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MAJOR ARTICLE

Towards Genotype-Specific Care for Chronic Hepatitis B: The First 6 Years Follow Up From the CHARM Cohort Study

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Objective. There is increasing evidence to suggest that, among those with chronic hepatitis B virus infection, the natural history and rate of progression to cirrhosis and hepatocellular carcinoma is influenced by hepatitis B virus genotype. The unique hepatitis B virus genotype C4 circulates among Indigenous Australians. The aim of this work is to describe the process of establishing this cohort and review the first 6 years of available data in an effort to understand the real-world clinical care and natural history of this subgenotype.

Method. We followed a longitudinal cohort of Indigenous Australians from the Northern Territory of Australia with established subgenotype C4 infections. We assigned phases of disease according to Gastroenterological Society of Australia and Asian Pacific Association for the Study of the Liver criteria using clinical and laboratory information that had been collected for clinical management.

Results. Of 193 patients followed over a median of 38 months, 58 (30%) individuals transitioned from 1 disease phase to another, 10 (5%) cleared hepatitis B e antigen, and 6 cleared hepatitis B surface antigen (3%). In this relatively young cohort (median age 40.3 years), 26 (13%) had cirrhosis by the end of the follow up period, with the majority of these being in the immune control phase of disease.

Conclusions. In this cohort of hepatitis B subgenotype C4 patients, we report an aggressive and dynamic clinical phenotype. High rates of cirrhosis at a young age appear to occur in the early phases of disease.

Key words: cirrhosis; genotype; hepatitis B; Indigenous.

INTRODUCTION

The worldwide literature increasingly supports the importance of hepatitis B virus (HBV) genotype with respect to the natural history of chronic hepatitis B (CHB) [1, 2], as well as the risk of cirrhosis [3, 4] and hepatocellular carcinoma (HCC) [5, 6]. Genotype C HBV, which predominates in Southeast Asia [7], has been associated with a higher risk of progression to cirrhosis [4], longer duration of hepatitis B e antigen (HBeAg) positivity [8, 9] and a higher incidence of HCC [10] compared to genotype B. Some genotypes, such as B5 (previously classified as B6), prevalent in Alaskan natives have been suggested to have a more benign course [11, 12]. There is no current evidence to support any significant difference in response to nucleotide/nucleoside antiviral therapy on the basis of genotype [13]; however, genotype C HBV appears to be less responsive to interferon therapy than genotypes A and B [14].

The Indigenous population of the Northern Territory (NT) of Australia have a high prevalence of CHB with a recently estimated seroprevalence of 6% [15]. To date, where the HBV genotype has been reported, NT Indigenous Australians have been infected exclusively with subgenotype C4 [16]. Hepatitis B subgenotype C4 only has ever been identified in Indigenous Australians and has molecular characteristics previously associated with more rapid progression to cirrhosis and an increased risk of HCC [17]. Genotype C4 has 2 distinct clades—C4a

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and C4b—that demonstrate clear geographical distribution, with the C4b clade in the East Arnhem and Katherine/Central Australia regions, while the C4a clade is found more commonly in the western NT regions [18]. The natural history of HBV genotype C4 is currently unknown.

The CHARM (Characterizing Hepatitis B in Northern Australia Through Molecular Epidemiology) study originally was established to determine the prevalent genotype(s) of HBV in the NT. We have expanded this study into a longitudinal cohort [16]. The aim of this work is to describe the process of establishing the cohort and review the first 6 years of available data in an effort to understand the real-world clinical care and natural history of HBV/C4 in Indigenous Australians.

METHODS

Ethical approval was obtained from the Human Research Ethics Committee of the NT Department of Health and Menzies School of Health Research (HREC-09/105). A prospective cohort study was conducted. Patients were recruited between June 2010 and September 2016 through the Royal Darwin Hospital Viral Hepatitis Service, which has regular outreach clinics to remote communities across the NT, spread over an area of over 1 million km^2. At the time of enrollment into the study, baseline information about location of birth and early life, mother's birth location, risk factors for viral hepatitis, treatment, and current liver disease was collected. The results of routine blood tests for full blood count, electrolytes, creatinine, liver function, coagulation profile, and serology for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus were recorded.

