)
GT1b		
1	L31M	L31M (79.6%)
2	L31M	L31M (99.5%)
3	L31M	L31M (99.1%)
9	No RAS detected	Y93H (8.2%)
14	Y93H	Y93H (68.3%)

5.2.6 Discussion

This is the first Australian data describing the frequency of baseline HCV GT1b and GT3 NS5A RAS amongst DAA-naïve individuals with chronic HCV infection, and contributes to recent data regarding HCV GT1a RAS (7).

HCV NS5A RAS were detected in 7.6% of people with GT1a HCV infection. The most prevalent GT1a NS5A RAS were M28V and L31M. Certain baseline NS5A RAS at positions M28, Q30, L31 and Y93 are relevant for GT1a patients treated with elbasvir and grazoprevir, and pre-treatment testing is recommended when this regimen is utilised in certain regions. Our data demonstrates a low prevalence of these substitutions in HCV infected Australian patients. The prevalence of GT1a NS5A RAS in this study was lower than recent Australian data and may be partly explained by the exclusion of secondary variants such as at residue H58, when identified in isolation (7). Large international DAA registration trials have demonstrated a higher prevalence of 11-23% in GT1a HCV (3,13,14), and 16% in a subset from Oceania (6). Smaller overseas studies have also reported higher GT1a NS5A RAS frequencies of 10-17% (15-17). Our lower prevalence supports Australian guidelines that baseline PBS to detect pre-existing RAS is not indicated (18).

The frequency of RAS among GT1b and GT3 HCV was similar to other international studies. Our analysis demonstrates baseline GT1b NS5A RAS frequency of 15.7%, similar to international data (16-33%) (3,6,14-16,19,20). The prominent GT1b NS5A RAS identified included Y93H (7.8%) and L31M (5.9%), concordant with other geographical regions.

The baseline frequency of GT3 NS5A RAS was similar to that in North America and Europe (12-19%) (3,14,16,21), however lower compared to other countries in the Asian-Pacific region. This includes China (>50%), reflecting their high prevalence of GT3b infection and likelihood of baseline dual A30K/L31M RAS (20). Y93H was the most prevalent GT3 NS5A RAS (7.5% including where present as a dual substitution) conferring significant resistance to all NS5A inhibitors *in vitro*, and reducing virological outcomes in GT3 patients treated with sofosbuvir/velpatasvir (3). A30K was identified in 6.5% of samples, including those with dual NS5A RAS. A30K has been associated with reduced SVR12 in non-cirrhotic GT3 infected patients treated with glecaprevir/pibrentasvir for 8 weeks, a regimen used commonly in Australia (22). Sequencing for GT1b and GT3 RAS at baseline is not currently recommended in treatment naïve individuals.

We evaluated 103 GT1 HCV samples using both NGS and PBS. The increased diagnostic yield of NGS was low, and only a small number of additional NS5A RAS at low frequencies were identified. North American and European guidelines designate that only RAS with a prevalence of >15% are clinically significant (23,24), greater than the prevalence of the additional RAS identified via NGS. Given the

overall low frequency of NS5A RAS in GT1 participants in Australia at baseline, and the minimal improvement in sensitivity, our data suggests that NGS does not have a role in routine pre-treatment assessment in DAA-naïve patients.

In summary, amongst DAA-naïve patients, baseline GT1a NS5A RAS were lower compared to international data, and comparable amongst GT1b and GT3 populations. Detection of HCV NS5A RAS by NGS at baseline only marginally increased diagnostic sensitivity and therefore has limited clinical applicability.

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6 Chapter 6: Retreatment with elbasvir, grazoprevir, sofosbuvir ± ribavirin is effective for GT3 and GT1/4/6 HCV infection after relapse

6.1 Introduction and summary

The introduction of DAAs has revolutionised HCV treatment. Despite excellent efficacy, with SVR12 rates exceeding 95% in clinical trials, some patients experience relapse after first-line DAA therapy (141, 164). When relapse does occur, it is associated with the selection of HCV RAS which reduce viral susceptibility to DAAs. Retreatment strategies should therefore include multiple agents with activity against at least three viral targets to overcome resistance and achieve cure (374). At the time that this study was conducted, no such therapy was licenced for use in Australia. As such, there was a need to evaluate salvage regimens in cirrhotic patients to minimise liver-related morbidity and mortality.

Despite ELB and GZR having significantly higher EC50 in vitro for GT3 infection, recent data had demonstrated that when combined with SOF and RBV, this regimen was highly effective for GT3 participants who were treatment naïve (404, 405). We therefore postulated that this regimen would also be effective for NS5A experienced patients with relapse, including those with GT3 infection, as the combination provided activity against NS5A, NS5B and NS3/4 PI, and was therefore well equipped to suppress the emergence of resistant species.

We evaluated the efficacy and safety of 12-16 weeks of SOF, ELB, GZR ± RBV for relapsed HCV infection following NS5A inhibitor exposure at four Melbourne-based hospitals. Our sample was significant for high rates of cirrhosis (63%) and GT3 infection (53%). We demonstrated high efficacy for both GT3 (95%) and non-GT3 infection (100%), and treatment was in general well tolerated. There was one case of suspected PI toxicity, without clinical decompensation, highlighting the need to carefully monitor PI use in those with advanced liver disease.

This study was published in *Liver International*.

Reference:

Papaluca T, Sinclair M, Gow P, Pianko S, Sievert W, Arachchi N, Cameron K, Bowden S, O'Keefe J, Doyle JS, Stoope M, Hellard M, Iser D, Thompson AJ. Retreatment with elbasvir, grazoprevir, sofosbuvir +/- ribavirin is effective for GT3 and GT1/4/6 HCV infection after relapse. *Liver International*. 2019 Dec;39(12):2285-90.

The text in its entirety is included in this chapter. The print version from the journal is included as an appendix.

6.2 Original Article

Retreatment with Elbasvir, Grazoprevir, Sofosbuvir +/- Ribavirin is effective for GT3 and GT1/4/6 HCV infection after relapse

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6.2.1 Abstract

Introduction:

Despite highly effective direct-acting antiviral (DAA) therapies for chronic hepatitis C virus (HCV) infection, some patients experience virological relapse. Salvage regimens should include multiple agents to suppress emergence of resistance-associated substitutions (RAS) and minimise treatment failure. The combination of sofosbuvir (SOF) and elbasvir/grazoprevir (ELB/GZR) $\pm$ ribavirin (RBV) is an effective retreatment strategy for HCV genotype (GT)1 and 4 infection. We hypothesized that SOF and ELB/GZR ($\pm$ RBV) would also be an effective salvage regimen for DAA-experienced GT3 patients.

Methods:

We evaluated the efficacy and safety of SOF/ELB/GZR $\pm$ RBV in DAA-experienced participants with chronic HCV infection who had prior relapse. Participants were treated at four hospitals between December 2016 and March 2018 for either 12- or 16-weeks. The primary endpoint was sustained virological response at week 12 post-treatment (SVR12) using intention-to-treat analysis.

Results:

There were 40 participants included in the analysis. The mean age was 53 years, 53% had GT3, 33% had GT1 infection, and 63% had cirrhosis. Fifty-eight percent were treated for 12 weeks, 42% were treated for 16 weeks, and 90% received RBV. The SVR12 rate was 98% overall, 100% in non-GT3 participants and 95% in GT3 participants. One GT3 cirrhotic participant relapsed. ELB/GZR was stopped at week 6 in one GT3 cirrhotic participant who switched to SOF/velpatasvir/RBV for a further 12 weeks and achieved SVR12. RBV dose reduction was required in two participants. Treatment was otherwise well tolerated.

Discussion:

The combination of SOF/ELB/GZR±RBV is effective and safe for difficult-to-cure patients who relapse after first-line DAA, including those with cirrhosis and GT3 infection.

6.2.2 Introduction

The introduction of highly effective and well tolerated direct acting antiviral (DAA) therapy has revolutionised hepatitis C virus (HCV) treatment. Despite excellent cure rates which exceed 95%, some patients experience virological relapse after treatment. Virological relapse is associated with the selection of HCV resistance associated substitutions (RAS), which reduce viral susceptibility to currently available DAAs (1). NS5A inhibitors are the backbone of most DAA regimens and selection of NS5A RAS is the most common reason for DAA failure. NS5A variants can persist for many years post-relapse (2). Variants in the NS5B region and/or NS3/4A region may also be selected with sofosbuvir (SOF) or protease inhibitor therapy, respectively. The ideal salvage regimen should involve combination therapy with at least two agents that are active against all the prevalent HCV quasiespecies. Triple combination therapy involving sofosbuvir (SOF), a protease inhibitor, and an NS5A inhibitor is therefore attractive as a salvage strategy.

Elbasvir (ELB) plus grazoprevir (GZR) is licenced for treatment of HCV genotype (GT)1 and 4 infection (3). Recent data have demonstrated that ELB/GZR in combination with SOF (± ribavirin (RBV)) is also an effective salvage treatment for cirrhotic DAA-experienced GT1/4 patients, those genotypes for which ELB/GZR has the greatest *in-vitro* and *in-vivo* activity (4). ELB and GZR alone have reduced effectiveness against HCV GT3 infection, with a significantly higher EC₅₀ than for GT1 HCV *in vitro* and suboptimal SVR12 rates in clinical trials (3, 5). Despite this, ELB/GZR in combination with SOF plus RBV was highly effective for the treatment of DAA-naïve GT3 patients; in a large prospective study SVR12 rates exceeded 94% in those treated for 12 to 16 weeks, including participants with cirrhosis (6).

In this context, we hypothesized that SOF plus ELB/GZR ± RBV (ELB/GZR/SOF±RBV) would be an effective salvage regimen for DAA experienced patients, irrespective of GT or underlying cirrhosis.

6.2.3 Patients and methods

Patients

From December 2016 a special access scheme supported by Merck, Sharp and Dohme Australia allowing access to ELB/GZR for use in combination with SOF/RBV for retreatment of patients who were non-responders to licensed DAA therapy. SOF/RBV were prescribed via the Pharmaceutical Benefit Scheme which reimburses the cost of HCV treatment for all Australians (7). Patients for whom this treatment was accessed from four tertiary centres in Melbourne, Australia were consecutively

treated with SOF 400mg daily, fixed dose combination ELB/GZR 100mg/50mg daily, with or without RBV (600-1200mg daily) for either 12 or 16 weeks. Duration of therapy was decided on an individual basis by the treating clinician at each participating site.

Eligibility criteria for the special access scheme included non-response following treatment with an approved NS5A inhibitor containing DAA regimen, defined as detectable HCV RNA at week 12 post treatment, and infection with HCV GT 1, 3, 4 or 6. All participants in this study were deemed compliant with their initial DAA treatment regimen. Exclusion criteria for this study included: decompensated chronic liver disease (Child Pugh Category B/C), active or suspected hepatocellular carcinoma, contraindicated drug-drug interactions and prior liver transplantation. Cirrhosis was determined by liver stiffness measurements using FibroScan (cut-off ≥ 12.5 kPa), prior liver biopsy or radiological and/or endoscopic evidence of portal hypertension. Portal hypertension was defined as the presence of oesophageal varices at endoscopy or splenomegaly, intra-abdominal varices or ascites identified on abdominal imaging.

Assessment

All participants were reviewed at baseline, treatment week 4, end-of-treatment (EOT) and 12 weeks post-treatment at a minimum. On-treatment virological response was defined as undetectable HCV RNA on PCR testing. The primary outcome was SVR12, defined as undetectable HCV RNA at 12 weeks post-EOT. All cirrhotic patients were enrolled in hepatocellular carcinoma (HCC) surveillance.

The secondary outcomes were frequency of treatment and non-treatment related adverse events and the frequency of denovo or recurrent HCC. Safety and tolerability were monitored at each clinical review and further investigations were arranged where deemed appropriate. All adverse events, laboratory abnormalities and treatment interruptions and/or discontinuations were recorded.

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines, and regulatory requirements. The study was approved by the St Vincent's Hospital Melbourne Human Research Ethics committee prior to governance review at each participating hospital. A waiver of consent was granted by the St Vincent's Hospital HREC and then ratified at each participating site.

Assessment for NS5A resistance-associated substitutions

A subset of participants were investigated for sequences encoding NS5A RAS at time of relapse, prior to treatment with the study regimen. Testing was performed at the Victorian Infectious Disease Reference Laboratory using Sanger sequencing with a prevalence threshold for detection of 20%.

Statistical Analysis:

Results are presented as median with interquartile range for continuous data and number (percentage) for categorical data. Analysis was performed using Stata 15.2 for Windows, StataCorp LLC, USA.

6.2.4 Results

Participant characteristics

Forty participants with chronic HCV infection and prior virological failure after HCV DAA therapy were included. Baseline characteristics are displayed in Table 6.1. The sample was predominately male (n=32, 90%), just over half had HCV GT3 infection (n=21, 53%) and majority were cirrhotic (n=25, 63%). Of those with cirrhosis, nine (36%) had portal hypertension.

Prior treatment regimens included SOF + ledipasvir (n=15, 37%), SOF + daclatasvir (n=22, 55%), SOF + velpatasvir (n=1, 3%), ELB/GZR (n=1, 3%) and paritaprevir/r + ombitasvir + dasabuvir (n=1, 3%). Treatment durations are detailed in Table 6.1. All participants had completed the entire course of the prior treatment and had detectable HCV RNA at 12 weeks post treatment completion. A mean duration of 10 months (range 5-19 months) had elapsed between EOT of the initial regimen and initiation of the study regimen. HCV NS5A sequencing results were available in 26/40 subjects, with the majority having NS5A RAS detectable (Supplementary Table 6.1).

Table 6.1: Baseline characteristics.

Baseline characteristics	N = 40
Age, mean, [IQR]	53 [46-60]
Male gender, n (%)	32 (80%)
ALT U/L, median, [IQR]	87 [51-124]
HCV RNA IU/mL, median, [IQR] ^a	1, 842, 281 [303, 500-7, 548, 579]
Platelet count, median, [IQR]	188 [155-237]
Albumin, median, [IQR]	39 [36-40]
Bilirubin, median, [IQR]	15 [12-24]
INR, median, [IQR]	1.1 [1.0-1.1]
HCV Genotype, n (%)	
1a	11 (28%)
1b	2 (5%)
3 ^b	21 (53%)
4 ^c	3 (7%)
6	3 (7%)
PR treatment experienced, n (%)	12 (30%)
Prior HCC, n (%)	2 (5%)
Active HCC, n (%)	0 (0%)
Liver stiffness measurements of non-cirrhotic participants, n	
<6 kPa	7/15
6-9.5 kPa	7/15
9.5-12.5 kPa	1/15
Cirrhotic, n (%)	25 (63%)
Child-Pugh score	
A5	18/25
A6	6/25
B7 ^d	1/25
MELD score, mean [IQR]	8 [7-10]
Portal hypertension, n, (%)	9/25 (36%)
Prior HCV treatments	
Sofosbuvir/Ledipasvir	15
Sofosbuvir/Daclatasvir	22
Sofosbuvir/Velpatasvir	1
Elbasvir/Grazoprevir	1
Paritaprevir/r, Ombitasvir, Dasabuvir	1
Duration of prior treatment	
8 wk	2
12 wk	20
24 wk	18

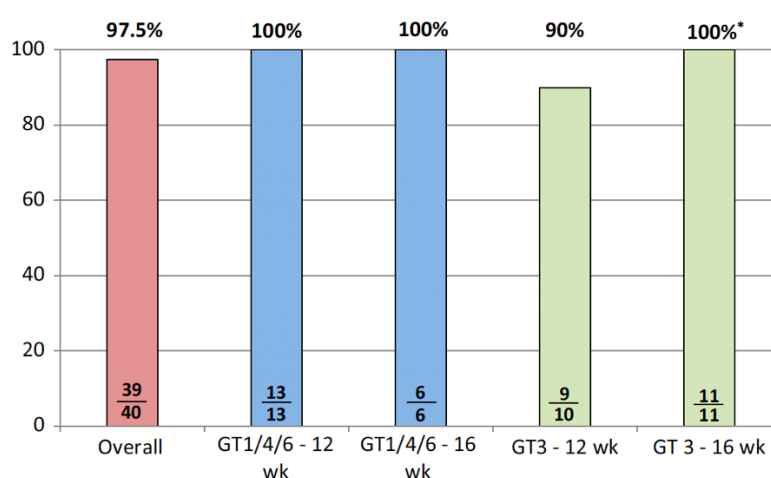
^aMedian and interquartile range presented for 30 of 40 participants. 10/40 were HCV PCR positive at baseline, however, quantitative HCV viral load testing was not performed. ^bGT subtyping was performed on 17 of 21 GT3 participants, who were subtype 3a. ^cGT subtyping was performed on two of three GT4 participants, who were

both subtype 4h. ^dOne Child-Pugh B7 participant was commenced on the study regimen beyond the inclusion criteria of the special access scheme for ELB/GZR, however, baseline hypalbuminaemia was attributed to poorly controlled type 2 diabetes with documented proteinuria. Legend: ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalised ratio; IQR, interquartile range; MELD, model for end stage liver disease; PR, pegylated interferon and ribavirin.

HCV DAA therapy and virological response

Of the 40 participants, 23 (58%) and 17 (42%) were treated with 12 weeks and 16 weeks of SOF/ELB/GZR±RBV respectively. Thirty-six (90%) received RBV; RBV was omitted in one GT3 participant with a history of prior RBV intolerance, and in three GT1/6 participants at the discretion of the treating physician due to concerns about tolerance. Treatment outcomes are displayed in Figure 1. Overall, the SVR rate was 97.5% (n=39/40). The SVR rate amongst HCV GT1, 4 and 6 participants was 100% (n=19/19), irrespective of treatment duration (12 or 16 weeks) or inclusion of RBV. Amongst HCV GT3 participants, overall SVR rate was 95% (n=20/21), 90% (9/10) for 12 weeks of therapy and 100% (10/10) for 16 weeks. This included an SVR12 rate of 100% (5/5) in non-cirrhotic GT3 participants, and 94% (n=15/16) among cirrhotic GT3 participants.

Figure 6.1: SVR12 rates by HCV genotype and duration of therapy.



*One participant stopped SOF/ELB/GZR/RBV at treatment week six because of increasing bilirubin and INR, was immediately changes to SOF/VEL/RBV for an additional 12 weeks and achieved SVR12. Ninety percent of participants received ribavirin, which was omitted for two GT1, one GT6 and one GT3 participant. Legend: GT, genotype; HCV, hepatitis C virus; SVR12, sustained virologic response at post-treatment week 12.

One episode of virological relapse occurred in a HCV GT3 cirrhotic participant (Child Pugh A5, MELD 6). NS5A RAS testing at relapse was significant for the Y93H RAS. The participant was treated with 12 weeks of SOF/ELB/GZR/RBV, but had detectable HCV RNA at SVR12 time point. A second HCV GT3 cirrhotic participant with portal hypertension (Child Pugh A6, MELD 9) prescribed ELB/GZR/SOF/RBV

for 16 weeks experienced an increase in bilirubin (30 to 50µmol/L) and INR (1.3 to 1.5) at treatment-week 6, and was immediately switched to SOF + velpatasvir + RBV for an additional 12 weeks. Bilirubin remained stable at 40-50µmol/L for the duration of RBV therapy before returning to baseline. The INR remained stable between 1.4 - 1.5 throughout the period of evaluation. There was no rise in the ALT or clinical decompensation event at any time. The participant went on to achieve SVR12.

One participant was commenced on ELB/GZR/SOF/RBV beyond the inclusion criteria of the ELB/GZR special access program. The participant was Child Pugh B7 at baseline, however the baseline hypoalbuminaemia (albumin 27g/L) was thought secondary to diabetic nephropathy and significant proteinuria. There was no adverse event, and the participant achieved SVR12.

SVR12 rate was 100% (n=9/9) amongst participants with no HCV NS5A RAS and 94% (n=16/17) in participants with an NS5A RAS detected at baseline. SVR12 was achieved in 92% (n=12/13) of participants with virus with the Y93H/D/S NS5A RAS at baseline.

Hepatocellular carcinoma

No *de novo* or recurrent hepatocellular carcinomas (HCC) were identified during the period of evaluation.

Adverse events

Adverse events are detailed in Table 6.2. One HCV GT3 cirrhotic participant discontinued therapy at treatment week 6 as described. There were 25 adverse events in 19 participants. Most were RBV-related haematological abnormalities, but also included fatigue, headache, gastrointestinal upset, insomnia and rash and were mild in severity. Two participants required dose reduction of RBV for management of anaemia.

Table 6.2: Adverse events

Adverse events, n (%)	N = 25
Haemoglobin decline	
20-40 g/L	9 (23)
>40 g/L	2 (5)
Requiring ribavirin dose reduction	2 (5)
Fatigue	5 (13)
Headaches	3 (8)
Gastrointestinal upset	3 (8)
Insomnia	1 (3)
Rash	1 (3)
Treatment discontinuation	1 (3)

6.2.5 Discussion

Whilst DAA therapy cures most people with HCV, a subset of patients do not respond to first line treatment. This hard-to-cure group is characterized by a high prevalence of HCV GT3 infection, cirrhosis, and portal hypertension (8). Our data suggests that the combination of SOF/ELB/GZR±RBV is highly effective for the retreatment of DAA non-responders, in both GT3 and GT1/4/6 HCV infection. The overall SVR12 rate was 98%, and 95% amongst GT3 cirrhotic participants, similar to approved pan-genotypic salvage regimens (9, 10).

A small study has previously reported the efficacy of this regimen for the treatment of non-responders with GT 1/4 HCV infection (4) and the SVR12 rate was 100% (per protocol) in 25 patients. Our study is the first description of efficacy among a group of non-responders that included with patients with GT3 HCV infection who had relapsed after treatment with an NS5A containing regimen. In our study, 38/40 patients (21 who were GT3) had relapsed following treatment with a regimen including SOF plus an NS5A inhibitor. Both ELB and GZR have an EC₅₀ for GT3 that is at least 10-fold greater than for GT1 (3). In clinical trials of treatment naïve patients, SVR12 rates were only 41% for HCV GT3 infected patients treated with ELB/GZR for 12 weeks (5). In contrast, combination therapy with SOF/ELB/GZR±RBV has been shown to be effective for treatment naïve patients with GT3 HCV, including patients with cirrhosis (6), with SVR12 rates > 90%. We now show a similar rate of SVR12 in treatment experienced patients. Our data support synergy between SOF and ELB/GZR and effectiveness for HCV GT3. Of note, the rate of SVR12 in our cohort is numerically higher than that reported in a cohort of participants (including HCV GT1, GT2 and GT3 infection) who were re-treated with SOF/velpatasvir/RBV for 24 weeks following relapse after a first course of SOF/velpatasvir (n=63/69, 91%) (11).

There was one episode of virologic relapse in a HCV GT3 participant. The participant had previously relapsed following treatment with pegylated interferon and ribavirin and subsequently SOF/daclatasvir for 24 weeks, was cirrhotic and had virus with the Y93H NS5A RAS detectable at retreatment baseline which is associated with high level resistance to NS5A inhibitors (12). The participant reported good adherence to 12 weeks of SOF/ELB/GZR/RBV600 before relapsing. It is possible that longer treatment duration for 16 weeks may have been more effective.

Combination SOF/ELB/GZR/RBV was safe. The most frequent adverse events were haematological abnormalities associated with RBV. A dose reduction was only required in two cases. The most serious adverse event occurred in a HCV GT3 participant with advanced cirrhosis. At TW6 the participant's bilirubin level had risen from 30 to 50µmol/L and INR from 1.3 to 1.5. The participant was switched to SOF/velpatasvir/RBV due to concern about protease inhibitor related hepatotoxicity. The participant's hyperbilirubinaemia at TW6 may be attributable to RBV-associated haemolysis, however this does not

account for their INR prolongation. HCV NS3/4A protease inhibitors are contraindicated in Child Pugh B/C cirrhosis, in whom accumulation may cause hepatotoxicity, and participant with Child Pugh A cirrhosis should be closely monitored. Notably, the participant went on to achieve SVR12, suggesting that 6 weeks of protease inhibitor exposure, with a longer tail of SOF/velpatasvir/RBV was sufficient to eradicate virus.

Following the introduction of HCV DAAs, there was concern regarding potential increases in HCC incidence and recurrence with antiviral therapy (14). More recent data suggests this correlation is unlikely (15). In the *Revenge* study, a comparable population with HCV GT1/4 infection was treated with SOF/ELB/GZR/RBV, in which 19% (5/26) of the participants developed HCCs during the period of evaluation (4). The authors attributed this to the study populations advanced age, duration of HCV infection and high prevalence of cirrhosis, rather than to the study regimen. This is supported by our data where no recurrent or *de novo* HCCs were identified during the period of evaluation.

Two DAA regimens have recently been approved by the Federal Drug Administration and European Medicines Agency for the treatment of DAA non-responders. These include the combination of SOF/velpatasvir/voxilaprevir, approved for the treatment of all HCV genotypes, and glecaprevir/pibrentasvir, approved for retreatment of people with GT1 HCV previously treated with a regimen including either (but not both) an NS5A inhibitor or an NS3/4A protease inhibitor; and non-GT1 HCV who have previously been treated with SOF/PR. Whilst these regimens are licensed in the US and some countries in Western Europe, they are not yet available in all regions due to licencing or funding restrictions. Our data supports the combination of SOF/ELB/GZR±RBV as a second pan-genotypic re-treatment option, combining drugs which are currently licensed and available in most regions. The role of the different re-treatment options is likely to vary between regions according to cost and drug availability.

Study limitations include an inability to define the optimal duration for SOF/ELB/GZR±RBV and the role of RBV, particularly for HCV GT3 participants, for which prospective randomised studies are required. Until such time, we recommend treating GT3 infected patients for 16 weeks in combination with RBV, where possible.

In conclusion, the combination of SOF/ELB/GZR±RBV was safe and effective salvage treatment for patients who had not responded to a previous NS5A-containing DAA therapy. It was effective for the treatment of hard-to-cure GT3 as well as GT1/4/6 HCV infection. The DAAs in this regimen are currently available in many regions, and represent another option for treating patients who do not respond to a first course of DAA therapy, with the aim of stopping the progression of liver disease and reducing HCV-related morbidity and mortality.

