Hepatocellular carcinoma screening reduces cancer-related mortality in patients with chronic hepatitis B infection: a case-control study

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Abstract

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Background: Patients with chronic hepatitis B (CHB) infection often undergo screening for hepatocellular carcinoma (HCC), but the efficacy of such screening remains unclear. We aimed to evaluate the impact of screening with ultrasound (USS) and/or serum alpha-fetoprotein (AFP) on HCC-related mortality in Veterans Affairs (VA) patients with CHB. **Methods:** We performed a matched case-control study. Patients with CHB and at least 4 years of VA care after CHB diagnosis were identified. Cases were patients who died of HCC between 01/01/2004 and 12/31/2017, while controls were patients who did *not* die of HCC. Cases and controls were matched by CHB diagnosis date, age, sex, race/ethnicity, cirrhosis status, antiviral therapy exposure, hepatitis B e antigen status, and viral load. We identified screening USS and AFPs obtained in the 4 years preceding HCC diagnosis in cases and the equivalent index date in controls. Using conditional logistic regression, we compared cases and controls with respect to receipt of screening. A lower likelihood of screening in cases corresponds to an association between screening and reduced risk of HCC-related mortality. **Results**: We identified 169 cases, matched to 169 controls. Fewer cases than controls underwent screening with either screening modality (33.7% versus 58.6%) or both modalities (19.5% versus 34.4%). In multivariable conditional logistic regression, screening with either modality was associated with a lower risk of HCC-related mortality (adjusted odds ratio [aOR] 0.21, 95% confidence interval [CI] 0.09-0.50), as was screening with both modalities (aOR of 0.13, 95% CI 0.04-0.43). **Conclusions:** HCC screening was associated with a substantial reduction in HCC-related mortality in VA patients with CHB.

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Disclaimer

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Abbreviations

AASLD - American Association for the Study of Liver Disease

AFP – alpha-fetoprotein

aOR – adjusted odds ratio

BMI – body mass index

CDW – Corporate Data Warehouse

CHB – chronic hepatitis B

CI – confidence interval

CT – computed tomography

DPP – detectable preclinical period

HBeAg – hepatitis B e antigen

HBsAg – hepatitis B surface antigen

HBV DNA – hepatitis B viral load

HCC – hepatocellular carcinoma

HCV – hepatitis C virus

HIV - human immunodeficiency virus

ICD - International Classification of Diseases

LIRADS - Liver Imaging and Reporting Data System

MRI – magnetic resonance imaging

USS – ultrasound

VA – Veterans Affairs

INTRODUCTION

Patients with chronic hepatitis B virus infection (CHB) are at high risk of developing hepatocellular carcinoma (HCC). The American Association for the Study of Liver Disease (AASLD) recommends that patients with CHB-related cirrhosis and high-risk patients without cirrhosis undergo HCC screening every 6 months using ultrasound scanning (USS) with or without serum alpha-fetoprotein (AFP)[1-3]. The goal of screening is to improve survival by detecting tumors at an early stage when curative or life-prolonging treatments are possible, such as percutaneous ablation, surgical resection, and liver transplantation.

Although routine HCC screening is accepted as standard-of-care in the management of most patients with CHB, the quality of the evidence in support of screening is low[4-6]. Two randomized trials have examined the impact of HCC screening on cancer-related mortality among patients with CHB[7, 8]. One trial observed that ultrasound screening was associated with lower mortality compared to no screening, but the validity of these findings has been called into question due to flaws in the design and analysis of the trial[5-7]. The other utilized AFP only and found little evidence of a mortality benefit associated with screening[8]. Observational studies are more abundant but are also hampered by several limitations[9-12]. Most such studies compare survival between screen-detected and symptomatic *cases,* and so are subject to lead-time, length-time, and selection bias, all of which result in an exaggeration of the potential benefit of screening. It is widely believed that conducting a randomized trial of screening in Western countries is not feasible due to ethical concerns and because potential participants are unlikely to consent to assignment to a no screening arm[13].

Case-control studies are an alternative method for evaluating the impact of screening on cancer-related mortality[14]. In case-control studies of cancer screening, cases and controls are sampled from a population of patients at risk of developing the cancer of interest. Patients with fatal cancer (cases) are compared to persons sampled from members of the population from which the cases arose (controls) with respect to receipt of the screening test during the period of time preceding cancer diagnosis when a tumor is presumed to be detectable by the screening modality. If a screening test reduces cancer-related mortality, cases should have a lower likelihood of receipt of screening than controls. Case-control studies have been used to evaluate the efficacy of screening for several other malignancies, including colorectal[15], esophageal[16], and cervical cancer[17].

A recent case-control study observed no reduction in HCC-related mortality in patients with cirrhosis who underwent USS or AFP-based screening[18]. However, these results may not apply to patients with CHB, as such patients were specifically excluded from that study. The performance characteristics of both USS and serum AFP may be superior in patients with CHB because the nodular liver parenchyma in cirrhosis can impede detection of small tumors by USS[19, 20], and because serum AFP may be elevated in patients with cirrhosis in the absence of HCC[21, 22]. Furthermore, non-cirrhotic patients with CHB may have more curative treatment options for HCC than patients with cirrhosis, in whom portal hypertension and impaired liver function are often contraindications to potentially-curative therapy.

Using a case-control design, our objective was to determine whether HCC screening is associated with a reduction in HCC-related mortality among patients with CHB.

METHODS

Overall study design

We conducted a matched case-control study of patients with CHB who received care through the United States Veterans Affairs (VA) healthcare system. Cases were patients with fatal HCC. Controls were patients who did not die of HCC and who did not have a diagnosis of HCC as of the date HCC was first suspected in their matched case (which we refer to as the index date) (**Figure 1**). Cases and controls were compared with respect to receipt of screening USS or AFP during the 4 years prior to the date HCC was first suspected in cases and the equivalent index date in controls. Four years was chosen to approximate the period of time between when HCC is first detectable by screening and when it would present clinically in the absence of screening, known as the detectable preclinical period (DPP). The DPP was estimated in prior studies by following untreated HCC patients with serial ultrasounds and determining the time it took for a tumor to grow from 1 cm (minimum size potentially detectable by USS) to 10 cm (the size generally expected to cause symptoms) based on the median tumor growth rate (which was a doubling time of 117 days)[23]. The median DPP for HCC was estimated to be ~ 3.2 years[23]. We analyzed screening tests performed up to 4 years prior to the index date as our primary analysis because the *maximal* DPP is felt to provide the least biased estimate of the true association between receipt of screening and cancer mortality[24]. We excluded all patients with hepatitis C virus (HCV) co-infection, as defined by a positive HCV viral load.