Blood for HBV viral load was obtained and, where able, genotype and full genome sequencing was performed at the Victorian Infectious Diseases Reference Laboratory in Melbourne. Viral sequences were determined with methods as previously published [16, 18]. We assessed whether the following mutations were associated with patients with cirrhosis as these mutations previously have been associated with more rapid disease progression: basal core promotor (BCP) G1613A, T1753A/G, A1762T, G1764A, C1766T, preS deletion, and core deletion [17]. If there was an insufficient amount of HBV DNA detected (<400 IU/ml), the sample was not genotyped.

Cirrhosis was considered to be present if either transient elastography (TE) using a Fibroscan (Echosens, Paris) had shown a median liver stiffness score of greater than 10kPa [19] or there was at least 1 abnormality suggestive of cirrhosis on at least 2 of the following 3 domains: clinical assessment, blood tests, and imaging. A portable Fibroscan 402 was used to determine TE, operated by trained individuals. Valid readings fulfilled the following criteria: the median of at least 10 readings; a success rate of measurements >60% and an interquartile range [IQR]/median ratio of <30%. Relevant findings in the (1) clinical domain contained the presence of >5 spider naevi, asterixis, ascites, or splenomegaly; (2) blood test domain comprised international normalized ratio >1.3 (and not on anticoagulation therapy), platelet count $<150 \times 10^9$/L, albumin <35g/dL or serum bilirubin >17Umol/L; and iii) imaging domain from either ultrasound or CT scan revealed splenomegaly, enlarged portal vein diameter, reverse flow in the portal vein, or enlarged caudate lobe of the liver. Advanced fibrosis was defined as a median liver stiffness score by Fibroscan >8kPa.

At the time of recruitment into the study, a management plan based on the stage of CHB infection and the severity of concurrent liver disease was recommended in line with Gastroenterological Society of Australia (GESA) [20] and Central Australian Rural Practitioners Association (CARPA) guidelines [21]. Clinical and laboratory data obtained during the course of standard clinical follow up subsequently were collected. This information included all repeat episodes of the clinical and laboratory parameters collected at the initial recruitment visit and, in addition, body mass index (BMI), alpha fetoprotein (AFP), Hepascore [22], Fibroscan results, and treatment. Clinical review and repeat blood tests were recorded if available at 6 monthly intervals from the date of recruitment and included in the analysis if they occurred within a 2-month window on either side of the allocated 6 monthly review date. If HCC screening had been completed as part of standard clinical care, these data were collected as well. Follow up occurred until September 2016, last record of results, or death, whichever was earlier.

Each patient was allocated a disease phase from the following phases: immune tolerance, immune clearance, immune control, immune escape, or resolved infection. The phase was allocated at the date of enrolment and at the last point of follow up using GESA criteria and the Asian Pacific Association for the Study of the Liver (APASL) definitions as described in Table 1. For the GESA criteria, an abnormal Alanine aminotransferase (ALT) was defined as ≥30 IU/L for men and ≥19 IU/L for women; for the APASL criteria, the abnormal ALT was ≥54 IU/L for both men and women [20, 23]. If both HBeAg and anti-HBe were negative or either 1 was equivocal, the individual case was reviewed manually and assigned a phase using all available information.

Data were entered into a purpose-built web-based database and analyzed using STATA version 14 (Statacorp, College Station, TX). Results are presented as mean+/- standard deviation for normally distributed parameters and median +/- interquartile range for non-normally distributed parameters. All patients recruited who had confirmed CHB were included in this longitudinal cohort, including those who did not have a sufficient viral load for genotyping.

RESULTS

Between June 2010 and September 2016, we recruited 193 participants from 38 remote communities in 6 regions of the NT. Of

Table 1. Definitions of the Phases of Chronic Hepatitis B and Recommended Follow Up using Gastroenterological Society of Australia and Asian Pacific Association for the Study of the Liver Definitions

