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MAY 3–6, 2025 | SAN DIEGO, CA

CLIMB: A MULTI-ANALYTE CFDNA-BASED BLOOD TEST FOR EARLY DETECTION OF HEPATOCELLULAR CARCINOMA

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PURPOSE / OBJECTIVES

Patients at high-risk for hepatocellular carcinoma (HCC) are recommended to undergo semi-annual abdominal ultrasound, which suffers from low sensitivity for small HCC nodules and poor adherence. To address these clinical needs, the multi-analyte HelioLiver Dx blood test was developed to aid in the detection of HCC for patients at high-risk for HCC due to liver cirrhosis.

The CLiMB trial (NCT03694600) was designed to compare the performance of the blood-based HelioLiver Dx test to ultrasound for the detection of HCC within a cirrhotic patient population.

TRIAL DESIGN

1,968 adult patients with liver cirrhosis were enrolled from 42 sites across the U.S. in a fully prospective, blinded, multicenter study, to compare the performance of the HelioLiver Dx test to ultrasound for HCC detection. Multiphasic MRI was used as the gold standard to determine patient HCC status.

- **Coprimary outcomes (Passed):** Superior sensitivity (> 5%) and non-inferior specificity (>-10%) of HelioLiver Dx compared to ultrasound
- **Secondary outcome (Passed):** HelioLiver Dx to possess superior sensitivity than ultrasound for detecting HCC lesions not more than 4 cm in diameter.

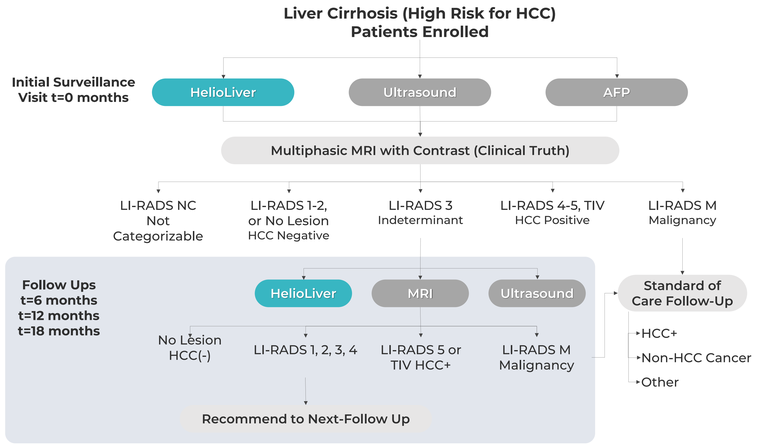

Figure 1. Study Design Schematic for Endpoints. Patients at high risk for HCC due to a diagnosis of liver cirrhosis were invited to participate. At the Initial Visit (t = 0), all patients provided blood samples for the HelioLiver blood test, ultrasound and the protein tumor marker alpha-fetoprotein (AFP). All participants also underwent multiphasic MRI with contract and the established Liver Imaging Reporting and Data System (LI-RADS) was used to determine if participants were positive or negative for HCC for study endpoints. Only participants with an indeterminant finding (LI-RADS 3) were sent to multiphasic MRI with contrast at a second, Follow Up Visit (t = 6 months) to resolve the participants HCC status. HCC, hepatocellular carcinoma, LI-RADS, Liver Imaging Reporting and Data System; NC, Not Characterizable, TIV, Tumor in vein.

TRIAL PARTICIPANTS

1,968 Participants

412 enrolled prior to June 20, 2020 assigned to Training Cohort

1,556 Assigned to Validation Cohort

271 not evaluable at Initial Visit (200 failed inclusion/exclusion criteria, 10 ultrasound not evaluable, 6 MRI not evaluable, 36 blood specimens not received / accepted, 19 HL Dx test not evaluable)

1,285 Evaluable at Initial Visit

46 HCC (42 at initial visit, 4 at follow-up) ← **45 HCC Indeterminant at Initial Visit*** → **1,222 non-HCC** (1,198 at initial visit, 24 at follow-up)

**17 lost to follow-up*

Figure 2. Disposition of Subjects. A total of 1,968 participants consented to the study. Data from the first 412 participants enrolled prior to June 20, 2020 were utilized as part of the training cohort for test development. Of the 1,285 evaluable participants, 46 (3.6%) were classified as having HCC.