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7 Chapter 7: Efficacy and safety of sofosbuvir/velpatasvir/voxilaprevir for HCV NS5A-inhibitor experienced patients with difficult to cure characteristics

7.1 Introduction and Summary

DAA therapy has revolutionised HCV treatment, and cure rates are greater than 95% in clinical trials (141, 164). There are, however, some patients who fail to achieve cure after first line DAA therapy. The combination of SOF/VEL/VOX includes three agents with activity against the NS5B, NS5A and NS3 proteins, respectively, and is now licenced for the management of HCV NS5A-experienced patients who have relapsed (374). At the time of this study, however, this regimen was not licenced or reimbursed for use in Australia.

The POLARIS-1 registration trial demonstrated excellent efficacy of combination SOF/VEL/VOX with cure rates exceeding 95%, which were sustained across all genotypes and fibrosis stage (374). Multiple real-world data however subsequently identified signals of reduced efficacy in those with GT3 infection, HCC and cirrhosis (375-377). In 2018, Gilead Sciences established an early access program making SOF/VEL/VOX available for Australians with relapsed HCV infection and advanced disease, including cirrhosis with PHT and prior LT. As such this population was well suited to investigating the signals of reduced efficacy identified in other real-world cohorts, while scrutinizing the safety of PI usage in those with advanced disease.

We therefore evaluated the safety and efficacy of SOF/VEL/VOX in a large cohort of patients with difficult to cure characteristics from 27 Australian hospitals. SVR12 rates overall were 90% and were sustained in those with GT3 infection (89%) and with cirrhosis (90%). We also noted that those with prior decompensation who had achieved recompensation during their initial DAA treatment tolerated therapy well and achieved cure, making this a reasonable treatment option for highly selected patients. There were, however, three episodes of hepatic decompensation events which further highlight the caution needed when using PIs in those with advanced disease.

This study has been accepted for publication in *Clinical Infectious Diseases*.

Reference:

Papaluca T, Roberts S, Strasser S, Stuart K, Farrell G, MacQuillan G et al. Efficacy and safety of sofosbuvir/velpatasvir/voxilaprevir for HCV NS5A-inhibitor experienced patients with difficult to cure characteristics. *Clinical Infectious Diseases*. 2020 September 5.

The text in its entirety is included in this chapter.

7.2 Original article

Efficacy and safety of sofosbuvir/velpatasvir/voxilaprevir for HCV NS5A-inhibitor experienced patients with difficult to cure characteristics

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7.2.1 Abstract

Background:

In clinical trials, HCV salvage treatment with Sofosbuvir/Velpatasvir/Voxilaprevir (SOF/VEL/VOX) achieved an SVR12 rate of >95% in NS5A-experienced participants. Lower SVR12 rates have been reported in real-world studies, particularly for genotype (GT)3 infection and cirrhosis. We determined the efficacy and safety of SOF/VEL/VOX in a large real-world cohort.

Methods:

We assessed the efficacy of salvage SOF/VEL/VOX for HCV infection in NS5A-inhibitor experienced participants with cirrhosis and portal hypertension, prior liver transplantation (LT) or severe extra-hepatic manifestations. SOF/VEL/VOX was available via an early access program. The primary outcome was SVR12. Secondary outcome was frequency of adverse events (AE).

Findings:

Ninety-seven participants were included. Median age was 58, 82% were male, 78% had cirrhosis, most with portal hypertension (61%, n=46/76), and 18% had prior-LT. Of the cirrhotic participants, 96% were Child-Turcotte-Pugh class A and 4% were class B. Of the 72% with GT3, 76% were also cirrhotic. By intention-to-treat analysis, SVR12 rate was 85% (n=82/97). Per protocol, the SVR12 rate was 90%, including 91% in GT1 (GT1a n=18/18, GT1b n=2/4), 89% in GT3 (n=59/66) and 100% in GT6 (n=3/3). SVR12 in participants with GT3 and cirrhosis was 90%. No predictors of non-SVR12 were identified. There were four serious AEs including one death and three hepatic decompensation events. NS5A resistance-associated substitutions detected at baseline did not affect SVR12.

Conclusion:

This real-world study confirms high efficacy of SOF/VEL/VOX for the treatment of difficult-to-cure NS5A-inhibitor experienced patients, including those with GT3 and cirrhosis. Treatment was well tolerated in most however serious AEs can occur in those with advanced liver disease.

7.2.2 Introduction

Hepatitis C virus (HCV) infection affects approximately 71 million people worldwide, and contributes significantly to liver related morbidity and mortality [1,2]. Direct acting antiviral (DAA) therapy has revolutionized HCV treatment, with cure rates exceeding 95% in registration trials [3-5]. Some patients, however, do not respond to their initial DAA therapy, more commonly those with HCV genotype (GT)3 infection and with cirrhosis [6,7]. Virological failure is associated with the selection of HCV resistance associated substitutions (RAS) and ideal retreatment regimens should target the NS5A, NS5B and NS3 proteins [8,9].

The efficacy of sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) for relapsed HCV infection following treatment with an NS5A-inhibitor (NS5Ai) containing DAA regimen has been investigated in multiple clinical trials [10]. In the POLARIS-1 registration trial, NS5A-experienced participants treated with SOF/VEL/VOX achieved a sustained virological response 12 weeks after end of treatment (SVR12) rate of 96%. Several real-world studies have subsequently suggested reduced efficacy in those with cirrhosis, prior SOF/VEL exposure and HCV GT3 infection [11-14]. This included a SVR12 rate of 69%

amongst the small number of participants with both HCV GT3 infection and cirrhosis in one Spanish cohort [11]. In most studies, a minority of participants had cirrhosis (35-41%) and only one included those with prior liver transplantation (LT), where complex drug-drug interactions must be considered [11-13].

In both registration and real-world trials, treatment with SOF/VEL/VOX was generally well tolerated and adverse events (AE) were limited. It is important to note that NS3/4A PIs are not recommended in Child-Turcotte-Pugh (CTP) B/C cirrhosis due to concerns about hepatotoxicity related to higher VOX drug exposures. The FDA has also recently cautioned practitioners to closely monitor patients with compensated cirrhosis during treatment with a HCV protease inhibitor (PI) due to the potential for hepatic decompensation, particularly in the setting of portal hypertension (PHT), hepatocellular carcinoma (HCC), or alcohol misuse [15]. As SOF/VEL/VOX is increasingly used as salvage treatment in patients with advanced disease to avoid LT, it is important that these risks are further explored and validated in real-world settings [16,17]. In this study, we have evaluated the efficacy and safety of SOF/VEL/VOX in a difficult to cure population with advanced liver disease or prior-LT.

7.2.3 Methods

This was a retrospective, nationwide study to evaluate the efficacy and safety of SOF/VEL/VOX for relapsed HCV infection amongst participants with advanced liver disease or LT, treated in 27 Australian centres. All participants were treated with fixed dose SOF 400mg, VEL 100mg and VOX 100mg for 12 weeks available via an early access program (EAP) supported by Gilead Sciences. The EAP permitted access to SOF/VEL/VOX before reimbursement on the national prescription drug scheme. As such, at this time, this regimen was only available to Australians via this EAP, where specific eligibility criteria were satisfied. Eligibility criteria for the EAP were: i) age greater than 18 years; ii) chronic HCV infection GT1-6; iii) prior treatment failure on a NS5A-inhibitor DAA containing regimen; iv) compensated liver disease with at least one of a) CTP class A cirrhosis and clinically significant portal hypertension (hepatic venous portal gradient (HVPg) >10mmHg or the presence of varices (radiologically / endoscopically) or platelets <100 x 10⁹/L, b) prior-LT, or c) severe extrahepatic manifestations; and v) eGFR ≥30mL/min/1.73m². Exclusion criteria for the EAP included i) hepatic decompensation (CTP class B/C), ii) contraindication or known hypersensitivity to the active substances or to any other component of the tablets and iii) concomitant prescription of rifampicin and/or rosuvastatin. Participants with HIV and/or hepatitis B virus (HBV) coinfection were eligible for inclusion. Combination therapy with ribavirin was permitted.

Australian hospitals with two or more participants treated via the Gilead Sciences EAP were invited to participate in this study. The primary outcome was the frequency of participants achieving SVR12,

defined as the absence of detectable HCV RNA at least 12 weeks after end-of-treatment (EOT). The secondary outcome was frequency of treatment related adverse events (AE). Participants were followed until the SVR12 time point.

Assessments

Participant data were collected at baseline, EOT, and SVR12 timepoints with SOF/VEL/VOX, as well as details of prior HCV treatments. Cirrhosis status was defined as transient elastography score ≥ 12.5 kPa, prior liver biopsy demonstrating METAVIR fibrosis score 4 or clinical/radiological evidence of cirrhosis. Clinical evidence of cirrhosis included signs of cirrhosis and baseline platelet count of $<100 \times 10^6$. PHT was defined by the presence of intra-abdominal and/or oesophageal varices, splenomegaly or ascites. CTP scores were recorded at all timepoints. Laboratory investigations recorded included full blood count, renal and liver biochemical tests, HCV RNA, HCV genotype, HIV and HBV serology. The presence of HCV NS5A RAS were investigated in a subset of participants where serum was available, utilising Sanger sequencing with a threshold limit of 20% for variant detection. HCV NS5A RAS were defined as polymorphisms encoded by the NS5A gene associated with at least two-fold reduced susceptibility to a registered NS5A inhibitor. Virological breakthrough (VBT) was defined as detectable HCV RNA on PCR testing at the EOT timepoint.

Adverse events (AE) related to treatment with SOF/VEL/VOX were recorded until the SVR12 timepoint. Serious AEs were particularly scrutinised including hepatic decompensation, and AEs leading to hospitalisation, treatment discontinuation and death.

Ethical considerations

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines, and regulatory requirements. The study was approved by the Human Research Ethics Committee at St Vincent's Hospital Melbourne.

Statistical analysis

Statistical analysis was performed using STATA 12.0 (StataCorp LP, TX, USA). Normally-distributed continuous data were analysed with the Student *t* test, whilst non-normally distributed data was analysed using the Mann-Whitney U test. Categorical data were described as number and percentage and were analysed using chi-square or Fisher's exact test, as appropriate. Variables associated with SVR12 were investigated using appropriate univariable statistical tests after considering distribution of data. A *p* value of <0.05 was deemed statistically significant.

7.2.4 Results

Patient population

From June 2018 to March 2019, 97 patients were commenced on SOF/VEL/VOX across 27 Australian hospitals. Baseline characteristics are described in Table 7.1. In brief, the cohort was predominantly male (82%), the median age was 58 years, and the most common genotype was HCV GT3 (72%). Most the cohort were cirrhotic (78%) or had previously undergone LT (18%). Of the cirrhotic participants, 50% were CTPA5, and 46% were CTPA6. Three participants were included with CTP class B7 (n=1) and B8 (n=2) as protocol deviations by the treating clinicians; none of these three participants were felt to be LT candidates. 61% of the cirrhotic participants had PHT. HIV (n=2) and HBV (n=3) coinfection were uncommon. A prior history of HCC was present in 19% (n=18), six of whom had undergone LT. There were no participants with HCC at baseline. SOF/VEL/VOX was prescribed with ribavirin (600-1200mg daily) for three participants.

Table 7.1: Baseline characteristics of participants commencing SOF/VEL/VOX.

Baseline Characteristics	N = 97
Age, median [IQR]	58 [53–64]
Male sex, n (%)	80 (82)
HCV viral load IU/mL, median, [IQR] (75/97)	654 000 [160 000–2 870 000]
HCV genotype, n (%)	
- Genotype 1a	19 (20)
- Genotype 1b	4 (4)
- Genotype 3	70 (72)
- Genotype 4	1 (1)
- Genotype 6	3 (3)
ALT, U/mL, median [IQR]	85 [48–170]
Albumin, g/L, median [IQR]	37 [33–40]
Bilirubin, umol/L, median [IQR]	14 [10–21]
Platelet count, median [IQR]	125 [90–178]
INR, median [IQR]	1.1 [1–1.2]
HIV infection, n (%)	2 (2)
HBV serology, n (%)	
HBsAg +	3 (3)
HBcAb +	29 (30)
Cirrhosis, n (%)	76 (78)
CPA5	38/76 (50)
CPA6	35/76 (46)
CPB7	1/76 (1)
CPB8	2/76 (3)
Cirrhosis with portal hypertension, n (%) ^a	46/76 (61)
Post liver transplantation, n (%)	17 (18)
Noncirrhotic, n (%)	4 (4)
Prior hepatocellular carcinoma n (%)	18 (19)
Pegylated interferon ± ribavirin ± protease inhibitor experienced, n (%)	24 (25)
2+ DAA treatments prior to SOF/VEL/VOX, n (%)	15 (15)

^a Portal hypertension defined as the presence of intra-abdominal and/or gastroesophageal varices, splenomegaly, or ascites. Legend: ALT; alanine aminotransferase; DAA, direct-acting antivirals; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalised ratio; IQR, interquartile range; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

Prior HCV treatment experience

All participants were HCV NS5Ai treatment experienced. The most recent HCV DAA treatments prescribed prior to SOF/VEL/VOX are detailed in 7.2. The most frequent common NS5Ai were daclatasvir (DCV, 56%), VEL (20%), and ledipasvir (LDV, 15%). 15% of participants (n=15) had received more than one NS5Ai-based treatment (range 1-4). Three participants were treated with an NS5Ai containing 'lead-in' DAA regimen immediately prior to commencing SOF/VEL/VOX to optimise liver function, including one who was downgraded from CTP class B to A during this treatment. Four other participants had a history of prior hepatic decompensation that had recompensated during their preceding first-line DAA treatment. 25% of participants had been treated with pegylated IFN- α + ribavirin with or without a first-generation PIs prior to their first all-oral DAA treatment course.

Table 7.2: Most recent DAA regimen prescribed prior to SOF/VEL/VOX.

Prior DAA course	N = 97
Sofosbuvir + daclatasvir $\pm$ ribavirin, n (%)	54 (56)
Sofosbuvir + velpatasvir $\pm$ ribavirin, n (%)	19 (20)
Sofosbuvir + ledipasvir $\pm$ ribavirin, n (%)	15 (15)
Elbasvir + grazoprevir, n (%)	3 (3)
Paritaprevir + ritonavir + ombitasvir + dasabuvir $\pm$ ribavirin, n (%)	2 (2)
Sofosbuvir + elbasvir + grazoprevir $\pm$ ribavirin, n (%)	2 (2)
Uprifosbuvir + ruzasvir, n (%)	2 (2)

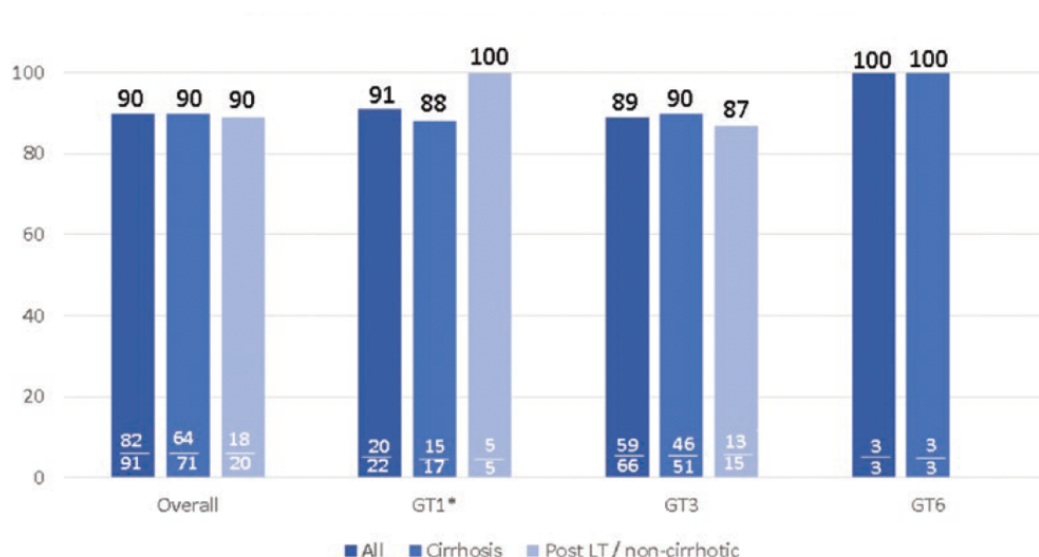
Legend: DAA, direct-acting antiviral; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

Efficacy

By intention to treat, 82 (85%) of the 97 participants commenced on SOF/VEL/VOX achieved SVR12, and SVR12 rates were 95% (n=18/19) for GT1a, 50% (n=2/4) for GT1b, 84% (n=59/70) for GT3, 0% (n=0/1) for GT4, and 100% (n=3/3) for GT6 infection. Three participants discontinued treatment due to AEs during treatment week (TW) one, two participants were lost to follow up after EOT and one participant died while receiving treatment. Of these six participants, four had GT3 infection. As such, complete data was available for per protocol analysis for 91 participants. Therefore, the per protocol SVR12 rate was 90% (n=82/91, Figure 1). Two participants had VBT at EOT, and seven relapsed. Per protocol, the SVR12 rate for participants with GT1a infection was 100% (n=18/18), 50% (n=2/4) for GT1b, 89% (n=59/66) for GT3, and 100% (n=3/3) for GT6. The SVR12 rate was 90% (n=64/71) for

cirrhotic participants, 88% (n=14/16) amongst those who were post-LT and 100% (n=4/4) in non-cirrhotic, non-LT participants. SVR12 was achieved in 90% (n=77/86) of participants previously treated with SOF+NS5Ai, and 100% amongst those previously treated with other combination regimens (n=5/5, Table 7.2).

Figure 7.1: SVR12 rates by genotype and cirrhosis status.



*GT1a SVR12 100% (n=18/18) and GT1b SVR12 50% (n=2/4). Legend: LT, liver transplantation; GT, genotype; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

Non-SVR participants

Nine participants had detectable HCV RNA at the SVR12 timepoint (Table 7.3). All nine participants reported adherence above 80%. Of the two participants with VBT, both had previously experienced VBT during their prior treatment with SOF + VEL. The remaining seven participants relapsed. Of the treatment failures, seven were cirrhotic, five of whom had PHT, and two were post-LT. Seven had HCV GT3 and two had HCV GT1b infection. HBV co-infection was present in one. HCV NS5A RAS testing was available for four non-SVR12 participants at baseline, in whom all had the Y93H RAS detected. One participant was diagnosed with *de novo* HCC at EOT. Two participants were treated with SOF/VEL/VOX following the recent diagnosis of non-Hodgkin lymphoma. Prior malabsorptive bariatric surgery was considered contributory to treatment failure in one participant. One participant was prescribed a proton-pump inhibitor with SOF/VEL/VOX but no other concomitant medications were recorded that may have caused significant drug-drug interactions (DDI). No clinical or virological characteristics were associated with likelihood of SVR12 on univariate analysis (Table 7.4).

Table 7.3: Characteristics of non-SVR12 participants.

Characteristic	#1	#2	#3	#4	#5	#6	#7	#8	#9
Sex	M	F	M	M	M	M	M	M	M
Age	64	51	57	62	66	50	46	64	62
Cirrhosis status	F4	F4	Post LT (no cirrhosis)	Post LT (no cirrhosis)	F4	F4	F4	F4	F4
Relapse vs VBT	Relapse	Relapse	Relapse	VBT	VBT	Relapse	Relapse	Relapse	Relapse
HCV GT	1b	1b	3	3	3	3	3	3	3
ALT	103	64	50	126	110	91	73	305	82
Albumin	37	36	44	32	42	38	35	33	37
Platelets	151	125	106	177	117	216	60	131	73
Bilirubin	14	30	8	7	14	12	26	13	21
Portal hypertension ^a	Y	N	N	N	Y	N	Y	Y	Y
CTP score	5	5	5~	5~	5	5	6	6	5
HIV / HBV coinfection	HBV	N	N	N	N	N	N	N	N
Ribavirin	N	N	N	N	N	N	N	N	N
NS5A RAS at baseline	Y93H	NA	NA	NA	Y93H L31I	Y93H	NA	NA	Y93H
Prior treatment	PrOD	SOF / LDV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / VEL
Prior VBT	N	N	N	Y	Y	N	Y	N	N
HCC at SVR12	N	N	N	N	N	N	N	Y	N
Non-HCC malignancy	N	NHL [#]	N	N	NHL [#]	N	N	N	N
DDI							PPI		
Other cofactors	Malabsorptive bariatric surgery								

^aPortal hypertension defined as the detection of intra-abdominal and/or gastroesophageal varices, splenomegaly, or ascites radiologically and/or endoscopically. ~Non-cirrhotic participants post LT. Legend: DAA, direct-acting antiviral; DCV, daclatasvir; DDI, drug-drug interactions; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LDV, ledipasvir; PrOD, paritaprevir/ritonavir/ombitasvir/dasabuvir; SOF, sofosbuvir; VBT, virological breakthrough; VEL, velpatasvir; NHL, non-Hodgkin's lymphoma; LT, liver transplantation.

HCV NS5A RAS testing

HCV NS5A RAS testing results were available for 54 (56%) participants. Overall, 91% had detectable NS5A RAS at baseline (n=49/54). Of those with detectable HCV NS5A RAS, 84% (n=41/49) had a single NS5A RAS, and 16% (n=8/49) had dual mutations identified (Table 7.5). The most frequent NS5A RAS was Y93H which was detected in 84% of positive samples. On per-protocol analysis, the presence of baseline HCV NS5A RAS was not associated with treatment failure (SVR12 94% vs 100%, p>0.05), nor was the presence of the Y93H RAS specifically (SVR 90% vs 100%, p=0.56).

Table 7.5: Baseline NS5A resistance associated substitutions (RAS), available for a subset of patients.

Baseline NS5A RAS	N = 54
No NS5A RAS detected	5 (9)
NS5A RAS detected	49 (91)
Single NS5A RAS detected	
Y93H	37/49 (76)
A30K	2/49 (4)
L31M/V	2/49 (4)
Dual NS5A RAS detected	
Y93H / A30K/H	3/49 (6)
A30K / L31M	2/49 (4)
L31M/I / H58P	2/49 (4)
Y93H / L31M	1/49 (2)

Table 7.4 Univariate analysis of factors associated with SVR12.

Variable	SVR12 (n = 82/91)	P value
Sex		.54
Male	68/76 (89%)	
Female	14/15 (93%)	
Age (years)	57.5	.86
> 65	9/10 (90%)	
≤ 65	73/81 (90%)	
Non-LT		.63
Cirrhosis	64/71 (90%)	
No cirrhosis ^a	4/4 (100%)	
Prior LT		.66
No	68/75 (91%)	
Yes	14/16 (88%)	
HCV viral load, IU/mL (n = 70)		.25
> 800 000	29/34 (85)	
≤ 800 000	34/36 (94)	
HCV genotype		.71
GT3 infection	59/66 (89%)	
Non-GT3	23/25 (92%)	
Baseline CTP score		.93
A5	31/36 (86%)	
A6	29/32 (91%)	
B7	1/1 (100%)	
B8	2/2 (100%)	
Portal hypertension ^b		.27
No	41/45 (91%)	
Yes	41/46 (89%)	
Albumin		.73
> 35	50/56 (89%)	
≤ 35	32/35 (91%)	
Platelet count		.86
Pl > 150	25/28 (89%)	
Pl ≤ 150	57/63 (90%)	
HCC, de novo / recurrent		.27
No	80/88 (91%)	
Yes	2/3 (66%)	
HCV NS5A (n = 50)		1.0
RAS present	44/47 (94%)	
No RAS	3/3 (100%)	
>1 prior NS5A-containing DAA course		.59
No	70/78 (90%)	
Yes	13/13 (100%)	
Prior peginterferon/ribavirin		.68
No	63/69 (91%)	
Yes	19/22 (86%)	

^aExcluding non-cirrhotic participants with prior LT. ^bPHT defined as the presence of intra-abdominal and/or gastroesophageal varices, splenomegaly or ascites. Legend: HCC, hepatocellular carcinoma; LT, liver transplantation; HCV, hepatitis C virus; GT, genotype; CTP, Child Pugh Turcotte; RAS, resistance associated substitution; DAA, direct-acting antiviral.

Safety

In most cases, treatment was well tolerated, however AEs that led to treatment discontinuation and hepatic decompensation were observed in a minority (Table 7.6). SOF/VEL/VOX was discontinued during TW1 in three participants – two developed severe abdominal pain, and one individual's eGFR declined from 48 to 17mL/min/m². There were three episodes of hepatic decompensation on treatment. Amongst the 18 participants with prior HCC (six of whom had undergone LT), one had recurrent HCC identified between EOT and SVR12 and one discontinued therapy during TW1 due to abdominal pain, but otherwise no AEs were recorded in this group.

The first participant who developed hepatic decompensation on-treatment had CTP class A5 cirrhosis with PHT at baseline secondary to HCV and prior alcohol misuse, with no history of hepatic decompensation. They did not attend review appointments whilst on treatment but presented at week one post-EOT with new ascites, bilirubin 119 umol/L and INR 1.5, thought to be treatment related. This participant did not consume alcohol whilst receiving SOF/VEL/VOX. Alternate causes of hepatic decompensation were excluded. This participant subsequently had gastric variceal haemorrhage at week five post EOT. SVR12 was achieved, however they had persistent ascites and were assessed for LT. The second participant was CTP class A6 with PHT, with no prior episodes of liver decompensation, at baseline. This participant, who had a second diagnosis of autoimmune thrombocytopaenia thought to be related to HCV, developed new grade 3 ascites and presumed encephalopathy at TW3 which was attributed to SOF/VEL/VOX. The participant was immunosuppressed and sepsis was considered as a secondary cause of decompensation, although bacterial cultures were negative. Treatment with SOF/VEL/VOX was continued with weekly review. At week six post EOT, the participant recompensated and diuretic and lactulose therapy were withdrawn. SVR12 was achieved. A third participant, who was CTP class A6 at baseline, presented at TW5 with oesophageal variceal haemorrhage attributed by their clinician to significant recidivist alcohol consumption. Variceal surveillance was up-to-date prior to treatment. Bleeding was controlled endoscopically, however the participant developed progressive hepatic decompensation and died two weeks later. At end of follow up, one recurrent and two de novo HCCs were identified which were detected between EOT and SVR12 timepoints. HCC surveillance was up to date in all three at baseline.

The three participants who were CTP class B at baseline tolerated treatment well and no AEs were reported, and their aggregate CTP scores were unchanged between baseline and SVR12 timepoints. The five participants that were CTP class A at baseline, but who had had prior clinical decompensation, and a further two treated with an immediate 'lead-in' regimen of SOF + NS5A-inhibitor also tolerated SOF/VEL/VOX well and no AEs were recorded. Amongst participants with LT, there was no adverse effects on graft function or DDIs recorded.