Phase	Definition	Follow Up Recommendations
GESA: Immune tolerance APASL: Immune tolerant	HBsAg positive, HBeAg positive, normal ALT, hepatitis B viral DNA >20 000 IU/ml	Minimum of 12 monthly liver function tests and hepatitis B viral load
GESA: Immune clearance APASL: Immune reactive	HBsAg positive, HBeAg positive, abnormal ALT[a]	GESA consider treatment for all; APASL consider treatment if ALT>2xULN or > F2 fibrosis
GESA: Immune control APASL: Low replicative	HBsAg positive, HBeAg negative, hepatitis B viral load <2000 IU/ml, normal ALT[b]	Minimum of 6 monthly liver function tests and annual hepatitis B viral load
GESA: Immune escape APASL: Reactivation	HBsAg positive, HBeAg negative, hepatitis B viral load >2000 IU/ml	GESA consider treatment for all; APASL consider treatment if ALT>2xULN or >F2[c] fibrosis
GESA: Resolved infection APASL: HBsAg sero-clearance	HBsAg negative, hepatitis B viral load not detected, having previously been HBsAg positive	
All patients over 50 years of age and anyone with either cirrhosis or a family history of hepatocellular carcinoma		6 monthly ultrasound and alpha fetoprotein in addition to standard care

Abbreviations: ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; GESA, Gastroenterological Society of Australia; HBsAg, Hepatitis B surface antigen; HBeAg, Hepatitis B e antigen; ULN, upper limit of normal.

[a] Abnormal ALT as per GESA >19 for a female and >30 for a male, as per APASL > laboratory defined ULN (≥ 54).

[b] … or alternative explanation for raised ALT.

[c] F2 fibrosis is defined as moderate fibrosis on transient elastography or on liver biopsy by portal fibrosis with infrequent septa.

these, 125 (65%) had sufficient detectable HBV DNA to enable genotyping and all were subgenotype C4. Sixty-eight subjects did not have sufficient HBV DNA detected to perform genotyping. Patients were followed for a median of 38 months (IQR, 17–58; range, 1–75) for a total of 605 person years. During follow up, 11 individuals passed away and none were lost to follow up. Baseline demographics and clinical parameters at study entry as well as the latest time point recorded are detailed in Table 2.

Participants' hepatitis B disease phase at study entry, as defined by GESA criteria, is shown in Figure 1 (comparison between GESA and APASL criteria is provided in Supplementary Figure 1). Over the reviewed time period 10 (5%) individuals changed their HBeAg status: 8 from positive to negative (with all 8 of these individuals developing anti-HBe), 1 positive to equivocal, and 1 negative to positive. Median age at HBeAg seroconversion was 32 years (range, 19–53 years). There was a total of 240 person years of follow up for individuals HBeAg positive at baseline, which equates to an HBeAg seroconversion rate of 3.33% (95% confidence interval [CI], 1.4–6.5) per year.

Six individuals cleared hepatitis B surface antigen (HBsAg) during the follow up period. The median age of HBsAg clearance was 43.5 years (range, 27–64 years). This equates to an overall seroconversion rate of 3.1% or a 1% (95% CI, 0.4%2.2%) per year rate. None of the participants who cleared HBsAg were on treatment. One case was anti-HBcIgM positive (27 years), which may have been due to acute HBV.

Fifty-eight individuals changed their phase of disease over the period of follow up; this includes 19 patients who have received or are receiving antiviral treatment. This is a 9.6% (95% CI, 7.3–12.4) per year rate of change in phase of disease. The median ALT level at study enrolment was 31 U/L at and final follow up was 29 U/L. Fibroscan results were available for 111 (57%) individuals and median liver stiffness score was 5.3kPa (range, 2–66.4). Liver stiffness scores stratified by baseline disease phase are presented in Figure 2.

In 16 participants, HBV treatment already had commenced or was initiated at the enrollment visit. At the end of follow up, 31 individuals were taking antiviral medication; no individual received interferon therapy (Table 2). At the final time point of follow up, 26 (13%) individuals had evidence of cirrhosis, and, of these, 13 (50%) were on treatment. The median age of those with cirrhosis was 48 years (IQR, 37–52 years). The most common phase of disease for patients with cirrhosis both at entry to the study and at the latest follow up point was the immune control phase. As a proportion, 5 of 12 (42%) patients in the immune escape phase had cirrhosis at latest follow up.

The median BMI for patients with cirrhosis was 26.2 (IQR, 24.2–27.8) and for patients without cirrhosis was 22.5 (IQR, 19.7–25.9). Of the patients with cirrhosis, 7 of 26 reported hazardous alcohol intake (as defined by >2 standard drinks per day) compared with 25 of 167 patients without cirrhosis (P = .13). There were no coinfections with hepatitis C virus or HIV.

