HCC surveillance in HBV:
Recognising and stratifying oncological risk

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Disclosures:

Advisory/ Speaker Bureau:

Arbutus/ ASC/ Aligos/Biotest/ Bluejay/ Janssen/ Roche/ DrugFarm/ Gilead/ GSK/ Grifols / PrecisionBio/ Merck/ Tune/ Vir

Acknowledgments:

A Villaneuva

Ed Gane

Paul Sidhu

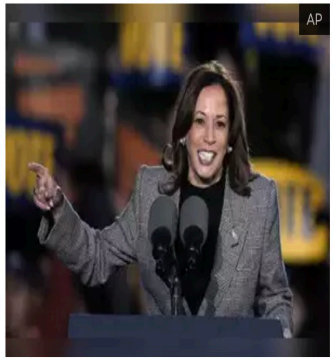
James Lok

Bad news for Trump, there has been a massive shift in momentum towards Harris in the past few days and she could win US elections

The Feed • Last Updated: Nov 05, 2024, 01:34:00 AM IST

Synopsis

Donald Trump was earlier leading in the US elections for the past couple of weeks, but is now seeing a sudden shift in momentum as Kamala Harris is picking up from where she left off, suggest recent poll data. Is there a big chance that the Democratic candidate can win the US elections?



The US elections 2024 proceedings are currently in full swing, with just more than a day to go for the polls to begin. As America goes to vote, a certain prediction market is claiming that Kamala Harris is leading in the polls, showing a huge turn of affairs. The sudden momentum shift towards Kamala Harris is more like a 'ferocious comeback', according to Thomas Peterffy, founder and chairman of Interactive Brokers.

This betting market is rooting for a Harris win

Videos

'Descendants of Razakars...': Fadnavis attacks Owaisi

Justice Sanjiv Khanna takes oath as new CJI

Predictions are not always easy

(Personal) perspective

screening vs surveillance

a huge amount of work but occasional patients picked up

but every 2-3 months advanced HCC in a young (African) male…not screened

Significant systems strain

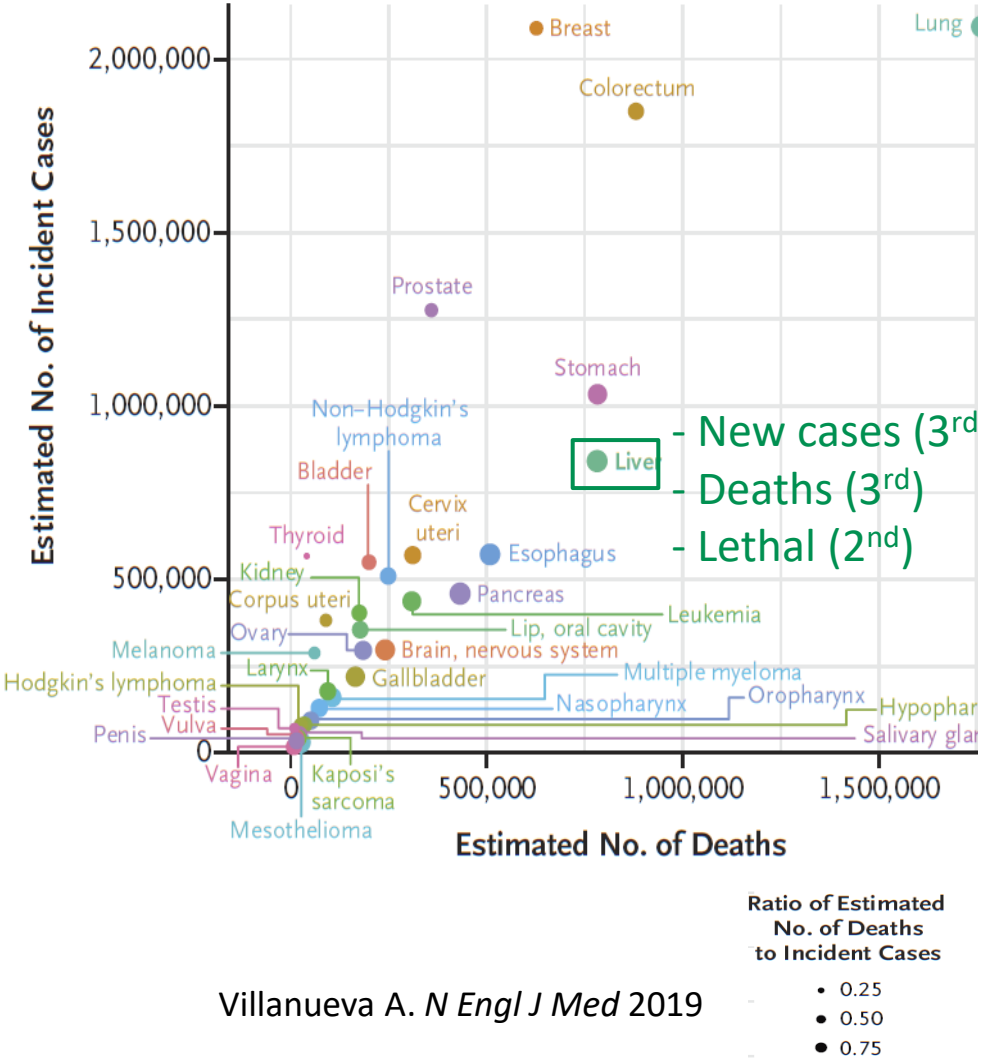
- >30% DNA rate
- False positives / anxiety
- Patient understanding
- Increasing admin
- Threshold (20%) outside criteria!
- Validity of scores on NUC treatment
- applicability