Data source

The VA is the largest provider of healthcare in the United States and utilizes a nationally-integrated electronic medical record system. The VA Corporate Data Warehouse (CDW) is a national repository of health information on all VA patients from October 1999 forward. Available data include demographic information, inpatient and outpatient encounters, International Classification of Diseases (ICD)-9 and -10 codes, diagnostic tests, procedures, and VA pharmacy records. The VA CDW was used to screen for potential cases and controls. The medical records of all potential cases and controls were then reviewed through the Compensation and Pension Record Interchange, an electronic interface providing online access to medical records from all VA facilities.

Cases

Using the VA CDW (**Figure 2**), we identified all patients who had a positive hepatitis B surface antigen (HBsAg) or detectable hepatitis B viral load (HBV DNA), an ICD-9 or -10 code corresponding to a diagnosis of HCC (155.0, C22.0) recorded at least twice, and who died between 01/01/2004 and 12/31/2017. The first ICD-9 or -10 code for HCC must have been recorded at least 4 years after the first positive HBsAg or viral load in the VA system to ensure that all cases had sufficient follow-up time before the diagnosis of HCC for screening to plausibly impact HCC-related mortality.

A physician investigator blinded to screening status reviewed the electronic medical records of all potential cases. Data from medical record review were abstracted onto a REDCap database. Cases were included only if they fulfilled all of the following criteria: confirmed CHB, fatal HCC defined as cancers that definitely or probably contributed to death, and at least 4 years of follow-up between their first available hepatitis B diagnostic test and the index date.

Confirmation of CHB diagnosis

Patients were confirmed as having CHB if they had: 1) 2 or more positive tests for hepatitis B infection (HBsAg or HBV DNA) separated by at least six months, or 2) 2 or more positive tests for hepatitis B infection not separated by at least 6 months or 1 positive test for hepatitis B infection while also having a clinical diagnosis of CHB. A clinical diagnosis of CHB was defined as: receipt of antiviral therapy for CHB, having cirrhosis attributed to CHB, or having a diagnosis of CHB as documented by a gastroenterology, hepatology, or infectious disease provider.

Confirmation of HCC diagnosis

Patients had to meet one of the following criteria to qualify as having HCC: 1) Liver nodule ≥10 mm that was hypervascular in the arterial phase with washout in the portal venous or delayed phase in either 4-phase multidetector computed tomography (CT) or dynamic contrast enhanced magnetic resonance imaging (MRI) scan, 2) Lesions reported to be “LI-RADS5” according to the Liver Imaging and Reporting Data System[25], or 3) Histology consistent with HCC.

Assignment of index date

For patients with confirmed HCC, we assigned an index date. The index date was the date that HCC was first suspected based on the earliest of the following: 1) date of first multiphasic imaging or histology diagnostic of HCC, 2) date of first symptoms of HCC, 3) date of first elevated AFP to greater than 20 ng/mL, or 4) date of first suspicious imaging of any kind. By definition, any USS or AFP obtained after the index date was not considered a screening test.

Confirmation of fatal HCC

Medical records were reviewed to verify that cases had fatal HCC. Whether HCC contributed to death was categorized as “definitely”, “probably”, “definitely not”, or “unable to determine”[18]. HCC that “definitely” contributed to death included cancers with extra-hepatic metastasis, local or vascular invasion, a large-volume tumor burden (defined as > 6 cm in size in any dimension or AFP > 1000 ng/mL), multifocal disease (defined as > 3 tumors in the liver) at the time of death, or if death was related to complications of HCC treatment. HCC was considered to have “probably” contributed to death if the cause of death was liver failure or other complications (such as pulmonary embolism) that might be related to HCC. Deaths “definitely not” attributable to HCC were deaths due to liver failure with concomitant small (< 3 cm) or completely ablated HCC and deaths due to completely unrelated causes (such as myocardial infarction or cerebrovascular accident). Criteria used to determine whether HCC contributed to death were validated by a pilot study of a set of 50 cases of fatal HCC reviewed independently by two authors. There was excellent inter-observer agreement (97.5% agreement, kappa = 0.94, $p < 0.001$) between the 2 authors in assigning whether HCC definitely contributed to the patient’s death using the aforementioned criteria.

Controls

Using the VA CDW (**Figure 2**), we identified potential controls who were restricted to patients who had a positive HBsAg or HBV DNA, fulfilled the matching criteria for their specified case, were in VA care and not diagnosed with HCC as of the index date of their matched case, and did not later die from HCC. Controls were assigned an index date identical to that of their matched case. The medical records of controls were reviewed to confirm a diagnosis of CHB using the same method as for cases, the absence of HCC as of the index date, and that HCC was not the cause of death for controls who died.

Matching criteria

Controls were matched to cases in a 1:1 ratio by factors associated with both fatal HCC and the likelihood of screening: 1) Date of first positive HBsAg or HBV DNA in the VA CDW, 2) Race/Ethnicity (categorized as non-Hispanic White, non-Hispanic Black, Hispanic, Asian / Pacific Islander / Alaska Native / American Indian), 3) Age (within 2 years), 4) Gender, 5) Presence/absence of cirrhosis (determined by ICD-9 codes 571.2 or 571.5) prior to the index date, 6) Receipt of hepatitis B antiviral medications prior to the index date, 7) hepatitis B e antigen (HBeAg) status, and 8) maximum viral load prior to the index date (<2000 or ≥2000 prior to index date for the HBeAg negative patients and <20,000 or ≥20,000 prior to index date for the HBeAg positive patients). Because cases and controls were matched for the date of the first positive HBsAg or HBV DNA, each case and his matched control were compared with respect to receipt of screening during identical calendar years when they were both in VA care.