Table 7.6: Twenty adverse events reported in 15 patients.

Adverse events, n (%)	N = 97
Treatment related	
Nausea	4 (4)
Fatigue	3 (3)
Abdominal pain	2 (2)
Diarrhea	2 (2)
Headache	1 (1)
Vertigo	1 (1)
Weight gain	1 (1)
Mood disturbance	1 (1)
Serious adverse events	
Hepatic decompensation	3 (3)
Death	1 (1)
Deteriorating renal function	1 (1)
Leading to discontinuation of treatment	
Treatment related	
Abdominal pain	2 (2)
Deteriorating renal function	1 (1)

7.2.5 Discussion

This is the first Australian real-world study to investigate the efficacy and safety of SOF/VEL/VOX amongst participants with relapsed HCV infection following treatment with an NS5Ai-containing DAA regimen, and advanced liver disease or LT. Whilst the per protocol SVR12 rate of 90% was lower than top-line results in the registration trial, our sample was notable for “difficult-to-cure” characteristics including cirrhosis, PHT and HCV GT3 infection.

The cohort is unique in its complexity including a high prevalence of cirrhosis (78%), the majority with PHT (61%, n=46/76), which was notably higher than in POLARIS-1 and other SOF/VEL/VOX real-world studies (prevalence of cirrhosis 34-46%) [10-13]. The majority of participants also had HCV GT3 infection, which has been associated with increased likelihood of relapse after SOF/VEL/VOX in some real-world data, and approximately one fifth had prior HCC [11]. It is the largest real world study of relapsed patients with GT3 HCV. Our cohort also included the largest number of LT recipients outside of registrations trials, demonstrating that LT recipients can be safely treated and potential DDI appropriately managed. As expected, where virological testing was available, there was a high prevalence of HCV NS5A RAS at baseline (90%, n=49/54), and 15% had previously failed multiple NS5Ai-containing DAA regimens, in some cases up to four. Despite these difficult-to-cure characteristics, we show that 12 weeks SOF/VEL/VOX can achieve high rates of cure in clinical practice.

Our analysis did not identify any association between treatment outcomes and HCV genotype. The SVR12 rate was 89% among patients with GT3 HCV. This SVR12 proportion is similar to that observed

in POLARIS-1, both in participants with GT3 HCV infection and cirrhosis, and in the overall cirrhotic population [10]. The rate of SVR12 observed in our GT3 patients is higher than that described by Llaneras and colleagues in Spain, who observed SVR12 rates of 80% (n=24/30) in GT3 infection and 69% (n=9/13) among GT3 cirrhotics, and from Degasperri and colleagues in Italy (SVR12 84%, (n=16/19) among GT3 cirrhotics) [11,13]. The SVR12 data in our cohort are more similar to those with GT3 cirrhosis reported by Belperio in a Veteran's Affairs cohort (SVR12 = 91%, n = 21/23) [12]. The reasons for the differences between SVR12 rates reported in these respective cohorts is not clear, but our data provides reassuring evidence that in a large real-world cohort of patients with GT3 cirrhosis and advanced disease, SVR12 rates remain high.

The high prevalence of participants with GT3 infection in this cohort, most of whom were cirrhotic, was notable. The community prevalence of GT3 HCV infection is approximately 39% in Australia [19]. The frequency of GT3 infection in our cohort exceeded 70%, consistent with GT3 remaining harder to cure than non-GT3 HCV after first-line DAA regimens, particularly in patients with cirrhosis (5). Most GT3 participants in our study were previously treated with SOF+DCV or SOF+VEL. GLE+PIB was not available in Australia at the time this study was conducted. European guidelines no longer recommend 12 weeks SOF+VEL as a first line treatment for GT3 infection and cirrhosis due to suboptimal outcomes in this group [18]. The high prevalence of GT3 in our study cohort highlight the importance of optimising first-line DAA therapy for patients with GT3 infection and cirrhosis to minimise relapse and the need for salvage treatment.

Overall safety of SOF/VEL/VOX was acceptable however there were three episodes of hepatic decompensation in previously well compensated CTP class A participants during treatment. As salvage triple therapy including PIs are increasingly prescribed to patients with advanced disease, the data highlight the need for regular clinical review and laboratory investigations to monitor for PI toxicity for at-risk patients during treatment. When toxicity is detected, treatment should be immediately discontinued. Where patients are LT candidates, we recommend review by a transplant board prior to commencing treatment.

Three participants with CTP class B disease were commenced on SOF/VEL/VOX, beyond the EAP criteria. Whilst no hepatic toxicity was noted in these cases, the use of NS3/4A PI in patients with CTP class B/C cirrhosis cannot be recommended. There were five other participants who were downstaged to CTP class A severity from CTP class B/C during prior treatment with SOF + NS5A (VEL/DCV), which was prescribed as a 'lead-in' regimen immediately prior to SOF/VEL/VOX in one; all tolerated treatment well with close monitoring. In patients who are not candidates for LT, we believe the lead-in strategy regimen of SOF + NS5Ai to optimise liver function and downstage to CTP class A, prior to introducing VOX, warrants prospective evaluation given our positive experience.

Baseline RAS testing was available for 54 participants and the presence of NS5A RAS was not associated with treatment failure. This is in keeping with results of a post-hoc analysis of SOF/VEL/VOX registration data demonstrating that multitargeted combination therapy can overcome baseline resistance quasispecies [20]. There is therefore a limited role for HCV NS5A sequencing in routine clinical management of these patients.

Second-line salvage therapies for the small number of patients who fail SOF/VEL/VOX are not well defined. As extension of treatment duration and regimen intensification with ribavirin was shown to improve SVR12 rates amongst DAA-experienced patients prior to the registration of relapse regimens including triple DAA therapy, we believe that both 24 weeks SOF/VEL/VOX±ribavirin or 16 weeks SOF+GLE/PIB±ribavirin present reasonable retreatment strategies for SOF/VEL/VOX failures [21]. Whilst ribavirin was included with SOF/VEL/VOX for three participants with GT3 and cirrhosis in our cohort, this number was too small to determine its effect.

In conclusion, SOF/VEL/VOX was an effective option for treatment of patients with difficult-to-cure characteristics who do not respond to a first-line DAA regimen, including patients with cirrhosis, GT3 HCV and prior-LT. Overall this treatment regimen was well tolerated, however serious AEs can occur in those with advanced liver disease.

7.2.6 References

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8 Chapter 8: Summary and future directions

8.1 Summary

There has been significant progress in the management of HCV infection over the last decade, primarily due to the introduction of DAA therapies. The persisting challenges, however, include how we best identify and treat the 71 million people living with HCV infection and manage the small minority who do not achieve cure following first-line therapy. This thesis identifies strategies to address these remaining challenges including the effective management of populations who have historically been more difficult to engage in HCV care including prisoners, PWIDs and other socially marginalised people. This thesis also advances our knowledge regarding the effectiveness and safety of second line salvage therapies for relapsed HCV infection in those with advanced liver disease, important to minimisation of liver related death and disability.

The evaluation of our prison-based, nurse-led, decentralised HCV program identified that DAA treatment within correctional settings is highly effective, safe and can reach prisoners in large numbers. HCV cure rates for those with complete follow up was similar to DAA registration trials and adverse events were infrequent. Our analysis also demonstrated that over 80% of the prisoners had never previously sought specialist HCV care, identifying the prison as a unique setting to engage those poorly served by existing healthcare models. Furthermore, as 68% of our cohort identified as current PWIDs our data highlights the important role the prison-based treatment can play in interrupting transmission and achieving the WHO elimination targets. Since the program's inception in 2015, 3,600 prisoners have been assessed and over 2,300 commenced on HCV treatment (406). Furthermore, over 30% of HCV treatments prescribed in Australia in 2019 were within the prison system (407). As such, prison-based HCV will continue to remain incredibly important to achieving HCV elimination.

One practical barrier to prison-based hepatitis C care is the need for liver fibrosis evaluation. Transient elastography is recommended as the standard of care test by most guidelines, however, it is expensive, operator dependent and there are access issues for bringing a transient elastography machine onsite. We identified that the use of simple algorithms including the APRI and FIB-4, calculated with routine blood test results, can achieve excellent NPVs amongst prisoners for excluding cirrhosis, thereby minimising the need for transient elastography. We also identified the utility of age thresholds in fibrosis assessment pathways which excluded cirrhosis with high confidence. The implementation of such fibrosis assessment pathways could significantly improve prison-based HCV treatment throughput which has important implications for both the individual and community more broadly.

Despite the effectiveness of prison-based HCV programs, there remain multiple policy, financial, logistic and prisoner-related barriers in many regions to their widespread implementation. We believe these data should support policy makers to develop and justify prison-based models of care to promote elimination of HCV as a public health threat.

Not all prisoners can be treated during incarceration. Linkage to hepatitis care post-release is therefore important. Prior data has identified the challenges of healthcare continuity amongst individuals following prison release, in part due to other immediate competing priorities. This thesis has identified that similarly linkage to HCV care following community re-entry is poor. Overall, only 25% of those released untreated had been prescribed HCV treatment within six months of re-entry and the sole predictor of DAA initiation was reincarceration. Our subsequent prospective randomised controlled trial evaluated a novel model of care which streamlined DAA initiation without significant fiscal or time expenditure and monitored for reincarceration, anticipating high recidivism rates in keeping with Australian data. Our decentralised, community-based treatment model was effective and 73% commenced treatment. The data also highlighted the importance of possessing accurate contact information for re-entering individuals to facilitate successful community engagement and the important role played by care navigators in initiating and sustaining contact to prioritise treatment amongst individuals' other important matters.

The second half of this thesis addressed HCV viral resistance, including both the prevalence of resistant quasiespecies at baseline amongst DAA naïve Australians, and the efficacy of HCV salvage regimens in DAA-experienced individuals with relapse. Australian HCV guidelines do not currently recommend routine NS5A RAS testing at baseline. This recommendation is supported by our data which demonstrated a lower NS5A RAS prevalence in GT1a infection at baseline than in other regions internationally. This is relevant as HCV GT1a NS5A RAS detected at baseline can significantly reduce treatment efficacy when particular DAA regimens are prescribed. The prevalence however was comparative for GT1b and GT3 infection. We also demonstrated that as baseline NS5A RAS prevalence is low, the use of next generation sequencing, a more expensive, more technically demanding technique, has limited utility over population-based sequencing for this indication.

The next study evaluated the efficacy and safety of SOF, ELB, GZR $\pm$ RBV for NS5A-inhibitor experienced participants with HCV relapse following first-line DAA treatment. Our study builds on the existing data from REVENGE and C-ISLE trials, and for the first time demonstrates high efficacy of SOF, ELB, GZR + RBV for DAA-experienced GT3 participants with cirrhosis, as well as for non-GT3 infection. Despite the high EC50 for ELB and GZR for HCV GT3, the synergy of ELB + GZR in combination with SOF was able to overcome resistance and achieve high rates of cure. This regimen therefore presents a

cost-effective pan-genotypic option for resource limited settings where generic SOF could be combined with ELB/GZR. Such strategies are important to minimise liver related morbidity and mortality in those with advanced disease.

The final study evaluated the efficacy and safety of SOF/VEL/VOX for relapsed HCV infection amongst NS5A-inhibitor experienced participants with difficult to cure characteristics. Several real-world studies had identified reduced efficacy of SOF/VEL/VOX in the setting of certain baseline characteristics including GT3 infection, cirrhosis, HCC and prior VEL treatment experience. Our study contributes to the global SOF/VEL/VOX post-marketing experience and provides reassuring evidence of high efficacy for relapsed HCV despite these patient characteristics, all of which were well represented in our cohort. We also demonstrated successful treatment of individuals with prior hepatic decompensation, some who received a non-PI containing lead-in regimen to optimise hepatic function prior to introduction of VOX. There were however three hepatic decompensation events which highlight the importance of careful monitoring when prescribing PIs to those with advanced disease.

8.2 Future directions

The knowledge gained from this thesis can be applied locally and internationally to encourage the propagation of similar prison-based HCV treatment services to make a meaningful impact on HCV prevalence and incidence. As prison-based treatments now account for over 25% of DAA prescriptions in Australia, these data highlight the important contribution the prison setting can play in engaging marginalised individuals and achieving the WHO viral hepatitis elimination targets globally.

The evaluation of the Statewide Hepatitis Service demonstrated that cure rates were high, and that prisoners could be treated in large numbers. The analysis, however, revealed that many individuals are released from prison untreated due to short sentence durations. Further evaluation is therefore required to determine how prison-based HCV treatment throughput can be enhanced. Victorian prisons currently have a policy of opt-in testing for HCV infection on reception and during subsequent transfers. The implementation of opt-out testing could significantly increase testing coverage and identify a greater number of prisoners living with HCV for treatment. The establishment of key performance indicators for HCV testing on prison reception could also aid in prioritising this important task amongst other reception priorities. Other potential strategies for evaluation include the implementation of point of care diagnostics at prison reception and streamlined treatment initiation to minimise the interval between incarceration and DAA commencement. Models of shared care by which treatment is initiated in prisons with community follow arranged with the existing network of clinical hepatitis nurse consultants also warrants evaluation. The remaining barriers to prison-based

HCV management will also be investigated. A qualitative study interviewing key stakeholders including prisoners, prison healthcare and security staff and program personnel will be undertaken to identify the key ongoing challenges to efficient and effective care.

More broadly, the findings of our prison evaluation should be used to advocate for similar programs internationally. It is estimated that HCV prevalence exceeds 15% in prisons globally and that prison-based treatments can significantly reduce community HCV incidence and prevalence. Future works must therefore investigate the cost-effectiveness of our model to highlight this as a viable and economical option for other jurisdictions and regions internationally. Another important consideration will be the investigation of HCV reinfection rates following successful treatment in prisons. It is anticipated that widespread prison-based treatment will confer a reduction in anticipated reinfection rates due to treatment as prevention, however this requires formal evaluation.

This thesis also presents the first randomised controlled data regarding the effectiveness of HCV transitional care for released prisoners. Extensive social, educational and psychological participant characteristics were not adequately captured, however, due to the time constraints of prison-based assessments. Therefore, ongoing qualitative assessment of individuals' experiences of HCV treatment, and community re-entry more broadly, will be investigated to enhance the model's effectiveness and acceptability. There is an existing network of hepatitis C clinical nurse consultants throughout Victoria, Australia, who have an important role in engaging primary health services and providing them with support and mentoring to treat people with HCV infection. They also collaborate with specialist HCV clinics in a shared-care model to assist with the engagement, assessment and treatment of people living with HCV. This existing network is well placed to provide transitional hepatitis C care for re-entering individuals, which has been demonstrated as effective in this thesis. Discussions with the Department of Health and Human Services are in progress to formalise this arrangement and to provide appropriate resourcing for the continuation of this important work.

This thesis reported the prevalence of HCV NS5A RAS in GT1 infection at baseline, and for the first time in Australia, GT3 infection. These data provide reassuring evidence which support Australian HCV guidelines that RAS testing is not required at baseline. In collaboration with VIDRL, we have also performed HCV NS5A and NS3 RAS sequencing in a large number of DAA-experienced patients with relapse. This data will contribute to a collaborative study with other Australian jurisdictions to investigate NS5A and NS3 RAS profiles amongst DAA-experienced individuals with relapse and their impact on retreatment strategies.

Our evaluation of SOF/VEL/VOX for relapsed HCV also raised important clinical queries for evaluation. Firstly, despite excellent treatment efficacy, a small number of individuals did not achieve SVR12.

Currently, retreatment strategies following unsuccessful second-line salvage therapy are not well defined. Prospective evaluation of extended treatment with SOF/VEL/VOX ± RBV or 16 weeks SOF + GLE/PIB ± RBV are therefore required. Given the small numbers of patients who fail both first- and second-line DAA therapy however, multicentre collaboration will be required. There are however commercial limitations to conducting this research as neither extended-duration SOF/VEL/VOX ± RBV or 16 weeks SOF + GLE/PIB ± RBV are reimbursed by the Australian Pharmaceutical Benefits Scheme. Our data also highlighted the effectiveness of SOF/VEL/VOX for participants with prior clinical decompensation who achieved recompensation during lead-in SOF + NS5A-inhibitor. This strategy too warrants prospective evaluation as a reasonable approach for carefully selected patients.

In conclusion, this thesis provides important insights in the management of populations with difficult to treat HCV infection, either due to challenges with engagement or based on their viral characteristics. The findings demonstrate that prisons offer an important opportunity to engage and treat marginalised individuals in a manner that is safe and effective, and though implementing new models of care which are dynamic and streamlined, engagement following community re-entry can also be enhanced. Finally, these data also identify high efficacy and novel strategies for prescribing second-line salvage therapy for relapsed HCV in those with advanced disease, which is important to minimise decompensation events and liver related mortality.

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10 Appendices

10.1 Chapter 2 supplemental data

Supplemental table 2.1: Treatment failures.

Supplemental Table 2.1 – Treatment failures				
Genotype	Cirrhosis	Therapy	Duration	Treatment Interruption *
1a	No	SOF / LDV	8 weeks	Yes
1a	No	SOF / LDV	8 weeks	Yes
1a	No	SOF / LDV	8 weeks	Yes
1a	No	SOF / LDV	8 weeks	Yes
1a	No	SOF / LDV	12 weeks	Yes
3a	No	SOF / DCV	12 weeks	Yes
3a	No	SOF / DCV	12 weeks	Yes
3a	No	SOF / DCV	12 weeks	No
3a	Yes	SOF / DCV	24 weeks	Yes
3a	Yes	SOF / DCV	24 weeks	No
3a	Yes	SOF / DCV	24 weeks	No

*Treatment interruption; three prisoners who had virological relapse had treatment interruptions of 1, 17, and 18 weeks, respectively. Legend: SOF, sofosbuvir; LDV, ledipasvir; DCV, daclatasvir.

Supplemental table 2.2: Assessments at each of the 14 individual prisons and the anticipated proportion of viraemic individuals treated.

Prison	Security ¹	Assessed	Eligible ²	Ineligible			Treatment started	SVR12 outcome			Treated later ⁵	Treatments per prison population ⁶	Proportion HCV infected prisoners treated ⁷	
				PCR neg	Inadequate time ³	Other ⁴		Neg	Pos	LTF			50% viraemic ⁸	75% viraemic ⁹
1	Max	110	75	16	19	0	43	29	1	13	32	10.1%	42%	28%
2	Min	32	11	7	14	0	10	5	1	4	1	5.8%	24%	16%
3	Max	67	37	17	13	0	29	26	0	3	8	7.6%	32%	21%
4	Min	55	27	8	19	1	21	13	0	8	6	7.6%	32%	21%
5	Med	75	44	12	19	0	37	27	0	10	7	4.7%	20%	13%
6	Med	109	78	6	23	2	63	52	2	9	15	9.4%	39%	26%
7	Min	46	31	3	11	1	27	19	0	8	4	7.3%	30%	20%
8	Min	55	37	5	13	0	31	18	3	10	6	6.9%	29%	19%
9	Med	125	71	19	35	0	51	40	1	10	20	8.9%	38%	25%
10	Max	4	3	0	1	0	2	2	0	0	1	0.7%	3%	2%
11	Max	54	29	8	16	1	13	10	1	2	16	1.7%	7%	5%
12	Max	166	87	33	44	2	61	43	2	16	26	5.7%	24%	16%
13	Min	16	9	4	2	1	7	3	0	4	2	13.0%	58%	37%
14	Min	35	23	6	6	0	21	14	1	6	2	8.9%	36%	24%
												Average	27%	18%

¹Security level of prison rated as maximum (Max), medium (Med) or Minimum (Min) security.

²Hepatitis C RNA PCR positive and adequate remaining incarceration duration to provide complete hepatitis C treatment

³Inadequate remaining period of incarceration to provide entire HCV treatment

⁴Prisoner refusal, drug-drug interactions resulting in treatment deferral

⁵Hepatitis C treatment initiated whilst incarcerated, but beyond the period of evaluation

⁶Number of prisoners treated at each prison site, relative to average prison population over period of evaluation as documented in 'Monthly Prisoner and Offender Statistics', Corrections Victoria, State Government of Victoria 2016, accessed at <http://www.corrections.vic.gov.au/home/prisoner+and+offender+statistics>

⁷Based on an average period of incarceration of 6 months, and HCV antibody prevalence of 24% from National Prison Entrants' Bloodborne Virus and Risk Behaviour Survey Report 2004, 2007, 2013 and 2016 accessed at <https://kirby.unsw.edu.au/report/national-prison-entrants-bloodborne-virus-and-risk-behaviour-survey-report-2004-2007-2010>

^{8,9} Assuming that of HCV seropositive prisoners, 50% or 75% respectively are also HCV RNA positive

10.2 Chapter 3 supplemental data

Supplemental table 3.1: Community contacts provided by participants for engagement following release.

Supplemental table 3.1: Points of contact provided by study participants	N = 38
- Personal number	12
- Parent	10
- Alternate family member	7
- Partner	6
- Peer	2
- Healthcare worker	1
Initial point of contact for participants who could be reached in the community	N = 15
- Parent	7
- Personal number	4
- Alternate family member	3
- Partner	1

10.3 Chapter 4 supplemental data

Supplemental table 4.1: The sensitivity, specificity, NPV and PPV of FIB-4 for advanced fibrosis (LSM $\geq$ 9.5kPa) compared with TE.

		Liver stiffness measurement					
	All prisoners (n=1007) n (%)	< 9.5kPa (n=791) n (%)	$\geq$ 9.5kPa (n=216) n (%)	Sensitivity %	Specificity %	PPV %	NPV %
For prediction of advanced fibrosis							
FIB-4							
≤ 1.45	713 (71)	635 (80)	78 (36)	64	80	47	89
> 1.45	294 (29)	156 (20)	138 (64)				
≤ 3.25	960 (95)	785 (99)	175 (81)	18	99	87	82
> 3.25	47 (5)	6 (1)	41 (19)				

Legend: PPV, positive predictive value, NPV, negative predictive value, TE, transient elastography, LSM, liver stiffness measurement.

Supplemental table 4.2: Sensitivity, specificity, PPV and NPV of fibrosis assessment pathway incorporating age and APRI thresholds applied to validation cohort.

		Liver stiffness measurement					
	All prisoners (n=189) n (%)	< 12.5kPa (n=169) n (%)	$\geq$ 12.5kPa (n=20) n (%)	Sensitivity %	Specificity %	PPV %	NPV %
For prediction of cirrhosis							
Age + APRI							
>35 years and APRI ≥ 1.0	33 (17)	18 (11)	15 (75)	75	89	45	97
≤ 35 or > 35 and APRI < 1.0	156 (83)	151 (89)	5 (25)				

Legend: PPV, positive predictive value, NPV, negative predictive value

10.4 Chapter 6 supplemental data

Supplemental table 6.1: Prevalence of NS5A RAS at baseline.

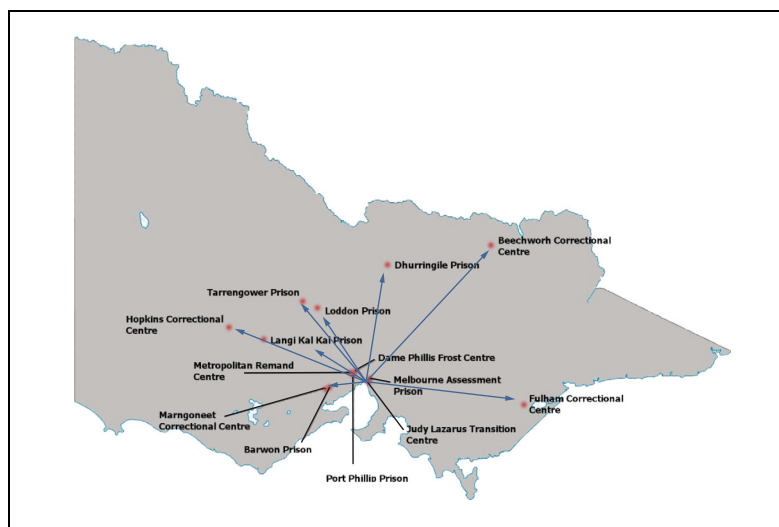
HCV NS5A RAS	N = 26*
Y93D/H/S	13 (4/8/1)
Q30E/R	2 (1/1)
L31M	1
A30K	1
Not detected	9

*HCV NS5A RAS testing was available for 26 of 40 participants at baseline. Legend: HCV, hepatitis C virus; RAS, resistance-associated substitution.

10.5 Print versions of the published manuscripts

Outcomes of treatment for hepatitis C in prisoners using a nurse-led, statewide model of care

Graphical abstract



Highlights

- Nurse-led care was associated with SVR12 rates of >95% in large numbers of prisoners.
- <20% of prisoners required specialist input.
- >80% of prisoner had never pursued specialist hepatitis C care in the community.

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Lay summary

There is a high burden of hepatitis C infection among prisoners worldwide. Prisoners who continue to inject drugs are also at risk of developing new infections. For this reason, the prison setting provides an opportunity to treat those people at greatest risk of infection and to stop transmission to others. We developed a new method of providing hepatitis C treatment to prisoners, in which nurses rather than doctors assessed prisoners locally at each prison site. Treatment was safe and most prisoners were cured. Such programs will contribute greatly to achieving the World Health Organization's hepatitis C elimination goals.



Outcomes of treatment for hepatitis C in prisoners using a nurse-led, statewide model of care

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Background & Aims: Treatment programs for people who inject drugs (PWID), including prisoners, are important for achieving hepatitis C elimination targets. There are multiple barriers to treatment of hepatitis C in prisons, including access to specialist physicians, testing and antiviral therapy, short prison sentences, and frequent inter-prison transfer. We aimed to assess the effectiveness of a nurse-led model of care for the treatment of prisoners with hepatitis C.

Methods: A statewide program for assessment and management of hepatitis C was developed in Victoria, Australia to improve access to care for prisoners. This nurse-led model of care is supported by telemedicine to provide decentralized care within all prisons in the state. We prospectively evaluated the feasibility and efficacy of this nurse-led model of care for hepatitis C within the 14 adult prisons over a 13-month period. The primary endpoint was sustained virological response at post-treatment week 12 (SVR12) using per protocol analysis.

