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ORIGINAL RESEARCH—CLINICAL

External Validation of LCR1-LCR2, a Multivariable Hepatocellular Carcinoma Risk Calculator, in a Multiethnic Cohort of Patients With Chronic Hepatitis B

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BACKGROUND AND AIMS: The liver cancer risk test (LCR1-LCR2) is a multianalyte blood test combining proteins involved in liver cell repair (apolipoprotein A1, haptoglobin), hepatocellular carcinoma (HCC) risk factors (gender, age, gamma glutamyl transpeptidase), a marker of fibrosis (alpha2-macroglobulin), and alpha-fetoprotein, a specific marker of HCC. The aim was to externally validate LCR1-LCR2 in hepatitis B. **METHODS:** Preincluded patients were from the Hepather cohort, a multicenter, multiethnic prospective study in 6071 patients. The coprimary study outcome was the negative predictive value of LCR1-LCR2 at 5 years for the occurrence of HCC and survival without HCC according to the predetermined LCR1-LCR2 cutoffs, adjusted for risk covariables and for chronic hepatitis B treatment and quantified using time-dependent Cox proportional hazards models. A post hoc analysis compared the number of patients needed to screen one cancer by LCR1-LCR2 and PAGE-B. **RESULTS:** A total of 3520 patients, 191 (5.4%) with cirrhosis, with at least 1 year of follow-up were included. A total of 76 HCCs occurred over a median (interquartile range) of 6.0 years (4.8–7.3) of follow-up. Among the 3367 patients with low-risk LCR1-LCR2, the 5-year negative predictive value was 99.3% (95% confidence interval = 99.0–99.6), with a significant Cox hazard ratio (6.4, 3.1–13.0; $P < .001$) obtained after adjustment for exposure to antivirals, age, gender, geographical origin, HBe-Ag status, alcohol consumption, and type-2 diabetes. LCR1-LCR2 outperformed PAGE-B for number of patients needed to screen mean (95% CI), 8.5 (3.2–8.1) vs 26.3 (17.5–38.5; $P < .0001$), respectively. **CONCLUSION:** The performance of LCR1-LCR2 to identify patients with chronic hepatitis B at very low risk of HCC at 5 years was externally validated. ClinicalTrials.gov: NCT01953458.

Keywords: Fibrosis Progression; Cirrhosis; LCR1-LCR2; FibroTest

Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death worldwide and the fastest growing cause of cancer deaths in the United States.[1] The prognosis of HCC remains poor except for the subset of patients who are diagnosed at an early stage of disease. The increase in the incidence of HCC shows the importance of effective surveillance strategies, particularly among emerging at-risk cohorts such as patients with chronic hepatitis B (HBV) and chronic hepatitis C (HCV) and a sustained virological response.[2–5]

The association between HBV infection and HCC has been established based on the increased incidence of HCC in areas where the virus is endemic,[6] prospective studies, and animal models of hepadna virus infections.[7] Finally, the incidence of HBV infection and related cirrhosis and HCC has significantly decreased as a result of routine vaccination[8] and by effective viral suppression with either interferon-α or nucleos(t)idic analogs, mostly entecavir and

***The lists of investigators in the HEPATHER cohort, and in the FibroFrance-Pitié Salpêtrière cohort group (GHPS) appear in File A1.**

Abbreviations used in this paper: **AFP, alpha-fetoprotein; CI, confidence interval; EASL, European Association for the Study of the Liver; HBV, chronic hepatitis B; HCC, Hepatocellular carcinoma; HCV, chronic hepatitis C; LCR, Liver Cancer Risk; NNS, number of patients needed to be screened; NPV, negative predictive value; SIR, standardized incidence ratio.**

Most current article

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tenofovir.[9] The benefit of these treatments is a significant histological improvement, resulting in the reduction of fibrosis progression or even a reversion of cirrhosis.[10]

This progress, the effectiveness of surveillance in patients with chronic liver diseases, remains a subject of debate.[1–4] The American Association for the Study of Liver Diseases only recommends surveillance every 6 months in HBV carriers with cirrhosis (called here the "standard surveillance") and in Asian male hepatitis B carriers over the age of 40 years and in female over age the age of 50 years, using ultrasound with or without serum alpha-fetoprotein (AFP) assessment.[2] The European Association for the Study of the Liver recommends the same surveillance extended to precirrhotic stages (F3).[3] The criticisms of this surveillance protocol include a small net benefit with increased harms from false positive results.[1–4] Recently, several HCC risk scores have been developed and validated to stratify the risk of the development of HCC, that do not use viral factors or ethnicity, such as PAGE-B in HBV.[3,11–18]

To increase the sensitivity without decreasing the specificity of surveillance, we recently constructed and internally validated LCR1-LCR2 in patients from the Groupe-Hospitalier-Pitié-Salpêtrière in Paris, France (Assistance Publique Hôpitaux de Paris, FibroFrance-GHPS cohort), called the "Original-Study".[12] LCR1 combined proteins involved in liver cell repair (apolipoprotein A1 and haptoglobin), known risk factors (gender, age, gammaglutamyltranspeptidase), and a marker of fibrosis (alpha2-macroglobulin), with a very high negative predictive value (NPV) for the occurrence of HCC.[12] The LCR1-LCR2 algorithm, which included AFP, had a 99.5% (99.0–99.7) NPV at 5 years in patients with mixed causes of liver disease. LCR1-LCR2 has also been externally validated in a case-control study of 149 prospectively enrolled patients in the Bondy cohort[19] and in 4903 patients with HCV from the national French cohort Hepather.[20]

The aim of the present study was to externally validate LCR1-LCR2 with the cutoffs identified in the Original-Study, in patients with HBV, whatever the stage of fibrosis at inclusion, by a longitudinal analysis of patients prospectively included in the large Hepather cohort.[9,20]

As in patients with HCV,[20] the present study confirmed the performance of LCR1-LCR2 in patients with HBV in the multiethnic cohort Hepather. The utility of LCR1-LCR2 is its ability to identify early, patients at a very low risk of HCC at 5 years.

Patients and Methods

Study Design and Participants

The ANRS CO22 Hepather cohort is a French national, multicenter, prospective, observational cohort study of patients with active or inactive hepatitis B virus or past or present hepatitis C infection, which started in August 2012.[9,20] This study is registered with ClinicalTrials.gov, number NCT01953458. Between 1 August 2012 and January 1, 2016, 14,389 HCV-positive patients and 6249 HBV-infected patients were enrolled to be followed up for a median of 6 years. Detailed demographics and clinical (including fibrosis staging and a history of the past treatment) and biological data were collected during the inclusion visit on an electronic case report form. HDV and HCV–coinfected patients (n = 90) were excluded. The main analysis excluded patients with a past history of HCC, decompensated cirrhosis, or liver transplantation. Follow-up included systematic visits (once a year) and spontaneous reports for particular events on specific data forms (eg, deaths, HCC, decompensated cirrhosis, and the onset of therapy). The study was observational, and the choice of the antiviral regimen, treatment timing, and screening for HCC or the progression of fibrosis was left up to the physician, but followed national French recommendations based on European Association for the Study of the Liver (EASL) guidelines.[3] Virological response was defined as serum HBV DNA <2000 IU/mL.[7,9]

In the current post hoc analysis, we selected all patients with chronic HBV infection at entry and with a reliable measurement of fibrosis using the FibroTest (BioPredictive, Paris, France), a validated routine biomarker of the stages of fibrosis, before inclusion. It was possible to assess the LCR1 value from the FibroTest components, which identified patients without cirrhosis but with a high risk of HCC, defined by the predetermined cutoff ≥ 0.0154. All of these at-risk patients were included, despite their absence of cirrhosis, as well as the patients with cirrhosis defined by the FibroTest >0.74, who were already known to be at risk of HCC,[1] were included if AFP was also available to assess LCR2.[12] To perform and preserve the independence of an external validation, the patients of the Hepather cohort followed at the Pitié-Salpêtrière expert center, where LCR1-LCR2 was constructed and internally validated, were not included. This is an ambispective study[21] because patients were included prospectively, but if patients were missing components of LCR1-LCR2, those data were assessed retrospectively, either when a frozen serum sample was available at inclusion or using the components of a previously performed routine FibroTest.