Determination of screening history in cases and controls

We abstracted the dates of all abdominal USS and serum AFPs performed during the 4 years preceding the index date for cases and controls. A trained research assistant blinded to case/control status abstracted USS reports along with progress notes before and after the date of each test onto a REDCap database. A physician-investigator blinded to case-control status assigned the indication for each test as screening or not after review of abstracted reports and progress notes. The indication was categorized as “definitely”, “probably”, “probably not”, or “definitely not” for screening, or “unable to determine” based on criteria detailed in **Supplemental Table 1**. Patients who received tests “definitely” or “probably” for screening were categorized as having a history of screening.

Statistical Analysis

Cases and controls were compared with respect to receipt of screening abdominal USS or serum AFP during the DPP (i.e. 4 years prior to the index date) using conditional logistic regression. Models were additionally adjusted for diabetes, alcohol use disorder, body mass index (BMI), human immunodeficiency virus (HIV) co-infection, and receipt of abdominal CT or MRI during the period of interest. Models were not adjusted for receipt of the other screening modality during the period of interest due to collinearity (almost all – 90.1% – patients who received screening ultrasound also received screening AFP).

We evaluated the following binary screening variables in different conditional logistic regression models to obtain odds ratios summarizing the association between screening and HCC-related mortality:

1) Screening with <u>either</u> USS and serum AFP versus no screening with either USS or serum AFP
2) Screening with <u>both</u> USS or serum AFP versus no screening with either USS or serum AFP
3) Screening with serum AFP only versus no screening with either USS or serum AFP

We were unable to evaluate the effect of screening with USS only because too few patients received USS screening alone without serum AFP.

In case-control studies of cancer screening effectiveness, receipt of screening during the DPP is modeled as a binary variable, i.e. we only determine whether a person had *any* screening. Cases and controls should not be compared with respect to the *number* of screening tests they received (i.e. screening intensity) because cases are expected to have fewer screening tests than controls even if no HCC treatment is administered or the treatment is completely ineffective, thus leading to a spurious association between low screening intensity and higher cancer mortality[26]. The reason cases are expected to have a lower intensity of screening is that a case diagnosed with HCC at time *t* (the matched index date) is unlikely to have undergone *multiple* screening tests during the time period before *t* because the first of these tests would likely have been positive (assuming the test is sensitive) and subsequent screening tests would not have been performed. In contrast, a matched control who does not have HCC may have had several screening tests during the time period before time *t*.

We planned 2 sensitivity analyses. First, we compared cases and controls with respect to receipt of screening during the 1, 2, and 3 years prior to the index date to determine whether our results were robust to different estimates of the DPP. Second, we excluded all patients who received abdominal CT or MRI in the 4 years prior to the index date because some patients may have undergone CT/MRI screening in lieu of USS or AFP-based screening and so would appear to be "unscreened". Lastly, we performed a subgroup analysis evaluating the association between screening and HCC-related mortality separately in patients with and without cirrhosis in order to explore whether the effectiveness of screening differs depending on the presence of underlying cirrhosis.

RESULTS

Identification of cases and controls

We identified 291 potential cases of fatal HCC after electronically searching the VA CDW. After medical record review, we excluded 9 patients who did not have HCC, 25 who did not die as a result of HCC, 19 who did not have CHB, 43 who did not have at least four years of follow-up after a positive hepatitis B test prior to the index date, 7 for missing diagnosis information, and 19 who could not be matched to a control fulfilling the matching criteria. This left 169 cases in our analysis (**Figure 2**) who were each matched to one control. The majority of case:control pairs (N = 94) were matched on all 8 criteria. The remaining pairs (N = 75) were matched after relaxing race/ethnicity matching and age to within 4 years due to a limited number of available controls.

Baseline characteristics of cases and controls

Cases and controls were well matched with respect to age at first positive HBV test (52 years), age at index date (60 years), sex, race/ethnicity, year of first positive hepatitis B test, interval between first positive HBV test and index date, cirrhosis, and prior receipt of hepatitis B antiviral therapy (**Table 1**). All cases and controls were male. Approximately one third of patients had cirrhosis. HBeAg was positive in 74.4% of cases and 76.9% of controls. Cases were slightly more likely than controls to have comorbid diabetes (28.4% versus 23.7%) or alcohol use disorders (42% versus 36.7%). Controls were more likely than cases to have undergone abdominal CT or MRI scans prior to the index date (44.4% versus 27.2% in the 0-4 years prior to the index date).

Characteristics of HCC in cases

The majority of fatal cases were diagnosed by imaging (94.7%) and nearly half of the patients had histologic confirmation of HCC (46.7%) (**Table 2**). At the time of diagnosis, 65.6% of patients had HCC beyond Milan criteria, 27.8% had evidence of vascular invasion, and 14.2% had evidence of extrahepatic metastases. While the majority of the fatal cases received some type of HCC directed treatment (69.8%), only 1.2% of patients underwent liver transplantation and 5.3% underwent surgical resection, while 34.3% underwent transarterial chemoembolization, 11.2% radiofrequency ablation, and 4.1% Y-90 radioembolization. Since by definition this was the subset of HCC cases in VA care that were *fatal*, it is not surprising that they presented in advanced stages and generally did not receive potentially curative treatments.

Association between screening and HCC-related mortality

In the 4 years prior to the index date, cases received 133 USS exams (including 81 "definitely" and 2 "probably" obtained for screening) and 193 serum AFP tests (143 "definitely" and 6 "probably" obtained for screening). Over the same interval, controls received 201 USS exams (148 "definitely" and 1 "probably" obtained for screening) and 382 serum AFP tests (314 "definitely" and 2 "probably" obtained for screening) (**Table 3**).