Of the 125 participants with sequence data available, 8.8% (3 of 34) participants with C4a clade had cirrhosis, compared with 16.5% (15 of 91) of participants with C4b clade (P = .28). Sixty-seven participants had full genome sequencing data available. Of those diagnosed with cirrhosis (13 of 67), 92% (12 of 13) had at least 1 mutation known to be associated with more rapid disease progression or the development of HCC, or both, compared with 55% (30 of 54) of those without cirrhosis (χ^2 test, P = .014). The most common mutations observed in those with cirrhosis were BCP G1764A (84.6%; 11 of 13), A1762T (69.2%; 9 of 13), C1766T (53.8%; 7 of 13) and core deletion (15.4%; 2 of 13).

Table 2. Baseline Demographics and Clinical Details at Study Entry and the Latest Time Point Recorded for the Chronic Hepatitis B Patients

N = 193	Study Entry		Latest Time-point	
Median age (IQR)	40.3 years (31.3–50.3)		40.2 years (31.7–51.5)	
Male	101 (52%)			
Indigenous Australian ethnicity	193 (100%)			
BMI median (IQR)	22.8 (19.9–26.4)			
Vaccine status				
Unknown	148 (76%)			
1 dose received	11 (6%)			
2 doses received	6 (3%)			
Fully vaccinated[a]	28 (15%)			
Alcohol use				
None	133 (69%)			
0–2 STD drinks[b] per day	21 (11%)			
3–4 STD drinks per day	12 (6%)			
>4 STD drinks per day	19 (10%)			
Unknown	8 (4%)			
HBeAg status				
Positive	66 (34%)		58 (30%)	
Negative	126 (65%)		134 (69%)	
Equivocal	0		1 (1%)	
Unknown	1 (1%)		0	
Anti-HBe status				
Positive	121 (63%)		127 (66%)	
Negative	67 (35%)		60 (31%)	
Equivocal	2 (1%)		6 (3%)	
Unknown	3 (1%)		0	
HBV DNA viral load (IU/ml) median (IQR)	684 (43-7,729,938)		<20 (<20–184)	
ALT U/L median (IQR)	31 (19–44)		29 (20–41)	
% participants with an ALT U/L > laboratory ULN (lower cut off)[c]	17 (61)		11 (63)	
Platelets (x 10^9/L) median (IQR)	239 (202–279)		235 (188–287)	
Albumin (g/L) median (IQR)	42 (40–44)		41 (39–44)	
Creatinine (μmol/L) median (IQR)	72 (61–87)		76 (63–90)	
Evidence of cirrhosis	21 (11%)		26 (13%)	
Median liver stiffness score (kPa)			5.3 (range, 2–66.4)	
Phase of disease	**GESA**	**APASL**	**GESA**	**APASL**
Immune tolerance	24 (12%)	44 (23%)	22 (11%)	49 (25%)
Immune clearance	42 (22%)	22 (11%)	35 (18%)	8 (4%)
Immune control	102 (53%)	118 (61%)	110 (57%)	121 (63%)
Immune escape	20 (10%)	4 (2%)	12 (6%)	1 (1%)
Resolved infection	4 (2%)	4 (2%)	10 (5%)	10 (5%)
Unable to phase[d]	1 (1%)	1 (1%)	4 (2%)	4 (2%)
Currently receiving antiviral treatment	16 (8.3%)		31 (16%)	
Entecavir	12 (75%)		23 (74%)	
Tenofovir	4 (25%)		8 (36%)	
Death during follow up[e]			11 (6%)	

Abbreviations: ALT, alanine aminotransferase; APASL, Asian Pacific Association for the Study of the Liver; BMI, body mass index; GESA, Gastroenterological Society of Australia; HBeAg, Hepatitis B e antigen; Anti-HBe, Hepatitis B e antibody; HBV, Hepatitis B virus; IQR, interquartile range; STD, standard; ULN, upper limit of normal.

[a] Fully vaccinated was defined as having received 3 vaccines.

[b] A standard drink was defined as 10g of alcohol.

[c] Laboratory upper limit of normal is 54 U/L and the lower cut off is 19 U/L for women and 30U/L for men.

[d] … due to lack of available e markers.

[e] Cause of death known for 4 individuals includes breast cancer, parotid cancer, vulvar cancer, and end stage renal disease.