Procedures

Blood and urine samples were obtained and stored in a centralized biobank (Cell & Co Biorepository, Pont du Château, France). Detailed demographic, clinical, and biological data were gathered during the inclusion visit using an electronic case-report form. Follow-up included systematic visits once a year and spontaneous reports for particular events, which were recorded on specific data forms.[7]

This study is observational, and decisions about treatment combination, treatment timing, and screening for progression of fibrosis were made by the clinician, but the choices were made according to French national recommendations, based on EASL guidelines.[3]

Fibrosis stages were assessed by the FibroTest (F0 to F4) and activity grades (A0 to A3), by the ActiTest according to the manufacturer's instructions using the equivalence with the histological METAVIR scoring system, standard validated cutoffs, and reliability criteria.[22–24] The FibroTest is approved by European guidelines, by American Association for the Study of Liver Diseases, and by World Health Organization for the diagnosis of the fibrosis stage in HBV.[3,7,24,25] Several of the expert centers routinely performed these tests. For the other patients, the

FibroTest was measured on the available centralized biobank, independently of patients' characteristics. Components of the FibroTest and LCR1-LCR2 were measured according to the manufacturer's instructions as of 1997 in expert centers[26] and 2002 in French private laboratories.[23]

Outcomes

The co-primary study outcome was the NPV of LCR1-LCR2 for the occurrence of HCC and survival without HCC at 5 years according to the predetermined LCR1-LCR2 cutoff, adjusted for HCC risk covariables and for the response to HBV treatment and quantified using time-dependent Cox proportional hazards models. This core analysis used the algorithm assuming that only patients with cirrhosis (FibroTest >0.74) and patients without cirrhosis (FibroTest ≤ 0.74) and a high risk of LCR1-LCR2 would need surveillance. The binary result of the LCR1-LCR2 algorithm, the risk of HCC, is qualified as "low" or "high". Data for incident HCC included the number of tumors at diagnosis, the largest nodule size, total size, and diagnostic imaging procedures.

The secondary outcome was the prognostic performance of LCR1-LCR2, using the same endpoints but adding patients with severe fibrosis (METAVIR stage F3) defined as numerous septa.[22] This analysis used the algorithm assuming that patients with cirrhosis or severe fibrosis (FibroTest >0.58) and high LCR1-LCR2 would need surveillance. This strategy for the surveillance including both stages F3-F4 is recommended by the EASL association,[3] by the American Gastroenterological Association in patients before and after DAA with a sustained virological response in HCV,[2] as well as in other published reviews.[1] LCR1-LCR2 does not take into account the stratification by F4 or F3 stages, and we anticipated that one interest could be a decrease in the need for assessment of AFP. Indeed, a subject with low LCR1 does not need to enter in the second step of the algorithm (LCR2 with AFP) owing to the high NPV of LCR1.[20]

Eight post hoc analyses of the performance were performed. First, LCR1-LCR2 was compared to standard surveillance (the reference) in patients with cirrhosis only and using AFP at the standard 20 ng/mL cutoff.[2] Second, we assessed the performance of LCR1-LCR2 in patients aged 50 years or older, which could improve the cost-effectiveness, as suggested in the Original-Study[12] and by other studies in treated patients.[9,27] These performances were then compared to the standard surveillance in the same age subset. Fourth, we assessed the NPV sustainability at 10 years and at 15 years as well as in patients with ≥ 90 days of follow-up. We also compared the risk of HCC according to LCR1-LCR2 to the risk of HCC in the general population obtained from national cancer registries, with standardization for age and gender.[28–30] In the seventh analysis, we compared in the same patients the performance of LCR1-LCR2 with those of PAGE-B and FIB-4 using the previously determined cutoffs from previous studies.[4,5,17,18]

In the eighth and final analysis, we combined the present external validation database with the updated Original-Study,[12] to check the NPV of LCR1-LCR2 in the pooled sample of patients with HBV.

Statistical Analysis

A post hoc calculation was based on the Original-Study of LCR1-LCR2 in which 32 HCCs occurred among 2031 cases in the HBV population without contemporaneous HCC.[12] One hundred events were considered to be an appropriate sample size for external validation of a multivariate prognostic model.[31] These results suggest that in the same context of use in expert centers in France, 6000 patients with HBV were necessary for external validation, which is the sample size of the Hepather cohort with and without HBV treatment.

Survival time was calculated as the time between the first assessment of LCR1-LCR2 and the date of the primary outcome, or the last follow-up visit, or the date of death, or 3 August 2021, whichever occurred first. HCCs occurring after less than 1 year of follow-up were excluded from the primary analysis.

We used a multivariate Cox proportional hazards model with exposure to HBV treatment modeled as a time-varying covariate in the previous analysis focusing on a comparison between HBV treatment and the patients with HCV in the same national cohort.[9,32] This analysis was adjusted for the baseline values of all predictor variables previously identified as associated with the occurrence of HCC: age, gender, geographical origin (European vs other), HBV DNA, HBV HBeAg positive status, current excessive alcohol consumption, past excessive alcohol use, arterial hypertension, body mass index, diabetes, and response to HBV treatment. The logistic regression model included age, gender, ActiTest, time since HBV diagnosis, time since the first treatment, and HBV treatment naïve at the inclusion assessed between groups in the weighted sample. The inverse probability of treatment weighting was used. Stabilized weights were calculated and the balance of baseline covariates was assessed between groups in the weighted sample.

Categorization of continuous covariates was based on clinically relevant thresholds determined a priori (all biological variables) or quartile limits (age, time since HBV diagnosis). To prevent an incorporation bias, the FibroTest, which defined the stages of fibrosis, was not included among the HCC risk covariables. All analyses were performed in duplicate, and the final decision was made by 2 authors (F.C. and S.P.), independently of the LCR1-LCR2 inventor team. LCR1-LCR2 values were assessed at the first available date. Other characteristics were measured at inclusion in the Hepather cohort.[9,32]

Here, the goal was to validate a predictive test for the incidence of HCC, and therefore, only HCC occurring after 1 year after the LCR1-LCR2 assessment was considered to be an incident HCC in the Original-Study.[12]

For the post hoc analyses, the same methods were used as those for the main endpoints. We did not perform cost-effectiveness analyses but assessed the number of patients needed to be screened (NNS) to identify one HCC. The standardized incidence ratio (SIR)[30] was estimated to determine whether the risk of HCC in patients with low LCR1-LCR2 was similar to the risk in the general population.[29] SIRs were compared with the chi^2 test.