Table 1. Characteristics of Evaluable Population.

Characteristics	HCC Positive (n = 46)	HCC Negative (n = 1,222)
Age (years), mean (SD)	64.6 (7.4)	59.4 (10.9)
Sex, n (%)		
Male	30 (65.2)	596 (48.8)
Female	16 (34.8)	626 (51.2)
Ethnicity, n (%)		
Hispanic	20 (43.5)	405 (33.1)
Non-Hispanic	26 (56.5)	814 (66.6)
Race, n (%)		
White	40 (87.0)	1039 (85.0)
Non-white	6 (13.0)	182 (15.0)
Obesity, n (%)	26 (56.5)	676 (55.2)
Study setting, n (%)		
Academic center (%)	8 (17.4)	243 (19.9)
Community-based center (%)	38 (82.6)	979 (80.1)
Liver disease etiology, n (%)		
NAFLD	23 (50.0)	626 (51.2)
ALD	10 (21.7)	262 (21.4)
Viral (HBV and/or HCV)	16 (34.8)	205 (16.8)
Other	3 (6.5)	204 (16.7)
Laboratory, median (IQR)		
AFP (ng/mL)	6.0 (4.0 - 14.7)	3.7 (2.5 – 5.8)
Size of largest HCC lesion, n (%)		
≤ 2 cm	21 (45.7)	
> 2 to ≤ 3 cm	13 (28.3)	
> 3 to ≤ 4 cm	3 (6.5)	
> 4 cm	9 (19.6)	

RESULTS

Table 2. Comparison of Test Performance

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV (%)	NPV (%)
HelioLiver Dx test alone	47.8 (32.9, 63.1)	87.6 (85.6, 89.4)	12.6	97.8
Ultrasound alone	28.3 (16.0, 43.5)	93.9 (92.5, 95.2)	14.9	97.2
Ultrasound + AFP (≥ 20 ng/mL)	34.8 (21.4, 50.3)	92.1 (90.5, 93.6)	14.3	97.4
Ultrasound + AFP (≥ 10 ng/mL)	39.1 (25.1, 54.6)	87.4 (85.4, 89.2)	10.5	97.4
AFP (≥ 20 ng/mL)	21.7 (11.0, 36.4)	98.0 (97.1, 98.7)	29.4	97.1
AFP (≥ 10 ng/mL)	32.6 (19.5, 48.0)	93.0 (91.5, 94.4)	15.0	97.3
HelioLiver Dx test and Ultrasound	52.2 (37.0, 67.1)	82.7 (80.4, 84.7)	10.2	97.9

PPV, Positive Predictive Value; NPV, Negative Predictive Value;; AFP, alfa-fetoprotein; HCC, hepatocellular carcinoma

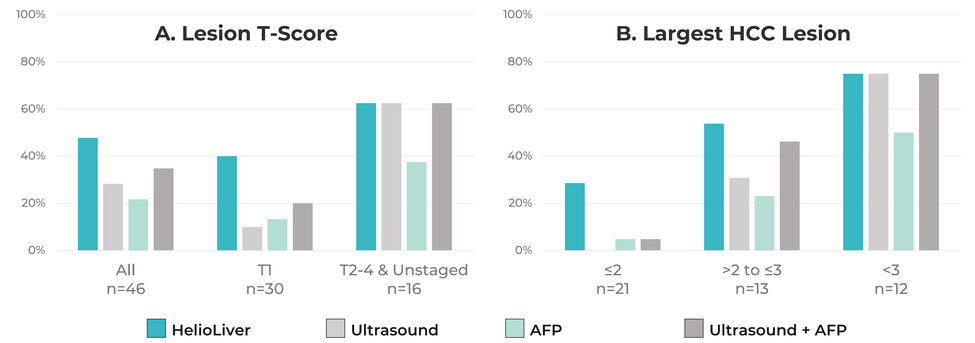

Figure 3. Test Sensitivities by HCC Lesion Characteristics. The sensitivities of the HelioLiver Dx test (blue), ultrasound alone (white), AFP at a cutoff of 20 ng/mL (green) and the combination of ultrasound and AFP at a cutoff of 20 ng/mL (grey) are by (A) T category of the TNM staging system, (B) largest HCC Lesion size as having HCC.