Results: There were 416 prisoners included in the analysis. The median age was 41 years, 90% were male, 50% had genotype 3 and 44% genotype 1 hepatitis C and 21% had cirrhosis. Injecting drug use was reported by 68% in the month prior to prison entry, 54% were receiving opioid substitution therapy, and 86% reported never previously engaging with specialist HCV care. Treatment duration was 8 weeks in 24%, 12 weeks in 59%, and 24 weeks in 17% of treatment courses. The SVR12 rate was 96% (301/313) per protocol. Inter-prison transfer occurred during 26% of treatment courses but was not associated with lower SVR12 rates. No treatment-related serious adverse events occurred.

Conclusion: Hepatitis C treatment using a decentralized, nurse-led model of care is highly effective and can reach large numbers of prisoners. Large scale prison treatment programs should be considered to support hepatitis C elimination efforts.

Lay summary: There is a high burden of hepatitis C infection among prisoners worldwide. Prisoners who continue to inject drugs are also at risk of developing new infections. For this

reason, the prison setting provides an opportunity to treat those people at greatest risk of infection and to stop transmission to others. We developed a new method of providing hepatitis C treatment to prisoners, in which nurses rather than doctors assessed prisoners locally at each prison site. Treatment was safe and most prisoners were cured. Such programs will contribute greatly to achieving the World Health Organization's hepatitis C elimination goals.

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Introduction

The World Health Organization (WHO) has set global targets for the elimination of viral hepatitis as a public health threat.¹ For hepatitis C, the goals are to reduce incidence by 80% and mortality by 65% by 2030. In Western countries where injecting drug use is the dominant risk factor for hepatitis C transmission,² eliminating incident infection will require coordinated efforts using harm reduction strategies as well as “treatment as prevention” to interrupt transmission among people who inject drugs (PWID). This population has not typically been well engaged with specialist care, highlighting the need to develop new models of care for hepatitis C among marginalized, high transmitting populations.

Prison systems provide a unique opportunity to scale-up hepatitis C treatment.³ The prevalence of hepatitis C is higher in prisoners than in the general population, reflecting the criminalization of drug use and the frequent detention of PWIDs.^{4,5} In Australia, 46% of prisoners self-report a lifetime history of injecting drug use, and the hepatitis C seroprevalence among incarcerated PWID is greater than 50%.⁶ Harm reduction strategies currently available in Australian prisons include bleach for cleansing of injection devices and opioid substitution therapy (OST). Despite this, ongoing incident hepatitis C infection has been reported among PWID in Australian prisons.⁷ The median sentence length in Australia is less than 6 months and there are high rates of recidivism, meaning that prisoners frequently cycle between incarceration and freedom, creating new networks for hepatitis C transmission.⁸ Mathematical modelling studies have demonstrated that the global elimination of HCV will require widespread treatment of key risk populations, in particular PWID.^{9,10} Treatment programs in prisons, where a

Keywords: Hepatitis C; People who inject drugs; Prisoner; Elimination; Direct acting antiviral.

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large numbers of PWID can be screened and treated, could therefore significantly contribute to the HCV elimination agenda.⁹

Despite WHO recommending universal screening of all prisoners for hepatitis C,³ rates remain low in many regions worldwide and treatment programs have been limited by multiple barriers.^{11–14} Historical barriers have included the nature of interferon-based therapy (parenteral, long duration, significant toxicity and poor efficacy) as well as systemic barriers (limited local access to clinicians, need for hospital-based specialist review, frequent transfer between prisons interrupting care, and budgetary policy requiring that funding for testing and treatment come from local prison budgets rather than national health schemes). The recent introduction of direct-acting antivirals (DAAs) for the treatment of hepatitis C has provided highly effective therapy that is simple and safe, with short treatment durations – ideal for the prison environment. However, DAAs are expensive, which has limited the implementation of prison treatment programs worldwide;¹⁵ less than 20% of surveyed European countries provided access to hepatitis C treatment in all prisons.¹⁴ Where treatment is available to people in custody, it has often been restricted to prisoners with advanced liver disease.^{16,17} Such a policy will withhold effective treatment from most people living with hepatitis C in prison, including those at risk of transmission.

In March 2016, the Australian Government approved universal access to DAAs for all Australians living with chronic hepatitis C, including prisoners. A decentralized nurse-led model of care was therefore developed to make viral hepatitis assessment and treatment available to all prisoners across all 14 prisons in the state of Victoria, Australia. We present the first evaluation of the feasibility and efficacy of this novel model of care for hepatitis C.

Patients and methods

Model of care

Australia has a policy of state-sponsored universal health care for all members of the general community, managed by the federal government. However, prisoners are not eligible for this scheme, and medical care during the period of incarceration becomes the budgetary responsibility of the local correctional service under the state government. Such a funding silo challenges the feasibility of DAA therapy given the high list price of these medications. The Australian Government, recognizing the importance of providing DAAs to prisoners if hepatitis C elimination is to be achieved, classified DAA treatment for hepatitis C under both the general drug schedule for the community, as well as a highly specialized schedule, which allows drugs to be provided to prisoners with the requirement that they are prescribed by a specialist and dispensed to prisons from a hospital pharmacy.

In this context, the Victorian Statewide Hepatitis Program was developed by St Vincent's Hospital, Melbourne under contract to the Department of Justice and Regulation, State Government of Victoria, Australia. At the time of evaluation, there were 14 prisons for adults (12 male, 2 female) in the state of Victoria, with capacity for 7,441 prisoners (Fig. 1). In the 12 month period from November 2015, more than 10,500 prisoners were received into the Victorian prison system.¹⁸ Each of the 14 Victorian prison sites was serviced by the program, as described below. The program staff included 2 full-time hepatitis program

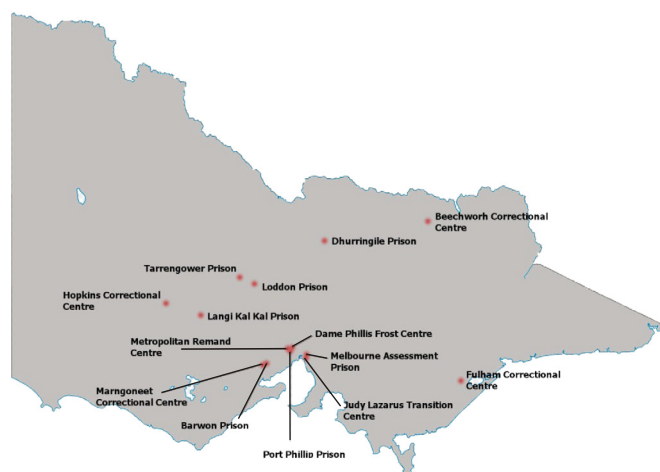


Fig. 1. Distribution of Victoria Prisons, Australia. (This figure appears in colour on the web.)

nurses, 3 part-time hepatologists (0.25 equivalent full-time (EFT) staffing in total), and a central pharmacist and pharmacy technician (time commitment increased from 0.2 to 0.6 EFT during the period of evaluation). A centralized electronic medical record was accessible at each prison as well as from St Vincent's Hospital. The medical record could be accessed by nursing, medical, and pharmacy staff to review and coordinate relevant investigations and track prisoner movements across the system to minimize interruptions of care from referral to the sustained virological response at post-treatment week 12 (SVR12) time-point.

Victorian prison healthcare policy is that all prisoners are offered opt-in screening for viral hepatitis at first prison reception and at each transfer between prison sites. This is the responsibility of the primary healthcare service at each prison. If a prisoner was seropositive for hepatitis C, or self-reported a previous diagnosis of hepatitis C, they were referred to the Statewide Hepatitis Program for protocol-driven face-to-face assessment by a program nurse at their residing prison. A program nurse visited each prison on a weekly, fortnightly or monthly basis. Assessment involved a detailed clinical questionnaire including evaluation of risk behavior, liver stiffness measurement using transient elastography (portable FibroScan™), and blood-based investigations, including general biochemistry, hepatitis C viral load and genotype, HBV and HIV serology. The presence of comorbid medical conditions was assessed, including psychiatric illness (self-reported). The evaluation of risk behavior included assessment of active injecting drug use, defined as injecting in the month prior to incarceration. Injecting drug use in prison was defined as the use of drugs requiring needles and/or other injecting apparatus in either the current or a previous incarceration. Significant alcohol intake included pre-imprisonment or historically significant daily drinking (more than 2 standard drinks, most days, for both men and women)¹⁹ or binge drinking (heavy intermittent alcohol intake).

Prisoners identified as having chronic hepatitis C infection were then triaged as either 'low risk' or 'higher risk'. 'Low risk' prisoners were either non-cirrhotic or had compensated cirrhosis without significant comorbidities, for whom the hepatologist prescribed DAA therapy after a paper-based consultation with a program nurse, without the physician having direct interaction with the prisoner. 'Higher risk' prisoners had evidence of cirrhosis (clinical/FibroScan), including all prisoners who had

decompensated cirrhosis and/or significant comorbid conditions potentially complicating hepatitis C treatment such as renal failure or HBV coinfection. 'Higher risk' prisoners were referred for either a telemedicine consult (conducted from their local prison) or face-to-face assessment by a hepatologist. Face-to-face consultation for prisoners located at one of the 13 peripheral prisons required prisoner transfer to the central prison. At clinical review, it was decided whether 'higher risk' prisoners were to commence DAA therapy immediately or required further assessment prior to treatment initiation.

Prisoners with hepatitis C and cirrhosis were enrolled in surveillance programs for hepatocellular carcinoma (HCC) and esophageal varices as recommended by consensus guidelines.^{20,21} A viral hepatitis education program for prisoners and prison staff, including primary care staff, drug and alcohol service providers and correctional officers, was provided at all 14 correctional facilities to support the clinical service.

Harm reduction

An OST program is available in Victorian prisons, which 11% of prisoners utilized in 2016.²² Bleach is available which can be used to clean injecting equipment. There is no provision of clean needles or syringes.

Treatment eligibility

To be eligible for treatment prisoners had to be 18 years of age or older, have evidence of chronic hepatitis C with detectable serum HCV RNA, and have an adequate sentence duration to facilitate complete hepatitis C treatment while incarcerated (between 8 to 24 weeks depending on the selected treatment regimen). It was not a requirement however, that prisoners remained in prison to complete blood tests at week 12 post-treatment. Prisoners commenced on DAA therapy who were released early (early parole) were provided with their remaining medication to complete treatment in the community. Prisoners for whom a short sentence duration made them ineligible for treatment whilst incarcerated were referred to a healthcare service for treatment upon release to the community.

Standard correctional pharmacy procedures required that medications be repackaged into blister packs at the central hospital pharmacy before being couriered to the prisons. Initial packs were prepared to include 28 days of therapy, and subsequent packs were distributed to each prison monthly thereafter, until therapy was completed. Two of the 14 prisons provided medications to prisoners in weekly packets while the remaining 12 prisons dispensed medications to the prisoners daily. Medications moved with patients at time of inter-prison transfer to minimize treatment interruption and prisoner location was confirmed in real time using the electronic medical record. When DAAs first became available on 1 March 2016, the program had capacity to initiate 6 prisoners per week. This subsequently increased to 16 prisoners per week in June 2016 with increased pharmacy staff resources.

Analysis

This analysis evaluated hepatitis C treatment outcomes among all prisoners who commenced therapy over the 13-month period between 1 November 2015, when the program started, and 1 December 2016.

All prisoners had serum HCV RNA tested at baseline, at the end-of-treatment (EOT), and at week 12 post-treatment. The pre-specified period of follow-up was to week 12

post-treatment. The primary endpoint was SVR12, which was defined as undetectable serum HCV RNA 12 weeks after the EOT. Secondary endpoints included EOT response (defined as undetectable serum HCV RNA at EOT), confirmed HCV reinfection (defined as detectable serum HCV RNA with HCV genotype switch between EOT and SVR12) and treatment-associated adverse events. Relapse was defined as prisoners who achieved an EOT response, but in whom serum HCV RNA was detectable at week 12 post-treatment, without HCV genotype switch.

We considered 2 analyses for virological outcome – the primary outcome was rate of SVR12 per protocol, including those prisoners with a serum HCV RNA result at 12 weeks post-treatment (complete follow-up). This analysis was performed to account for the high number of prisoners 'lost to freedom'; that is prisoners released to freedom after the EOT but prior to SVR12, as well as a small number of prisoners who were granted parole during their treatment course. We also considered overall SVR12 rate by intention to treat.

Data were described using median and interquartile ranges (IQR). Categorical data were described as number and percentage. Comparisons between groups were made using appropriate statistical tests after considering distribution of data.

Ethical considerations

This study was approved by the St Vincent's Hospital Melbourne Human Research Ethics Committee.

Results

Prisoner characteristics

During the 13-month period of evaluation, 949 prisoners were assessed by the Statewide Hepatitis Program, of whom 562 (59%) prisoners were eligible for treatment, and 416 (44%) were commenced on therapy during the period of evaluation. Reasons for treatment ineligibility are detailed (Table S2). Baseline characteristics are described in detail in Table 1. In brief, the cohort was predominately male (90%), the median age was 41 [34–46] years, 80% were Caucasian, 12% were Indigenous, and 4% were Asian. Cirrhosis was present in 21%. HIV and HBV coinfection were uncommon, but past HBV infection (anti-HBc positivity) was present in 24%. The majority of prisoners responded to detailed questions about their past and/or current injecting practices. Injecting drug use was reported by 68% (n = 262/386) in the month prior to incarceration and the most common drug of choice was heroin (60%, n = 120/200). Mental health comorbidities were reported by 70%. Only 14% of prisoners had previously seen a specialist to discuss their hepatitis C; 94% were treatment naïve (Table 1).

HCV DAA therapy and virological response

The DAA regimens prescribed are listed (Table 2). The rate of SVR12 was 96% (n = 301/313) per protocol (Table 3). SVR12 rates exceeded 95% for all genotypes, including genotype 1a (96%, n = 137/143), genotype 1b (100%, n = 11/11), genotype 2 (100%, n = 5/5), genotype 3a (96%, n = 147/153) and genotype 6 (100%, n = 1/1). There was no difference in SVR12 between cirrhotic and non-cirrhotic prisoners (97% vs. 94%, *p* = 0.51). SVR12 rates by ITT analysis were lower (SVR12 72%, n = 301/416), (Table 3); the most common reason for not achieving SVR12 was loss to follow-up due to being released to freedom (90%, n = 103/115).

Table 1. Prisoner characteristics.

Characteristics	N = 416
Age, yr, median [IQR]	41 [34–46]
Male sex, n (%)	376 (90)
Ethnicity, n (%)	
Caucasian	332 (80)
Indigenous	51 (12)
Asian	15 (4)
Other	18 (4)
Body mass index, kg/m ² , mean [IQR]	31 [27–34]
HCV genotype, n (%)	
Genotype 1a	184 (44)
Genotype 1b	14 (3)
Genotype 2	8 (2)
Genotype 3	208 (50)
Genotype 6	2 (1)
HCV viral load, IU/ml, median [IQR]	685,000 [192,000–2,630,000]
ALT, U/ml, median [IQR]	88 [55–145]
Platelet count, median [IQR]	218 [176–256]
HBV serology, n (%)	
HBsAg ⁺	6 (2)
Anti-HBs ⁺	310 (75)
Anti-HBc ⁺	98 (24)
HIV serology, n (%)	8 (2)
Liver stiffness measurement [~] , n (%)	
<9.5 kPa	278 (71)
9.5–12.5 kPa	40 (10)
>12.5 kPa	71 (19)
Cirrhosis, n (%)	86 (21)
Compensated	77 (19)
Decompensated	9 (2)
Opiate substitution therapy	228 (54)
Comorbid psychiatric illness [*]	291 (70)
Psychotropic medication	208 (50)
Significant alcohol history [#] , n (%)	243 (58)
Injecting drug use, n (%)	
PWID, current/previous	389 (94)
Injecting prior to incarceration ⁺	262/386 (68)
Injecting in prison, current/previous [^]	130/228 (57)
Previous specialist care for HCV, n (%)	59 (14)
Treatment experienced, n (%)	25 (6)

ALT, alanine aminotransferase; HBsAg, HBV surface antigen; PWID, people who inject drugs.

[~] Valid liver stiffness measurements using transient elastography were obtained in 94% (n = 389). The remaining 6% (n = 27) of prisoners were either cirrhotic (n = 10) (evidenced by clinical decompensation and/or radiological evidence of portal hypertension) or had unsuccessful elastography due to body habitus (n = 17).

^{*} Self-reported or recorded from medical record at initial assessment.

[#] Recent (pre-imprisonment) or historically significant daily (more than 2 standard drinks, most days, for men and women) or binge drinking (significant intermittent alcohol intake).

⁺ Injecting drug use within 1 month of incarceration [^]injecting drug use in the current or previous incarceration.

Table 2. Treatment regimens and durations.

Treatment regimen	(N = 416)
Sofosbuvir 400 mg/ledipasvir 90 mg, n (%)	
8 weeks	100 (24)
12 weeks	77 (18)
24 weeks	4 (1)
Sofosbuvir 400 mg/daclatasvir 90 mg ± RBV, n (%)	
12 weeks	147 (35)
24 weeks	65 (16)
Sofosbuvir 400 mg/RBV 1,000–1,200 mg, n (%)	5 (1)
Paritaprevir 150 mg/ritonavir 100 mg/ombitasvir 25 mg/dasabuvir 250 mg, n (%)	16 (4)
Sofosbuvir 400 mg/PegIFN ± RBV, n (%)	2 (1)

RBV, ribavirin.

Treatment failure was due to virological relapse in 11 prisoners (Table S1) and confirmed hepatitis C reinfection in one prisoner. Three of the 11 prisoners who relapsed had treatment interruptions of greater than 1-week duration, including 2 prisoners prescribed sofosbuvir plus daclatasvir for 24 weeks who received only 6 and 7 weeks respectively due to being released on parole; both were HCV PCR positive on re-incarceration within the follow-up period. The one prisoner with confirmed reinfection, who had risk factors for reinfection, was diagnosed by recurrent HCV viremia between EOT and SVR12, with a switch from genotype 1a to genotype 3 between EOT and SVR12 time points.

Treatment-related adverse events were infrequent (7.9%, 37 events in 33 participants, Table 4). One prisoner (0.2%) discontinued treatment at week 8 of 12 due to insomnia (SVR12 achieved). There were no treatment-related deaths. Nine prisoners had decompensated liver disease at treatment initiation (Child Pugh B, n = 4 and Child Pugh C, n = 5). The SVR12 rate was 71% (n = 5/7) amongst this group. Two prisoners experienced virological relapse and 2 prisoners were released to freedom prior to SVR12. One Child Pugh C prisoner achieved SVR12 and was referred to a liver transplantation service and wait-listed for transplantation while incarcerated. This patient underwent successful orthotopic liver transplantation following release from prison. No *de novo* HCCs were diagnosed during the period of evaluation.

There were 6 prisoners who were HCV-HBV co-infected. All were non-cirrhotic. One prisoner who was HBeAg-positive with a high serum HBV DNA level was initially commenced on antiviral therapy for hepatitis B (tenofovir 300 mg daily), and then subsequently hepatitis C, 4 weeks later. Five prisoners were HBeAg-negative and had serum HBV DNA levels <2,000 IU/ml at baseline. These prisoners were monitored with liver function tests and serial measurements of HBV DNA levels. Two prisoners experienced on-treatment increases in serum HBV DNA levels without biochemical flare and antiviral therapy for HBV was not commenced. Past HBV infection was present in 24% of the cohort. There were no cases of on-treatment or post-treatment hepatitis flare to suggest HBV reactivation.

Nurse-led care was delivered effectively across the statewide network

Most prisoners (82%, n = 340) were suitable for treatment based on nurse-led evaluation only and did not require a formal hepatologist assessment ('low risk'). The remaining 76 prisoners (18%) were categorized as 'higher risk' and required either face-to-face (13%, n = 55), telemedicine hepatologist consultation (3%, n = 13), or both (2%, n = 8) (Fig. 2). Rates of SVR12 were comparable in 'low risk' and 'higher risk' prisoners, both per protocol (96% vs. 93%, *p* = 0.27), and by ITT (74% vs. 67%, *p* = 0.26) analysis (Fig. 2).

The decentralized, nurse-led model of care removed the requirement for most prisoners to attend a central prison or hospital for hepatitis C assessment and treatment. However, 26% of prisoners still had at least one prison transfer while receiving DAA therapy (n = 110, range 0–6). The frequency of prison transfers while receiving hepatitis C treatment was not associated with diminished SVR12 outcomes (SVR12 96–100%, Fisher's exact test *p* = 0.89) (Fig. 3).

The cascade of care from referral is presented in detail for each prison in Table S2. Assessing the cascade of care following referral to the program, 562 (59%) were eligible for treatment

Table 3. Virological response.

Virological response EOT, n (%)	Overall (N = 416)	Per protocol (n = 364)
HCV PCR negative	364 (88)	364 (100)
Lost to freedom*	52 (12)	–
Virological breakthrough	0 (0)	0 (0)
Treatment-related death	0 (0)	–
SVR12, n (%)	(N = 416)	(n = 313)
HCV PCR negative	301 (72)	301 (96)
Genotype 1a	137/184 (74)	137/143 (96)
Genotype 1b	11/14 (79)	11/11 (100)
Genotype 2	5/8 (63)	5/5 (100)
Genotype 3	147/208 (71)	147/153 (96)
Genotype 4/5	–	–
Genotype 6	1/2 (50)	1/1 (100)
Cirrhosis		
Yes	59/86 (69)	59/63 (94)
No	242/330 (73)	242/250 (97)
Lost to freedom	103 (25)	–
Virological relapse	11 (2.8)	11 (3.5)
Reinfection	1 (0.2)	1 (0.5)
Treatment-related death	0 (0)	–

EOT, end-of-treatment; SVR12, sustained virological response at post-treatment week 12.

* Lost to freedom; prisoners either released on-treatment or after end-of-treatment but prior to week 12 post-treatment.

Table 4. Adverse events.

Adverse events, n (%)	N = 416
Treatment-related	
Headache	12 (2.9)
Gastrointestinal symptoms	9 (2.2)
Lethargy	8 (1.9)
Rash	4 (1.0)
Myalgia	3 (0.7)
Serious adverse events	0 (0)
Treatment-related deaths	0 (0)
Leading to discontinuation of treatment	
Treatment-related	
Insomnia	1 (0.2)

and 416 (44%) were started on therapy. The majority of those ineligible had an insufficient remaining period of incarceration to complete treatment (61%, n = 235/387). During the period of evaluation, the average time from assessment to treatment was 4.6 months, reflecting in large part the fact that assessments commenced in November 2015, 4 months prior to reimbursement of DAAs in March 2016. The period between assessment and initiating treatment has since fallen to an average of 3 weeks.

Discussion

This is the first large scale hepatitis C prison management program to be implemented across an entire jurisdiction. The

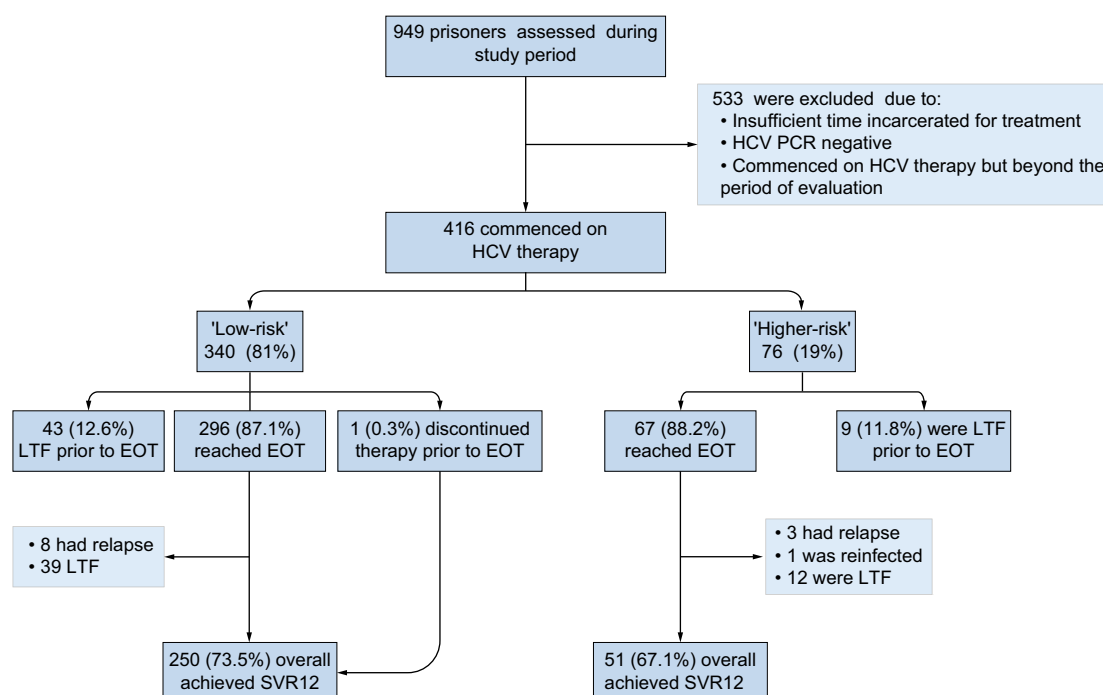


Fig. 2. Decentralized, nurse-led model of care. EOT, end-of-treatment; LTF, loss to freedom; SVR, sustained virological response at post-treatment week 12.

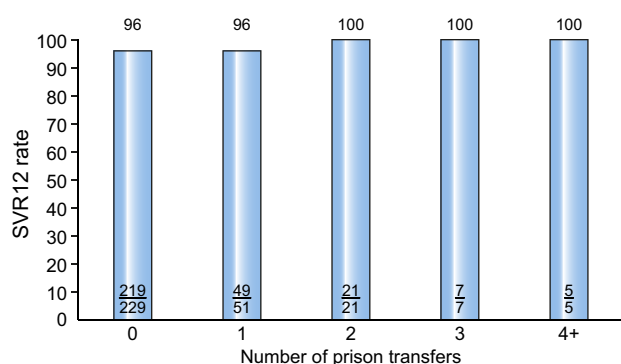


Fig. 3. Treatment outcome (SVR12 rate) by number of inter-prison transfers while receiving HCV DAA therapy. DAA, direct-acting antiviral; SVR12, sustained virological response at post-treatment week 12.

decentralized, nurse-led model of care implemented across 14 prisons has overcome many of the traditional systemic barriers to providing effective and efficient hepatitis C treatment to large numbers of prisoners, including the need for specialist consultation and hospital transfer, and treatment interruption from prisoner transfer between correctional facilities with partitioned clinical services. Importantly hepatitis C cure rates achieved by this new model were high.