Baseline characteristics were compared using the Mann-Whitney test for quantitative variables or Fisher's exact test for categorical variables. Kaplan-Meier curves were drawn to compare the survivals without HCC. Incidence and 95% confidence intervals (CIs) were estimated with an exact method based on the Poisson distribution and NNS by the Z test. For the pooled analysis of the updated Original-Study cohort which started in 1997, the sustained viral response was not limited to direct acting antiviral treatments. Analyses were performed in duplicate, blind to LCR1-LCR2 values, with SAS,

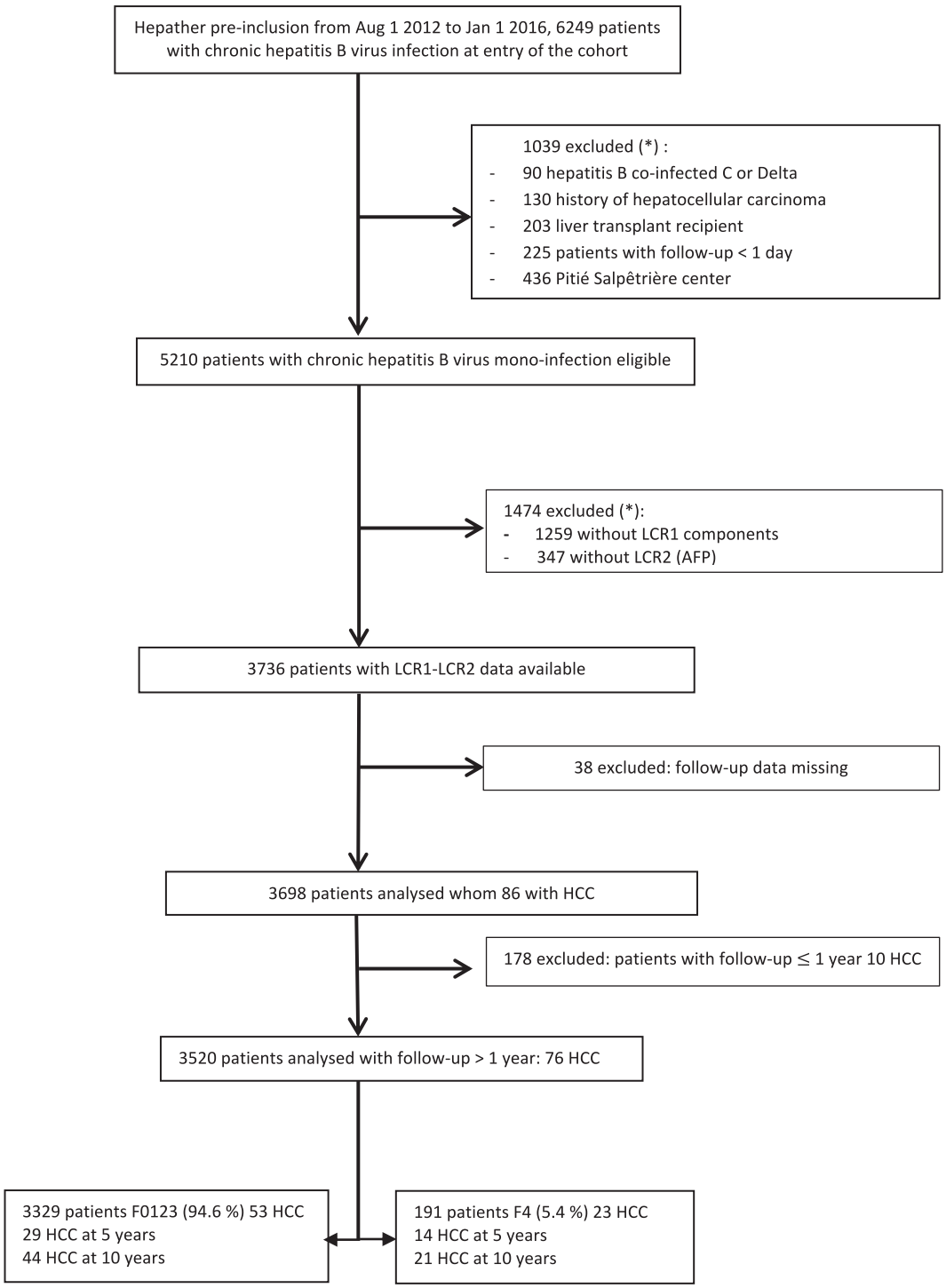


Figure 1. Flow of participants through the study. Patients could be excluded for several reasons.

version 9.4. NCSS-12.0 and R softwares, including timeROC library.

Role of the Funding Source

The funder contributed to the study design and the writing of the report. The funder had no role in data collection. Data analysis or data interpretation was performed in duplicate both by the founders and nonfounders. The corresponding author had full access to all data in the study, and FC and SP had made the final decision to submit for publication. All authors had access to the study data and reviewed and approved the final manuscript.

High Similarity Rates and Repeated Validations of the LCR1-LCR2 Algorithm

Three publications of internal and external validations of the LCR1-LCR2 algorithm, and 2 detailed publications of the patients

Table 1. Characteristics in Hepather Patients Included in the External Validation of the LCR1-LCR2 Algorithm

Characteristics	Hepather cohort included	Missing data
Number of patients	3520	0
HCC	76	0
LCR1-LCR2 algorithm		
Low risk (%)	3367 (95.6%)	0
High risk	153 (4.4%)	0
Follow-up time (y), median [IQR]	6.0 [4.8–7.3]	0
Age at inclusion	44.0 [34.8–55.4]	0
Age at FibroTest time	43.0 [34.0–54.2]	0
Men	2221 (63.1%)	0
Body mass index (kg/m^2)	24.9 [22.3–27.8]	23
Smoker		
At inclusion	631 (17.9%)	3
In the past	1301 (37.0%)	3
Geographical origin		26
France or Eastern Europe	1100 (31.5%)	
Asia	573 (16.4%)	
North Africa	398 (11.4%)	
Sub-Saharan	1160 (33.2%)	
Other	263 (7.5%)	
Past excessive alcohol use	336 (9.6%)	0
Time since HBV infection (y)	37.2 [28.9–46.5]	1608
HBV-DNA (log10 UI/mL) median	2.8 [1.9–3.5]	1859
HBeAg		460
Negative	2662 (87.0%)	
Positive	398 (13.0%)	
Fibrosis at inclusion using Hepather criteria		756
F0	205 (7.4%)	
F1	1759 (63.6%)	
F2	336 (12.6%)	
F3	194 (7.0%)	
F4	260 (9.4%)	
Fibrosis at the first FibroTest assessment	0.24 (0.12–0.44)	2
F0 ($\leq$0.21)	1579 (44.9%)	
F1 ($>$0.21)	1182 (33.6%)	
F2 ($>$0.48)	279 (7.9%)	
F3 ($>$0.58)	289 (8.2%)	
F4 ($>$0.74)	191 (5.4%)	
Activity at the first FibroTest assessment		321
A0 ($\leq$0.21)	2582 (81.0%)	
A1 ($>$0.21)	402 (12.6%)	
A2 (0.48)	61 (1.9%)	
A3 (0.58)	144 (4.5%)	
ALT (UI/L) at inclusion	28.0 [20.0–39.0]	93
Steatosis at the first SteatoTest2	0.26 (0.17–0.40)	717
S0 $<$5% ($\leq$0.40)	2121 (75.7%)	
S1 $<$33%($<0 = 0.56$)	401 (14.3%)	
S2 $<$66% ($\leq$0.62)	105 (3.8%)	
S3 $\geq$66% ($>$0.62)	178 (6.2%)	
Type-2 diabetes	251 (17.1%)	0
Arterial hypertension	630 (17.9%)	1
AFP class (ng/mL)	2.3 [1.7–3.6]	0
$<$6	3155 (89.6%)	
6 to $<$10	217 (6.2%)	
10 to $<$20	96 (2.7%)	
20 to $<$120	49 (1.4%)	
$>$120	3 (0.1%)	
HBV treatment		
Naive at inclusion	1695 (48.2%)	

IQR, interquartile range.