SUMMARY / CONCLUSION

- The HelioLiver Dx test met both the coprimary and secondary study endpoints. HelioLiver Dx detected more HCC lesions overall compared to ultrasound and was able to detect more early-stage and smaller HCC lesions in a cohort of patients with diverse etiologies of liver cirrhosis.
- HelioLiver Dx had a sensitivity of 47.8% (95%CI 32.9 to 63.1) for all HCC lesions compared to ultrasound, which had a sensitivity of 28.3% (95%CI 16.0 to 43.5).
- Similarly, for lesions 4 cm or smaller, HelioLiver Dx had a superior sensitivity at 37.8% (95%CI 22.5 to 55.2) compared to ultrasound at 13.5% (95%CI 4.5 to 28.8).
- For T1 lesions (59%), HelioLiver Dx's sensitivity was fourfold higher at 44.4% (95%CI 25.5 to 64.7) compared to11.1% (95%CI 2.4 to 29.2) for ultrasound. The overall specificity of HelioLiver Dx and ultrasound were 87.6%(95%CI 85.6 to 89.4) and 94.1% (95%CI 92.6 to 95.4), respectively.
- An accurate and convenient blood-based test may reduce HCC related mortality and lead to better patient outcomes, through consistent adherence, easier access, superior sensitivity, and improved early HCC detection.

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CELL-FREE DNA METHYLATION BIOMARKERS FOR EVALUATING PROGNOSIS AND TUMOR AGGRESSIVENESS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC)

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INTRODUCTION

Hepatocellular carcinoma (HCC), one of the leading causes of cancer-related mortality worldwide, is a heterogeneous, complex disease where patient assessment is often complicated by comorbidities and other clinical factors. Multiple index scores and staging systems have been developed to assess HCC severity and prognosis. However, reliable prognostic biomarkers for HCC are lacking.

AIM

In this study, we trained a model that utilizes cell-free DNA (cfDNA) methylation-based features to distinguish between patients with HCC versus those with cirrhosis and applied the model to an independent dataset to show that methylation features have prognostic value and can be used to predict patient outcomes.

METHODS AND MATERIALS

- Longitudinal study of 108 HCC patients monitored from initial diagnosis (pre-treatment). A small proportion of the patients had prior treatment history and II patients received liver-directed therapy (LDT).
- Patient categories – Dropout (Progression of disease), Transplant (patients that eventually received a transplant), Active (active follow-up). For the transplant group, the methylation scores shown here are pre-transplant.

STUDY CRITERIA

Inclusion

- Adult patients with biopsy-confirmed or radiographic HCC diagnosis according to LIRADS criteria
- Deemed non-resectable with disease staging of BCLC A-B modified to include patients with ECOG < 2
- Including selected candidates with Child-Pugh C or ALBI 3 deemed by the multidisciplinary tumor board to be acceptable candidates for Liver-directed therapy (LDT)
- Able to and providing consent to a longitudinal, blood-based HCC biomarker profiling study
- Selected to receive LDT as a bridge to liver transplantation or definitive treatment plan

Exclusion

- Concomitant malignancy under an active treatment plan

PATIENT CHARACTERISTICS

Table 1 - Cohort Demographics, Baseline Hepatology, and Tumor Characteristics Prior to Treatment