Sixty-six participants (34%; 66 of 193) qualified for HCC screening based on being over 50 years old or having cirrhosis. Of these, 39% (26 of 66) had an ultrasound and AFP in the past 6 months before analysis and 50% (33 of 66) had HCC screening in the last year.

Of those recruited in the last year before analysis, 65% (11 of 17) had HCC screening in the last 6 months. There were no diagnoses of HCC during the follow up period and none of the deaths in the study period were due to a known HCC (cause of death was available for 4 of 11 deaths).

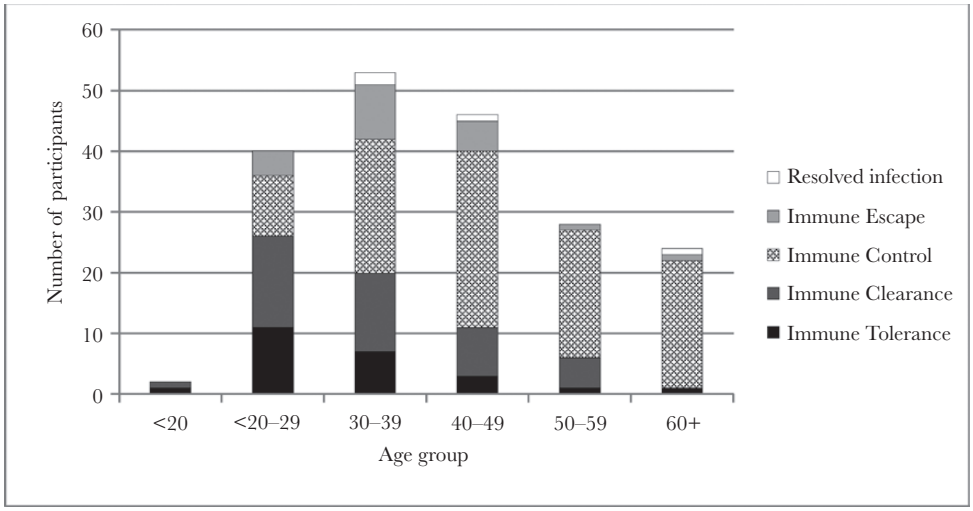

Figure 1. Disease Phase at Study Entry Using Gastroenterological Society of Australia Criteria by Age Group

DISCUSSION

This work describes the clinical follow up over a median period of 38 months for patients with HBV subgenotype C4. Using the lower cut-off values for abnormal ALT to define the phase of disease, 30% (58 of 193) of individuals transitioned into a different phase of disease over a relatively short period of follow up. This highlights the importance of viewing CHB as a dynamic disease requiring regular reassessment to evaluate the need for treatment and not labelling patients as "inactive carriers," implying a benign static prognosis.

The rate of seroconversion from HBeAg positive to anti-HBe positive has been reported to be 8%–12% per year in a summary of all genotypes [24] and 6% per year in the REVEAL group, which only included genotypes B and C [25]. We document a HBeAg seroconversion rate of 3.3% (95% CI, 1.4–6.5) per year in our C4 patients, lower than that in the literature for other genotypes. The age distribution of individuals in each phase of disease at study entry is consistent with published evidence that genotype C is associated with a significantly older age of HBeAg seroconversion [26]. In our cohort, the median age of HBeAg seroconversion was 32 years, with 4 individuals seroconverting after the age of 40 years. One individual seroreverted to HBeAg positive, which also has been described more commonly in genotype C disease [27]. Seroconversion of HBeAg after the age of 30 is associated with a significantly higher incidence of cirrhosis and HCC [28].

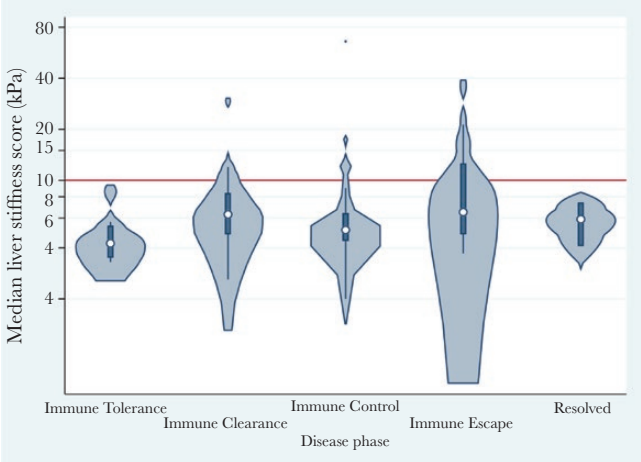

Figure 2. Median Liver Stiffness Scores Grouped By Disease Phase at Entry Using Gastroenterological Society of Australia Criteria In these violin plots, the median is represented by the white circle and the box indicates the interquartile ranges; the violin plot itself represents the estimated kernel density. The horizontal line at 10 kPa indicates the liver stiffness score cut off for cirrhosis.