Table 2. Standardized Ratio Incidence for the LCR1-LCR2 Algorithm and Standard Surveillance (Cirrhosis Only) and According to Surveillance Option and HCC Incidence

Surveillance option	Cases	Incidence HCC	
		HCC	Standardized ratio incidence (SIR)
5-y follow-up, 1-y HCC exclusion	n	n	Low risk/high risk 95% CI
Primary outcome 5-y LCR1-2	3520	43	12.08 [7.81–17.83]/42.86 [25.39–67.74]
Secondary outcome F3F4 only	480	29	25.0 [13.30–42.75]/48.48 [27.70–78.74]
Post hoc analyses			
Standard, cirrhosis only	191	14	22.26 [4.48–65.07]/52.0 [25.92–93.04]
50 y or older, LCR1-2	1181	38	10.36 [6.33–16.01]/43.90 [26.01–69.39]
50 y or older, cirrhosis only	131	13	16.15 [1.81–58.31]/53.0 [26.41–94.80]
Other follow-ups LCR1-2			
10-y follow-up,	3520	65	10.49 [7.27–14.66]/54.0 [36.71–76.70]
Maximum follow-up,	3520	76	11.60 [8.33–15.76]/59.75 [41.62–83.11]
5-y follow-up, 90-d HCC exclusion	3616	50	12.33 [8.12–17.94]/55.0 [34.83–82.47]

and methods of the Hepather cohort, were previously published (Supplementary Material File A2).[12,19,20] Therefore, there were high similarity rates between the present publication and the 5 other publications, which is not a plagiarism but the consequence of the recommended methodology for the validation of diagnostic or prognostic tests.

Results

Flow of Participants in the Study

Between August 1, 2012, and January 1, 2016, 6249 patients with HBV virus infection at entry had been recruited to the ANRSCO22 Hepather cohort. A total of 5210 patients with monoinfection were eligible for the core study on HCC risk factors, and 3520 patients remained for the LCR1-LCR2 external validation with a follow-up of more than 1 year (Figure 1). The characteristics of included patients are presented in Table 1. A total of 1690 patients were not included in the present diagnostic study, mostly due to missing LCR1 components, n = 1259, or missing AFP, n = 347. Compared to not included patients, excluded patients had more risk factors associated with HCC, in particular, age, cirrhosis stage, HBeAg status, past excessive alcohol use, type-2 diabetes, and arterial hypertension, but the geographical origins were similar (Table A1).

Primary Outcomes

A total of 3520 patients, 191 (5.4%) with baseline cirrhosis, were included in the study. Twenty-three percent of patients were treated with virological response for a median of 6 (interquartile range = 4.8–7.3) years. A total of 43 HCCs occurred at 5 years and 76 at the end of follow-up (Table 2).

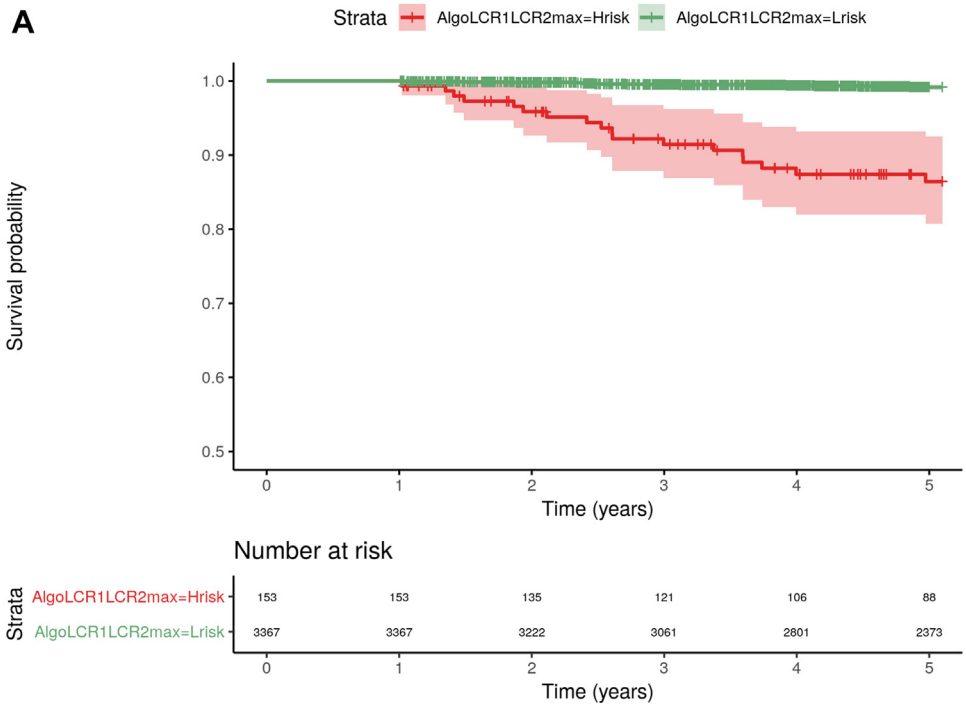
Among the 3367 patients with low-risk LCR1-LCR2, only 25 HCCs occurred at 5 years for a NPV of 99.3% (95% CI = 99.0–99.6) vs 18 out of 153 with high-risk LCR1-LCR2, for a lower survival without HCC of 86.4% (80.6–92.3; $P < .001$) (Figure 2A). The diagnostic performances were presented in Table 3. The false negative rate was 58.1% (25/43; 95% CI = 43.3–72.9). The NNS was 8.5 (Figure 2B).

The multivariable LCR1-LCR2 Cox hazard ratio, the primary endpoint, was still highly significant 6.4 (95% CI = 3.1–13.0; $P < .001$) after adjustment for exposure to antivirals, age, gender, geographical origin, HBeAg status, alcohol consumption, type-2 diabetes, and arterial hypertension. Without adjustment, the univariate hazard ratio for high- vs low-risk LCR1-LCR2 was slightly higher, 17.8 (95% CI = 9.7–32.7; $P < .001$) (Table 4).

The characteristics of incident HCCs according to low- or high-risk LCR1-LCR2 cutoffs are reported in Tables A2 and A3. Most incident HCCs were potentially curable; all were smaller than 30 mm, with nodular macroscopic patterns, 91.7% in patients with low LCR1-LCR2 vs 88.6% in those with high LCR1-LCR2. The 25 patients with HCC and low LCR1-LCR2 had a higher prevalence of F1 and F2 stages, 88% males, 21% HBeAg status, 16% T2 diabetes, with more AFP between 6 and 20 ng/mL than patients without HCC and low LCR1-LCR2 (Table A2). The other comparisons of patient characteristics between low- and high-risk populations are presented in Table A3. The comparison of patients' characteristics between the low- and high-risk populations according to the LCR1-LCR2 algorithm is presented in Table A4. There was no significant difference in the balance of baseline covariates according to low- and high-LCR1-LCR2 groups (Table A5).

Secondary Outcomes

A total of 480 patients out of 3520 (16.4%) had severe fibrosis or cirrhosis (F3–F4) (Table 1). Twenty-nine HCCs occurred among F3F4 at 5 years (Figure 3), vs 112 among patients with cirrhosis (Figure 2B). In comparison to the "cirrhosis-only" option, an assessment of LCR2 in F3 and cirrhosis decreased the needed number of LCR2 by 32.0%, from 2702 with cirrhosis only as the first step, to 1043 for F3F4.