Although many countries continue to mandate specialist physician management for hepatitis C therapy initiation,²³ our data demonstrate that nurse-led treatment is safe and effective in the correctional setting, with only 18% of assessed prisoners requiring specialist consultation. Whilst inter-prison transfer was frequent (driven by movement in the justice system rather than related to prisoners need to access hepatitis C services), it did not lead to treatment interruption or diminished efficacy. The model of care allows high treatment numbers with existing resources now permitting 16 new treatment starts per week. The key features of the program that likely support its success include the nurse-led model delivering DAA therapy locally, the statewide coverage across all correctional facilities, the use of information technology including telemedicine and a central electronic medical record, a centralized pharmacy distribution with real-time prisoner tracking, and federal government policy supporting prisoner access to DAAs.

An important benefit of the program is that it engages a marginalized population in hepatitis C treatment and primary healthcare more broadly. Many prisoners had a history of current or recent injecting prior to incarceration, and most had psychiatric comorbidities. As expected, a high proportion (86%) had never previously consulted a clinician about hepatitis C treatment. PWID are the group at greatest risk of hepatitis C infection in many developed countries and are traditionally difficult to engage in health care. Prison-based hepatitis C treatment programs can therefore play a vital role in increasing care and treatment in this group.

There is now a global focus on how to best utilize public health platforms to eliminate hepatitis C as a public health threat. Mathematical modelling has estimated that for Victoria to achieve the WHO elimination targets by 2030,¹ treatment rates among current PWID will need to be scaled up to approximately 59/1,000, or around 1,300 active PWID per year.^{9,24} Sustaining this level of treatment uptake in the community will require increased testing and retention of PWID

in care,⁹ including the treatment of prisoners, many of whom have a history of current or recent injecting.

The success of our program has been recognized by a recent increase in resourcing to increment treatment start numbers to 20 per week (1,040/year). This treatment rate means the program would potentially contribute >50% of the overall yearly elimination target for treatment scale-up among PWID in Victoria, based on our data that 68% of prisoners with hepatitis C were considered current PWID. This highlights the vital contribution that prison treatment programs can make as part of a broader elimination agenda.

Limitations of this study include the large proportion of prisoners 'lost to freedom', most of whom were released from prison between EOT and SVR12. The study, however, evaluates a proposed model of care to increase treatment scale-up amongst a priority population, rather than to assess DAA effectiveness, which is already established.^{25,26} Despite a high proportion of prisoners who were 'lost to freedom', approximately 90% did complete DAA therapy while incarcerated and most are anticipated to have achieved cure. Previous Australian studies have demonstrated a hepatitis C incidence of >10% annually amongst incarcerated PWID.⁷ In our study there was only one confirmed case of hepatitis C reinfection identified, although the duration of follow-up was only 12 weeks post-treatment; with a longer duration of follow-up a higher number of reinfections is anticipated. It is possible that a number of the cases of relapse were in fact reinfections with the same hepatitis C genotype; regardless, the number of relapses was also low. The program supports retreatment of those reinfected to engage prisoners at highest risk of transmission, key for reducing prison hepatitis C prevalence and achieving community elimination targets. Finally, detailed data concerning the rate of opt-in screening at reception, overseen by the prison medical staff, was not available, this step in the cascade of care preceding referral to the program. The estimate of the percentage of viremic prisoners treated was informed by Australian data estimating hepatitis C seroprevalence within prisons, the proportion therefore anticipated to be hepatitis C RNA positive and the short average duration of imprisonment (Table S2). Although the average time between assessment and treatment was 4.6 months at the time of evaluation, there was a 4-month disparity between commencement of clinical assessments in November 2015 and hepatitis C treatment availability in March 2016. The period between assessment and initiating treatment has since fallen to an average of 3 weeks, with 75% of prisoners assessed now starting DAA therapy.

Future work will explore refinements to prison-based models of care. This will include evaluating the role for peer workers to increase prisoner engagement and the role for point-of-care testing to increase screening uptake and reduce time from assessment to treatment start. This will also examine the success of linkage to community care for prisoners released to freedom, including treatment completion rates for those who are released on-treatment, as well as treatment uptake in those referred to community treatment centers for DAA therapy post-release. In addition, long-term follow-up studies monitoring HCV reinfection are underway.

In conclusion, DAA therapy for hepatitis C using a decentralized, nurse-led model of care was highly effective in increasing hepatitis C treatment access to a large population of prisoners, with high rates of hepatitis C cure. Given the high prevalence of hepatitis C among prisoners, with many reporting current

or recent injecting drug use, large scale prison treatment programs should be promoted to benefit both the individual prisoner and also hepatitis C elimination efforts in the broader community.

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Conflict of interest

TP: honoraria from Merck. LM: honoraria from Gilead, Merck and AbbVie. JH: investigator initial research funding to institution from Gilead. JD: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS. Honoraria to institution from Gilead, Merck, and BMS. AP: no disclosures. ARL: investigator initiated research funding to institution from Gilead and Merck. MS: no disclosures. MH: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS. DI: honoraria from AbbVie, BMS, Gilead and Merck. AJT: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS; advisory board – Gilead, AbbVie, BMS, Merck, Eisai. All other authors declare no conflicts of interest.

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

Authors' contributions

TP was responsible for study design, analysis, acquisition and interpretation of data, and drafting and revision of the manuscript. LM, AC and LG were responsible for acquisition of the data and revision of the manuscript content. PD was responsible for substantial contribution to the study conception and design and revision of the manuscript content. DW, RW and NS were responsible for analysis and interpretation of the data and revision of the manuscript content. JH was responsible for substantial contribution to the conception and design of the study and for revision of the manuscript content. JD and AP were responsible for substantial contribution of study design and data interpretation and revision of the manuscript content. AL was responsible for substantial contribution to the conception and design of the study and revision of the manuscript. MS and MH were responsible for analysis and interpretation of the data and revision of the manuscript. DI was responsible for study design, analysis, acquisition and interpretation of the data and revision of the manuscript. AT was responsible for a substantial contribution to the conception and design, acquisition, analysis, or interpretation of the data and drafting and revision of the manuscript content. All authors were responsible for giving final approval of the version to be published, and agree to be

accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2019.01.012>.

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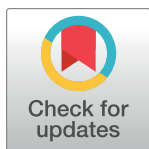
RESEARCH ARTICLE

Non-invasive fibrosis algorithms are clinically useful for excluding cirrhosis in prisoners living with hepatitis C

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Data Availability Statement: The data included in this analysis includes sensitive information regarding prisoners under the care of the Department of Justice and Regulation, Victorian

Abstract

Background and aims

Prison-based HCV treatment rates remain low due to multiple barriers, including accessing transient elastography for cirrhosis determination. The AST-to-platelet ratio index (APRI) and FIB-4 scores have excellent negative predictive value (NPV) in hospital cohorts to exclude cirrhosis. We investigated their performance in a large cohort of prisoners with HCV infection.

Methods

This was a retrospective cohort study of participants assessed by a prison-based hepatitis program. The sensitivity, specificity, NPV and positive predictive value (PPV) of APRI and FIB-4 for cirrhosis were then analysed, with transient elastography as the reference standard. The utility of age thresholds as a trigger for transient elastography was also explored.

Results

Data from 1007 prisoners were included. The median age was 41, 89% were male, and 12% had cirrhosis. An APRI cut-off of 1.0 and FIB-4 cut-off of 1.45 had NPVs for cirrhosis of 96.1% and 96.6%, respectively, and if used to triage prisoners for transient elastography, could reduce the need for this investigation by 71%. The PPVs of APRI and FIB-4 for cirrhosis at these cut-offs were low. Age ≤ 35 years alone had a NPV for cirrhosis of 96.5%. In those >35 years, the APRI cut-off of 1.0 alone had a high NPV $>95\%$.

Government. As such, in keeping with Department of Justice regulations, this data cannot be made publicly available. Data queries regarding the hepatitis C non-invasive fibrosis algorithm dataset can be forwarded to Bernadette De Graff, Clinical Research Compliance Manager, Department of Gastroenterology, St Vincent's Hospital Melbourne, Bernadette.degraff@svha.org.au.

Funding: TP received funding from an Australian Government Research Training Program Scholarship and the Department of Gastroenterology, St Vincent's Hospital Melbourne; [https://urldefense.com/v3/__https://www.education.gov.au/research-training-program_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdVSrZNSo\\$](https://urldefense.com/v3/__https://www.education.gov.au/research-training-program_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdVSrZNSo$) AJT and MH received funding from the National Health and Medical Research Council of Australia (NHMRC) Practitioner Fellowships 1142976 and 1112297. [https://urldefense.com/v3/__https://www.nhmrc.gov.au/_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdT5HBwvc\\$](https://urldefense.com/v3/__https://www.nhmrc.gov.au/_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdT5HBwvc$) JH is funded by a University of Melbourne CR Roper Fellowship and a NHMRC program grant. [https://urldefense.com/v3/__https://staff.unimelb.edu.au/mdhs/research-development/research-collaboration-and-funding/faculty-trust-fellowships/cr-roper-fellowship_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6Apxdbmg15Ep\\$](https://urldefense.com/v3/__https://staff.unimelb.edu.au/mdhs/research-development/research-collaboration-and-funding/faculty-trust-fellowships/cr-roper-fellowship_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6Apxdbmg15Ep$) This work was supported by NHMRC Program grant 1132902 and Partnership grant 1116161. [https://urldefense.com/v3/__https://www.nhmrc.gov.au/_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdT5HBwvc\\$](https://urldefense.com/v3/__https://www.nhmrc.gov.au/_!!LUUsMDrd6!yF7XCdsDwModOT7ENzQHtsvv3IAco8amgz6Bf0hZtRF8uM2Os3mbmU1by4H_6ApxdT5HBwvc$) The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have no disclosures in relation to this work.

Conclusion

APRI and FIB-4 scores can reliably exclude cirrhosis in prisoners and reduce requirement for transient elastography. This finding will simplify the cascade of care for prisoners living with hepatitis C.

Introduction

The advent of direct acting antivirals (DAAs) has revolutionised the treatment of chronic hepatitis C virus (HCV) infection. People living with HCV can now be treated with all oral, short duration, and well tolerated DAA regimens which achieve cure rates in excess of 95% [1,2]. In this context, the World Health Organization (WHO) has established targets for HCV elimination, including an 80% reduction in incident infections and a 65% reduction in HCV related mortality by 2030 [3]. Mathematical modelling has demonstrated that to achieve these targets, concerted efforts must be made to treat key populations with high risk for transmission including people who inject drugs (PWID) [4].

Prisons provide an excellent opportunity to identify and treat people living with HCV given the over-representation of people with injecting drug use histories and the associated high prevalence of chronic HCV [5]; HCV seroprevalence exceeds 15% in prisons globally, and in Australia is as high as 50% amongst incarcerated PWIDs [6,7]. Multiple data have now demonstrated that prison-based HCV treatment is safe and effective and can reach prisoners in large numbers [8–10]. Despite this, rates of HCV treatment in prisons worldwide remain low due to multiple barriers [11]. These include frequent prisoner transfer interrupting treatment, short sentence durations, the need for hospital transfer for specialist review and limited resourcing for HCV testing and treatment [12,13]. One important barrier is access to TE (e.g. FibroScan®) for cirrhosis determination in the correctional setting [14].

Cirrhosis determination prior to HCV DAA initiation is important for decision-making regarding treatment regimen and duration, and to identify those who require ongoing specialist referral for cirrhosis management and hepatocellular cancer (HCC) surveillance [15]. TE is the most accurate non-invasive tool for cirrhosis determination and has largely replaced liver biopsy in the community [16]. Access to TE within the correctional sector however is challenging and remains a significant barrier to care due to its high cost, limited availability of equipment within prison health services, frequent need for prisoner transfer and issues with security [10,17].

Based on routine blood tests, the AST-to-platelet ratio index (APRI) and the FIB-4 score were developed to predict advanced fibrosis and cirrhosis, using community recruited samples [18–21]. Both scores have subsequently been validated and demonstrate excellent negative predictive value for excluding advanced fibrosis or cirrhosis. Indeed, the WHO recommends both APRI and FIB-4 as cost-effective tools for the assessment of hepatic fibrosis in resource-limited settings [22]. Prisoner populations, however, are characterised by male gender, younger age, as well as multiple high-risk behaviours that differ considerably from the hospital populations used to validate these algorithms. Validation of simple serum-based fibrosis markers for use in the correctional setting to triage prisoners at risk of cirrhosis would be clinically useful and help improve the cascade of care by reducing treatment costs and the time delay and logistical challenges associated with TE.

We have therefore evaluated the accuracy of non-invasive fibrosis algorithm scores including APRI and FIB-4 as a practical triage tool for excluding cirrhosis in the correctional setting. This analysis was conducted using a large cohort of prisoners with HCV infection with a view

to simplifying clinical pathways by reducing the number of prisoners requiring TE, therefore improving the HCV cascade of care.

Materials and methods

Participants

This was a retrospective cohort study investigating the performance of the APRI and FIB-4 in a prison setting. The cohort provided prospectively collected data from all prisoners with HCV infection evaluated through the Statewide Hepatitis Program in Victoria, Australia. Prisoners evaluated for HCV infection were consecutively recruited from November 2015. This model of care has been described in detail previously [8]. Prisoners who self-reported a prior HCV diagnosis or who were identified as HCV seropositive whilst incarcerated were referred to the Statewide Hepatitis Program for face-to-face protocol-driven assessments with a program nurse. Clinical assessments were conducted at all 16 adult prisons throughout Victoria, which are serviced by the program. Pathology testing was performed by the diagnostic laboratory affiliated with each correctional facility. A database was created containing participants' baseline clinical characteristics, blood test results and liver stiffness measurement (LSM) median scores, interquartile ranges (IQR) and IQR:median ratios. Blood-based investigations that were recorded included haemoglobin, white blood cell count, platelet count, creatinine, urea, sodium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, albumin, international normalised ratio (INR), hepatitis B/C serology, HIV serology, and HCV genotype and viral load.

Prisoners were excluded if they had insufficient blood test results to calculate the APRI or FIB-4 scores within 6 months of TE, if TE had not been performed, if IQR:median ratio exceeded 0.3 or the absence of current HCV infection, defined as detectable HCV RNA (Fig 1).

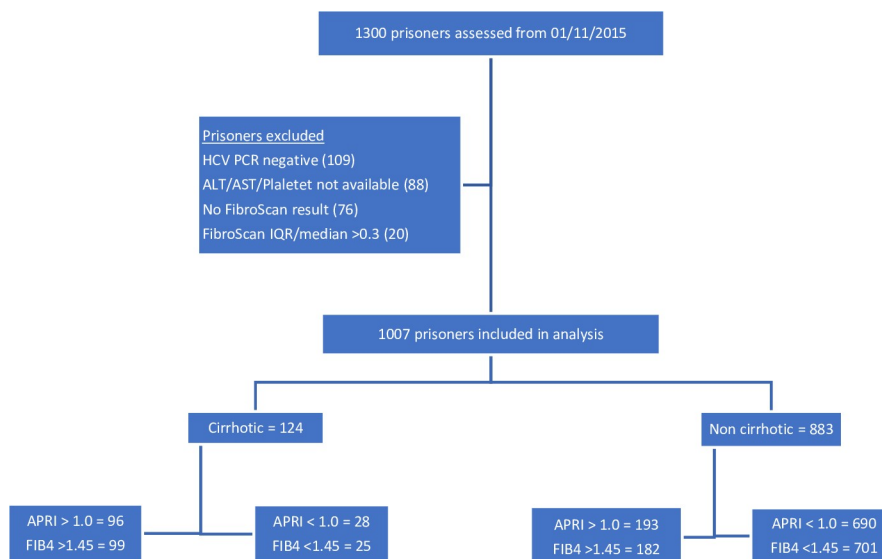


Fig 1. Consort diagram. Legend IQR, interquartile range.

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Liver cirrhosis determination

TE was used for cirrhosis determination at each prisoner's initial clinical assessment to guide appropriate management. Cirrhosis was defined as a median LSM of ≥ 12.5 kPa, in keeping with Australian HCV consensus guidelines [23,24]. Blood-based and radiologically investigations were also arranged at this review, where appropriate. TE was performed using FibroScan[®] (Echosens) with the 'M' or 'XL' probe, as determined by the participant's body mass index (BMI) and body habitus. Measurements were collected in the mid-axillary line until ten valid readings were recorded. The LSM results was deemed valid only when the IQR/median ratio was less than 0.3 and when performed prior to HCV DAA initiation. All transient elastography was performed by three hepatology clinical nurse consultants who had performed at least 100 supervised examinations for credentialing. The APRI and FIB-4 scores were then determined for each prisoner. The APRI was calculated as $[(\text{AST}/\text{Upper limit of normal of AST}) \times 100]/[\text{Platelets}(10^9/\text{L})]$ [18]. The FIB-4 score was calculated as $[(\text{age} \times \text{AST})/(\text{platelets}(10^9/\text{L}) \times \text{ALT}^{1/2})]$ [19].

Statistical methodology and analysis

Statistical analyses were performed using STATA 12.0 (StataCorp LP, College Station, TX, USA). Categorical data were described as number and percentage and interval data as median and IQR.

Data were used to determine the sensitivity, specificity, PPV and NPV of the APRI and FIB-4 scores to diagnose or exclude cirrhosis using standard analytical approaches. Performance of APRI and FIB-4 were assessed against gold-standard TE assessments. Cut-offs of 1.0 and 2.0 for APRI, and 1.45 and 3.25 for FIB-4 were selected on the basis of having been previously validated in other data for the diagnosis of cirrhosis and advanced fibrosis (METAVIR F3/4), respectively [18–20]. Two analyses for FIB-4 at these thresholds were considered, including for advanced fibrosis ($\text{LSM} \geq 9.5$ kPa) (for which this algorithm was originally validated) and for cirrhosis (≥ 12.5 kPa). The reduction in requirement for TE was also determined should prisoners be triaged for TE using these algorithms cut-offs.

In addition to APRI and FIB-4, given the young average age of prisoner populations globally and the slow natural history of HCV infection [25], the sensitivity, specificity, NPV and PPV of binary age thresholds alone to predict or exclude cirrhosis were also analysed, to determine if stratifying fibrosis assessment by age could minimise the need for TE while maintaining high NPV. The age thresholds were determined by the data and the quartile values (25th, 50th and 75th) were selected for analysis. The cohort was then separated by these age values and we determined the predictive value of APRI at a cut off of 1.0 in these groups separately to contribute to new clinical pathways for fibrosis assessment for the entire prison cohort incorporating age categories. This novel fibrosis assessment pathway was then validated using data from an additional cohort of prisoners.

Ethics statement

This study was approved by the St Vincent's Hospital Human Research Ethics Committee. The study was granted a waiver of consent as all data were analysed anonymously. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines, and regulatory requirements.

Results

Prisoner characteristics

In total, data from 1300 was assessed. Two hundred and ninety three prisoners were excluded including 109 who were HCV PCR negative, 88 who had insufficient blood test results to

calculate APRI and/or FIB-4 ($n = 88$), 76 who did not undergo TE ($n = 76$) and 20 who had LSM IQR:median >0.3 ($n = 20$) (Fig 1). As such, 1007 were included in the analysis. Baseline characteristics are described in Table 1. In short, prisoners were prominently male (89%), the median age was 41 [35–47] years, HCV GT3 infection was the most common genotype (46%), and there were low rates of HBV and HIV coinfection (1%). One hundred and twenty-four individuals (12%) were cirrhotic, as defined by TE (LSM ≥ 12.5 kPa, $n = 124/1007$). The median APRI score was 0.66 [0.41–1.09] and median FIB-4 score was 1.02 [0.69–1.54] (Table 2). The median time between the collection of blood-based investigations and transient elastography was 34 days [IQR 13–86 days].

Performance of APRI or FIB-4 scores for cirrhosis in prisoners

The performance of the APRI and FIB-4 scores for cirrhosis (LSM ≥ 12.5 kPa) at previously validated thresholds are presented in Table 3. An APRI score cut-off of 1.0 had a sensitivity and specificity for cirrhosis of 77.4% and 78.1%, respectively. The NPV of APRI <1.0 to exclude cirrhosis was 96.1%. If this cut-off was incorporated into prison fibrosis assessment protocols where only prisoners with an APRI ≥ 1.0 were referred for TE, the need for FibroScan[®] would reduce by 71% ($n = 718/1007$). The PPV at this threshold was low (33.2%), meaning that these prisoners would still require TE for cirrhosis confirmation. At the higher APRI cut-off of 2.0, the PPV was higher (48.4%), but resulted in lower NPV (91.4%) and sensitivity (36.3%)—using an APRI cut-off of 2.0 alone to exclude cirrhosis would have meant that 64% ($n = 79/124$) cases of cirrhosis were missed (S1 Table).

Similar patterns were observed using different FIB-4 cut-offs, with the lower cut-off score of 1.45 associated with a high NPV for the exclusion of cirrhosis, and a low PPV (Table 3). At a higher cut-off score of 3.25, the specificity of the score was enhanced, however the sensitivity and NPV were significantly reduced (Table 3).

Table 1. Prisoner baseline characteristics.

Baseline characteristics	n = 1007
Age, median [IQR]	41 [35–47]
BMI, median [IQR]	28.4 [25.3–32.2]
Male sex, n (%)	899 (89)
Indigenous Australian, n (%)	133 (13)
HCV viral load IU/mL, median [IQR]	757,000 [193,250–3,130,750]
HCV Genotype, n (%)	
• GT1a	435 (43)
• GT1b	33 (3)
• GT2	26 (3)
• GT3	466 (46)
• GT4	2 (0.5)
• GT6	8 (0.5)
• NA	37 (4)
HBsAg positive, n (%)	14 (1)
HIV Ab positive, n (%)	12 (1)
ALT, median [IQR]	81 [53–132]
AST, median [IQR]	52 [36–79]
Platelets, median [IQR]	234 [192–276]
Cirrhotic, n (%)	124 (12)

Baseline characteristics of prisoner population at initial assessment. Legend, BMI, body mass index, GT, genotype.

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Table 2. Results of cirrhosis determination using APRI, FIB-4 and elastography.

Cirrhosis determination	n = 1007
APRI score, n (%)	
• < 1.0	718 (71)
• 1.01–2.0	196 (19)
• > 2.0	93 (9)
FIB-4 score, n (%)	
• < 1.45	726 (72)
• 1.45–3.25	236 (23)
• > 3.25	45 (45)
Liver Stiffness Measurements, n (%)	
• < 6 kPa	356 (36)
• 6–9.4 kPa	435 (43)
• 9.5–12.4 kPa	92 (9)
• ≥ 12.5 kPa	124 (12)

Results of cirrhosis determination using APRI, FIB-4 and elastography.

<https://doi.org/10.1371/journal.pone.0242101.t002>

The performance of FIB-4 for diagnosing or excluding advanced fibrosis (LSM ≥ 9.5 kPa) at previously validated threshold was also analysed (S1 Table).

Age thresholds for the exclusion of cirrhosis

The sensitivity, specificity, NPV and PPV of age thresholds alone for cirrhosis were determined. The binary age thresholds selected for analysis were 35, 41 and 47 years, representing the quartile values for the cohort. The binary variable of prisoner age ≤ 35 achieved a NPV for cirrhosis of 96.5%, due to the low prevalence in this group (3.5%, n = 9/258) (Table 4). If no formal fibrosis assessment was performed for prisoners aged ≤ 35, nine cases of cirrhosis would be missed, representing 1% of the total cohort. The NPV of the age thresholds 41 and 47 years alone to exclude cirrhosis decreased to 94.7% and 91.6%, respectively, due to the increasing prevalence of cirrhosis in these age groups (Table 4). Furthermore, as the age threshold

Table 3. Accuracy of APRI and FIB4 in predicting cirrhosis as compared to transient elastography.

	All prisoners (n = 1007) n (%)	Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		< 12.5 kPa (n = 883) n (%)	≥ 12.5 kPa (n = 124) n (%)				
For prediction of cirrhosis							
APRI							
≤ 1.0	718 (71)	690 (78)	28 (23)	77	78	33	96
> 1.0	289 (29)	193 (22)	96 (77)				
≤ 2.0	914 (91)	835 (95)	79 (64)	36	95	48	91
> 2.0	93 (9)	48 (5)	45 (36)				
FIB4							
≤ 1.45	713 (71)	688 (78)	25 (20)	80	78	34	97
> 1.45	294 (29)	195 (22)	99 (80)				
≤ 3.25	960 (95)	871 (99)	89 (72)	28	99	75	91
> 3.25	47 (5)	12 (1)	35 (28)				

Sensitivity, specificity, NPV and PPV of the APRI and FIB-4 scores to diagnose or exclude cirrhosis (LSM ≥ 12.5 kPa) represented as percentages. Legend, NPV, negative predictive value, PPV, positive predictive value.

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Table 4. Performance of age categories for predicting cirrhosis as compared to transient elastography.

	All prisoners (n = 1007) n (%)	Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		< 12.5kPa (n = 883) n (%)	≥ 12.5kPa (n = 124) n (%)				
For prediction of cirrhosis							
Age							
≤ 35	258 (26)	249 (28)	9 (7)	92	28	26	97
> 35	749 (74)	634 (72)	115 (93)				
≤ 41	528 (52)	500 (57)	28 (23)	77	57	20	95
> 41	479 (48)	383 (43)	96 (77)				
≤ 47	752 (75)	689 (78)	63 (51)	49	78	24	92
> 47	255 (25)	194 (22)	61 (49)				

Ability of age alone to diagnose or exclude cirrhosis, with TE as the reference standard. Legend, NPV, negative predictive value, PPV, positive predictive value.

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increased, the number of prisoner with cirrhosis who were missed, increased. We also calculated the NPV using the combination of age and APRI (APRI cut-off of 1.0 for prisoners aged ≤35 and >35 to exclude cirrhosis (Table 4)). Amongst the age ≤35 years subgroup, an APRI cut-off of 1.0 was associated with a NPV of 99.0%, however would result in 26% of this age group requiring TE who had an APRI >1.0. In those >35 years old, the NPV of APRI 1.0 remained satisfactory and was >95%, which could reduce the need for TE by 70% in this age group (Table 5).