Cases were far less likely to have received screening with either USS or AFP (33.7%) than controls (58.6%). In multivariable conditional logistic regression, screening with either USS or AFP was strongly associated with reduced HCC-related mortality (aOR 0.21, 95% CI 0.09-0.50) (**Table 4**).

Cases were also far less likely to have received screening with both USS and AFP (19.5%) than controls (34.3%). In multivariable conditional logistic regression, screening with both tests was strongly associated with reduced HCC-related mortality (**Table 4**).

With respect to screening with AFP only, cases were again less likely to have received screening (12.4%) than controls (20.1%). In multivariable analysis, screening with AFP only was associated with reduced HCC-related mortality (aOR 0.22, 95% CI 0.05-1.00). While a smaller proportion of cases (1.8%) than controls (4.1%) received screening with USS only, there were too few patients to assess the association between screening and HCC-related mortality in multivariable analysis.

In sensitivity analyses, we compared cases and controls with respect to receipt of screening during the 1, 2, and 3 years prior to the index date to test whether our results were robust to different assumptions about the duration of the DPP. Screening with either USS or AFP was associated with a reduced risk of HCC-related mortality during the 3 years (aOR 0.24, 95% CI 0.11-0.52), 2 years (aOR 0.26, 95% CI 0.13-0.55), and 1 year (aOR 0.42, 95% CI 0.22-0.81) prior to the index date (**Table 4**).

In a second sensitivity analysis, we excluded patients who received abdominal CT or MRI in the 4 years prior to the index date (**Supplemental Table 2**). Screening with either USS or AFP remained strongly associated with reduced HCC-related mortality (aOR 0.20, 95% CI 0.05-0.78).

Lastly, in an exploratory subgroup analysis, screening with USS or AFP was associated with a reduced risk of mortality from HCC in persons without cirrhosis (29.9% of 107 cases versus 49.5% of 107 controls had been screened during the four years prior to index date, aOR 0.07, 95% CI 0.02-0.29) and also in persons with cirrhosis (40.3% of 62 cases versus 74.2% of 62 controls, aOR 0.17, 95% CI 0.03-0.91).

DISCUSSION

Using a matched case-control study design, we observed that HCC screening with USS and serum AFP was associated with a large reduction in HCC-related mortality among patients with CHB in VA care in the United States. Our results provide strong support for efforts to increase the uptake of screening in patients with CHB.

Although routine HCC screening is considered standard-of-care for patients with cirrhosis and for select patients with CHB without cirrhosis, major professional organizations have not reached consensus regarding the benefits of screening. The three major liver societies (AASLD, European Association for the Study of the Liver, and the Asian-Pacific Association for the Study of the Liver) all recommend HCC screening in patients with cirrhosis and in high-risk patients with CHB[2, 27, 28]. In contrast, the National Cancer Institute states that available evidence does not suggest a mortality benefit from screening whereas there is evidence of potential harm[29]. Neither the United States Preventative Services Task Force nor the American Cancer Society have formal positions on HCC screening.

The primary reason for a lack of consensus is that available evidence on HCC screening is of low quality[4-6]. The optimal study design to evaluate the effectiveness of a screening test would randomize participants to screening or no screening and compare them with respect to the occurrence of cancer-related mortality. Current AASLD guidelines cite 2 randomized controlled trials to support screening recommendations. However, closer scrutiny of these trials reveals limitations in study design that threaten their validity. In a cluster randomized trial by Zhang et al., patients with CHB in China who were assigned to receive semi-annual USS and AFP-based screening were found to have a lower risk of HCC-related mortality compared to persons not so assigned (relative risk 0.62, 95% CI 0.41 – 0.98)[7]. However, the method of allocation of study participants to the two trial arms was not clearly described, and persons not assigned to the screening arm were not actively followed in the same way as those in the intervention arm. In a second randomized trial by Chen et al., screening with serum AFP did not appreciably reduce mortality (relative risk 0.83, 95% CI 0.68 – 1.03)[8], but the screening modality employed an AFP assay (reverse passive hemagglutination) that is less sensitive than AFP assays currently in use (immunoassay)[5].

In the absence of high quality randomized controlled trials, many observational studies have been conducted to evaluate the efficacy of HCC screening[12]. Most studies compared survival between cases with screen-detected versus symptomatically diagnosed HCC, or alternatively between cases who were compliant or non-compliant with screening prior to HCC diagnosis. However, these types of studies are highly susceptible to lead-time, length-time, and selection bias[30]. Some studies employed methods to correct for lead-time bias in their analysis[9-12, 31-35], but only a small number of patients with CHB were included in these studies[9-11, 33-35]. Moreover, results of cohort studies that correct for lead time bias vary widely depending on assumptions of the tumor growth rate and the duration of follow-up[10, 31, 36]. Furthermore, they remain susceptible to length-time bias.

In case-control studies of cancer screening, the screening history of cases – defined as patients who died of the cancer of interest – is compared to that of controls selected from the population from which the cases arose who did not die of the relevant cancer. Therefore, since cases are specifically selected from the population of patients with CHB and *fatal* HCC, they are relatively more likely to have presented with advanced tumors and to not have received curative treatments. As such, the tumor characteristics and HCC treatment histories of cases presented in **Table 2** do not reflect the average VA patient with CHB who develops HCC. The rationale for selecting as controls patients who did not die of HCC is to avoid lead-time bias. It may be tempting to assume that controls ought to be patients with CHB and HCC who had not (yet) succumbed to this cancer by the end of the study period. However, choosing "HCC survivors" as controls is inappropriate because some number of survivors at any point in time will later die from HCC but are alive now only because screening pushed their diagnosis forward in time. Consequently, choosing such patients as controls would overestimate the proportion of controls who had undergone screening. It should be noted that – while we avoided selecting "HCC survivors" as controls – we did not prohibit including as controls patients who developed HCC after the index date but who did not die of HCC by the end of the study period (**Figure 1**). Just as "survivors" in a randomized controlled trial of screening efficacy may include patients who do not develop HCC and those who do, controls in our study could have developed HCC or not, provided 1) controls who developed HCC did not die of it by the end of the study period, as they would then be eligible to be a case and 2) controls were not diagnosed with HCC prior to the index date of their matched case as this would truncate their screening history.