Variable	Cohort
Demographics	**n = 108**
Age (years), median (IQR)	64 (60 - 67)
Legal sex (male), number (%)	84 (78)
Declared race/ethnicity (Caucasian), number (%)	77 (71)
Hepatology	
Cirrhosis etiology (HCV), number (%)	65 (60)
Child-Pugh Score (CP-A), number (%)	71 (66)
ALBI grade (2b-3), number (%)	61 (56)
HCC	
Surgical Track, number (%)	53 (49)
BCLC stage (BCLC-A), number (%)	89 (82)
ECOG score (ECOG-0), number (%)	70 (65)
HCC burden (Solitary), number (%)	70 (65)
Largest HCC size (cm), median (IQR)	3.0 (2.3 - 4.1)
Albumin (g/dL), median (IQR)	3.5 (3.1 - 3.8)
AFP (ng/mL), median (IQR)	13 (4 - 89)
GALAD score, median (IQR)	-0.3 (-2.1 - 2.7)
Methylation summary score (ratio), median (IQR)	0.62 (0.43 - 0.88)
Treatment	
History of liver-directed therapy, number (%)	25 (23)
Liver-directed therapy treatment date, range	06/13/17 - 08/25/2022
Liver-directed therapy modality, number (%)	
MWA (Microwave ablation)	31 (29)
DEE-TACE (Drug-eluting bead transarterial chemoembolization)	27 (25)
90Y (90Yttrium transarterial radioembolization)	50 (46)

EXPERIMENTAL & COMPUTATIONAL PROCEDURE

Enzymatic methyl conversion of cfDNA combined with a target capture panel to generate **cfDNA methylation sequencing data**

↓

Model trained on processed data and methylation features from HCC patients and cirrhosis patients to **predict/detect HCC**

↓

Model applied to longitudinal HCC patient cohort (independent of the training cohort) to generate a model score – "**Methylation score**"

Figure 1. Generating methylation scores for each sample

RESULTS

Methylation score is shown to be the best predictor of patient outcomes with an AUC of 0.897.

Metric	AUC
Methyl Score	0.897
GALAD	0.706
Child-Pugh	0.426
MELD	0.409
ALBI	0.532
AFP	0.657
Lesion Size	0.753

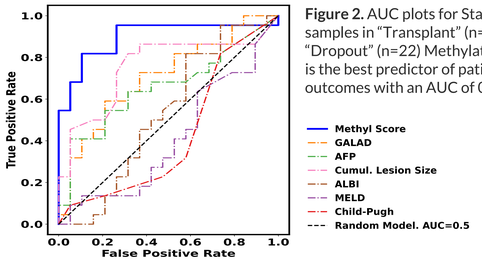


Figure 2. AUC plots for Stage 1 samples in "Transplant" (n=19) vs "Dropout" (n=22) Methylation score is the best predictor of patient outcomes with an AUC of 0.897.

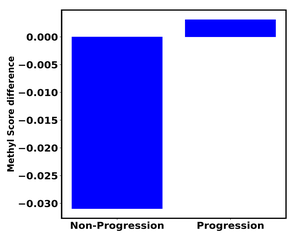
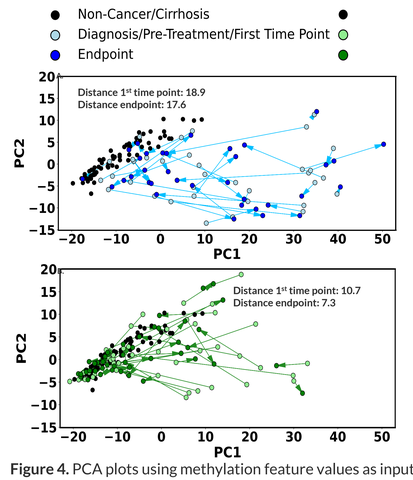


Figure 3. Median difference in methylation scores at diagnosis/ pre-treatment and at endpoint for Non-Progression ("Active" and "Transplant" groups) vs. Progression ("Dropout" group). These results suggest that a decrease in methylation score may be indicative of an improvement in the patient's condition.



Distance values indicated are the median values for the distance between the centroid of the cirrhosis group and each patient's first and last timepoint

Figure 4. PCA plots using methylation feature values as input to show the diagnosis/pre-treatment timepoint and endpoint for each patient. Methylation profiles of the "Dropout" group (in plot A in blue) are less similar to the cirrhosis group (in black) compared to the methylation profiles of the "Transplant" & "Active" groups (in plot B in green). For many patients in the "Active" & "Transplant" groups, their endpoint methylation profiles tend to approach those of cirrhosis patients, whereas the "Dropout" group's profiles are still easily distinguishable from the cirrhosis group at the study endpoint.