Finally, a novel fibrosis assessment algorithm of performing TE only in prisoners >35 years and only when APRI ≥1.0 would achieve a NPV of >95% for cirrhosis and reduce the need for TE by 78% of the entire cohort (Fig 2). The NPV for cirrhosis was sustained at 97% when this fibrosis assessment pathway was applied to a validation cohort of 189 prisoners, 11% (n = 20/189) of whom were cirrhotic, and referral for TE would be reduced by 83% (n = 156/189) (S2 Table).

Discussion

HCV treatment within prisons is important for global viral elimination, however the requirement for TE for fibrosis assessment presents barriers to efficient HCV care in custodial settings. Our data demonstrate that the APRI and FIB-4 serum scores can be useful to stratify risk of cirrhosis in a prison population, using validated thresholds from hospital-based cohorts.

Table 5. Performance of APRI in different age categories for predicting cirrhosis as compared to transient elastography.

	All prisoners (n = 1007) n (%)	Liver stiffness measurement		Sensitivity %	Specificity %	PPV %	NPV %
		< 12.5kPa (n = 883) n (%)	≥ 12.5kPa (n = 124) n (%)				
For prediction of cirrhosis							
Age + APRI							
≤ 35							
APRI ≤ 1.0	192 (19)	190 (21)	2 (2)	78	76	11	99
APRI > 1.0	66 (7)	59 (7)	7 (6)				
> 35							
APRI ≤ 1.0	526 (52)	500 (57)	26 (21)	78	79	40	95
APRI > 1.0	223 (22)	134 (15)	89 (71)				

The performance of APRI in age categories ≤ 35 and >35 to diagnose or exclude cirrhosis. Legend, NPV, negative predictive value, PPV, positive predictive value.

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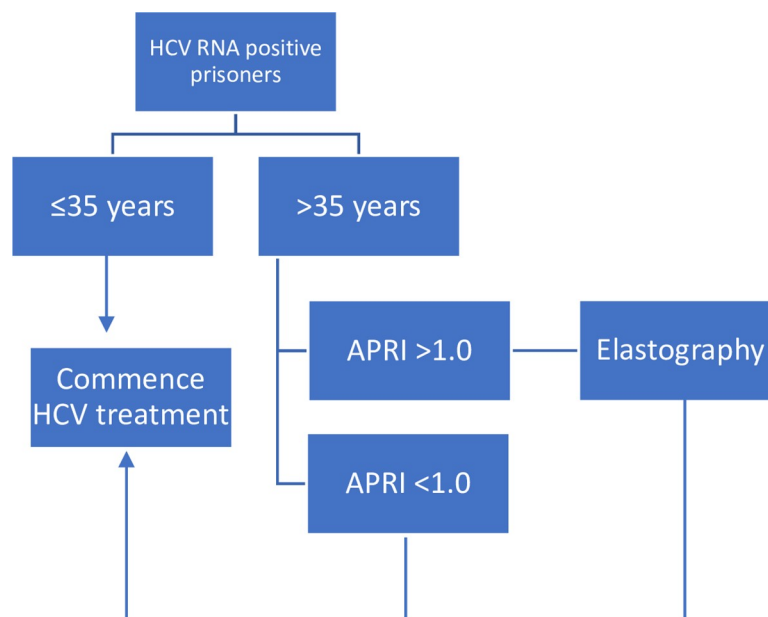


Fig 2. A novel clinical pathway for fibrosis assessment prior to HCV treatment in prisons. This pathway achieves a NPV for cirrhosis of >95% in all prisoners and minimises the need for FibroScan by 78%.

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When applied to our cohort of prisoners with HCV infection, APRI and FIB-4 thresholds of 1.0 and 1.45, respectively, were associated with NPVs for cirrhosis in excess of 96%. The sensitivity and PPV for both algorithms, however, was low. The data, therefore, demonstrate that the strength of these algorithms lies in the exclusion, rather than confirmation, of cirrhosis. The NPV of binary age thresholds for excluding cirrhosis were also investigated. The NPV of age ≤ 35 alone exceeded 96%, and could be incorporated into fibrosis assessment protocols to improve HCV treatment throughput. The use of APRI and FIB-4 have been prioritised by the WHO for cirrhosis determination in low- and middle-income countries due to their availability and low cost [18]. Similarly, prison health services provide complex care to prisoners in large numbers, including for HCV infection, yet operate under significant fiscal restrictions. Therefore, the use of these indices to minimise cost and streamline care should be expanded.

Both APRI (threshold of 1.0) and FIB-4 (threshold 1.45) had NPVs of >96% for cirrhosis. We believe that this NPV is such that a second test for fibrosis determination is not required. The use of these thresholds to determine need for TE would result in a 70% reduction in the number of prisoners being referred for TE. As such, these indices could help simplify pathways of care for HCV in prisons and promote increased treatment capacity. While mobile FibroScan[®] units are available, high equipment costs and mandatory training requirements mean that it is not feasible to have them routinely available in many prisons. As there are significant costs and logistics associated with transporting prisoners to tertiary hospitals or a central prison where a FibroScan[®] might be available, the reliance on TE for fibrosis assessment represents a significant challenge for expanding HCV treatment coverage in prisons. Using these algorithms to reduce the need for TE in the majority while maintaining a high NPV is significant, as delays in facilitating this investigation are at odds with the short average duration of incarceration, meaning some prisoners would be released without treatment. The NPV of APRI and FIB-4 are influenced by cirrhosis prevalence, and our cohort of predominately young male prisoners is well suited for their use.

Our data also demonstrate that age ≤ 35 years has a very high NPV for cirrhosis (96.5%), and that there is little clinical utility in applying blood-based algorithms or TE to this population. Evaluation of liver fibrosis in those ≤ 35 years might be restricted to people with clinical risk factors for cirrhosis, such as multiple spider naevi. In prisoners > 35 years there is a higher prevalence of cirrhosis, and in this group formal fibrosis evaluation is important. APRI < 1.0 had a NPV for cirrhosis of $> 95\%$ in prisoners > 35 years, reducing the need for TE by 70% in this age group. Therefore, LSM could be reserved for those prisoners > 35 years who have APRI > 1.0 . Combined, this approach achieves a NPV of $> 95\%$ across the entire cohort and minimised the need for TE by 78% (Fig 2). When applied to a validation cohort, the NPV of this approach was sustained and $< 20\%$ would require referral for TE.

Despite the high NPV of the FIB4, APRI and age ≤ 35 to exclude cirrhosis, a small number of cirrhotic prisoners were miscategorised. Using a FIB-4 cut off of 1.45 for TE, 2% ($n = 25/1007$) of the overall cohort and 20% ($n = 25/124$) of cirrhotics were incorrectly identified as non-cirrhotic. This was similar for an APRI threshold of 1.0 (3% miscategorised, $n = 28/1007$). All miscategorised prisoners were Child-Turcotte-Pugh class A and had preserved platelet counts. As such, the main implication for those miscategorised is exclusion from HCC surveillance. Achieving cure for these prisoners however will lead to significant reductions in their HCC risk and more broadly, non-HCC liver related morbidity and mortality [26]. Therefore, the reduction in prison-based HCV treatment throughput if TE is required for all prisoners must be considered. By stratifying the need for TE using fibrosis algorithms, it is anticipated that program efficiencies would be streamlined and that a greater number of prisoners would be treated, preventing the progression to cirrhosis and HCC in many.

The data demonstrate that the strength of APRI and FIB-4 are for excluding, rather than diagnosing cirrhosis. The higher APRI and FIB4 scores at thresholds of 2.0 and 3.25, which are recommended in WHO guidelines, were analysed for their ability to diagnose cirrhosis. Whilst achieving high specificity at these thresholds ($> 95\%$ for both APRI 2.0 and FIB4 3.25), their sensitivity was low and the majority of prisoners with cirrhosis were not diagnosed. As such, these algorithms perform poorly when used to diagnose cirrhosis within custodial settings and offer little clinical utility in prison HCV assessment pathways.

There is now increased focus on how best to utilise public health platforms to achieve the WHO elimination goals by 2030. Mathematical modelling has demonstrated to achieve this agenda, PWIDs must be prioritised as they contribute most to incident HCV infection [4]. The effectiveness of prison-based HCV care has now been widely described [8–10]. However, the propagation of similar programs worldwide requires simplification of HCV assessment algorithms to reduce cost, particularly for other countries operating within tight fiscal budgets. Our analysis demonstrates that APRI and FIB-4 maintain a high NPV for cirrhosis when used at their validated thresholds in the prison setting. Our new algorithm incorporating age as a categorical variable and only performing non-invasive blood test algorithms to exclude cirrhosis in those > 35 years exclusively is a practical way to more aggressively reduce the number requiring LSM while maintaining a high NPV. Such strategies will expedite HCV assessment and minimise costs and therefore have an important role in the prison settings in the era of viral hepatitis elimination.

Supporting information

S1 Table. Accuracy of FIB4 in predicting significant fibrosis as compared to transient elastography.
(DOCX)

S2 Table. Performance of novel fibrosis assessment pathway applied to a validation cohort.
(DOCX)

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Effective prison-based treatment and linkage to care after release

We read with interest Lara Tavoschi and colleagues' Article on the management of infectious diseases in European prisons.¹ The authors describe the high burden of communicable diseases, including hepatitis C virus (HCV), in European prisons and how prioritising health care in this sector has benefits for both prisoners and the broader community. Similarly, in Australia, there is a disproportionately high HCV seroprevalence among people in prison, reaching up to 50% among those who inject drugs.²

WHO has prioritised the elimination of HCV as a public health threat by 2030,³ and the prison setting must be used to engage prisoners living with HCV in care to achieve these targets. The high prevalence in prisons of both HCV infection and people who inject drugs, and the short average length of incarceration, make prisons ideal for diagnosing infection and providing short-duration, highly effective direct-acting antiviral (DAA) therapy. In this setting, we recently described a nurse-led model of care for HCV treatment in prison that was safe and achieved cure rates greater than 95% among prisoners.⁴ These data endorse Tavoschi and colleagues' recommendations and show that system-wide prison programmes are feasible and effective. Importantly, more than 80% of these prisoners had never engaged in specialist HCV care in the community,

and 68% reported injecting in the month before incarceration. Prison-based health care can engage people who do not traditionally participate in HCV care and interrupt onward-transmission networks.

Another important health-care gap is linkage to care after release from prison. In a pilot study, we evaluated the rate of successful linkage to HCV care among recently released prisoners. All prisoners had a comprehensive HCV assessment while incarcerated and were planned for treatment before release. On release, participants were referred to a local primary care practice with a detailed summary of their clinical work-up to streamline DAA prescription. There were no cost barriers to treatment uptake. The rate of treatment uptake was determined by registration with the national drug authorisation registry, required before DAA treatment can be dispensed. Among 75 former prisoners, only 19 (25%) were prescribed DAAs within 6 months of release. Of these, seven (37%) commenced treatment in prison when re-incarcerated during this period. Re-incarceration was the only predictor significantly associated with DAA initiation.

This period of time immediately after release from prison can be challenging, characterised by competing priorities including renegotiating social networks, finding stable accommodation, and managing recidivism.⁵ There is a need for new models of care to optimise linkage to care after release from prison for this vulnerable population.

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Living in the shadows of hepatitis

Viral hepatitis is a substantial public health problem with an annual mortality rate of 1·34 million deaths, which is similar to mortality from HIV (1·1 million) and tuberculosis (1·4 million).¹ The most common causes of viral hepatitis are the five distinct hepatotropic viruses: hepatitis A to E. Hepatitis B and C have been shown to attack the liver and cause both acute and chronic diseases. Globally, an estimated 257 million people have chronic hepatitis B infections and another 71 million suffer from hepatitis C infections.

The prevalence of viral hepatitis infections varies by geographical region, but Middle East and North Africa (MENA) has been identified as the region most affected by viral hepatitis worldwide. The main culprits for this widespread prevalence of viral hepatitis are factors such as socioeconomic conditions, migration, vaccination, and treatment policies. The MENA region shows wide range of viral hepatitis causes, viraemic prevalence, and diversity in hepatitis B and C genotype distributions.² Although hepatitis A and



Short communication

Prevalence of baseline HCV NS5A resistance associated substitutions in genotype 1a, 1b and 3 infection in Australia

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ABSTRACT

Background: Direct-acting antivirals (DAA) have revolutionised hepatitis C virus (HCV) treatment, and most regimens include an NS5A inhibitor. Certain amino-acid substitutions confer resistance to NS5A inhibitors, termed resistance-associated substitutions (RAS). If present at baseline, they can reduce virological response rates. Population-based sequencing (PBS) is generally used for baseline sequencing, however next generation sequencing (NGS) reduces the threshold for detection of sequences encoding RAS from 20% to 5%. We determined the prevalence of NS5A RAS at baseline amongst Australian chronically infected with genotype (GT)1a, GT1b and GT3 HCV, using both PBS and NGS.

Methods: Samples from DAA-naïve individuals were received at the Victorian Infectious Disease Reference Laboratory between June 2016 and December 2018. All samples were analysed for NS5A RAS using PBS. A subset of GT1 HCV samples were processed using NGS technology (Vela Diagnostics, Singapore) to determine the improvement in sensitivity.

Results: In total, 672 samples were analysed using PBS. The baseline prevalence of NS5A RAS was 7.6% for GT1a (n = 25/329), 15.7% for GT1b (n = 8/51) and 15.1% for GT3 (n = 44/292). NGS only marginally increased sensitivity for NS5A RAS at baseline in GT1a (16% vs 17%) and GT1b (29% vs 36%).

Conclusion: The prevalence of NS5A RAS in GT1a HCV in Australia was low compared with international data, and was similar to other reported international prevalence for GT1b and GT3 infection. NGS at baseline only marginally increased sensitivity for the detection of NS5A RAS in patients with GT1 HCV and cannot be recommended for routine use at baseline in clinical practice.

1. Background

It is estimated that hepatitis C virus (HCV) infection affects 71 million people globally [1]. Direct-acting antiviral (DAA) therapy targeting the NS3, NS5A and the NS5B proteins has revolutionised HCV treatment, and cure rates exceed 90% in real world settings. Amongst those infected, the poor fidelity of the HCV viral polymerase generates a range of genetic variants, some which result in amino-acid polymorphisms. These can reduce viral susceptibility to DAAs and are known as resistance-associated substitutions (RAS); they have been detected at baseline and virological relapse.

The presence of HCV NS5A RAS at baseline can affect treatment outcomes and certain NS5A RAS have been implicated in diminished sustained virological response rates (SVR) [2–4]. As such, guidelines

recommend HCV NS5A RAS testing at treatment baseline for particular regimens, including elbasvir/grazoprevir; for this regimen, the detection of specific NS5A RAS requires extension of treatment duration and addition of ribavirin [5]. The prevalence of baseline HCV RAS varies among geographical regions. In Australia there are limited data regarding the prevalence of baseline genotype (GT)1a and GT1b NS5A RAS [6]. Recent data has demonstrated a baseline GT1a NS5A RAS prevalence of 11.9% in one Australian region [7], however there are no Australian prevalence data regarding baseline HCV GT3 or GT1b NS5A RAS, nor data evaluating the utility of next generation sequencing (NGS) at baseline.

Population-based sequencing (PBS) for detection of NS5A RAS has a threshold limit of approximately 20%. The added yield of NGS, as well as the clinical relevance of low frequency variants remains unclear [8].

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In this context, we determined the frequency of NS5A RAS in DAA-naïve HCV GT1a, 1b and 3 in Australia, using both PBS and NGS.

2. Objectives

We tested for NS5A RAS in a large population of DAA-naïve individuals who had a baseline serum sample sent for sequencing studies prior to DAA treatment in Victoria, Australia. We restricted analysis to individuals infected with HCV GT1a, GT1b or GT3 infection. All subjects were tested for NS5A RAS using PBS. A subset of participants with GT1a and GT1b HCV were also tested for NS5A RAS using NGS.

3. Study design

3.1. Clinical samples

All serum samples received at the Victorian Infectious Disease Reference Laboratory between June 2016 and December 2018 were investigated. HCV genotype was previously determined with the Abbott RealTime genotyping II assay or the Versant HCV Genotype 2.0 assay (Siemens Healthcare Diagnostics, Surrey, UK). All participants were verified as DAA-naïve from their medical record. RNA was extracted from 200 µL of patient serum and eluted to 100 µL by an automated method, using the Abbott m2000 instrument (Abbott MolecularAbbott Park, IL) and the Sample Preparation System_{RNA} kit (AbbottMolecular).

3.2. Population sequencing approach

The NS5A region (639 nt) was amplified in a nested PCR using primers with Superscript III one-step RT-PCR system with Platinum Taq (Invitrogen, Waltham, MA) and Taq DNA Polymerase (Qiagen, Hilden, Germany). Purified PCR product was sequenced using ABI-Prism 3730 Genetic Analyser (Applied Biosystems, Life Technologies, Ltd, Paisley, UK). Using the ABI sequence analysis program SeqScape version 2.1.1 (Applied Biosystems), a consensus sequence was generated and aligned to a stored GenBank HCV reference sequence (Subtype 1a H77, subtype 1b Con 1, and subtype 3a HPCCK3A).

3.3. Next generation sequencing approach

A subset of HCV GT1 samples were also tested using the NGS platform *Sentosa*[®] SQ HCV Genotyping assay (Vela Diagnostics, Singapore). With an initial input of 530 µL of serum, RNA was isolated from serum samples by an automated method using the Vela Diagnostics *Sentosa*[®] SX101 instrument. The PCR amplification step was performed off-board and the plate returned to the *Sentosa*[®] SX101 instrument for normalisation, enzymatic shearing, purification, adapter ligation and library pooling. After emulsion PCR and enrichment of the ion sphere particles, the amplicons were then subjected to NGS on the *Sentosa*[®] SQ301 Sequencer, with the data analysed by the *Sentosa*[®] SQ Suite software. The threshold for detection of this platform is 5%.

3.4. Definition of NS5A resistance associated substitutions

HCV NS5A RAS were defined as polymorphisms encoded by the NS5A gene associated with at least two-fold reduced susceptibility to available NS5A inhibitors in *in-vitro* cell-based replicon assays [9–11]. All changes at positions 24, 28, 30, 31, 58, 92 and 93 were investigated.

3.5. Ethical considerations

This study was approved by the St Vincent's Hospital Melbourne Human Research Ethics committee (HREC/17/SVHM/143).

Table 1

Frequency of NS5A RAS at treatment baseline amongst subjects chronically infected with GT1a, 1b and 3 HCV using a population sequencing approach.

Frequency of baseline HCV RAS, N (%)	NS5A RAS	GT1a	GT1b	GT3
Samples with NS5A RAS identified		25/329 (7.6)	8/51 (15.7)	44/292 (15.1)
Single RASs at NS5A position:				
28	M28L	1 (0.3)		
	M28T	2 (0.6)		
	M28V	9 (2.7)		
30	A30K			15 (5.1)
	A30S			4 (1.4)
	A30V			1 (0.3)
	Q30H	1 (0.3)		
	Q30R	1 (0.3)		
31	L31M	6 (1.8)	3 (5.9)	
	L31V	1 (0.3)		
92	A92T		1 (2.0)	
93	Y93H	1 (0.3)	4 (7.8)	18 (6.2)
	Y93N	1 (0.3)		
	Y93S	1 (0.3)		
Dual RAS at NS5A position				
28, 58	M28 V, H58Q	1 (0.3)		
30, 31	A30 K, L31M			2 (0.7)
93, 30	Y93H, A30K			2 (0.7)
	Y93H, A30T			2 (0.7)

Prevalence of NS5A RAS detected via population sequencing at baseline, presented by genotype, as frequencies and (percentages). Abbreviations: GT, genotype, RAS, resistance-associated substitution, HCV hepatitis C Virus.

4. Results

4.1. Prevalence of NS5A RAS using PBS

In total, 672 samples were analysed. There were 329 HCV GT1a samples, and 26 NS5A RAS were identified in 25 patient samples (7.6%, $n = 25/329$) (Table 1). The most frequent GT1a NS5A RAS were M28 V (2.7%, $n = 9/329$) and L31 M (1.9%, $n = 6/329$). Dual NS5A RAS were identified in one sample (Table 1).

There were 51 HCV GT1b samples. NS5A RAS were identified in 15.7% (8/51) (Table 1). Y93H was most frequently identified (7.8%, $n = 4/51$), then L31 M (5.9%, $n = 3/51$) and A92 T (2.0%, $n = 1/51$).

There were 292 GT3 HCV samples (subtype 3a accounts for almost all GT3 infection in Australia [12]) and 47 NS5A RAS were identified in 44 patient samples (15.1%, $n = 44/292$) (Table 1). The most prevalent single RAS included Y93H (6.2%, $n = 18/292$) and A30 K (5.1%, $n = 15/292$). Dual RAS were identified in six samples (Table 1).

4.2. Benefit of NGS at baseline in GT1a and 1b samples

Eighty-nine GT1a and 14 GT1b samples were also analysed using NGS, of which 75 and 10 respectively had no NS5A RAS detected by PBS. NGS confirmed that the RAS detected by PBS were the dominant quasiespecies (Table 2). NGS detected additional low frequency variants in 2/89 GT1a samples (in one of which no RAS was detected by PBS) and 1/14 GT1b samples, Table 2).

5. Discussion

This is the first Australian data describing the frequency of baseline HCV GT1b and GT3 NS5A RAS amongst DAA-naïve individuals with chronic HCV infection, and contributes to recent data regarding HCV GT1a RAS [7].

HCV NS5A RAS were detected in 7.6% of people with GT1a HCV

Table 2

Positive NS5A RAS results among a subset of GT1a and GT1b HCV, including the increased sensitivity of NGS over PBS methods.

Sample code	Population based sequencing	Next Generation sequencing
GT1a		
8	M28V	M28V (52.3%)
11	M28V	M28V (91.1%)
15	L31M	L31M (99.65%)
21	Y93H	Y93H (16.4%)
36	L31M	L31M (28.3%)
37	L31M	L31M (99.54%)
39	M28T	M28T (99.78%)
45	M28V	M28V (96.7%) Q30R (5.6%)
50	Q30H	Q30H (99.7%)
53	M28T	M28T (17.0%)
62	M28V	M28V (99.1%)
83	L31M	L31M (45.2%)
86	No RAS detected	L31M (5.4%)
87	L31M	L31M (98.9%)
88	M28V	M28V (66.4%)
GT1b		
1	L31M	L31M (79.6%)
2	L31M	L31M (99.5%)
3	L31M	L31M (99.1%)
9	No RAS detected	Y93H (8.2%)
14	Y93H	Y93H (68.3%)

Comparison between the frequency of detection of NS5A RAS between PBS and NGS methodologies. GT1a samples 45 and 86 and GT1b sample 9 (highlighted in blue) represent serum samples where a single or dual NS5A RAS were identified by NGS (including the percentage of the prevalent virus) which was not detected by PBS.

infection. The most prevalent GT1a NS5A RAS were M28 V and L31 M. Certain baseline NS5A RAS at positions M28, Q30, L31 and Y93 are relevant for GT1a patients treated with elbasvir and grazoprevir, and pre-treatment testing is recommended when this regimen is utilised in certain regions. Our data demonstrates a low prevalence of these substitutions in HCV infected Australian patients. The prevalence of GT1a NS5A RAS in this study was lower than recent Australian data and may be partly explained by the exclusion of secondary variants such as at residue H58, when identified in isolation [7]. Large international DAA registration trials have demonstrated a higher prevalence of 11–23% in GT1a HCV [3,13,14], and 16% in a subset from Oceania [6]. Smaller overseas studies have also reported higher GT1a NS5A RAS frequencies

of 10–17% (15–17). Our lower prevalence supports Australian guidelines that baseline PBS to detect pre-existing RAS is not indicated [18].

The frequency of RAS among GT1b and GT3 HCV was similar to other international studies. Our analysis demonstrates baseline GT1b NS5A RAS frequency of 15.7%, similar to international data (16–33%) [3,6,14–16,19,20]. The prominent GT1b NS5A RAS identified included Y93H (7.8%) and L31 M (5.9%), concordant with other geographical regions.

The baseline frequency of GT3 NS5A RAS was similar to that in North America and Europe (12–19%) [3,14,16,21], however lower compared to other countries in the Asian-Pacific region. This includes China (> 50%), reflecting their high prevalence of GT3b infection and

likelihood of baseline dual A30 K/L31 M RAS [20]. Y93H was the most prevalent GT3 NS5A RAS (7.5% including where present as a dual substitution) conferring significant resistance to all NS5A inhibitors in vitro, and reducing virological outcomes in GT3 patients treated with sofosbuvir/velpatasvir [3]. A30 K was identified in 6.5% of samples, including those with dual NS5A RAS. A30K has been associated with reduced SVR12 in non-cirrhotic GT3 infected patients treated with glecaprevir/pibrentasvir for 8 weeks, a regimen used commonly in Australia [22]. Sequencing for GT1b and GT3 RAS at baseline is not currently recommended in treatment naïve individuals.

We evaluated 103 GT1 HCV samples using both NGS and PBS. The increased diagnostic yield of NGS was low, and only a small number of additional NS5A RAS at low frequencies were identified. North American and European guidelines designate that only RAS with a prevalence of > 15% are clinically significant [23,24], greater than the prevalence of the additional RAS identified via NGS. Given the overall low frequency of NS5A RAS in GT1 participants in Australia at baseline, and the minimal improvement in sensitivity, our data suggests that NGS does not have a role in routine pre-treatment assessment in DAA-naïve patients.

In summary, amongst DAA-naïve patients, baseline GT1a NS5A RAS were lower compared to international data, and comparable amongst GT1b and GT3 populations. Detection of HCV NS5A RAS by NGS at baseline only marginally increased diagnostic sensitivity and therefore has limited clinical applicability.

Author contribution statement

All authors contributed substantially to either the conception, design, acquisition, analysis or interpretation of the data and the drafting or revising the intellectual content and approved the final version being considered for publication. All authors agree to be accountable for all aspect of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Retreatment with elbasvir, grazoprevir, sofosbuvir $\pm$ ribavirin is effective for GT3 and GT1/4/6 HCV infection after relapse

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Abstract

Introduction: Despite highly effective direct-acting antiviral (DAA) therapies for chronic hepatitis C virus (HCV) infection, some patients experience virological relapse. Salvage regimens should include multiple agents to suppress emergence of resistance-associated substitutions (RAS) and minimise treatment failure. The combination of sofosbuvir (SOF) and elbasvir/grazoprevir (ELB/GZR) $\pm$ ribavirin (RBV) is an effective retreatment strategy for HCV genotype (GT)1 and 4 infection. We hypothesised that SOF and ELB/GZR ($\pm$ RBV) would also be an effective salvage regimen for DAA-experienced GT3 patients.