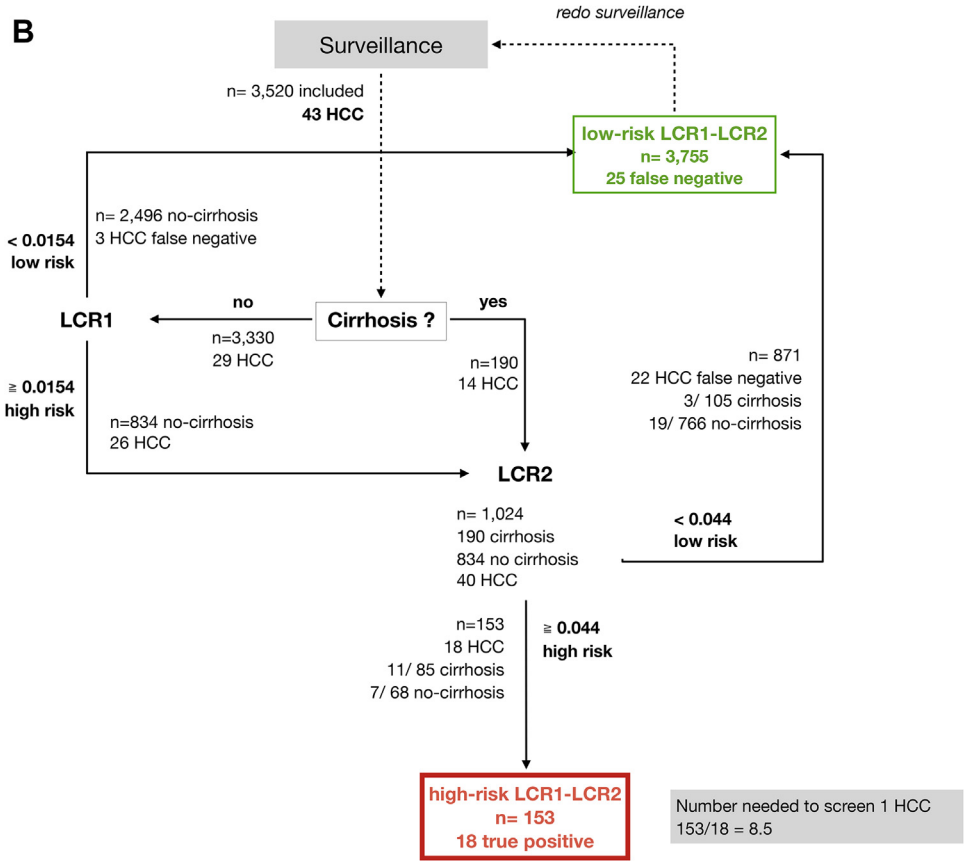


Figure 2. Survival without HCC according to LCR1-LCR2 cut-offs. Main outcome. (A) Survival without HCC. (B) Number of patients needed to screen one HCC.

Table 3. Standard Predictive Values, Sensitivity, and Specificity of the LCR1-LCR2 Algorithm According to the Surveillance Option

	LCR1-LCR2 and standard surveillance performances			
	Negative predictive value	Positive predictive value	Sensitivity	Specificity
Follow-up	%	%	%	%
Primary outcome 5-y LCR1-2	99.3 (99.0–99.6)	11.8 (10.7–12.9)	41.9 (40.3–43.5)	96.1 (95.5–96.7)
Secondary outcome F3F4 only	99.3 (99.0–99.6)	11.8 (10.7–12.9)	41.9 (40.3–43.5)	96.1 (95.5–96.7)
Post hoc analyses				
Standard, cirrhosis only	98.9 (98.5-99.2)	30.0 (6.7-65.3)	7.0 (1.5-19.1)	99.8 (99.6-99.9)
50 y or older, LCR1-2	98.1 (97.3–98.9)	23.2 (16.4-31.1)	52.5 (39.3-60.7)	90.5 (88.7-92.2)
50 y or older, cirrhosis only	96.3 (95.0-97.4)	14.7 (8.6–20.8)	36.1 (24.2-49.4)	90.3 (88.4-91.9)
10-y follow-up, LCR1-2	99.0 (98.7–99.3)	20.3 (19.0–21.6)	47.7 (46.1–49.4)	96.5 (95.9–97.1)
Maximum follow-up, LCR1-2	98.8 (98.4–99.2)	22.9 (21.5–24.3)	46.1 (44.5–47.8)	96.6 (96.0–97.2)
5-y HCC exclusion 90-d LCR1-2	99.2 (98.9–99.5)	14.1 (13.0–15.2)	46.0 (44.4–47.6)	96.1 (95.5–96.7)

Eight Post Hoc Analyses

#1. For standard surveillance, focusing on cirrhosis only (Figure 4A) and compared to LCR1-LCR2 (Figure 2B), the sensitivity was only 7% (3/43 95% CI = 1.5-19.1), 6 times lower than using LCR1-LCR2 41.9% (95% CI 40.3-43.5) (Table 3), the only benefit being a reduced NNS 3.3 vs 8.5, respectively (Figures 3 and 4A).

#2. For LCR1-LCR2 in patients 50 years or older and compared to the overall population (Figure 2B), there was no significant difference in the false negative rate 13.6% (16/118; 95% CI = 67.9–62.8) or in the NNS (10.0) (Figure 4B and Table 3)

#3. Standard surveillance in patients 50 years or older (Figure 4C): For LCR1-LCR2 in patients 50 years or older (Figure 4B and Table 3) and compared to standard surveillance in the same age subset (Figure 4C and Table 3), the false negative rate was significantly lower and the lower NNS persisted.

#4. NPV sustainability at 10 years and at 15 years: In the 3367 patients with low-risk LCR1-LCR2, survival without HCC observed at 5 years on univariate analysis (99.4% [95% CI = 99.1–99.6]) (Figure 2A) persisted at 10 years (98.2% [97.4–99.0]) and at 15 years (95.4% [92.9–97.9]) (Figure A1 and Table 3).

Table 4. Factors Associated With Survival Without HCC: Univariate and Multivariate Analyses

	Time-dependent hazard ratio (HR)					
	Univariate			Multivariate		
Characteristics	HR	95% CI	*P*-value	HR	95% CI	*P*-value
LCR1-LCR2 algorithm (high vs low risk)	17.83	9.73–32.69	<.0001	**6.40**	**3.14–13.02**	**<.001**
Gender (men vs women)	3.63	1.53–8.59	.0034	1.81	0.72–4.55	.2045
Age (y) at FT time	1.09	1.06–1.12	<.0001			
≤50 (reference)	1			1		
>50	14.99	5.90–38.08	<.0001	9.86	3.25–29.90	<.001
Geographical origin (European vs other)	3.32	1.80–6.12	.0006	1.07	0.54–2.11	.8518
Past excessive alcohol use (yes vs no)	4.22	2.20–8.08	<.0001	1.80	0.88–3.68	.1107
Ever smoked (yes vs no)	3.20	1.71–5.99	.0003	1.62	0.80–3.28	.1761
Treatment-naive vs treated	0.30	0.15–0.60	.0006	1.37	0.57–3.29	.4789
HBV-DNA						
Log HBV-DNA ≤3	1			1		
Log HBV-DNA >3	2.19	0.59–8.08	.2407	0.54	0.14–2.09	.3745
HBeAg status (positive vs negative)	1.19	0.46–3.08	.7203	1.41	0.53–3.78	.4927
Diabetes (yes vs no)	1.35	0.48–3.79	.5635	0.42	0.12–1.40	.1571
Arterial hypertension (yes vs no)	2.44	1.31–4.58	.0052	0.85	0.42–1.71	.6429
ActiTest (A2A3 vs A0A1)	1.90	0.75–4.84	.1777	0.52	0.18–1.49	.2238
Response to HBV treatment						
Nontreated	1			1		
Treated	3.92	1.40–10.96	.0094	3.38	0.81–14.10	.0945