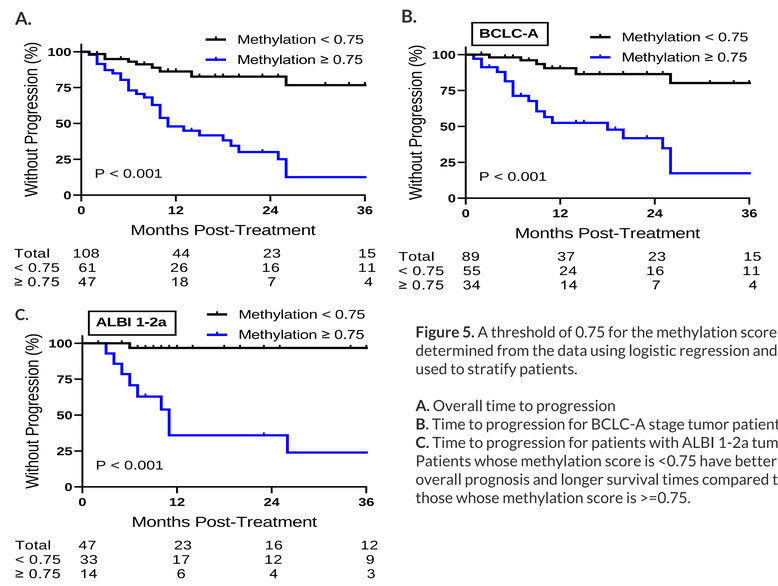


Figure 5. A threshold of 0.75 for the methylation score was determined from the data using logistic regression and was used to stratify patients.

A. Overall time to progression
B. Time to progression for BCLC-A stage tumor patients
C. Time to progression for patients with ALBI 1-2a tumors. Patients whose methylation score is <0.75 have better overall prognosis and longer survival times compared to those whose methylation score is >=0.75.

Table 2. Cox Regression for Sample Baseline Variables Associated with Time to Progression

Variable	Univariate P-value, ROC	Univariate HR (95%CI)	Multi-variate P-value	Multivariate HR (95%CI)
Demographics				
Age (years), per unit	0.293			
Legal sex, male vs. female	0.406			
Declared race/ethnicity, Caucasian vs. Other	0.423			
Hepatology				
Cirrhosis etiology, HCV vs. Other	0.344			
Child-Pugh Score, CP-A vs. CP B-C	0.161			
ALBI grade, 2b-3 vs. 1-2a	< 0.001[B]	3.2 (1.6 - 7.1)	0.012	2.5 (1.2 - 5.6)
HCC				
BCLC stage, BCLC-B vs. BCLC-A	< 0.001[A]	3.8 (1.9 - 7.6)	0.009	2.8 (1.3 - 5.7)
ECOG score, ECOG 1 vs. 0	0.611			
HCC burden, Multifocal vs. Solitary	0.032[A]			
Largest HCC size (cm), per unit	0.001[A]			
Albumin (g/dL), median (IQR)	0.001[B]			
AFP (ng/mL)	0.006, 150 ng/mL			
> 150 ng/mL vs. ≤ 150 ng/mL	< 0.001	3.6 (1.9 - 6.9)	0.959	
GALAD score	< 0.001, 1.7			
> 1.7 vs. ≤ 1.7	0.005	2.5 (13. - 4.8)	0.085	
Methylation summary score (ratio), per unit	< 0.001, 0.75			
> 0.75 vs. ≤ 0.75	< 0.001	5.4 (2.6 - 12)	0.001	3.5 (1.6 - 8.3)

[A] To avoid collinearity, the multivariate model was restricted to BCLC as the sole measure of tumor burden. [B] To avoid collinearity, the multivariate model was restricted to ALBI grade over albumin alone. [C] The GALAD and methylation score HR were evaluated per unit change in the multivariate model.

CONCLUSION

With biopsy contraindicated in early- to intermediate-stage disease (BCLC A-B), circulating mediators (biomarkers / ctDNA) provide the best potential targets for assessing prognosis, identifying treatment responders, and ultimately developing personalized.