We've all made interesting decisions…

BK Kim https://doi.org/10.3350/cmh.2024.0499
Clinical and Molecular Hepatology 2024;30:656-658

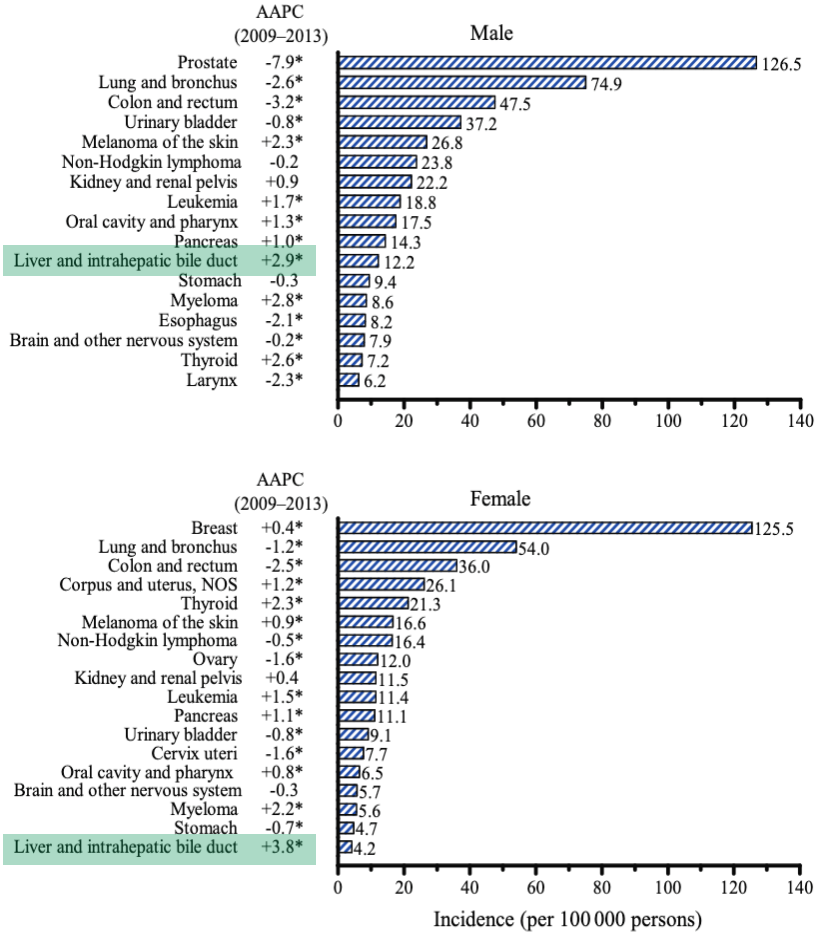
Disease burden and trends: Liver cancer

Incidence and mortality by tumor type



Villanueva A. *N Engl J Med* 2019

Cancer **incidence** in the US (2009-2013)



Jemal A et al. *JNCI* 2017

Is HCC Surveillance justified?
WHO Criteria for Screening for Disease

- High burden of disease in at-risk population
- At-risk population easily identifiable
- Understanding of natural history of the disease
  - *Long preclinical latent period allows early diagnosis*
- Available acceptable screening methods
  - *low morbidity & high sensitivity and specificity*
- Standardised recall procedures
- Safe, effective treatment
- Screening affordable and cost-effective

[Phil Johnson speaks eloquently on this point]

Permission Ed Gane

Wilson J, and Junguer G. **Geneva: World Health Organization**; 1968

Levels of perception
Clinical *vs* epidemiological

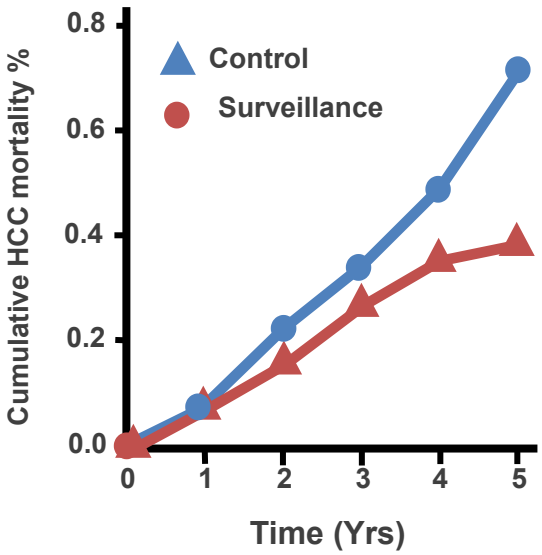
‘Show me the numbers Mrs Landingham, that’s how you win the argument and convince me,

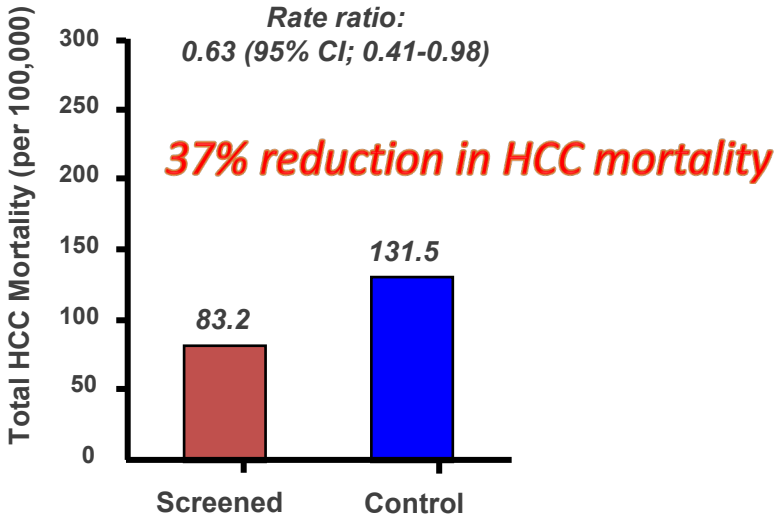
Show me the numbers…’