Six individuals became anti-HBs positive, hence "clearing" their CHB infection at a rate of 1% (95% CI, 0.4–2.2) per year, which is line with commonly quoted clearance rates of 1%–2% per year but lower than the 2.3% documented in a large Taiwanese cohort [29].

Importantly, in this cohort ALT levels infrequently were raised (median, 28 U/L) and using the standard local laboratory cut-off of 54 U/L as per the APASL recommendations would alter the disease phase classification of a large number of patients. The lower, gender-specific ALT level cut-offs were based initially on a group of Italian blood donors [30]; subsequently, other larger studies support these lower "normal" values [31, 32]. These revised cut-off levels also are accepted now and recommended by European and American professional bodies [33, 34]. A large study based in Hong Kong [35] compared histology from liver biopsy to ALT levels in a group of 211 HBeAg positive CHB patients and 108 anti-HBe positive individuals. This study concluded that ALT did not predict significant liver injury with 22.5% of HBeAg positive patients with normal ALT having F3 or greater fibrosis on biopsy. It also is important to note that there is not a validated normal ALT range for

Indigenous Australians. Differences in criteria for ALT is the major reason that there is a significant difference between individuals allocated to immune tolerance and clearance when using the APASL versus the GESA criteria. In the Australian context, this is particularly important as an abnormal ALT is 1 of the qualifying criteria for accessing government-funded antiviral treatment.

We previously have reported that the majority of C4 hepatitis B viruses that have had full genome sequence analysis have mutations that have been associated with increased rates of progression to cirrhosis and HCC [17]. Thirteen percent of this group of relatively young patients had evidence of cirrhosis and another 7 (4%) had evidence of advanced fibrosis. In this study, there appears to be a significant difference between patients with cirrhosis and at least 1 mutation previously associated with more rapid disease progression, or the development of HCC, or both (χ^2, $P = .014$). We observed a higher prevalence of cirrhosis in participants with HBV clade C4b compared with C4a (16.5% and 8.8%, respectively). Although this finding did not reach statistical significance, it is an observation that requires further study to assess if this could be a contributing factor for the higher prevalence of cirrhosis in some regions of the NT.

We used a median liver stiffness score of greater than 10kPa to define cirrhosis in this cohort. There has been much debate regarding the optimum cut-off to be used to define cirrhosis using TE, with a range of cut-offs being described in the literature. European guidelines recommend a cut-off of >12kPa for severe fibrosis/cirrhosis if ALT is elevated and of >9kPa for those with a normal ALT [36]. A Taiwanese study used >10kPa as their cut-off for F4 fibrosis (cirrhosis) for those with CHB specifically. They found a sensitivity and specificity of 0.8 and 0.77, respectively, using >10kPa as the liver stiffness measurement [37]. Although these findings were based on relatively small numbers, patients with CHB in the NT of Australia are more similar to Southeast Asian populations based on hepatitis B genotype compared to European cohorts. Using the higher cut-off of >12kPa would have reduced the number of our participants with cirrhosis from 26 to 22.

The small number of patients with cirrhosis (n = 26) precluded using a multivariate logistic regression model incorporating variables such as age, gender, clade, alcohol use, and BMI [38]. However, we did observe relatively small numbers of participants with high BMI (median BMI in patients with cirrhosis of 26.2 compared with 22.5 in patients without cirrhosis). There also was no significant difference in reports of hazardous alcohol intake when comparing those with or without cirrhosis.