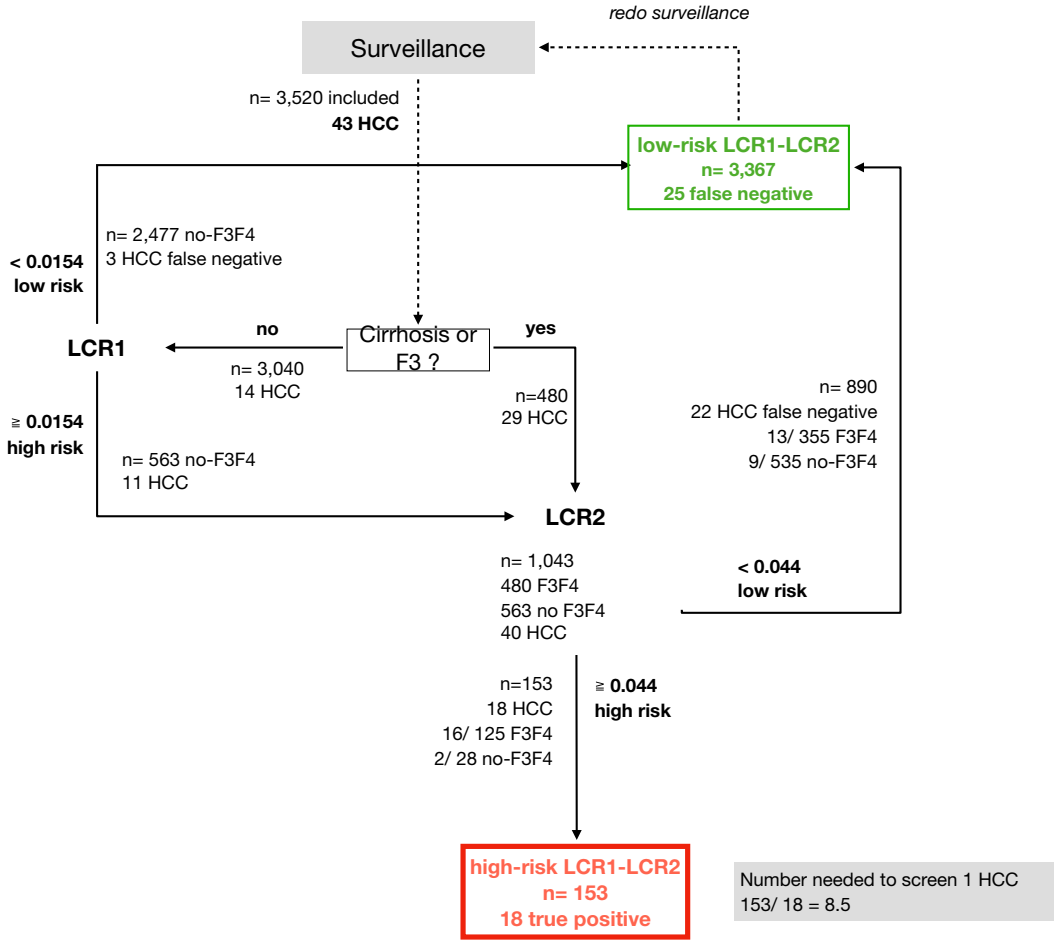


Figure 3. Survival without HCC in patients with surveillance of both severe fibrosis (F3) and cirrhosis (F4). Secondary outcome. Relative number of LCR1 and LCR2 assessments and number of patients needed to screen one HCC.

#5. LCR1-LCR2 in patients with ≥90 days of follow-up: The performance of LCR1-LCR2 at 5 years was similar when patients with less than 90 days of follow-up were excluded. Only 30 HCCs occurred at 5 years in the 3784 patients with low LCR1-LCR2, vs 151 out of 1194 with high LCR1-LCR2, for a NPV of 99.2% (95% CI = 98.9–99.5). The positive predictive value was 12.6% (95% CI = 10.8–14.7). The diagnostic performances are presented in Table 3.

#6. Comparison of the HCC standardized risk ratio in the low-LCR1-LCR2 subset vs the risk observed in the general population: The incidence of HCC standardized by gender and age in the patients with low LCR1-LCR2 was still significantly higher, SIR = 12.1 (95% CI = 7.8–17.8; $P <$ .001), than the incidence in the French general population. The SIR of patients with a high-risk LCR1-LCR2 was still 3.5 times higher, 42.9 (95% CI = 25.4–67.7), than that in patients with a low LCR1-LCR2 (Table A6).

#7. Direct comparisons of surveillance using PAGE-B, FIB4, FibroTest, or LCR1-LCR2: A total of 1518 patients had PAGE-B assessed less than 60 days from/after LCR1-LCR2, and 1514 had an assessment of FIB-4. LCR1-LCR2 (Figure A2A) outperformed the other risk markers with an NNS = 4.8 vs 26.3, 20.3, and 7.8 for PAGE-B (Figure A2B), FIB4 (Figure A2C), and cirrhosis by the FibroTest (Figure A2D), respectively (Tables A7 and A8). The NNS was significantly lower with a stratification based on the risk of LCR1-LCR2 than stratification according to the stage of cirrhosis using the FibroTest (P = .03) (Table A9).

#8. Updated results of the original GHPS cohort and pooled analysis: A subset of 1215 patients with hepatitis B included in the Original-Study[12] was updated in October 2020. The participants' characteristics are presented in Table A10. Three HCCs occurred at 5 years in 1156 patients with a low LCR1-LCR, vs 12 in 59 patients with a high LCR1-LCR2, or 99.6% (99.1–1.00) vs 93.7% (90.0–97.3; $P <$ .001) survival without HCC, respectively, Table A11 and Figure A3A. These results were similar to those of the present external validation.

A total of 4735 pooled patients were analyzed. The characteristics of these patients are presented in Table A12. Thirteen HCCs occurred at 5 years in 212 patients with a low LCR1-LCR, vs 28 in 4523 patients with a high LCR1-