Methods: We evaluated the efficacy and safety of SOF/ELB/GZR $\pm$ RBV in DAA-experienced participants with chronic HCV infection who had prior relapse. Participants were treated at four hospitals between December 2016 and March 2018 for either 12- or 16-weeks. The primary endpoint was sustained virological response at week 12 post-treatment (SVR12) using intention-to-treat analysis.

Results: There were 40 participants included in the analysis. The mean age was 53 years, 53% had GT3, 33% had GT1 infection and 63% had cirrhosis. Fifty-eight percent were treated for 12 weeks, 42% were treated for 16 weeks and 90% received RBV. The SVR12 rate was 98% overall, 100% in non-GT3 participants and 95% in GT3 participants. One GT3 cirrhotic participant relapsed. ELB/GZR was stopped at week 6 in one GT3 cirrhotic participant who switched to SOF/velpatasvir/RBV for a further 12 weeks and achieved SVR12. RBV dose reduction was required in two participants. Treatment was otherwise well tolerated.

Discussion: The combination of SOF/ELB/GZR $\pm$ RBV is effective and safe for difficult-to-cure patients who relapse after first-line DAA, including those with cirrhosis and GT3 infection.

KEYWORDS

hepatitis C, resistance-associated substitution, retreatment, salvage, virological relapse

Abbreviations: DAA, direct-acting antiviral; ELB, elbasvir; EOT, end of treatment; GT, genotype; GZR, grazoprevir; HCC, hepatocellular carcinoma; HCV, hepatitis C Virus; INR, international normalised ratio; MELD, model for end stage liver disease; NS5A, non-structural protein 5A; RAS, resistance-associated substitutions; RBV, ribavirin; RNA, ribonucleic acid; SOF, sofosbuvir; SVR12, sustained virological response week 12.

1 | INTRODUCTION

The introduction of highly effective and well-tolerated direct-acting antiviral (DAA) therapy has revolutionised hepatitis C virus (HCV) treatment. Despite excellent cure rates which exceed 95%, some patients experience virological relapse after treatment. Virological relapse is associated with the selection of HCV resistance-associated substitutions (RAS), which reduce viral susceptibility to currently available DAAs.¹ NS5A inhibitors are the backbone of most DAA regimens and selection of NS5A RAS is the most common reason for DAA failure. NS5A variants can persist for many years post-relapse.² Variants in the NS5B region and/or NS3/4a region may also be selected with sofosbuvir (SOF) or protease inhibitor therapy respectively. The ideal salvage regimen should involve combination therapy with at least two agents that are active against all the prevalent HCV quasiespecies. Triple combination therapy involving sofosbuvir (SOF), a protease inhibitor and an NS5A inhibitor is therefore attractive as a salvage strategy.

Elbasvir (ELB) plus grazoprevir (GZR) is licensed for treatment of HCV genotype (GT)1 and 4 infection.³ Recent data have demonstrated that ELB/GZR in combination with SOF ($\pm$ ribavirin (RBV)) is also an effective salvage treatment for cirrhotic DAA-experienced GT1/4 patients, those genotypes for which ELB/GZR has the greatest in vitro and in vivo activity.⁴ ELB and GZR alone have reduced effectiveness against HCV GT3 infection, with a significantly higher EC₅₀ than for GT1 HCV in vitro and suboptimal SVR12 rates in clinical trials.^{3,5} Despite this, ELB/GZR in combination with SOF plus RBV was highly effective for the treatment of DAA-naïve GT3 patients; in a large prospective study SVR12 rates exceeded 94% in those treated for 12 to 16 weeks, including participants with cirrhosis.⁶

In this context, we hypothesised that SOF plus ELB/GZR $\pm$ RBV (ELB/GZR/SOF $\pm$ RBV) would be an effective salvage regimen for DAA-experienced patients, irrespective of GT or underlying cirrhosis.

2 | PATIENTS AND METHODS

2.1 | Patients

From December 2016, a special access scheme supported by Merck Sharp and Dohme Australia allowing access to ELB/GZR for use in combination with SOF/RBV for retreatment of patients who were non-responders to licensed DAA therapy. SOF/RBV were prescribed via the Pharmaceutical Benefit Scheme which reimburses the cost of HCV treatment for all Australians.⁷ Patients for whom this treatment was accessed from four tertiary centres in Melbourne, Australia were consecutively treated with SOF 400 mg daily, fixed dose combination ELB/GZR 100 mg/50 mg daily, with or without RBV (600–1200 mg daily) for either 12 or 16 weeks. Duration of therapy was decided on an individual basis by the treating clinician at each participating site.

Eligibility criteria for the special access scheme included non-response following treatment with an approved NS5A inhibitor-containing DAA regimen, defined as detectable HCV RNA at week 12 post-treatment, and infection with HCV GT 1, 3, 4 or 6.

Keypoints

A small number of patients are not cured after their first course of hepatitis C treatment. Retreatment of these patients should include a combination of drugs to treat resistant hepatitis C virus. The combination of sofosbuvir, elbasvir, grazoprevir and ribavirin appears to be effective for retreatment of these patients, including for genotype 3 for which some of these drugs are less effective when used alone. In this study, there was a high rate of cure and treatment overall was safe. This combination could provide an alternate retreatment strategy in carefully selected settings.

All participants in this study were deemed compliant with their initial DAA treatment regimen. Exclusion criteria for this study included: decompensated chronic liver disease (Child-Pugh Category B/C), active or suspected hepatocellular carcinoma, contraindicated drug-drug interactions and prior liver transplantation. Cirrhosis was determined by liver stiffness measurements using FibroScan (cut-off ≥ 12.5 kPa), prior liver biopsy or radiological and/or endoscopic evidence of portal hypertension. Portal hypertension was defined as the presence of oesophageal varices at endoscopy or splenomegaly, intra-abdominal varices or ascites identified on abdominal imaging.

2.2 | Assessment

All participants were reviewed at baseline, treatment week 4, end of treatment (EOT) and 12 weeks post-treatment at a minimum. On-treatment virological response was defined as undetectable HCV RNA on PCR testing. The primary outcome was SVR12, defined as undetectable HCV RNA at 12 weeks post-EOT. All cirrhotic patients were enrolled in hepatocellular carcinoma (HCC) surveillance.

The secondary outcomes were frequency of treatment and non-treatment related adverse events and the frequency of denovo or recurrent HCC. Safety and tolerability were monitored at each clinical review and further investigations were arranged where deemed appropriate. All adverse events, laboratory abnormalities and treatment interruptions and/or discontinuations were recorded.

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines, and regulatory requirements. The study was approved by the St Vincent's Hospital Melbourne Human Research Ethics committee prior to governance review at each participating hospital. A waiver of consent was granted by the St Vincent's Hospital HREC and then ratified at each participating site.

2.3 | Assessment for NS5A resistance-associated substitutions

A subset of participants was investigated for sequences encoding NS5A RAS at time of relapse, prior to treatment with the study

regimen. Testing was performed at the Victorian Infectious Disease Reference Laboratory using Sanger sequencing with a prevalence threshold for detection of 20%.

2.4 | Statistical analysis

Results are presented as median with interquartile range for continuous data and number (percentage) for categorical data. Analysis was performed using Stata 15.2 for Windows, StataCorp LLC.

TABLE 1 Baseline characteristics

Baseline characteristics	N = 40
Age, mean, [IQR]	53 [46-60]
Male gender, n (%)	32 (80%)
ALT U/L, median, [IQR]	87 [51-124]
HCV RNA IU/mL, median, [IQR] ^a	1, 842, 281 [303, 500-7, 548, 579]
Platelet count, median, [IQR]	188 [155-237]
Albumin, median, [IQR]	39 [36-40]
Bilirubin, median, [IQR]	15 [12-24]
INR, median, [IQR]	1.1 [1.0-1.1]
HCV Genotype, n (%)	
1a	11 (28%)
1b	2 (5%)
3 ^b	21 (53%)
4 ^c	3 (7%)
6	3 (7%)
PR treatment experienced, n (%)	12 (30%)
Prior HCC, n (%)	2 (5%)
Active HCC, n (%)	0 (0%)
Liver stiffness measurements of non-cirrhotic participants, n	
<6 kPa	7/15
6-9.5 kPa	7/15
9.5-12.5 kPa	1/15
Cirrhotic, n (%)	25 (63%)
Child-Pugh score	
A5	18/25
A6	6/25
B7 ^d	1/25
MELD score, mean [IQR]	8 [7-10]
Portal hypertension, n, (%)	9/25 (36%)
Prior HCV treatments	
Sofosbuvir/Ledipasvir	15
Sofosbuvir/Daclatasvir	22
Sofosbuvir/Velpatasvir	1
Elbasvir/Grazoprevir	1
Paritaprevir/r, Ombitasvir, Dasabuvir	1

(Continues)

TABLE 1 (Continued)

Baseline characteristics	N = 40
Duration of prior treatment	
8 wk	2
12 wk	20
24 wk	18

Abbreviations: ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalised ratio; IQR, interquartile range; MELD, model for end stage liver disease; PR, Pegylated interferon and ribavirin; RNA, ribonucleic acid.

^aMedian and interquartile range presented for 30 of 40 participants.

Ten of 40 participants were HCV PCR positive at baseline, however, quantitative HCV viral load testing was not performed.

^bGT subtyping was performed on 17 of 21 GT3 participants, all who were subtype 3a.

^cGT subtyping was performed on two of three GT4 participants, who were both subtype 4h.

^dOne Child-Pugh B7 participant was commenced on the study regimen beyond the inclusion criteria of the special access scheme for ELB/GZR, however, baseline hypoalbuminaemia was attributed to poorly controlled type 2 diabetes with documented proteinuria.

3 | RESULTS

3.1 | Participant characteristics

Forty participants with chronic HCV infection and prior virological failure after HCV DAA therapy were included. Baseline characteristics are displayed in Table 1. The sample was predominately male (n = 32, 90%), just over half had HCV GT3 infection (n = 21, 53%) and majority were cirrhotic (n = 25, 63%). Of those with cirrhosis, nine (36%) had portal hypertension.

Prior treatment regimens included SOF + ledipasvir (n = 15, 37%), SOF + daclatasvir (n = 22, 55%), SOF + velpatasvir (n = 1, 3%), ELB/GZR (n = 1, 3%) and paritaprevir/r + ombitasvir + dasabuvir (n = 1, 3%). Treatment durations are detailed in Table 1. All participants had completed the entire course of the prior treatment and had detectable HCV RNA at 12 weeks post-treatment completion. A mean duration of 10 months (range 5-19 months) had elapsed between EOT of the initial regimen and initiation of the study regimen. HCV NS5A sequencing results were available in 26 of 40 subjects, with the majority having NS5A RAS detectable (Table S1).

3.2 | HCV DAA therapy and virological response

Of the 40 participants, 23 (58%) and 17 (42%) were treated with 12 weeks and 16 weeks of SOF/ELB/GZR ± RBV respectively. Thirty-six (90%) received RBV; RBV was omitted in one GT3 participant with a history of prior RBV intolerance, and in three GT1/6 participants at the discretion of the treating physician because of concerns about tolerance. Treatment outcomes are displayed in Figure 1. Overall, the SVR rate was 97.5% (n = 39/40). The SVR rate amongst HCV GT1, 4 and 6 participants was 100% (n = 19/19), irrespective of treatment duration (12 or 16 weeks) or inclusion of RBV. Amongst

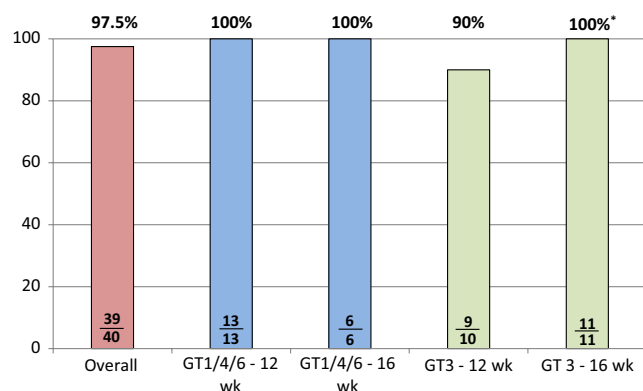


FIGURE 1 SVR12 rates by HCV genotype and duration of therapy. Abbreviation: GT, genotype. *One participant stopped SOF/ELB/GZR/RBV at TW6 because of increasing bilirubin and INR, was immediately changed to SOF/VEL/RBV for an additional 12 wk and achieved SVR12. Ninety percent participants received ribavirin, which was omitted in two GT 1, one GT 6 and one GT 3 participant

HCV GT3 participants, overall SVR rate was 95% ($n = 20/21$), 90% (9/10) for 12 weeks of therapy and 100% (10/10) for 16 weeks. This included an SVR12 rate of 100% (5/5) in non-cirrhotic GT3 participants, and 94% ($n = 15/16$) amongst cirrhotic GT3 participants.

One episode of virological relapse occurred in a HCV GT3 cirrhotic participant (Child-Pugh A5, MELD 6). NS5A RAS testing at relapse was significant for the Y93H RAS. The participant was treated with 12 weeks of SOF/ELB/GZR/RBV, but had detectable HCV RNA at SVR12 time point. A second HCV GT3 cirrhotic participant with portal hypertension (Child-Pugh A6, MELD 9) prescribed ELB/GZR/SOF/RBV for 16 weeks experienced an increase in bilirubin (30 to 50 $\mu\text{mol/L}$) and INR (1.3 to 1.5) at treatment week 6, and was immediately switched to SOF + velpatasvir + RBV for an additional 12 weeks. Bilirubin remained stable at 40–50 $\mu\text{mol/L}$ for the duration of RBV therapy before returning to baseline. The INR remained stable between 1.4 and 1.5 throughout the period of evaluation. There was no rise in the ALT or clinical decompensation event at any time. The participant went on to achieve SVR12.

One participant was commenced on ELB/GZR/SOF/RBV beyond the inclusion criteria of the ELB/GZR special access program. The participant was Child-Pugh B7 at baseline, however their baseline hypoalbuminaemia (albumin 27 g/L) was thought to be related to their diabetic nephropathy and significant proteinuria, rather than their underlying liver disease. There was no adverse event, and the participant achieved SVR12.

SVR12 rate was 100% ($n = 9/9$) amongst participants with no HCV NS5A RAS and 94% ($n = 16/17$) in participants with an NS5A RAS detected at baseline. SVR12 was achieved in 92% ($n = 12/13$) of participants with virus with the Y93H/D/S NS5A RAS at baseline.

3.2.1 | Hepatocellular carcinoma

No de novo or recurrent hepatocellular carcinomas (HCC) were identified during the period of evaluation.

3.3 | Adverse events

Adverse events are detailed in Table 2. One HCV GT3 cirrhotic participant discontinued therapy at treatment week 6 as described. There were 25 adverse events in 19 participants. Most were RBV-related haematological abnormalities, but also included fatigue, headache, gastrointestinal upset, insomnia and rash and were mild in severity. Two participants required dose reduction of RBV for management of anaemia.

4 | DISCUSSION

Whilst DAA therapy cures most people with HCV, a subset of patients do not respond to first-line treatment. This hard-to-cure group is characterised by a high prevalence of HCV GT3 infection, cirrhosis and portal hypertension.⁸ Our data suggests that the combination of SOF/ELB/GZR $\pm$ RBV is highly effective for the retreatment of DAA non-responders, in both GT3 and GT1/4/6 HCV infection. The overall SVR12 rate was 98%, and 95% amongst GT3 cirrhotic participants, similar to the approved pan-genotypic salvage regimens.^{9,10}

A small study has previously reported the efficacy of this regimen for the treatment of non-responders with GT 1/4 HCV infection⁴ and the SVR12 rate was 100% (per protocol) in 25 patients. Our study is the first description of efficacy amongst a group of non-responders that included with patients with GT3 HCV infection who had relapsed after treatment with an NS5A-containing regimen. In our study, 38/40 patients (21 who were GT3) had relapsed following treatment with a regimen including SOF plus an NS5A inhibitor. Both ELB and GZR have an EC_{50} for GT3 that is at least 10-fold greater than for GT1.³ In clinical trials of treatment naïve patients, SVR12 rates were only 41% for HCV GT3 infected patients treated with ELB/GZR for 12 weeks.⁵ In contrast, combination therapy with SOF/ELB/GZR $\pm$ RBV has been shown to be effective for treatment naïve patients with GT3 HCV, including patients with cirrhosis,⁶ with SVR12 rates >90%. We now show a similar rate of SVR12 in treatment-experienced patients. Our data support synergy between SOF and ELB/GZR and effectiveness for HCV GT3. Of note, the rate of

TABLE 2 Adverse events

Adverse events, n (%)	N = 25
Haemoglobin decline	
20–40 g/L	9 (23)
>40 g/L	2 (5)
Requiring ribavirin dose reduction	2 (5)
Fatigue	5 (13)
Headaches	3 (8)
Gastrointestinal upset	3 (8)
Insomnia	1 (3)
Rash	1 (3)
Treatment discontinuation	1 (3)

SVR12 in our cohort is numerically higher than that reported in a cohort of participants (including HCV GT1, GT2 and GT3 infection) who were re-treated with SOF/velpatasvir/RBV for 24 weeks following relapse after a first course of SOF/velpatasvir ($n = 63/69$, 91%).¹¹

There was one episode of virological relapse in a HCV GT3 participant. The participant had previously relapsed following treatment with pegylated interferon and ribavirin and subsequently SOF/daclatasvir for 24 weeks, was cirrhotic and had virus with the Y93H NS5A RAS detectable at retreatment baseline which is associated with high-level resistance to NS5A inhibitors.¹² The participant reported good adherence to 12 weeks of SOF/ELB/GZR/RBV600 before relapsing. It is possible that longer treatment duration for 16 weeks may have been more effective.

The combination of SOF/ELB/GZR/RBV was safe. The most frequent adverse events were haematological abnormalities associated with RBV. A dose reduction was only required in two cases. The most serious adverse event occurred in a HCV GT3 participant with advanced cirrhosis. At TW6 the participant's bilirubin level had risen from 30 to 50 $\mu\text{mol/L}$ and INR from 1.3 to 1.5. The participant was switched to SOF/velpatasvir/RBV because of concern about protease inhibitor-related hepatotoxicity. The participant's hyperbilirubinaemia at TW6 may be attributable to RBV-associated haemolysis, however, this does not account for their INR prolongation. HCV NS3/4A protease inhibitors are contraindicated in Child-Pugh B/C cirrhosis, in whom accumulation may cause hepatotoxicity,¹³ and participant with Child-Pugh A cirrhosis should be closely monitored. Notably, the participant went on to achieve SVR12, suggesting that 6 weeks of protease inhibitor exposure, with a longer tail of SOF/velpatasvir/RBV was sufficient to eradicate virus.

Following the introduction of HCV DAAs, there was concern regarding potential increases in HCC incidence and recurrence with antiviral therapy.¹⁴ More recent data suggests this correlation is unlikely.¹⁵ In the *Revenge* study, a comparable population with HCV GT1/4 infection was treated with SOF/ELB/GZR/RBV, in which 19% (5/26) of the participants developed HCCs during the period of evaluation.⁴ The authors attributed this to the study populations advanced age, duration of HCV infection and high prevalence of cirrhosis, rather than to the study regimen. This is supported by our data where no recurrent or de novo HCCs were identified during the period of evaluation.

Two DAA regimens have recently been approved by the Federal Drug Administration and European Medicines Agency for the treatment of DAA non-responders. These include the combination of SOF/velpatasvir/voxilaprevir, approved for the treatment of all HCV genotypes, and glecaprevir/pibrentasvir, approved for the retreatment of people with GT1 HCV previously treated with a regimen including either (but not both) an NS5A inhibitor or an NS3/4A protease inhibitor; and non-GT1 HCV who have previously been treated with SOF/PR. Whilst these regimens are licensed in the US and some countries in Western Europe, they are not yet available in all regions because of licensing or funding restrictions. Our data supports the combination of SOF/ELB/GZR $\pm$ RBV as a second pan-genotypic retreatment option, combining drugs which are currently licensed

and available in most regions. The role of the different retreatment options is likely to vary between regions according to cost and drug availability.

Study limitations include an inability to define the optimal duration for SOF/ELB/GZR $\pm$ RBV and the role of RBV, particularly for HCV GT3 participants, for which prospective randomised studies are required. Until such time, we recommend treating GT3-infected patients for 16 weeks in combination with RBV, where possible.

In conclusion, the combination of SOF/ELB/GZR $\pm$ RBV was safe and effective salvage treatment for patients who had not responded to a previous NS5A-containing DAA therapy. It was effective for the treatment of hard-to-cure GT3 as well as GT1/4/6 HCV infection. The DAAs in this regimen are currently available in many regions, and represent another option for treating patients who do not respond to a first course of DAA therapy, with the aim of stopping the progression of liver disease and reducing HCV-related morbidity and mortality.

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CONFLICT OF INTEREST

TP: honoraria from Merck for speaking duties; SP: advisory board—Gilead and Merck. Honoraria from AbbVie, Gilead and Merck; JD: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS. Honoraria to institution from Gilead, Merck and BMS; MH: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS; DI: honoraria from AbbVie, BMS, Gilead and Merck; AJT: investigator initiated research funding to institution from Gilead, Merck, AbbVie and BMS; advisory board—Gilead, AbbVie, BMS, Merck, Eisai.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Efficacy and Safety of Sofosbuvir/Velpatasvir/Voxilaprevir for Hepatitis C Virus (HCV) NS5A-Inhibitor Experienced Patients With Difficult to Cure Characteristics

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Background. In clinical trials, hepatitis C virus (HCV) salvage treatment with sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) achieved an SVR12 rate of >95% in NS5A-experienced participants. Lower SVR12 rates have been reported in real-world studies, particularly for genotype (GT)3 infection and cirrhosis. We determined the efficacy and safety of SOF/VEL/VOX in a large real-world cohort.

Methods. We assessed the efficacy of salvage SOF/VEL/VOX for HCV infection in NS5A-inhibitor experienced participants with cirrhosis and portal hypertension, prior liver transplantation (LT) or severe extra-hepatic manifestations. SOF/VEL/VOX was available via an early access program. The primary outcome was SVR12. Secondary outcome was frequency of adverse events (AE).

Findings. Ninety-seven participants were included. Median age was 58, 82% were male, 78% had cirrhosis, most with portal hypertension (61%, n = 46/76), and 18% had prior-LT. Of the cirrhotic participants, 96% were Child-Turcotte-Pugh class A, and 4% were class B. Of the 72% with GT3, 76% were also cirrhotic. By intention-to-treat analysis, SVR12 rate was 85% (n = 82/97). Per protocol, the SVR12 rate was 90%, including 91% in GT1 (GT1a n = 18/18, GT1b n = 2/4), 89% in GT3 (n = 59/66) and 100% in GT6 (n = 3/3). SVR12 in participants with GT3 and cirrhosis was 90%. No predictors of non-SVR12 were identified. There were 4 serious AEs including 1 death and 3 hepatic decompensation events. NS5A resistance-associated substitutions detected at baseline did not affect SVR12.

Conclusions. This real-world study confirms high efficacy of SOF/VEL/VOX for the treatment of difficult-to-cure NS5A-inhibitor experienced patients, including those with GT3 and cirrhosis. Treatment was well tolerated in most; however, serious AEs can occur in those with advanced liver disease.

Keywords. hepatitis C; relapse; cirrhosis; genotype 3; sofosbuvir/velpatasvir/voxilaprevir.

Hepatitis C virus (HCV) infection affects approximately 71 million people worldwide and contributes significantly to liver-related morbidity and mortality [1, 2]. Direct acting antiviral (DAA) therapy has revolutionized HCV treatment, with

cure rates exceeding 95% in registration trials [3–5]. Some patients, however, do not respond to their initial DAA therapy, more commonly those with HCV genotype (GT)3 infection and with cirrhosis [6, 7]. Virological failure is associated with the selection of HCV resistance associated substitutions (RAS), and ideal retreatment regimens should target the NS5A, NS5B, and NS3 proteins [8, 9].

The efficacy of sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX) for relapsed HCV infection following treatment with an NS5A-inhibitor (NS5Ai) containing DAA regimen has been investigated in multiple clinical trials [10]. In the POLARIS-1

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registration trial, NS5A-experienced participants treated with SOF/VEL/VOX achieved a sustained virological response 12 weeks after end of treatment (SVR12) rate of 96%. Several real-world studies have subsequently suggested reduced efficacy in those with cirrhosis, prior SOF/VEL exposure, and HCV GT3 infection [11–14]. This included a SVR12 rate of 69% among the small number of participants with both HCV GT3 infection and cirrhosis in one Spanish cohort [11]. In most studies, a minority of participants had cirrhosis (35–41%), and only 1 included those with prior liver transplantation (LT), where complex drug-drug interactions must be considered [11–13].

In both registration and real-world trials, treatment with SOF/VEL/VOX was generally well tolerated, and adverse events (AE) were limited. It is important to note that NS3/4A PIs are not recommended in Child-Turcotte-Pugh (CTP) B/C cirrhosis due to concerns about hepatotoxicity related to higher VOX drug exposures. The Food and Drug Administration (FDA) has also recently cautioned practitioners to closely monitor patients with compensated cirrhosis during treatment with an HCV protease inhibitor (PI) due to the potential for hepatic decompensation, particularly in the setting of portal hypertension (PHT), hepatocellular carcinoma (HCC), or alcohol misuse [15]. As SOF/VEL/VOX is increasingly used as salvage treatment in patients with advanced disease to avoid LT, it is important that these risks are further explored and validated in real-world settings [16, 17].

In this study, we have evaluated the efficacy and safety of SOF/VEL/VOX in a difficult to cure population with advanced liver disease or prior-LT.