The main finding of our study is that receipt of either screening AFP or USS (or of both modalitites) was associated with a substantially lower risk of HCC-related mortality, which was robust to multiple sensitivity analyses (**Table 4, Supplemental Table 2**). We were unable to examine USS alone as a screening modality because very few patients received USS alone. This pattern of low USS utilization is consistent with prior studies of HCC screening in the VA[31]. This is an important limitation, as USS is the main method of screening recommended by most professional societies. A greater number of patients underwent screening with AFP only, which is also consistent with prior studies[31]. The direction of the association between AFP only and HCC mortality suggests a possible benefit to AFP-based screening, but there were still too few patients in this group to evaluate the independent effect of this screening modality with any statistical precision.

Our findings provide support for current screening recommendations as well as efforts to improve screening uptake in patients with CHB. Uptake of HCC screening in patients with CHB is low in most real-world populations[31, 37, 38]. Overall, the proportions of patients who underwent screening in our study are consistent with previously reported screening utilization rates in the VA and in other healthcare systems in the United States. The low rate of screening utilization in our study, however, does not necessarily mean that an evaluation of screening effectiveness is not possible in this setting. In order to be able to compare the benefit of screening versus no screening, a substantial proportion of patients under study must fall into the “no screening” group. Power to detect an association between screening and mortality risk is in fact optimized when screening rates approach 50%. The magnitude and statistical precision of the associations in our study clearly demonstrate that it is possible to detect a benefit to screening if one truly exists even in the context of low screening utilization.

Results of this study notably contrast with those of our prior study examining the effect of screening in patients with cirrhosis who did not have CHB[18], where we found that screening was not associated with a reduction in HCC-related mortality. One potential explanation for the discordant results is that patients in the present study were not required to have cirrhosis to be included as a case or control. In fact, only 36.7% of patients had cirrhosis in this study (**Table 1**). This is relevant because the presence of cirrhosis could diminish the benefits of HCC screening. Screening may be associated with reduced HCC mortality in our current study but not in our prior study because: 1) USS and AFP have relatively higher sensitivity in non-cirrhotic patients, and are thus better able to detect HCC at earlier stages; and 2) patients with CHB without cirrhosis have more curative treatment options than patients with cirrhosis due to the presence of portal hypertension, which is often a contraindication to treatment. Another explanation for the difference between our two studies is that, even among patients with cirrhosis, screening efficacy may differ depending on the underlying cause of cirrhosis. Patients with CHB as the primary etiology of liver disease may have less advanced pathology than those with cirrhosis from other etiologies due to the availability of highly effective antiviral therapy for HBV. Effective viral suppression can stabilize liver function for many years. Indeed, a prospective cohort study of patients with Child’s Pugh Class A cirrhosis due to CHB or HCV (in the pre-direct acting antiviral era) found that those with CHB were less likely to develop primary liver cancer, experience decompensation, and had better survival than those with HCV[39]. A key difference between the two populations was that substantially more patients with CHB were on antiviral therapy with full suppression of viremia. It is possible that patients with CHB-related cirrhosis are more likely than patients with other etiologies of cirrhosis to benefit from screening because they have better preserved liver function and are more likely to be candidates for curative treatment. Therefore, although screening may be associated with a reduced risk of fatal HCC in CHB patients with cirrhosis based on our subgroup analysis, this benefit would not necessarily extend to patients with cirrhosis due to other reasons.

It is important to recognize several limitations of our study. First, in case-control studies of cancer screening, it is not possible to compare cases to controls with respect to the intensity of screening. Second, it is possible that some tests were misclassified as screening because the indication for each test was not always explicitly stated in the medical records. This was less of a concern for USS tests because the indication was recorded in each report, whereas provider progress notes were the only source of information for AFP tests. It should be noted, however, that the impact of such misclassification would be to produce an *underestimate* of the benefit of screening. Third, we were not always able to match cases and controls by date of CHB diagnosis because laboratory tests obtained before October 1999 – the date the VA CDW was created – were not available to us. The next best choice was to match by the date of the first positive hepatitis B test after October 1999. Fourth, all patients in our study were male, reflecting the demographics of the underlying VA population. However, to our knowledge, there are no reasons to believe that the effect of screening on HCC-related mortality ought to differ depending on sex. Lastly, it is possible that patients engaged in HCC screening may also practice positive health behaviors or possess other determinants of health that could have contributed to a portion of their decreased risk of fatal HCC.

In summary, in a matched case-control study, HCC screening with AFP and USS was associated with a reduced risk of HCC-related mortality among patients with CHB. Our results suggest that currently recommended screening approaches for patients with CHB are appropriate.