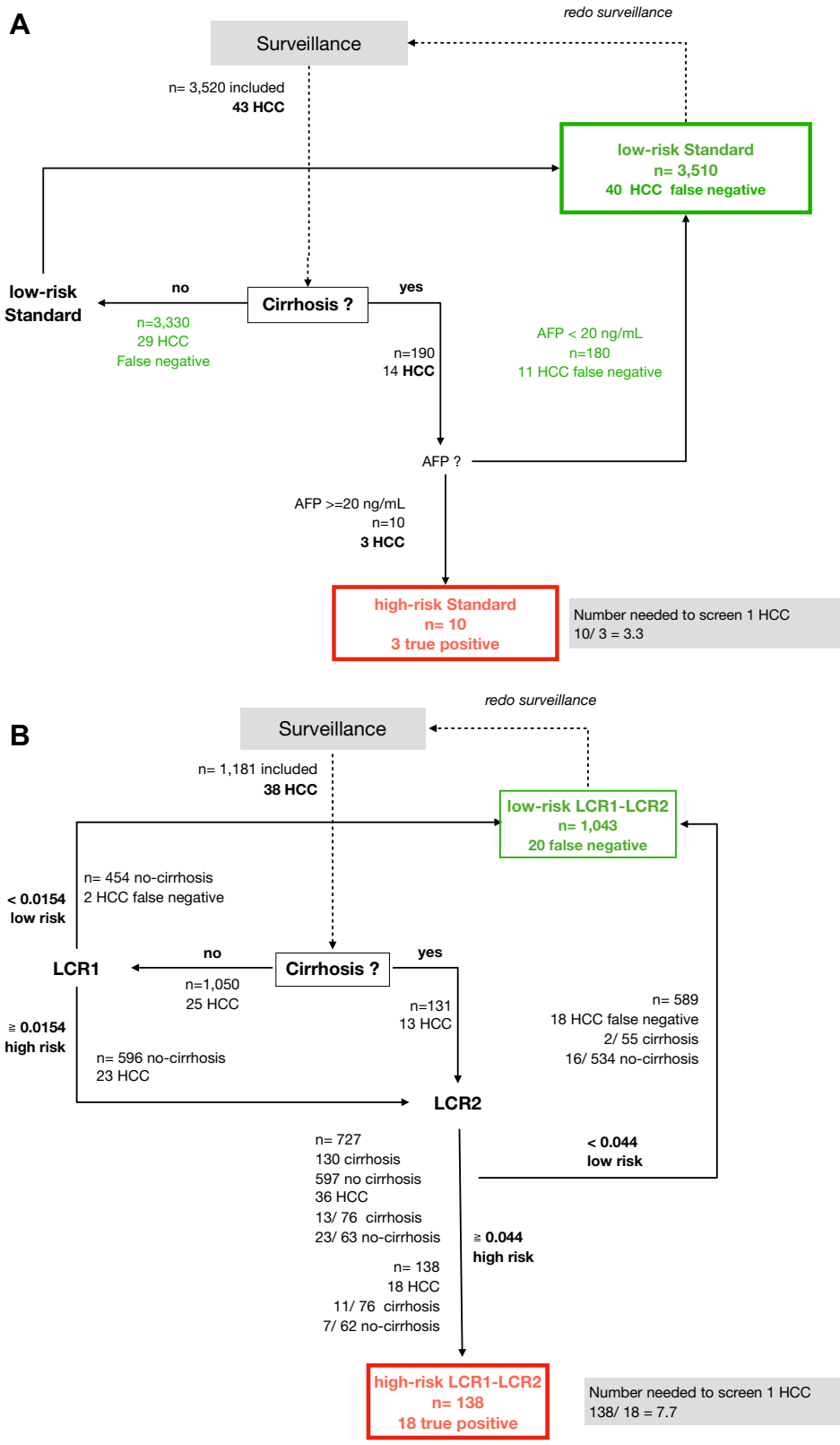


Figure 4. Standard surveillance and LCR1-LCR2 post hoc analyses. (A) Standard surveillance in cirrhosis only. NNS was reduced and false negatives were increased compared to LCR1-LCR2. (B) LCR1-LCR2 in patients 50 years or older. Number of patients needed to screen one HCC. There was no significant difference in the NNS and in the false negative rate compared to LCR1-LCR2. (C) Standard surveillance in patients 50 years or older. Number of patients needed to screen one HCC. NNS was reduced and false negatives were increased compared to LCR1-LCR2 in the same age subset.

LCR2 ($P < .001$). Survival without HCC in low-risk LCR1-LCR2 was 99.3% (99.1–99.6) at 5 years and 84.0% (87.5–89.3) at 15 years (Figure A3B).

The STROBE and STARD check lists are presented in Tables A13 and A14, respectively.

Discussion

This study provided an external validation of the prognostic performance of the LCR1-LCR2 algorithm, which included AFP, in patients with chronic hepatitis B (CHB) and

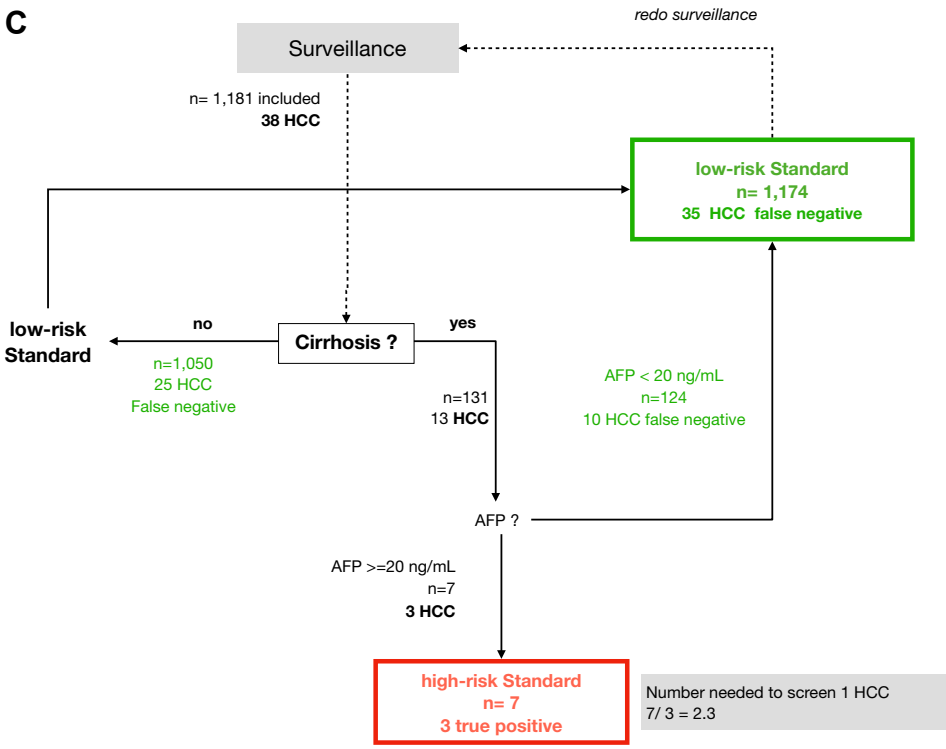


Figure 4. (*continued*).

showed that the NPV of LCR1-LCR2 was 99.3% (99.0–99.6) at 5 years. These results confirmed the performance of LCR1-LCR2 that was observed in 9892 patients with mixed causes of liver disease[12] and externally validated in 149 prospective patients in the Bondy Cohort[19] and in 4903 patients with HCV for the national French cohort Hepather.[20] Our study had several limitations and strengths compared to other HCC risk scoring systems.

HCC Risk Scoring Systems

Numerous HCC risk scoring systems have been developed for CHB patients who are undergoing long-term NUC treatment.[17,32–39] These new scores are specifically for patients receiving direct acting antivirals. Most of these systems were developed in Asian populations and include patients with and without cirrhosis. The PAGE-B score was first developed in a European cohort.[18] Patients are divided into 3 HCC classes according to platelets, age, and gender. Although the sensitivity is high with a cutoff of 10 (100%), the specificity is low (19.6%).[18] The EASL has endorsed the application of PAGE-B in patients with noncirrhotic HBV to trigger HCC surveillance.[3]

Recent studies on HCC risk stratification have used biomarkers with low performances for the diagnosis of fibrosis and necroinflammatory activity in patients with chronic viral hepatitis. Only 3 out of 15 studies used transient elastography.[17] The present study has the advantage of homogenous fibrosis staging, which is a major independent HCC risk factor, and the grading of activity because all included patients had a FibroTest and an ActiTest, 2 biomarkers that have been extensively validated in chronic viral hepatitis. While serum fibrosis scores such as FIB4 or aspartate aminotransferase to platelet ratio index are possible markers, their performances are significantly lower than those of the FibroTest both in chronic hepatitis C and CHB. In a direct comparison using the intention-to-diagnose approach, the FibroTest outperformed transient elastography, FIB4, and aspartate aminotransferase to platelet ratio index for the diagnosis of the fibrosis stage.[40] Our study also showed that LCR1-LCR2 had a significantly lower NNS than FIB4.

Limitations Due to the Small Number of Events

Despite the large prospective Hepather cohort, we did not reach the optimal power to assess the performance of LCR1-LCR2 at 5 years. The power of the study was relatively low for this external validation at 5 years with 43 HCC events, which is less than the recommended 100.[31]

In our study, 10 HCCs occurring in the year following inclusion were excluded, which is the more conservative method to exclude contemporaneous cancers. Furthermore, when the original study was merged with the validation study, a total of 106 HCCs occurred at the end of follow-up with a sustained proportional risk (Figure A3B).

We were able to confirm the consistency of the NPV after 10 years of follow-up with 65 HCCs and 400 patients still at risk despite treatment (Figure A1). Assessment of the relative risk and a high NPV is not enough to confirm the efficiency of this test as an updated screening tool. However, the present study provides external validation of LCR1-LCR2 for the first step of risk stratification, showing that 99.3% (99.0–99.6) of the population of interest could be reassured. The very high NPV of our results suggests that

for at least 5 years, patients with low LCR1-LCR2 do not require surveillance every 6 months by ultrasound with or without AFP.[2] At 10 years, the sustainability of the NPV was excellent, still 99.0% (98.7–99.3) (Table 3, Figure A1). At inclusion, the median HBV DNA is 2.8 log IU/mL; 48% were untreated, and 5.4% had cirrhosis. This cohort had a relatively low risk of HCC, and most of the patients did not need standard HCC surveillance. This suggests validating these results in patients who required viral treatment as they are having a higher risk of HCC as shown in Table 4.