METHODS

This was a retrospective, nationwide study to evaluate the efficacy and safety of SOF/VEL/VOX for relapsed HCV infection among participants with advanced liver disease or LT, treated in 27 Australian centers. All participants were treated with fixed dose SOF 400 mg, VEL 100 mg, and VOX 100 mg for 12 weeks available via an early access program (EAP) supported by Gilead Sciences. The EAP permitted access to SOF/VEL/VOX before reimbursement on the national prescription drug scheme. As such, at this time, this regimen was only available to Australians via this EAP, where specific eligibility criteria were satisfied. Eligibility criteria for the EAP were (i) age >18 years; (ii) chronic HCV infection GT1–6; (iii) prior treatment failure on a NS5A-inhibitor DAA containing regimen; (iv) compensated liver disease with at least one of (a) CTP class A cirrhosis and clinically significant portal hypertension (hepatic venous portal gradient (HVPG) > 10mmHg or the presence of varices (radiologically / endoscopically) or platelets $<100 \times 10^9/L$, (b) prior-LT, or (c) severe extrahepatic manifestations; and (v) eGFR $\geq 30\text{mL/min/1.73m}^2$. Exclusion criteria for the EAP included (i) hepatic decompensation (CTP class B/C), (ii) contraindication

or known hypersensitivity to the active substances or to any other component of the tablets, and (iii) concomitant prescription of rifampicin and/or rosuvastatin. Participants with human immunodeficiency virus (HIV) and/or hepatitis B virus (HBV) coinfection were eligible for inclusion. Combination therapy with ribavirin was permitted.

Australian hospitals with 2 or more participants treated via the Gilead Sciences EAP were invited to participate in this study. The primary outcome was the frequency of participants achieving SVR12, defined as the absence of detectable HCV RNA at least 12 weeks after end-of-treatment (EOT). The secondary outcome was frequency of treatment related adverse events (AE). Participants were followed until the SVR12 time point.

Assessments

Participant data were collected at baseline, EOT, and SVR12 timepoints with SOF/VEL/VOX, as well as details of prior HCV treatments. Cirrhosis status was defined as transient elastography score ≥ 12.5 kPa, prior liver biopsy demonstrating METAVIR fibrosis score 4, or clinical/radiological evidence of cirrhosis. Clinical evidence of cirrhosis included signs of cirrhosis and baseline platelet count of $<100 \times 10^6$. PHT was defined by the presence of intra-abdominal and/or esophageal varices, splenomegaly, or ascites. CTP scores were recorded at all timepoints. Laboratory investigations recorded included full blood count, renal and liver biochemical tests, HCV RNA, HCV genotype, HIV and HBV serology. The presence of HCV NS5A RAS were investigated in a subset of participants where serum was available, utilizing Sanger sequencing with a threshold limit of 20% for variant detection. HCV NS5A RAS were defined as polymorphisms encoded by the NS5A gene associated with at least 2-fold reduced susceptibility to a registered NS5A inhibitor. Virological breakthrough (VBT) was defined as detectable HCV RNA on PCR testing at the EOT timepoint.

Adverse events (AE) related to treatment with SOF/VEL/VOX were recorded until the SVR12 timepoint. Serious AEs were particularly scrutinized including hepatic decompensation, and AEs leading to hospitalization, treatment discontinuation, and death.

Ethical Considerations

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, Good Clinical Practice Guidelines, and regulatory requirements. The study was approved by the Human Research Ethics Committee at St Vincent's Hospital Melbourne.

Statistical Analysis

Statistical analysis was performed using STATA 12.0 (StataCorp LP, College Station, TX, USA). Normally distributed continuous data were analyzed with the Student *t* test, whereas

non-normally distributed data were analyzed using the Mann-Whitney *U* test. Categorical data were described as number and percentage and were analyzed using χ^2 or Fisher exact test, as appropriate. Variables associated with SVR12 were investigated using appropriate univariable statistical tests after considering distribution of data. A *P* value of < .05 was deemed statistically significant.

RESULTS

Patient Population

From June 2018 to March 2019, 97 patients were commenced on SOF/VEL/VOX across 27 Australian hospitals. Baseline characteristics are described in Table 1. In brief, the cohort was predominantly male (82%), the median age was 58 years, and the most common genotype was HCV GT3 (72%). Most of the cohort were cirrhotic (78%) or had previously undergone LT

Table 1. Baseline Characteristics of Participants Commencing SOF/VEL/VOX

Baseline Characteristics	N = 97
Age, median [IQR]	58 [53–64]
Male sex, n (%)	80 (82)
HCV viral load IU/mL, median, [IQR] (75/97)	654 000 [160 000–2 870 000]
HCV genotype, n (%)	
- Genotype 1a	19 (20)
- Genotype 1b	4 (4)
- Genotype 3	70 (72)
- Genotype 4	1 (1)
- Genotype 6	3 (3)
ALT, U/mL, median [IQR]	85 [48–170]
Albumin, g/L, median [IQR]	37 [33–40]
Bilirubin, umol/L, median [IQR]	14 [10–21]
Platelet count, median [IQR]	125 [90–178]
INR, median [IQR]	1.1 [1–1.2]
HIV infection, n (%)	2 (2)
HBV serology, n (%)	
HBsAg +	3 (3)
HBcAb +	29 (30)
Cirrhosis, n (%)	76 (78)
CPA5	38/76 (50)
CPA6	35/76 (46)
CPB7	1/76 (1)
CPB8	2/76 (3)
Cirrhosis with portal hypertension, n (%) ^a	46/76 (61)
Post liver transplantation, n (%)	17 (18)
Noncirrhotic, n (%)	4 (4)
Prior hepatocellular carcinoma n (%)	18 (19)
Pegylated interferon ± ribavirin ± protease inhibitor experienced, n (%)	24 (25)
2+ DAA treatments prior to SOF/VEL/VOX, n (%)	15 (15)

Abbreviations: ALT, alanine aminotransferase; DAA, direct acting antiviral; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalized ratio; IQR, interquartile range; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

^aPortal hypertension defined as the presence of intra-abdominal and/or gastroesophageal varices, splenomegaly, or ascites.

(18%). Of the cirrhotic participants, 50% were CTPA5, and 46% were CTPA6. Three participants were included with CTP class B7 (n = 1) and B8 (n = 2) as protocol deviations by the treating clinicians; none of these 3 participants were felt to be LT candidates. In total, 61% of the cirrhotic participants had PHT. HIV (n = 2) and HBV (n = 3) coinfection were uncommon. A prior history of HCC was present in 19% (n = 18), 6 of whom had undergone LT. There were no participants with HCC at baseline. SOF/VEL/VOX was prescribed with ribavirin (600–1200 mg daily) for 3 participants.

Prior HCV Treatment Experience

All participants were HCV NS5Ai treatment experienced. The most recent HCV DAA treatments prescribed prior to SOF/VEL/VOX are detailed in Table 2. The most frequent common NS5Ai were daclatasvir (DCV, 56%), VEL (20%), and ledipasvir (LDV, 15%). And 15% of participants (n = 15) had received more than 1 NS5Ai-based treatment (range 1–4). Three participants were treated with an NS5Ai containing “lead-in” DAA regimen immediately prior to commencing SOF/VEL/VOX to optimize liver function, including 1 who was downgraded from CTP class B to A during this treatment. Four other participants had a history of prior hepatic decompensation that had recompensated during their preceding first-line DAA treatment. In total, 25% of participants had been treated with pegylated IFN- α + ribavirin with or without a first-generation PIs prior to their first all-oral DAA treatment course.

Efficacy

By intention to treat, 82 (85%) of the 97 participants who commenced on SOF/VEL/VOX achieved SVR12, and SVR12 rates were 95% (n = 18/19) for GT1a, 50% (n = 2/4) for GT1b, 84% (n = 59/70) for GT3, 0% (n = 0/1) for GT4, and 100% (n = 3/3) for GT6 infection. Three participants discontinued treatment due to AEs during treatment week (TW) 1, 2 participants were lost to follow-up after EOT, and 1 participant died while receiving treatment. Of these 6 participants, 4 had GT3 infection. As such, complete data were available for per protocol analysis for 91 participants. Therefore, the per protocol SVR12 rate was 90% (n = 82/91, Figure 1). Two participants had VBT at EOT, and 7 relapsed. Per protocol, the SVR12

Table 2. Most Recent DAA Regimen Prescribed Prior to SOF/VEL/VOX

Prior DAA course	N = 97
Sofosbuvir + daclatasvir ± ribavirin, n (%)	54 (56)
Sofosbuvir + velpatasvir ± ribavirin, n (%)	19 (20)
Sofosbuvir + ledipasvir ± ribavirin, n (%)	15 (15)
Elbasvir + grazoprevir, n (%)	3 (3)
Paritaprevir + ritonavir + ombitasvir + dasabuvir ± ribavirin, n (%)	2 (2)
Sofosbuvir + elbasvir + grazoprevir ± ribavirin, n (%)	2 (2)
Uprifosbuvir + ruzasvir, n (%)	2 (2)

Abbreviations: DAA, direct acting antiviral; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

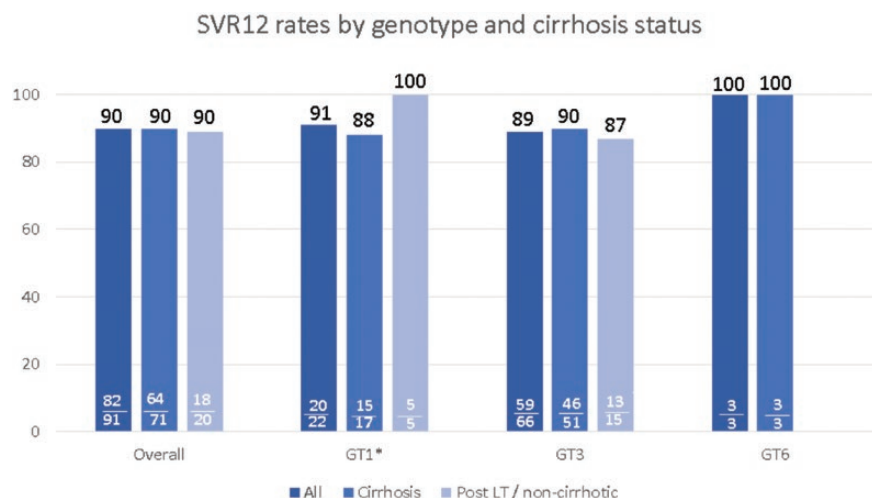


Figure 1. SOF/VEL/VOX per protocol treatment outcomes, presented by cirrhosis and post LT status. *GT1a SVR12 100% (n = 18/18) and GT1b SVR12 50% (n = 2/4). Abbreviations: LT, liver transplantation; SOF/VEL/VOX, sofosbuvir/velpatasvir/voxilaprevir.

rate for participants with GT1a infection was 100% (n = 18/18), 50% (n = 2/4) for GT1b, 89% (n = 59/66) for GT3, and 100% (n = 3/3) for GT6. The SVR12 rate was 90% (n = 64/71) for cirrhotic participants, 88% (n = 14/16) among those who were post-LT, and 100% (n = 4/4) in noncirrhotic, non-LT participants. SVR12 was achieved in 90% (n = 77/86) of participants previously treated with SOF + NS5Ai and 100% among those previously treated with other combination regimens (n = 5/5, Table 2).

Non-SVR Participants

Nine participants had detectable HCV RNA at the SVR12 timepoint (Table 3). All 9 participants reported adherence above 80%. Of the 2 participants with VBT, both had previously experienced VBT during their prior treatment with SOF + VEL. The remaining 7 participants relapsed. Of the treatment failures, 7 were cirrhotic, 5 of whom had PHT, and 2 were post-LT. Seven had HCV GT3, and 2 had HCV GT1b infection. HBV

Table 3. Characteristics of Non-SVR12 Participants

Characteristic	#1	#2	#3	#4	#5	#6	#7	#8	#9
Sex	M	F	M	M	M	M	M	M	M
Age	64	51	57	62	66	50	46	64	62
Cirrhosis status	F4	F4	Post LT (no cirrhosis)	Post LT (no cirrhosis)	F4	F4	F4	F4	F4
Relapse vs VBT	Relapse	Relapse	Relapse	VBT	VBT	Relapse	Relapse	Relapse	Relapse
HCV GT	1b	1b	3	3	3	3	3	3	3
ALT	103	64	50	126	110	91	73	305	82
Albumin	37	36	44	32	42	38	35	33	37
Platelets	151	125	106	177	117	216	60	131	73
Bilirubin	14	30	8	7	14	12	26	13	21
Portal hypertension ^a	Y	N	N	N	Y	N	Y	Y	Y
CTP score	5	5	5~	5~	5	5	6	6	5
HIV / HBV coinfection	HBV	N	N	N	N	N	N	N	N
Ribavirin	N	N	N	N	N	N	N	N	N
NS5A RAS at baseline	Y93H	NA	NA	NA	Y93H L31I	Y93H	NA	NA	Y93H
Prior treatment	PrOD	SOF / LDV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / DCV	SOF / VEL
Prior VBT	N	N	N	Y	Y	N	Y	N	N
HCC at SVR12	N	N	N	N	N	N	N	Y	N
Non-HCC malignancy	N	NHL [#]	N	N	NHL [#]	N	N	N	N
DDI							PPI		
Other cofactors				Malabsorptive bariatric surgery					

Abbreviations: DAA, direct acting antiviral; DCV, daclatasvir; DDI, drug-drug interactions; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LDV, ledipasvir; PrOD, paritaprevir/ritonavir/ozmbitasvir/dasabuvir; SOF, sofosbuvir; VBT, virological breakthrough; VEL, velpatasvir.

^aPortal hypertension defined as the detection of intra-abdominal and/or gastroesophageal varices, splenomegaly or ascites radiologically and/or endoscopically ~non-cirrhotic participants post LT.

[#] Non-Hodgkin's Lymphoma.

coinfection was present in 1 participant. HCV NS5A RAS testing was available for 4 non-SVR12 participants at baseline, in whom all had the Y93H RAS detected. One participant was diagnosed with de novo HCC at EOT. Two participants were treated with SOF/VEL/VOX following the recent diagnosis of non-Hodgkin lymphoma. Prior malabsorptive bariatric surgery was considered contributory to treatment failure in 1 participant. One participant was prescribed a proton-pump inhibitor with SOF/VEL/VOX, but no other concomitant medications were recorded that may have caused significant drug-drug interactions (DDI). No clinical or virological characteristics were associated with likelihood of SVR12 on univariate analysis (Table 4).

HCV NS5A RAS Testing

HCV NS5A RAS testing results were available for 54 (56%) participants. Overall, 91% had detectable NS5A RAS at baseline ($n = 49/54$). Of those with detectable HCV NS5A RAS, 84% ($n = 41/49$) had a single NS5A RAS, and 16% ($n = 8/49$) had dual mutations identified (Table 5). The most frequent NS5A RAS was Y93H, which was detected in 84% of positive samples. On per-protocol analysis, the presence of baseline HCV NS5A RAS was not associated with treatment failure (SVR12 94% vs 100%, $P > .05$), nor was the presence of the Y93H RAS specifically associated (SVR 90% vs 100%, $P = .56$).

Safety

In most cases, treatment was well tolerated; however, AEs that led to treatment discontinuation and hepatic decompensation were observed in a minority (Table 6). SOF/VEL/VOX was discontinued during TW1 in 3 participants; 2 developed severe abdominal pain, and 1 individual's eGFR declined from 48 to 17 mL/min/m². There were 3 episodes of hepatic decompensation on treatment. Among the 18 participants with prior HCC (6 of whom had undergone LT), 1 had recurrent HCC identified between EOT and SVR12, and 1 discontinued therapy during TW1 due to abdominal pain, but otherwise no AEs were recorded in this group.

The first participant who developed hepatic decompensation on treatment had CTP class A5 cirrhosis with PHT at baseline secondary to HCV and prior alcohol misuse, with no history of hepatic decompensation. They did not attend review appointments while on treatment but presented at week 1 post-EOT with new ascites, bilirubin 119 $\mu\text{mol/L}$ and international normalized ratio (INR) 1.5, thought to be treatment related. This participant did not consume alcohol while receiving SOF/VEL/VOX. Alternate causes of hepatic decompensation were excluded. This participant subsequently had gastric variceal hemorrhage at week 5 post-EOT. SVR12 was achieved; however, they had persistent ascites and were assessed for LT. The second participant was CTP class A6 with PHT, with no prior episodes of liver decompensation, at baseline. This participant, who had

Table 4. Univariate Analysis of Factors Associated With SVR12

Variable	SVR12 ($n = 82/91$)	<i>P</i> value
Sex		.54
Male	68/76 (89%)	
Female	14/15 (93%)	
Age (years)	57.5	.86
>65	9/10 (90%)	
≤65	73/81 (90%)	
Non-LT		.63
Cirrhosis	64/71 (90%)	
No cirrhosis ^a	4/4 (100%)	
Prior LT		.66
No	68/75 (91%)	
Yes	14/16 (88%)	
HCV viral load, IU/mL ($n = 70$)		.25
>800 000	29/34 (85%)	
≤800 000	34/36 (94%)	
HCV genotype		.71
GT3 infection	59/66 (89%)	
Non-GT3	23/25 (92%)	
Baseline CTP score		.93
A5	31/36 (86%)	
A6	29/32 (91%)	
B7	1/1 (100%)	
B8	2/2 (100%)	
Portal hypertension ^b		.27
No	41/45 (91%)	
Yes	41/46 (89%)	
Albumin		.73
>35	50/56 (89%)	
≤35	32/35 (91%)	
Platelet count		.86
PI >150	25/28 (89%)	
PI ≤150	57/63 (90%)	
HCC, de novo recurrent		.27
No	80/88 (91%)	
Yes	2/3 (66%)	
HCV NS5A ($n = 50$)		1.0
RAS present	44/47 (94%)	
No RAS	3/3 (100%)	
>1 prior NS5A-containing DAA course		.59
No	70/78 (90%)	
Yes	13/13 (100%)	
Prior peginterferon/ribavirin		.68
No	63/69 (91%)	
Yes	19/22 (86%)	

Abbreviations: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LT, liver transplantation; RAS, resistance associated substitution.

^aExcluding noncirrhotic participants with prior LT.

^bPHT defined as the presence of intra-abdominal and/or gastroesophageal varices, splenomegaly or ascites.

a second diagnosis of autoimmune thrombocytopenia thought to be related to HCV, developed new grade 3 ascites and presumed encephalopathy at TW3, which was attributed to SOF/VEL/VOX. The participant was immunosuppressed, and sepsis was considered as a secondary cause of decompensation, although bacterial cultures were negative. Treatment with SOF/

Table 5. Baseline NS5A Resistance Associated Substitutions (RAS), Available on a Subset of Participants

Baseline NS5A RAS	N = 54
No NS5A RAS detected	5 (9)
NS5A RAS detected	49 (91)
Single NS5A RAS detected	
Y93H	37/49 (76)
A30K	2/49 (4)
L31M/V	2/49 (4)
Dual NS5A RAS detected	
Y93H / A30K/H	3/49 (6)
A30K / L31M	2/49 (4)
L31M/I / H58P	2/49 (4)
Y93H / L31M	1/49 (2)

VEL/VOX was continued with weekly review. At week 6 post EOT, the participant recompensated, and diuretic and lactulose therapy were withdrawn. SVR12 was achieved. A third participant, who was CTP class A6 at baseline, presented at TW5 with esophageal variceal hemorrhage attributed by their clinician to significant recidivist alcohol consumption. Variceal surveillance was up to date prior to treatment. Bleeding was controlled endoscopically; however, the participant developed progressive hepatic decompensation and died 2 weeks later. At end of follow-up, 1 recurrent and 2 de novo HCCs were identified, which were detected between EOT and SVR12 timepoints. HCC surveillance was up to date in all 3 at baseline.

The 3 participants who were CTP class B at baseline tolerated treatment well, and no AEs were reported; their aggregate CTP scores were unchanged between baseline and SVR12 timepoints.

Table 6. Twenty Adverse Events Reported in 15 Patients.

Adverse events, n (%)	N = 97
Treatment related	
Nausea	4 (4)
Fatigue	3 (3)
Abdominal pain	2 (2)
Diarrhea	2 (2)
Headache	1 (1)
Vertigo	1 (1)
Weight gain	1 (1)
Mood disturbance	1 (1)
Serious adverse events	
Hepatic decompensation	3 (3)
Death	1 (1)
Deteriorating renal function	1 (1)
Leading to discontinuation of treatment	
Treatment related	
Abdominal pain	2 (2)
Deteriorating renal function	1 (1)

In 3 cases, adverse events led to treatment discontinuation.

The 5 participants who were CTP class A at baseline but who had had prior clinical decompensation, and a further 2 participants who were treated with an immediate “lead-in” regimen of SOF + NS5A-inhibitor, also tolerated SOF/VEL/VOX well; no AEs were recorded. Among participants with LT, there was no AEs on graft function or DDIs recorded.

DISCUSSION

This is the first Australian real-world study to investigate the efficacy and safety of SOF/VEL/VOX among participants with relapsed HCV infection following treatment with an NS5Ai-containing DAA regimen, and advanced liver disease or LT. Although the per protocol SVR12 rate of 90% was lower than top-line results in the registration trial, our sample was notable for “difficult-to-cure” characteristics including cirrhosis, PHT, and HCV GT3 infection.

The cohort is unique in its complexity including a high prevalence of cirrhosis (78%), the majority with PHT (61%, $n = 46/76$), which was notably higher than in POLARIS-1 and other SOF/VEL/VOX real-world studies (prevalence of cirrhosis 34–46%) [10–13]. The majority of participants also had HCV GT3 infection, which has been associated with increased likelihood of relapse after SOF/VEL/VOX in some real-world data, and approximately one-fifth had prior HCC [11]. It is the largest real-world study of relapsed patients with GT3 HCV. Our cohort also included the largest number of LT recipients outside of registrations trials, demonstrating that LT recipients can be safely treated and potential DDI appropriately managed. As expected, where virological testing was available, there was a high prevalence of HCV NS5A RAS at baseline (91%, $n = 49/54$), and 15% had previously failed multiple NS5Ai-containing DAA regimens, in some cases up to 4. Despite these difficult-to-cure characteristics, we show that 12 weeks SOF/VEL/VOX can achieve high rates of cure in clinical practice.

Our analysis did not identify any association between treatment outcomes and HCV genotype. The SVR12 rate was 89% among patients with GT3 HCV. This SVR12 proportion is similar to that observed in POLARIS-1, both in participants with GT3 HCV infection and cirrhosis, and in the overall cirrhotic population [10]. The rate of SVR12 observed in our GT3 patients is higher than that described by Llaneras and colleagues in Spain, who observed SVR12 rates of 80% ($n = 24/30$) in GT3 infection and 69% ($n = 9/13$) among GT3 cirrhotics, and from Degasperis and colleagues in Italy (SVR12 84% [$n = 16/19$] among GT3 cirrhotics) [11, 13]. The SVR12 data in our cohort are more similar to those with GT3 cirrhosis reported by Belperio in a Veteran's Affairs cohort (SVR12 = 91%, $n = 21/23$) [12]. The reasons for the differences between SVR12 rates reported in these respective cohorts is not clear, but our data provide reassuring evidence that in a large real-world cohort of

patients with GT3 cirrhosis and advanced disease, SVR12 rates remain high.

The high prevalence of participants with GT3 infection in this cohort, most of whom were cirrhotic, was notable. The community prevalence of GT3 HCV infection is approximately 39% in Australia [18]. The frequency of GT3 infection in our cohort exceeded 70%, consistent with GT3 remaining harder to cure than non-GT3 HCV after first-line DAA regimens, particularly in patients with cirrhosis (5). Most GT3 participants in our study were previously treated with SOF + DCV or SOF + VEL. GLE + PIB was not available in Australia at the time that this study was conducted. European guidelines no longer recommend 12 weeks SOF + VEL as a first-line treatment for GT3 infection and cirrhosis due to suboptimal outcomes in this group [19]. The high prevalence of GT3 in our study cohort highlight the importance of optimizing first-line DAA therapy for patients with GT3 infection and cirrhosis to minimize relapse and the need for salvage treatment.

Overall safety of SOF/VEL/VOX was acceptable; however, there were 3 episodes of hepatic decompensation in previously well-compensated CTP class A participants during treatment. As salvage triple therapy including PIs are increasingly prescribed to patients with advanced disease, the data highlight the need for regular clinical review and laboratory investigations to monitor for PI toxicity for at-risk patients during treatment. When toxicity is detected, treatment should be immediately discontinued. Where patients are LT candidates, we recommend review by a transplant board prior to commencing treatment.

Three participants with CTP class B disease were commenced on SOF/VEL/VOX, beyond the EAP criteria. Although no hepatic toxicity was noted in these cases, the use of NS3/4A PI in patients with CTP class B/C cirrhosis cannot be recommended. There were 5 other participants who were down-staged to CTP class A severity from CTP class B/C during prior treatment with SOF + NS5A (VEL/DCV), which was prescribed as a “lead-in” regimen immediately prior to SOF/VEL/VOX in one; all tolerated treatment well with close monitoring. In patients who are not candidates for LT, we believe the lead-in strategy regimen of SOF + NS5Ai to optimize liver function and downstage to CTP class A, prior to introducing VOX, warrants prospective evaluation given our positive experience.

Baseline RAS testing was available for 54 participants, and the presence of NS5A RAS was not associated with treatment failure. This is in keeping with results of a post hoc analysis of SOF/VEL/VOX registration data demonstrating that multitargeted combination therapy can overcome baseline resistance quasiespecies [20]. There is thus a limited role for HCV NS5A sequencing in routine clinical management of these patients.

Second-line salvage therapies for the small number of patients who fail SOF/VEL/VOX are not well defined. As extension of treatment duration and regimen intensification with ribavirin

was shown to improve SVR12 rates among DAA-experienced patients prior to the registration of relapse regimens including triple DAA therapy, we believe that both 24 weeks SOF/VEL/VOX ± ribavirin or 16 weeks SOF + GLE/PIB ± ribavirin present reasonable retreatment strategies for SOF/VEL/VOX failures [21]. Although ribavirin was included with SOF/VEL/VOX for 3 participants with GT3 and cirrhosis in our cohort, this number was too small to determine its effect.

In conclusion, SOF/VEL/VOX was an effective option for treatment of patients with difficult-to-cure characteristics who do not respond to a first-line DAA regimen, including patients with cirrhosis, GT3 HCV, and prior LT. Overall, this treatment regimen was well tolerated; however, serious AEs can occur in those with advanced liver disease.

Notes

Author contributions. All authors were involved in (i) substantial contribution to the conception and design; or the acquisition, analysis, or interpretation of the data and (ii) drafting and/or revision of the manuscript content, (iii) final approval of the version to be published, and (iv) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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