J Bartlett West Wing

Is HCC Surveillance justified?
First (and last) RCT of HCC screening

18,816 people with HBV infection in Shanghai randomised to:
Surveillance with US + AFP Q6M (n = 9373) vs nil (n = 9443)
Surveillance adherence only 58%





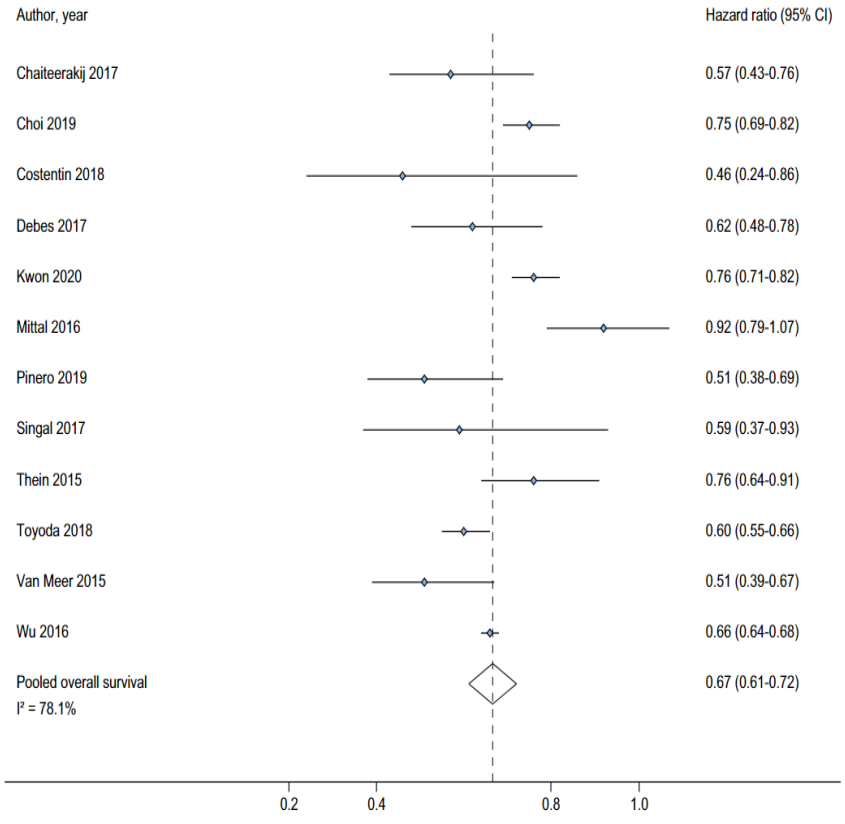
	Surveillance	Control
HCCs <5cm	46%	0
Resection	57%	8%

Permission Ed Gane

Zhang BH, et al. **J Cancer Res Clin Oncol**. 2004;130:417-422.

Surveillance in HCC
Evidence and approach

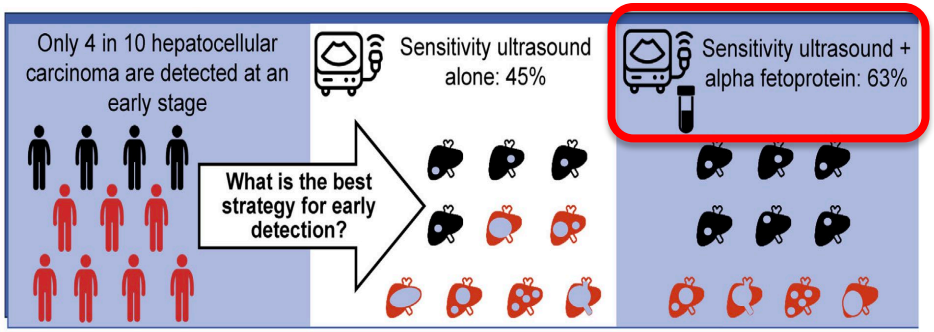
Surveillance improves survival



HR 0.67

Surveillance performance (Ultrasound +/- AFP)