Cost-Effectiveness

Specific studies must be performed to compare the cost-effectiveness of these biomarkers for stratifying the risk of HCC in patients with chronic viral hepatitis. However, the cost-effectiveness of this algorithm can be assessed in relation to the components of the FibroTest, which is already recommended for surveillance in patients with HBV, whatever the stage, and AFP, which is only required in 29% of patients (Figure 2B) together with ultrasound. Even if all patients are treated by antivirals, the stage of fibrosis must still be assessed.[3] Thus, a single blood test that assesses both the stage of fibrosis and the activity grade by the validated FibroTest-ActiTest (to define significant chronic hepatitis) as well as the risk of HCC would help both patients and clinicians. It could reduce the number of patients requiring repeated imaging. The decrease in the number of AFP and imaging tests, together with the components of the FibroTest, should reduce the cost of this algorithm. Our ambispective (both prospective and retrospective) analysis was useful to assess health outcomes with a long inclusion period and exposures.[21]

Several Other Limitations in the Original-Study Were Corrected

Compared to the Original-Study, this study focused on patients with HBV, with increased power in this subset, external validation in a large group, more HCC risk factors,[12] and using time-dependent multivariate analysis to take into account the impact of virological treatment on survival without HCC.[9] There was no significant difference in the balance of baseline covariates according to low- and high-LCR1-LCR2 groups. We acknowledge that the date of HBV infection was missing for 1608 patients, mostly related to patients born outside Europe, possibly due to familial transmission. Furthermore, we also applied the STROBE and STAR guidelines and assessed eight post hoc analyses including the performance at 10 years and a 90-day HCC exclusion to prevent an "immortal person-time" bias.[32]

LCR1-LCR2 Is Validated in Patients With Hepatitis B With or Without Cirrhosis

Despite the effectiveness of NUC, the incidence of HCC in patients with HBV with and without cirrhosis is a major issue.[1–5,7,9] The latest best practices state that "patients with severe liver fibrosis (F3) or cirrhosis (F4) at the time of treatment represent the highest-risk group for HCC after a treatment-induced sustained virological response. These patients should stay in HCC surveillance".[3] The present study validated LCR1-LCR2 in 2 screening steps in patients with and without cirrhosis.

Limitations Due to Excluded Patients

Multiethnic Population. Although our population included patients living in Europe, only 31.5% were of European origin, while 47.7% were of Sub-Saharan or Asian origin. Furthermore, the test performance was still significant after multivariate analyses including geographical origin. Thus, these results should be widely applicable.

A Robust Blood Test for Mixed Chronic Liver Diseases. In the results of the Original-Study, the NPVs of LCR1-LCR2 were similar in patients with alcoholic liver disease and non-alcoholic fatty liver disease and hepatitis B.[12] This is an advantage because patients with hepatitis B have a long period of possible exposure to these risks before and after sustained virological responder. This also supports generalizing the results of the present study.

A Sensitive Test Which Could be Updated With Other Specific Markers of HCC Than AFP. LCR1-LCR2 was constructed to be highly sensitive using the combined components and specific with the use of AFP. This notion could be extended to combinations with other specific HCC markers such as AFP-L3, decarboxy-prothrombin, or glycans.[22]

Other Results That Must Be Clarified. The incidence of HCC standardized by gender and age in patients with a low-risk LCR1-LCR2 is still significantly higher, SIR = 9.80 (6.27–14.58 < 0.001), than the incidence in the French general population. There is no clear explanation for this difference, including an underestimation of the incidence of HCC in the general population or an overestimation in patients with HBV. However, the SIR of patients with high-risk LCR1-LCR2 was still 6 times higher, 56.8 (46.8–68.3), than that in patients with low-risk LCR1-LCR2. One explanation could be the recent increase in the use of ultrasonography and AFP in patients with HBV. In the short term, screening increases the incidence and advances both the year and age of diagnosis, while in the long term, screening removes patients who were detected early from upcoming incidences.

Treatment Effect. A previous study of the present cohort selected and evaluated patients who had been receiving tenofovir or entecavir for a median of 2.7 years as well as 45% of these patients who had been receiving HBV treatment with nucleotide/nucleoside analogs for even longer at the start of follow-up.[9] Prior HBV treatment was strongly associated with the duration of viral suppression. This effect was controlled using the method accounting for left truncation in exposure adjustments or inverse probability of treatment weighting, thus limiting the risk of a selection bias. However, prior treatment could explain our

relatively low rates of HCC or other liver-related complications compared to studies focusing on incident HCC in tenofovir or entecavir users. While the geographical, clinical, and pathological profiles of the patients as well as the durations of follow-up were only slightly different for the nucleos(t)ide analogs in our real-life cohort, the distribution of these analogs and the follow-up durations in the Asian studies were imbalanced owing to the very early registration of these treatments.[9] It is important to note the low rate of HCC in our treated series (around 0.24/y) compared to other series, with figures ranging from 1.5% to 4.4%/y in nucleos(t)ide analog–treated compared to 5.2%–7.7% in untreated patients with cirrhosis. These differences are probably due to the lower rate of patients with cirrhosis (5.4%) or extensive fibrosis (8.2%) in our series and the different durations of exposure to nucleos(t)ide analogs. Viral suppression was associated with a constant decrease in the rate of complications, including HCC.[9] In addition, differences may be partially due to the different geographic origins of the patients. Asian patients are mainly infected at birth and African patients, during early childhood, while infection in Northern countries is mainly in teenagers and young adults. Thus, the risk of HCC is earlier in Africans or Asians than that in European patients.[9] In our study, the influence of geographical origin on the risk of HCC was significant on univariate analysis. On multivariate analysis, the only significant factors were the age >50 years and the LC1-LCR1 algorithm (Table 4). Because of the limited power of our study in relation to these factors, no firm conclusions can be drawn for nonsignificant factors.

Finally, we agree with others that stopping surveillance of low-risk groups is difficult and that intensifying screening programs in intermediate- or high-risk groups is a challenge that could improve compliance to surveillance recommendations.[3–5,11,17,25,26,28] In conclusion, LCR1-LCR2 is a robust blood test for use in the assessment of the risk of developing HCC in patients with HBV.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2022.02.008.

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T.P. is acting as the submission's guarantor.

Authors' Contributions:
Thierry Poynard contributed to experiment conception and design. Thierry Poynard, Valentina Peta, Victor de Ledinghen, Fabien Zoulim, Didier Samuel, Dominique Thabut, Hélène Fontaine, Philippe Mathurin, Vlad Ratziu, and Stanislas Pol contributed to experiment performance. Thierry Poynard, Olivier Deckmy, Jean Marc Lacombe, and Fabrice Carrat contributed to data analysis. Thierry Poynard, Chantal Housset, Jean Marc Lacombe, Stanislas Pol, and Fabrice Carrat contributed to drafting of the article. All authors approved the final version of the manuscript.

Conflicts of Interest:
These authors disclose the following: Thierry Poynard is the inventor of FibroTest, LCR1, and LCR2 and the founder of BioPredictive; the patents belong to the public organization Assistance Publique-Hôpitaux de Paris. Valentina Peta and Olivier Deckmyn are full-time employees of BioPredictive. The remaining authors disclose no conflicts.

T.P. is the inventor of LCR1-LCR2 and the founder of BioPredictive, the company that markets these tests. Patents belong to the French Public Organization Assistance Publique-Hôpitaux de Paris. T.P. plays a role in the study design, analysis, and preparation of the manuscript. T.P. and O.D. play also a role in the statistical analysis, but to preserve independence in this validation study, the statistical analysis was duplicated by independent academic authors F.C. and J.M.L. and supervised by F.C. O.D. and V.P. are BioPredictive employees and organized the anonymized assessments of the BioPredictive tests and did not play a role in the study design, data collection, decision to publish, or preparation of the manuscript and only provided financial support in the form of authors' salaries.

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Ethical Statement:
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
Data, analytic methods, and study materials will be made available to other researchers by contacting Fabrice Carrat (fabrice.carrat@iplesp.upmc.fr). Almost all details were already available in the supplementary files.