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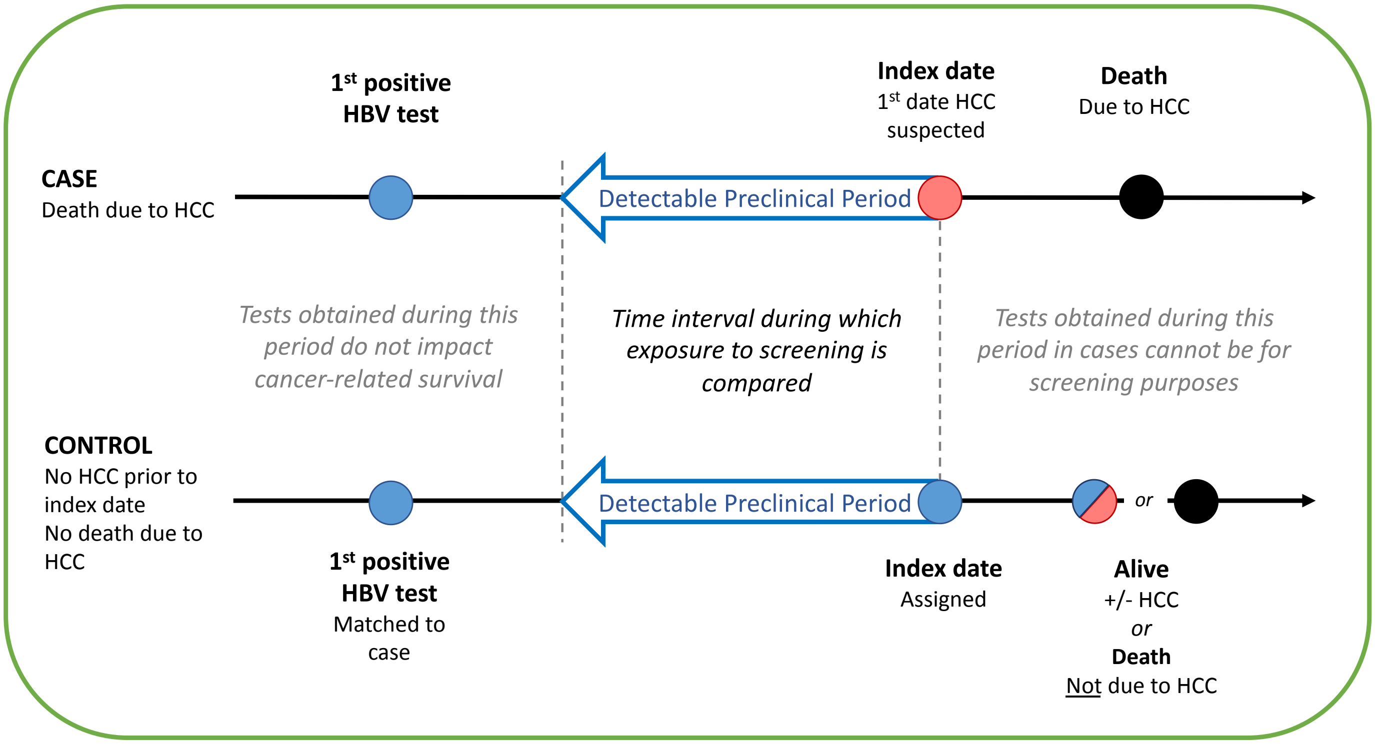
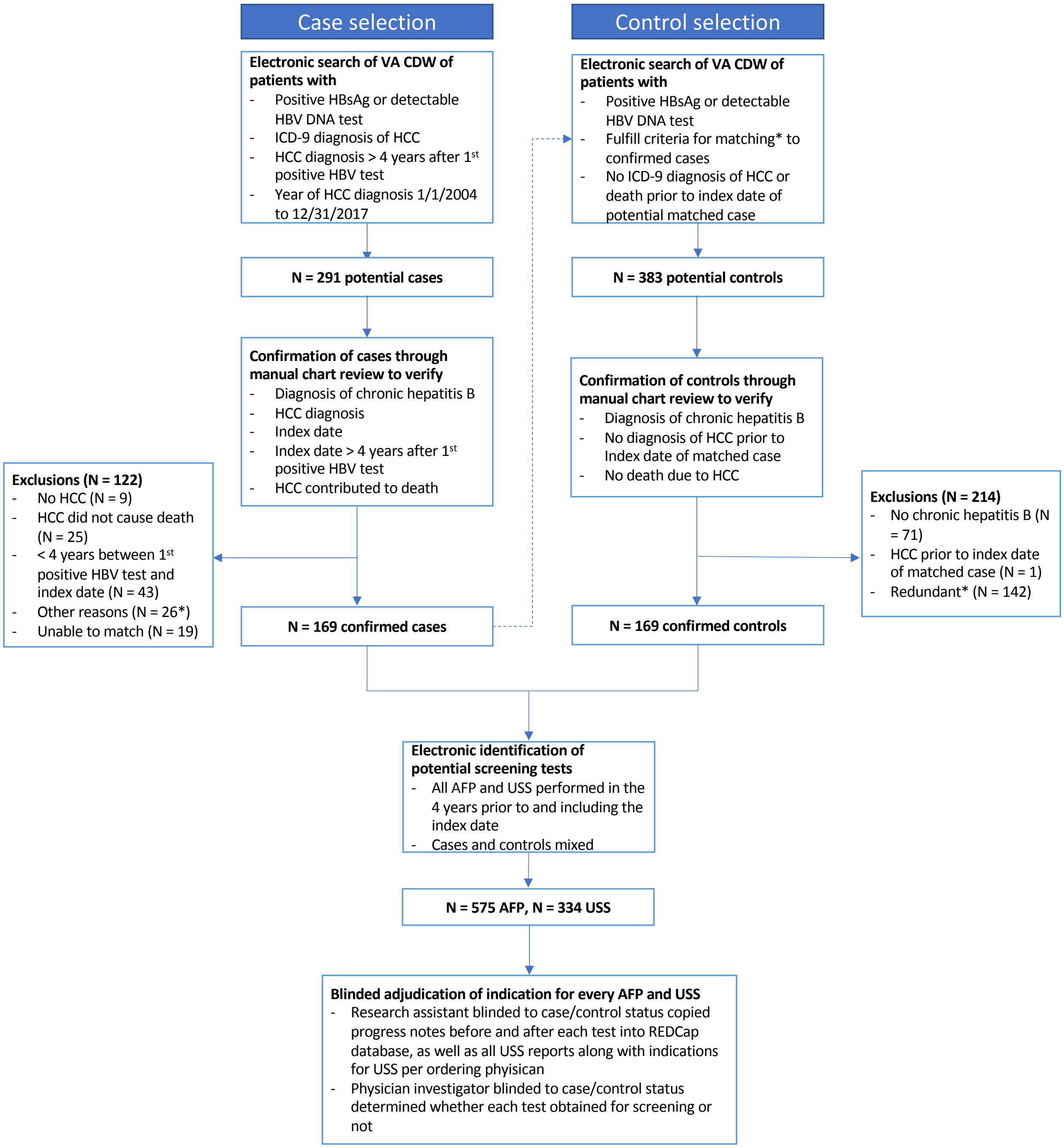
Figure 1. Schematic representation of the method used to match controls to cases, illustrating the Index Date and Detectable Preclinical Phase (DPP). The DPP comprised an identical period of calendar years for the case and control within each matched pair (e.g. 2010-2014), during which both case and control were in VA care.