Author and year	Study period	Study location	Study design	No. of patients (% cirrhosis)	No. of HCC (% early HCC)	Imaging modality	Sensitivity any stage HCC, % (sensitivity early HCC, %)	Specificity HCC, %	Follow-up, *mo*
Oka 1990[a]	1983–1988	Japan	Prospective	140 (100)	40 (NR)	Ultrasound	82.5 (NR)	NR	Median 36 (2–72)
Pateron 1994[b]	1986–1990	France	Prospective	118 (100)	14 (35.7)	Ultrasound	78.6 (21.4)	96.2	Median 36 (4–48)
Solmi 1996[a]	1988–1993	Italy	Prospective	360 (70.6)	24 (NR)	Ultrasound	100 (NR)	NR	Mean 56 (18–72)
Tradati 1998	1992–1996	Italy	Prospective	40 (100)	6 (33.3)	Ultrasound	100 (33.3)	NR	48
Giardina 1998	1988–1995	Italy	Prospective	132 (100)	19 (NR)	Ultrasound	100 (NR)	NR	Mean 64 (6–86)
Larcos 1998[a]	Not reported	Australia	Retrospective	232 (>50)	6 (33.3)	Ultrasound	100 (33.3)	91.6	96
Izzo 1998[a]	1993–1996	Italy	Prospective	325 (>50)	67 (NR)	Ultrasound	86.6 (NR)	NR	NR
Chalassani 1999	1994–1997	United States	Retrospective	285 (100)	27 (NR)	Ultrasound	59.3 (NR)	92.8	Median 15 (6–42)
Shimauchi 2000[a]	1990–1995	Japan	Retrospective	78 (100)	21 (NR)	Ultrasound	76.2 (NR)	NR	Mean 42 ± 1.6
Henrion 2000[b]	1995–1998	France	Prospective	141 (100)	6 (100)	Ultrasound	66.7 (66.7)	NR	Median 34 (3–42)
Tong 2001	1991–1999	United States	Prospective	173 (100)	31 (NR)	Ultrasound	100 (NR)	NR	Mean 35 (12–103)
Bolondi 2001	1989–1998	Italy	Prospective	313 (100)	61 (82.0)	Ultrasound	100 (82.0)	94.8	Mean 56 (6–100)
Caturelli 2002	1992–1997	Italy	Prospective	1599 (>50)	269 (NR)	Ultrasound	100 (NR)	98.6	84
Chen 2002	1991–1998	Taiwan	Prospective	292 (100)	49 (NR)	Ultrasound	61.2 (NR)	NR	Mean 84
Santagostino 2003	1996–2001	Italy	Prospective	66 (100)	8 (25.0)	Ultrasound	100 (25)	NR	72
Sangiovanni 2004	1987–2001	Italy	Prospective	417 (100)	112 (24.1)	Ultrasound	100 (24.1)	NR	Mean 148 (1–213)
Van Thiel 2004[a]	1998–2003	United States	Retrospective	100 (100)	20 (NR)	CT	60 (NR)	100	Mean 96
						Ultrasound	70 (NR)	93.8	
Mok 2005[a]	1997–2004	Hong Kong	Prospective	940 (100)	32 (NR)	Ultrasound	34.4 (NR)	98.6	Median 49
Shah 2006	2001–2004	United States	Prospective	310 (100)	22 (NR)	MRI	77.3 (NR)	82.6	Mean 22
Paul 2007	2001–2004	India	Prospective	194 (100)	9 (44.4)	Ultrasound	100 (44.4)	NR	Median 26 (0–181)
Sato 2009[a]	1994–2004	Japan	Retrospective	1431 (>50)	243 (NR)	Ultrasound	90.9 (NR)	NR	Mean 73
Luo 2010[a]	Not reported	China	Prospective	93 (100)	16 (NR)	Ultrasound	75 (NR)	NR	NR
Qian 2010[b]	1998–2004	Australia	Retrospective	268 (91.4)	22 (81.8)	Ultrasound	81.8 (68.2)	70.7	Mean 36
Lok 2010[b]		United States	Prospective	116 (56.9)	39 (61.5)	Ultrasound	56.4 (35.9)	NR	46
Trinchet 2011[b]	2000–2009	France	Prospective	1278 (100)	123 (74.8)	Ultrasound	88.6 (65.0)	89.7	Mean 47
Singal 2012[b]	2004–2006	United States	Prospective	442 (100)	41 (73.2)	Ultrasound	43.9 (31.7)	91.5	Median 42 (7–79)
Pocha 2013	2002–2011	United States	Prospective	163 (100)	17 (58.8)	CT	77.8 (62.5)	95.9	Mean 31
						Ultrasound	88.9 (55.5)	87.5	
Mancebo 2013[a]	1992–2010	Spain	Prospective	450 (100)	62 (66.1)	Ultrasound	88.7 (NR)	NR	Median 42
Frey 2015	2011–2012	Switzerland	Retrospective	285 (87.0)	9 (88.9)	Ultrasound	100 (88.9)	87.3	24
Pinero 2015	2005–2011	Argentina	Retrospective	572 (100)	56 (NR)	Ultrasound	33.9 (NR)	99.6	Median 3 (1–7)
Chang 2015[c]	2002–2010	Taiwan	Retrospective	1597 (100)	363 (58.7)	Ultrasound	92.0 (NR)	74.1	Median 57 (17–144)
Kim 2016[c]	2011–2014	Korea	Prospective	407 (100)	43 (97.7)	MRI	86.0 (83.7)	94.2	18
						Ultrasound	27.9 (25.6)	90.1	



Singal T et al. *J Hepatol* 2022;
Tzartzeva et al. *Gastroenterology* 2018

HCC Surveillance: International Society Guidance

	AASLD (2018)	EASL (2018)	APASL (2017)
Screening population	All HBsAg positive patients with: • Cirrhosis • Asian or black men over 40 • Asian women over 50 • First degree relative with history of HCC • Co-infection with HDV infection	Cirrhotic patients, Child Pugh A and B. Cirrhotic patients, Child Pugh C, awaiting liver transplant. Non-cirrhotic HBV patients at intermediate or high risk of HCC (PAGE-B score of 10-17 and ≥18 points respectively, Caucasian patients). Non-cirrhotic F3 patients, based on individual risk assessment.	Cirrhotic patients. Non-cirrhotic HBsAg positive patients if: • Asian females aged >50 years • Asian males aged >40 years • African patients aged >20 years • Family history of HCC
Modality of screening	Ultrasound every 6 months. Insufficient evidence for or against addition of AFP monitoring.	Ultrasound every 6 months. No recommendation can be made about utility of AFP for early tumour detection when used to complement ultrasound surveillance.	Combination of ultrasound and serum AFP every 6 months. Measurement of AFP alone is not recommended for routine surveillance of HCC.
Other comments	Advises against screening patients with cirrhosis and Child Pugh C, unless they are on the transplant waiting list.	Acknowledges lack of a universal prognostic model to assess risk of developing HCC.	Surveillance strategies in those with decompensated Child Pugh C cirrhosis may not be cost-effective, unless they are awaiting liver transplantation.