The majority of cirrhotic individuals in our C4 cohort were in the immune control phase both at study entry and final follow up, but 1 was in the immune tolerance and 7 in the immune clearance phase. This is at odds with standard thinking about the natural history of CHB, which is that significant fibrosis and cirrhosis are uncommon in the early phases [24]. Alternatively, the majority of liver damage in C4 CHB may be occurring in early disease phases and persisting when HBV viral loads are low and ALT has normalized. In reviewing 1387 patients with CHB over 1 year of follow up, Kumar et al [39] also found that of those with persistently normal ALT levels (<40 IU/ml), 40% of HBeAg positive patients and 13% of HBeAg negative patients had greater than grade 2 fibrosis on liver biopsy. These patients were predominantly nongenotype C HBV meaning these figures are likely to be even higher in those with genotype C.

At the latest follow up time point, 16% of participants were on treatment for hepatitis B. In the most recent nationwide mapping in 2017, the proportion of patients with CHB who were receiving treatment in Australia was 8.3%, with the proportion in the NT overall being 5.2% [40]. It is recommended that all patients with cirrhosis with any detectable viral load are commenced on antiviral treatment [33]; this again highlights the importance of regular reassessment of patients living with CHB to assess for changes in status that may indicate the need for treatment. In our cohort, only 50% of participants with cirrhosis were on treatment. Two participants with cirrhosis had undetectable HBV DNA and, therefore, did not meet criteria for treatment in Australia. During our analysis, if we found that a patient with CHB and cirrhosis was not on treatment this was fed back to the treating physicians in the communities for treatment to be initiated. The low treatment rate we observed may be a reflection of the high number of patients with cirrhosis being in immune control phase (not usually a phase where treatment would be recommended) and highlights the importance of education in the remote communities to offer treatment to all CHB patients with cirrhosis regardless of disease phase.

The main limitation of this study is that all follow up data were collected retrospectively and, therefore, were incomplete. This now has motivated a shift to the collection of prospective follow up data. We have included patients who were already on or have commenced treatment in the study as it would clearly be unethical to not commence treatment when it is warranted, but this will modify the natural history of the disease. In diagnosing cirrhosis, we did not use the gold standard of liver biopsy, so the prevalence of cirrhosis could have been overestimated.

A relatively small proportion of participants underwent HCC screening (39%). This reflects the need for systematic follow up of these populations and the need for a hepatitis B register to enable governance in remote and logistically challenging settings. The Hep B PAST (Partnership Approach to Sustainably eliminating chronic hepatitis B in the NT) was developed to improve the cascade of care for individuals living with CHB in the NT. The success of this is reflected in increased HCC screening rates the last year of follow up (65%). We did not document any incident cases of HCC in this cohort; we suggest that this likely is a consequence of the short duration of follow up to date plus the initial low rates of HCC screening—noting that rates

of HCC are high overall in the NT Indigenous Australian population [41].

Another limitation of the study is that the data were collected from a relatively small sample size and HBV genotype was only available for 65% of participants. We cannot be certain that all participants were infected with subgenotype C4; however, of those tested, 100% had this subgenotype. It is difficult to ascertain whether these results are generalizable to the Australian population, because it is unclear how far outside of the NT subgenotype C4 extends at this stage.

This study is the first to report clinical follow up of a cohort of individuals with subgenotype C4 CHB. Our results support the molecular virological findings previously reported, suggesting that C4 has an aggressive phenotype associated with a relatively high prevalence of cirrhosis. Our data also highlight the dynamic nature of CHB, reinforcing the need for regular monitoring and re-evaluation of the need for treatment. We show that, in subgenotype C4 patients, the majority of those with cirrhosis were already in this state by the immune control phase; therefore, in order to try and prevent or modify this outcome, earlier assessment and initiation of treatment may be required.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. J.D. was involved in study concept and design, recruitment, analysis, and writing the first draft. E.L.S. was responsible for analysis and developing subsequent drafts of the paper. M.L. performed laboratory work on the participant samples, reviewed and provided input into subsequent drafts of the paper, and performed phylogenetic analysis. R.E., T.S., and K.J. did laboratory work, phylogenetic analysis, reviewing and provided input into subsequent drafts of the paper. K.M. collected data, analyzed data, and provided input into subsequent drafts of the paper. P.B. designed, collected data, analyzed data, and provided input into subsequent drafts of the paper. B.C.C. designed, analyzed, and provided input into subsequent drafts of the paper. S.L. was involved in study concept and design, laboratory work, analysis, and providing input into subsequent drafts. Finally, J.S.D. and S.Y.C.T. were involved in study concept and design, recruitment, analysis, and providing input into subsequent drafts.

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