Questions? contactus@heliogenomics.com, shivani@heliogenomics.com

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Simulation of longitudinal ultrasound versus a multi-analyte blood-based test for detecting hepatocellular carcinoma in patients with cirrhosis

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INTRODUCTION

- Patients with liver cirrhosis have a high risk of developing Hepatocellular Carcinoma (HCC).
- At risk-patients are recommended to undergo surveillance via semi-annual abdominal abdominal ultrasound (US).
- However, ultrasound-based surveillance is limited by poor adherence and suboptimal sensitivity for early-stage HCC.
- Ultrasound performance reported in literature[1] is mostly in a surveillance setting and may be over-inflated due to factors such as verification bias (i.e. failure to verify negatives using a standard reference test such as MRI or CT).
- **A cross-sectional multi-center, fully prospective called CLiMB recently demonstrated that a blood-based test, HelioLiver Dx, has significantly higher sensitivity than ultrasound for early-stage HCC detection using multiphasic MRI as a reference standard.**

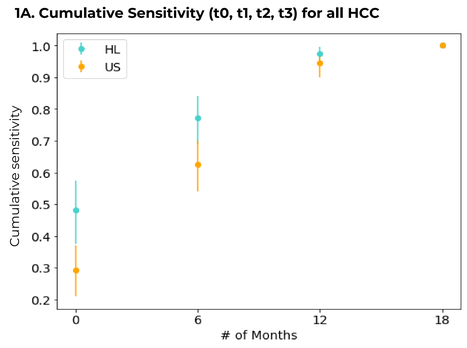
AIM

- The CLiMB study was cross-sectional in nature, so longitudinal test performance was not reported.
- **In this study, we performed Monte Carlo simulations to assess and compare the longitudinal performance of the HelioLiver Dx test and ultrasound for detection of HCC at an early stage, defined by the Milan Criteria.**

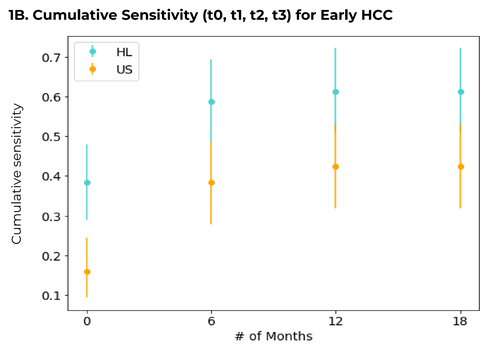
METHODS

- Patients diagnosed with HCC in the CLiMB study were binned into 4 groups : 21 patients HCC <2cm, 13 with 2cm-3cm, 5 with 3cm-5cm, 7 with HCC >5cm
- Sensitivity was calculated for both ultrasound and HelioLiver Dx based on the CLiMB trial results.
- Monte Carlo simulations were performed, using multiple surveillance adherence rates as well as binned test sensitivities described above, to compare the expected performance of ultrasound and HelioLiver Dx over time (at 6, 12 and 18 months).
- For each simulation, for each set of variables, 100 random trials were performed to calculate the mean cumulative sensitivity and its confidence intervals, assuming a cohort of 100 patients with baseline tumor size distribution as observed in the CLiMB study.
- A tumor doubling time of 6 months was used for the first set of simulations for all HCC patients.
- In a sensitivity analysis, tumor doubling times of 3 months for aggressive tumors, 6 months for average tumors and 12 months for indolent tumors were used to evaluate the impact of differential tumor growth rates on test performance.
- At each iteration, each patient/sample is assigned to one of the groups with probabilities - Aggressive=0.3, Average=0.4, Indolent=0.3. The groups are assigned independently.
- In a sensitivity analysis, we modeled different adherence rates for ultrasound and HelioLiver Dx.[2,3]

RESULTS



Modality	t0	t1=6mo	t2=12mo	t3=18mo
HELIOLIVER DX	0.482	0.772	0.976	1
ULTRASOUND	0.292	0.626	0.945	1



Modality	t0	t1=6mo	t2=12mo	t3=18mo
HELIOLIVER DX	0.384	0.587	0.612	0.612
ULTRASOUND	0.159	0.383	0.423	0.423