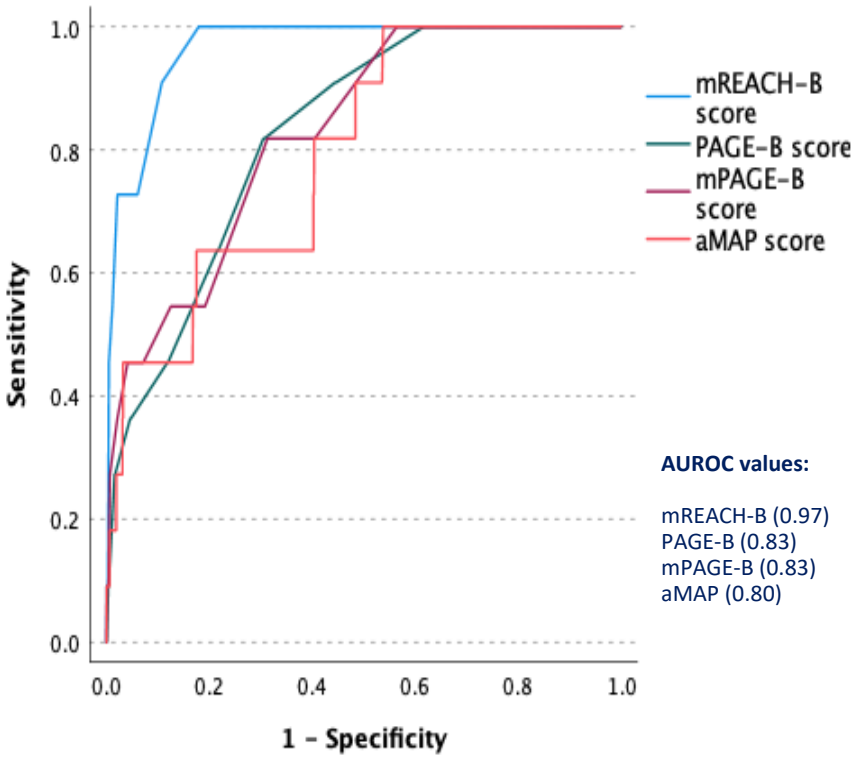
Seem very blunt

HCC Surveillance: risk prediction scores

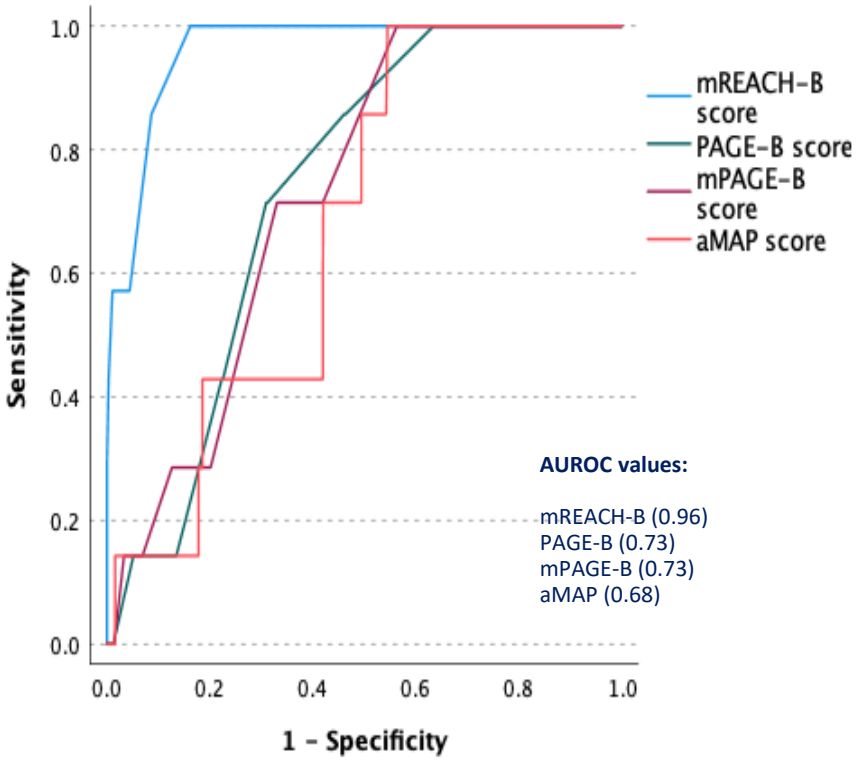
	PAGE-B	mPAGE-B	aMAP	mREACH-B	CAMD
Patients, n	1325 (Derivation) 490 (Validation)	2001 (Derivation) 1000 (Validation)	17 374 (Total) 75.3% chronic HBV infection	192 (Derivation)	23 851 (Derivation) 19 321 (Validation)
Population	Europe, Turkey	South Korea	299 centres, across 24 countries	South Korea	Taiwan, Hong Kong
Risk score parameters	Age, gender, platelets	Age, gender, platelets, albumin	Age, gender, platelets, albumin, bilirubin	Age, gender, liver stiffness, ALT, HBeAg	Age, gender, cirrhosis, diabetes mellitus
Performance (AUROC, c-index)	0.81 at 5 years (Derivation) 0.82 at 5 years (Validation)	0.81 at 5 years (Derivation) 0.82 at 5 years (Validation)	0.82 at 5 years (Derivation) 0.82-0.87 at 5 years (Validation)	0.81 at 3 years (Derivation)	0.82 at 3 years (Derivation) 0.75 at 3 years (Validation)
Interpretation of results	Scores range from 0 to 25. Low risk (≤9 points), intermediate (10-17), high risk (≥18 points).	Scores range from 0 to 21. Low risk (≤8 points), intermediate (9-12), high risk (≥13 points).	Scores range from 0 to 100. Low risk (<50), intermediate risk (50-60), high risk (>60).	Scores range from 0 to 16. LSM weighted as follows: <8.0kPa (0), 8.0-13.0kPa (2), >13.0kPa (4).	Scores range from 0 to 19. Low risk (<8 points), intermediate risk (8-13 points) and high risk (>13 points).