Abbreviations: HCC – hepatocellular carcinoma, HBV – hepatitis B virus

Figure 2. Flowchart illustrating the steps for identification and confirmation of cases and matched controls.



Abbreviations: VA CDW – Veterans Affairs Corporate Data Warehouse, HBsAg – hepatitis B surface antigen, HBV DNA – hepatitis B viral load, HCC – hepatocellular carcinoma, HBV – hepatitis B virus, AFP – alpha fetoprotein, USS – ultrasound

* Redundant refers to controls who met matching criteria but were not used after 1:1 matching of all 169 cases

Table 1. Characteristics of cases and their matched controls

	Controls N = 169	Cases N = 169
Male, %	100	100
Age at first positive HBV test, mean (yrs)	52.0	52.2
Age at index date, mean (yrs)	59.9	60.3
Year of first positive HBV test, %		
1999-2001	29.6	29.6
2001-2003	45.6	45.6
2004-2006	17.2	17.2
2007-2011	7.7	7.7
Time interval between first positive HBV test and index date, yrs	8.0	8.1
Index Date Year, %		
2004-2007	18.3	18.3
2008-2010	33.1	33.1
2011-2013	34.9	34.9
2014-2017	13.6	13.6
Race/Ethnicity, %		
White, non-Hispanic	46.2	44.4
Black, non-Hispanic	39.1	34.9
Other	14.8	20.7
Cirrhosis, %	36.7	36.7
HBeAg positive, %	76.9	74.4
Maximum HBV DNA viral load 5 years prior to index date ≥ 2000 (e Ag negative) or ≥ 20,000 (e Ag positive)	46.2	53.8
HBV antiviral treatment, %	46.2	46.2
Facility complexity,%		
Ambulatory (Basic or Advanced)	3.7	5.1
Inpatient Standard	3.7	2.6
Inpatient Intermediate	13.5	14.1
Inpatient Complex	79.1	78.2
BMI, mean (Kg/m^2)	27.9	27.1
Diabetes, %	23.7	28.4
Alcohol Use Disorders, %	36.7	42
HIV Coinfection, %	13.0	14.2
CT or MRI before index date, %		
0-2 years	29.6	21.3
0-3 years	37.9	25.4
0-4 years	44.4	27.2

Abbreviations: HBV – hepatitis B virus, HBeAg – hepatitis B e antigen, HBV DNA – hepatitis B viral load, BMI – body mass index, HIV – human immunodeficiency virus, CT – computed tomography, MRI – magnetic resonance imaging

Table 2. Characteristics of HCC among cases

	Cases N (%)
Method of HCC diagnosis*	
Imaging (CT/MRI)	160(94.7)
Histology	79(46.7)
Treatment of HCC*	
Liver transplantation	2(1.2)
Surgery (partial hepatectomy)	9(5.3)
Systemic chemotherapy (sorafenib)	72(42.6)
Trans-arterial chemoembolization	58(34.3)
Radiofrequency ablation	19(11.2)
Y-90 radioembolization	7(4.1)
Percutaneous ethanol injection	1(0.6)
Cryoablation	0(0.0)
Other Treatment	22(13.0)
Any one of the above treatments	118(69.8)
Stage of HCC at Diagnosis	
Maximum dimension of largest tumor (cm), mean (SD)	6.4(4.4)
Number of tumors, mean (SD)	2.2(1.7)
Number of tumors (%)	
1	86(57.0)
2-3	28(18.5)
≥4	37(24.5)
Size of largest tumor (%)	
0-3 cm	32(18.9)
3 to <5 cm	41(24.3)
5 to <6 cm	8(4.7)
6 to <7 cm	10(5.9)
≥7 cm	78(46.2)
Within Milan Criteria (%)†	70(34.4)
Beyond Milan Criteria (%)	117(65.6)
Vascular Invasion, %	47(27.8)
Metastasis, %	24(14.2)
HCC Contributed to patient's death*	
Metastatic HCC	58(34.3)
Multifocal HCC (>3 lesions)	70(41.4)
Local or vascular invasion by HCC	70(41.4)
Large Volume HCC (>6cm or AFP>1000)	124(73.4)
Death due to complications of HCC treatment	5(3.0)

*The categories for "method of HCC diagnosis", "treatment of HCC" and "HCC contributed to patient's death" are NOT mutually exclusive.
†Milan Criteria: One tumor <5 cm or 2-3 tumors each of which is < 3cm

Abbreviations: HCC – hepatocellular carcinoma, CT – computed tomography, MRI – magnetic resonance imaging, SD – standard deviation, AFP – alpha fetoprotein

Table 3. Distribution of categorization of USS scans and serum AFP tests during the 0-4 years prior to index date

	Controls	**Cases**
USS		
All USS	201	133
Definitely screening	148 (73.6%)	81 (60.9%)
Probably screening	1 (0.5%)	2 (1.5%)
Probably not screening	8 (4.0%)	8 (6.02%)
Definitely not screening	40 (19.9%)	38 (28.6%)
Unable to determine	4 (2.0%)	4 (3.0%)
AFP		
All AFP	382	193
Definitely screening	314 (82.2%)	143 (74.1)
Probably screening	2 (0.5%)	6 (3.1)
Probably not screening	1 (0.3%)	7 (3.6%)
Definitely not screening	57 (15%)	31 (16.1%)
Unable to determine	8 (2.1%)	6 (3.1%)

Abbreviations: USS – ultrasound, AFP – alpha fetoprotein

Table 4. Comparison of cases and controls with respect to occurrence of screening (defined as definitely or probably screening) prior to the index date.