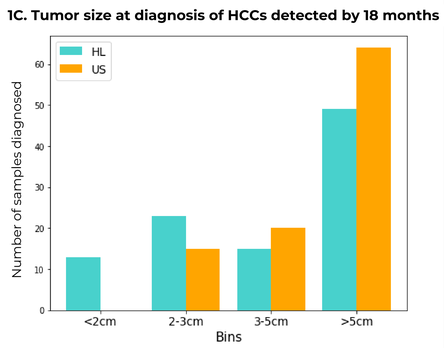


Figure 1. Baseline case assuming 100% adherence for both HelioLiver Dx (HL) and Ultrasound (US).
A) Cumulative sensitivities at t0, t1=6months, t2=12 months and t3=18 months. By 18 months, all HCCs were diagnosed by both modalities.
B) Cumulative sensitivities at each test time point for early-stage lesions. For each timepoint, sensitivity was calculated as the the number of early-stage tumors detected divided by total number of early-stage tumors at the first timepoint.
C) Size distribution for when HCCs were diagnosed by each modality. HL detects tumors earlier than US.

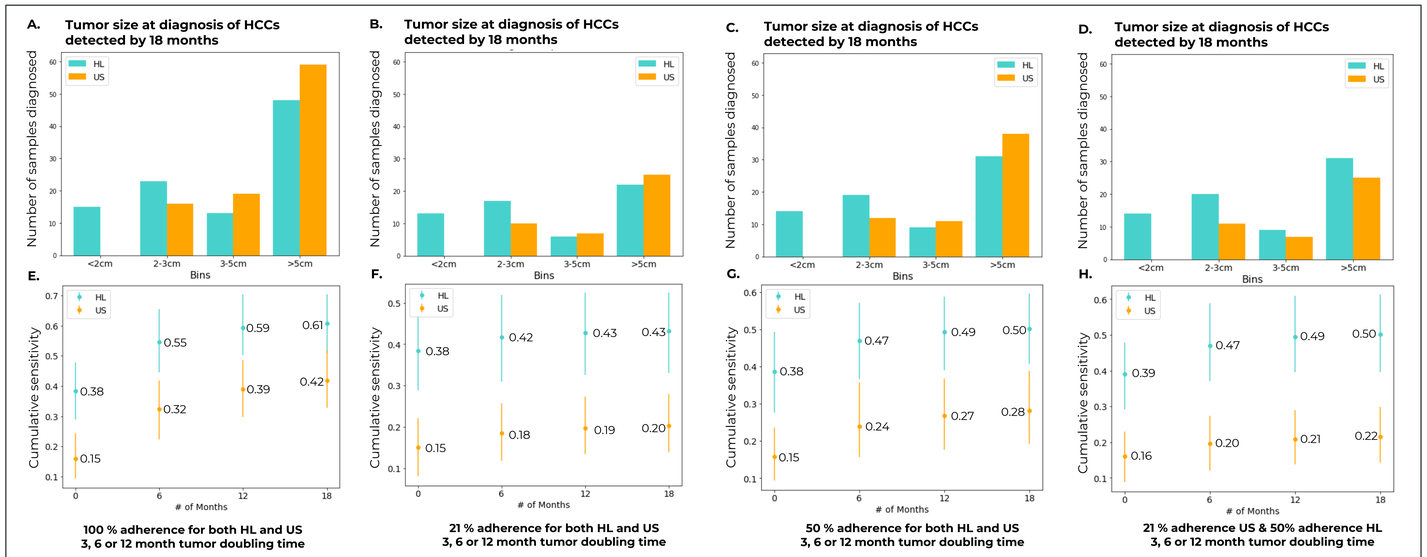


Figure 2. Sizes at which HCCs are detected by each testing modality and early-stage sensitivities for different adherence rates and tumor doubling times A,E) 100% adherence rate B,F) 21 % adherence rate C,G) 50% adherence rate. D,H) 21% adherence rate for US and 50% adherence rate for HelioLiver Dx (based on a expected increase using blood-based biomarkers

CONCLUSIONS

Our results reinforce that surveillance using a blood-based test, **such as HelioLiver Dx, can increase the proportion of patients with HCC detected at an early stage** compared to ultrasound.

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