Risk stratification scores – real world experience @Kings
A lot of work to reassure ourselves

Study cohort (n=2014)



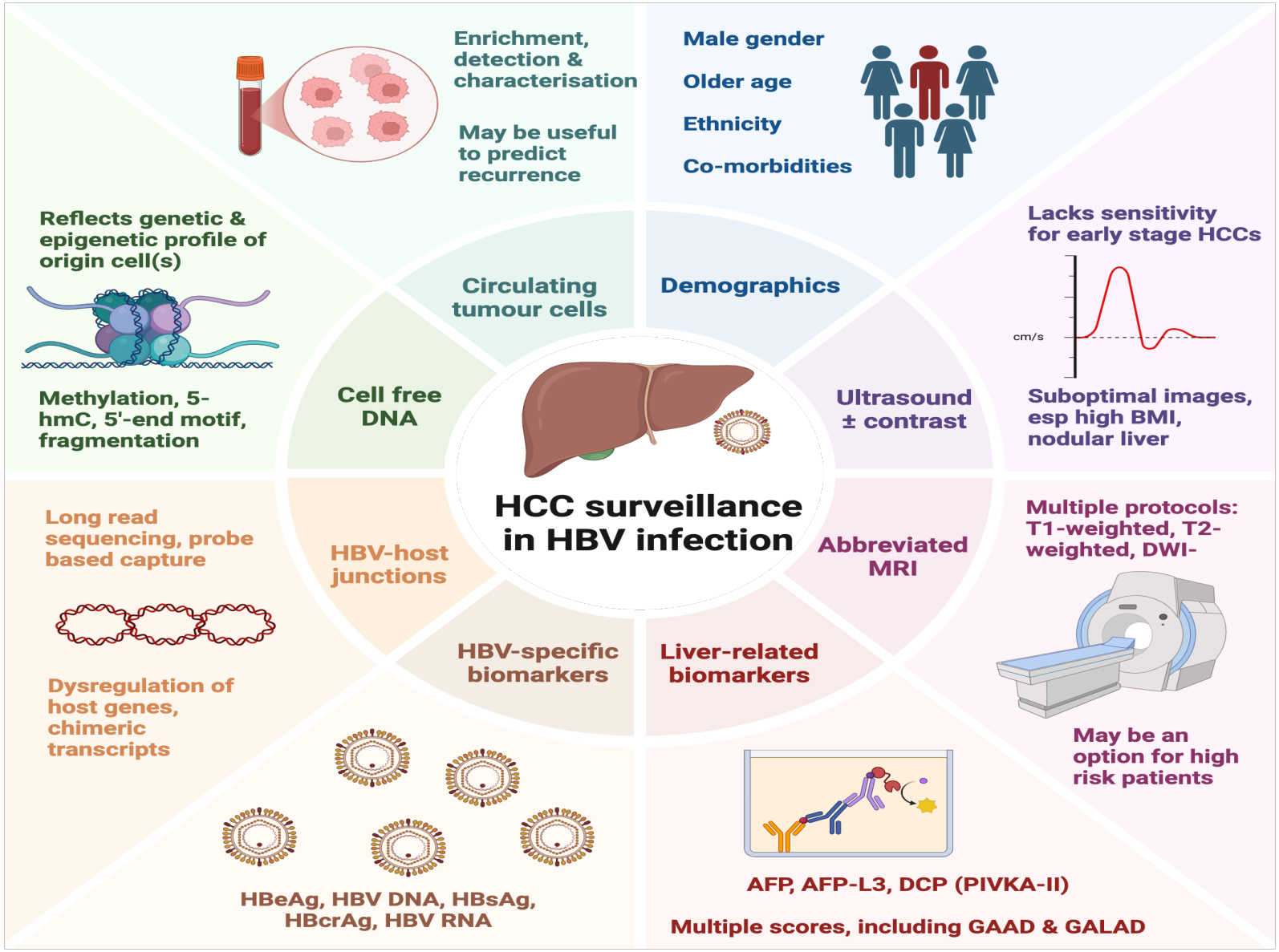
Sub-Saharan population (n=1210)



Risk predication scores have excellent NPV: >98%

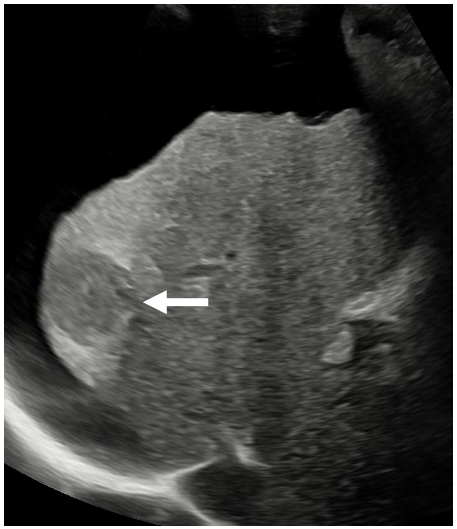
BUT they are poor at identifying high-risk cohorts