	Controls N=169	Cases N=169	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)
0-4 years prior to the index date				
No screening	70 (41.4%)	112 (66.3%)	1	1
Either USS or AFP	99 (58.6%)	57 (33.7%)	0.19 (0.10-0.38)	0.21 (0.09-0.50)
AFP only	34 (20.1%)	21 (12.4%)	0.33 (0.12-0.92)	0.22 (0.05-1.00)
USS only[b]	7 (4.1%)	3 (1.8%)	-	-
Both USS and AFP	58 (34.3%)	33 (19.5%)	0.12 (0.04-0.34)	0.11 (0.03-0.40)
0-3 years prior to index date				
No screening	76 (45.0%)	117 (69.2%)	1	1
Either USS or AFP	93 (55.0%)	52 (30.8%)	0.24 (0.13-0.44)	0.24 (0.11-0.52)
AFP only	32 (18.9%)	21 (12.4%)	0.40 (0.16-1.03)	0.37 (0.10-1.38)
USS only[b]	6 (3.6%)	4 (2.4%)	--	--
Both USS and AFP	55 (32.5%)	27 (16.0%)	0.17 (0.07-0.41)	0.12 (0.04-0.38)
0-2 years prior to index date				
No screening	86 (50.9%)	123 (72.8%)	1	1
Either USS or AFP	83 (49.1%)	46 (27.2%)	0.26 (0.14-0.48)	0.26 (0.13-0.55)
AFP only	32 (18.9%)	19 (11.2%)	0.29 (0.11-0.80)	0.31 (0.09-1.08)
USS only[b]	8 (4.7%)	4 (2.4%)	--	--
Both USS and AFP	43 (25.4%)	23 (13.6%)	0.24 (0.11-0.55)	0.15 (0.05-0.42)
0-1 year prior to index date				
No screening	103 (60.9%)	129 (76.3%)	1	1
Either USS or AFP	66 (39.1%)	40 (23.7%)	0.41 (0.24-0.71)	0.42 (0.22-0.81)
AFP only	33 (19.5%)	18 (10.7%)	0.45 (0.20-0.99)	0.54 (0.21-1.35)
USS only[b]	10 (5.9%)	7 (4.1%)	--	--
Both USS and AFP	23 (13.6%)	15 (8.9%)	0.31 (0.11-0.85)	0.22 (0.06-0.73)

[a]Adjusted for age, race/ethnicity, BMI, diabetes, alcohol use disorder, HIV coinfection, HBV e Ag positive, HBV DNA maximum viral load, years from HBV diagnosis to index date, and receipt of CT or MRI in the window.
[b]Unable to calculate odds ratio because too few patients received USS only.

Abbreviations: OR – odds ratio, CI – confidence interval, USS – ultrasound, AFP – alpha fetoprotein

Supplemental Table 1. Criteria for determining the indication for ultrasounds and AFPs ordered during the detectable preclinical period in cases and the equivalent interval in controls.

Assignment of Indication	Indication reported in ultrasound report or provider progress note
a. Definitely screening*	HCC (or hepatoma, or liver cancer) screening or surveillance; *In patients with no new symptoms, signs or tests suggestive of HCC:* R/O HCC, R/O hepatoma, HBV Surveillance; Cirrhosis surveillance; R/O focal liver lesions; Hepatitis B Virus; Hepatitis B Virus, Pls evaluate liver. "Abdominal aortic aneurysm screening" "Follow-up renal cysts"
b. Definitely non-screening	Elevated (or worsening) liver function tests (or LFTs); Ascites; Weight loss; Abdominal pain; Abdominal tenderness; Abdominal mass; Abdominal distension; Abdominal bloating; Enlarged liver; Palpable liver; Failure to thrive; Jaundice; Increased bilirubin; Variceal Bleeding; *As a follow-up of another positive test suggestive of HCC:* Abnormal AFP (for ultrasound); Liver abnormality on abdominal ultrasound (for AFP); Liver abnormality on abdominal CT or MRI scan; Any test done in the Emergency Room or as an Inpatient.
c. Probably screening	Screen for HCC and patient also has ascites (when the ascites is long-standing or unchanged)**†**; Serum AFP test with provider note that does not specifically mention screening but also does not mention any new symptoms or signs or concerns for HCC; Serum AFP test with no associated progress note by the ordering provider within 6 months and no evidence of new symptoms or signs suggestive of HCC ;
d. Probably non-screening	R/O HCC – but unclear if patient has symptoms or signs suspicious of HCC R/O cirrhosis (in patient with HBV infection who does not yet have known cirrhosis)
e. Unable to determine	No clear indication recorded. Neither "screening" nor "non-screening" indications are clearly reported. No provider progress notes available by the physician who ordered the test.

* Only record as "definitely screening" if there is no simultaneous "non-screening" indication.
† This refers to tests where the provider seems to be ordering the USS with the intention of screening for HCC but also wants a comment on the degree of ascites that is long-standing and being treated, without any obvious concern that development of HCC might have contributed to the ascites.

Supplemental Table 2. Sensitivity analysis after excluding patients who underwent CT or MRI in the DPP. Cases and controls compared with respect to occurrence of screening with either USS or AFP at given time intervals prior to the index date.

	Controls N (%)	**Cases N (%)**	**Unadjusted OR (95% CI)**	**Adjusted[a] OR (95% CI)**
0-4 years prior to index date				
Either USS or AFP	44 (26.0%)	24 (14.2%)	0.21 (0.07-0.62)	0.20 (0.05-0.78)
0-3 years prior to index date				
Either USS or AFP	44 (26.0%)	24 (14.2%)	0.27 (0.11-0.67)	0.20 (0.06-0.66)
0-2 years prior to index date				
Either USS or AFP	47 (27.8%)	25 (14.8%)	0.38 (0.17-0.81)	0.34 (0.13-0.90)
0-1 year prior to index date				
Either USS or AFP	40 (23.7%)	25 (14.8%)	0.54 (0.28-1.03)	0.55 (0.24-1.20)

[a]Adjusted for age, race/ethnicity, BMI, diabetes, alcohol use disorder, HIV coinfection, HBV e Ag positive, HBV DNA maximum viral load, years from HBV diagnosis to index date, and receipt of CT or MRI in the window.

Abbreviations: OR – odds ratio, CI – confidence interval, USS – ultrasound, AFP – alpha fetoprotein