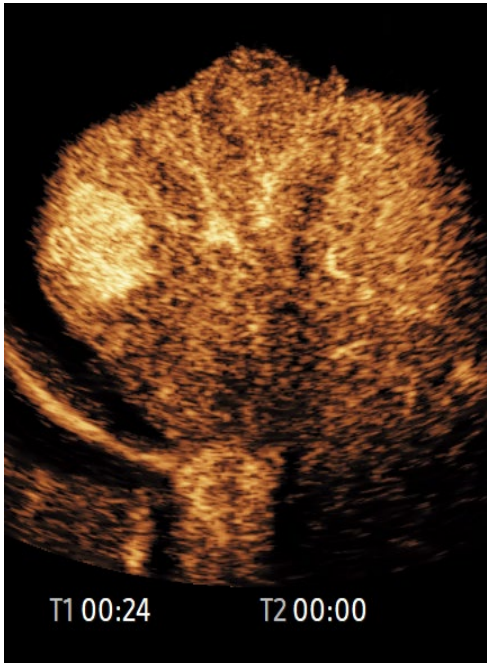
Brown et al. EASL 2019



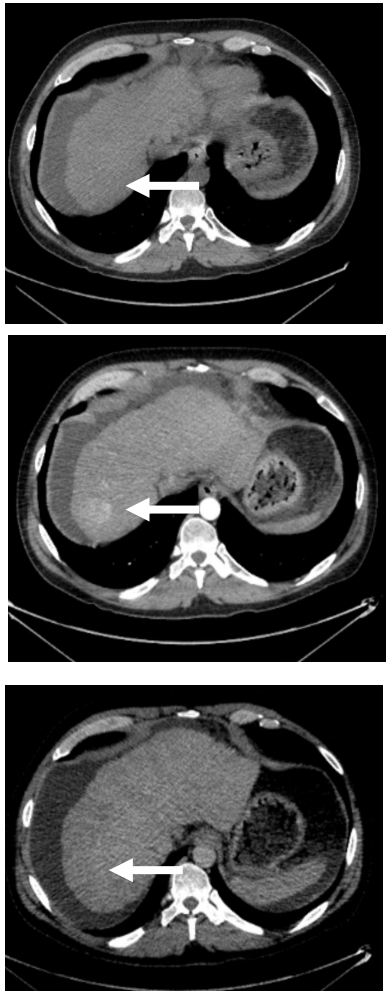
HBV is an oncological virus
We should do better

Ultrasound in Early Detection of Hepatocellular Carcinoma





Venous Phase



- AASLD guidelines recommend US surveillance for HCC and diagnosis with CT/MRI
- 2017 AASLD guidelines acknowledge but does not recommend CEUS for diagnosis of HCC
- EASL and APASL recommends CEUS in the diagnosis of HCC
- But not 2023 AASLD…

Heimbach J, Kulik LM, Finn R et all. AASLD guidelines for the treatment of hepatocellular carcinoma.
Hepatology https://doi.org/10.1002/hep.29086

Ultrasound in Early Detection of Hepatocellular Carcinoma

Key differences of CEUS compared to CT and MRI

- Permits ***real-time imaging***, eliminates possibility of arterial phase misiming
- May allow detection of ***arterial phase hyperenhancement*** (APHE missed on CT or MRI)
- Uses purely intravascular microbubble contrast agents, which affects washout and ‘capsule' characterization.
- ***CEUS washout is true washout***.
- CEUS characterization of washout requires ***assessment of onset (late vs. early***) and ***degree (mild vs. marked),*** not just presence.
- ***Allows multiple injections of contrast agent***, permitting more complete characterization
- ***Does not depict vascular pseudolesions*** such as arterioportal shunts, a frequent cause of diagnostic confusion on CT and MRI
- ***Any CEUS enhancing observation is a true lesion***
- Has fewer ancillary features
- Permits characterization of limited number of targeted observations per examination
- Requires higher level of expertise for optimal performance

Contrast Enhanced Ultrasound ACR Classification

CEUS Diagnostic Table

Arterial phase hyperenhancement (APHE)	No APHE		APHE (not rim [b], not peripheral discontinuous globular [c])	
Nodule size (mm)	< 20	≥ 20	< 10	≥ 10
No washout of any type	CEUS LR-3	CEUS LR-3	CEUS LR-3	CEUS LR-4
Late and mild washout	CEUS LR-3	CEUS LR-4	CEUS LR-4	CEUS LR-5

a. CEUS LR-M criteria – any of following:

- rim APHE **OR**
- early (< 60 s) washout **OR**
- marked washout

b. rim APHE indicates CEUS LR-M

c. peripheral discontinuous globular indicates hemangioma (CEUS LR-1)

With permission P Sidhu

DOI: 10.1097/HEP.0000000000000558

ORIGINAL ARTICLE

Contrast-enhanced ultrasound liver imaging reporting and data system: clinical validation in a prospective multinational study in North America and Europe

Andrej Lyshchik[1] | Corinne E. Wessner[1] | Kristen Bradigan[1] | John R. Eisenbrey[1] | Flemming Forsberg[1] | Misung Yi[2] | Scott W. Keith[2,3] | Yuko Kono[4] | Stephanie R. Wilson[5] | Alexandra Medellin[5] | Shuchi K. Rodgers[1,6] | Virginia Planz[7] | Aya Kamaya[8] | Lisa Finch[9] | David T. Fetzer[10] | Annalisa Berzigotti[11] | Paul S. Sidhu[12,13] | Fabio Piscaglia[14,15] | CEUS LI-RADS Trial Group

Results of a recent Multicenter Trial

nonacademic centers in North America and Europe. Patients at risk for HCC with at least 1 liver observation not previously treated, identified on ultrasound (US), or multiphase CT or MRI performed as a part of standard clinical care were eligible for the study. All participants were examined with CEUS of the liver within 4 weeks of CT/MRI or tissue diagnosis to characterize up to 2 liver nodules per participant using ACR CEUS Liver Imaging Reporting and Data System. Definite HCC diagnosis on the initial CT/MRI, imaging follow-up, or histology for CT/MRI-indeterminate nodules were used as reference standards. A total of 545 nodules had confirmed reference standards in 480 patients, 73.8% were HCC, 5.5% were other malignancies, and 20.7% were nonmalignant. The specificity of CEUS LR-5 for HCC was 95.1% (95% CI 90.1%–97.7%), sensitivity 62.9% (95% CI 57.9%–67.7%), positive predictive value 97.3% (95% CI 94.5%–98.7%), and negative predictive value 47.7% (95% CI 41.7%–53.8%). In addition, benign CEUS characterization (LR-1 or LR-2) had 100% specificity and 100% positive predictive value for nonmalignant liver nodules.

Conclusions: CEUS Liver Imaging Reporting and Data System characterization provides an accurate categorization of liver nodules in participants at risk for HCC.

Ultrasound in Early Detection of Hepatocellular Carcinoma

CONCLUSION

CEUS LI-RADS provides an accurate categorization of liver nodules in patients at risk for HCC. The CEUS LR-5 categorization, designed to be highly specific at the sacrifice of sensitivity to allow for management decisions based on imaging diagnosis alone, demonstrated 95.1% specificity for HCC with a PPV of 97.3%. Conversely, benign CEUS LI-RADS characterization (LR-1 or LR-2) had 100% specificity and 100% PPV for diagnosis of nonmalignant liver nodules. Finally, CEUS LR-5 categorization performed exceptionally well in separating HCC from non-HCC malignancy.

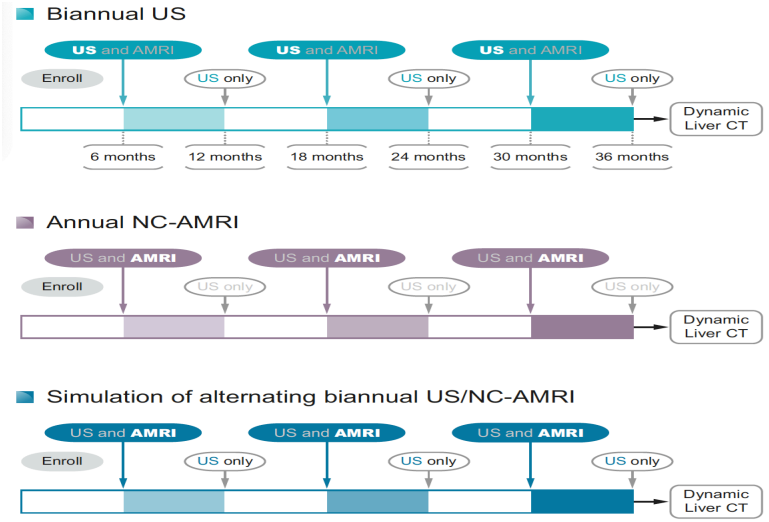
Results of this trial confirm the diagnostic performance of CEUS and provide the medical community with valuable information that can be used to determine the appropriate placement of CEUS in diagnostic and management algorithms for patients at risk for HCC.

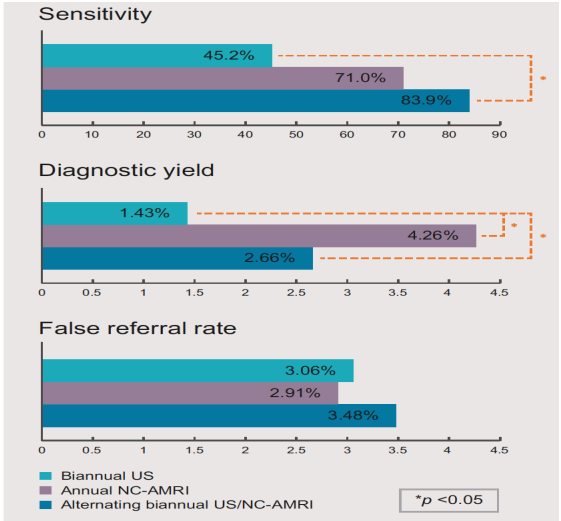
Hepatology. 2024;79:380–391

What about the potential of abbreviated MRI

Table 1. Ongoing trials testing abbreviated MRI for HCC surveillance.

Trial identification	Country	Patients phenotype	Planned inclusion	Randomization	Comparison	Health economic endpoint	HCC risk stratification	Completion date
	USA	High risk cirrhosis	4,700	Yes	US + AFP	No	Yes	2031
NCT03731923	South Korea	High risk HBV or HCV	257	No (single arm	US	No	No	2031
NCT05095714	France	High risk cirrhosis (non-viral, viral inactive)	944	Yes	US	Yes	Yes	2028
NCT05657249	South Korea	High risk HBV or HCV	199	No (single arm	US	No	Yes	2028
NCT06312826	South Korea	High risk cirrhosis	806	Yes	US	No	Yes	2027
NCT05828446	Switzerland	Cirrhosis, high risk HBV	330	No (single arm)	US	No	No	2027
NCT04455932	Australia, New Zealand	Cirrhosis (and poor US visualization)	476	No (single arm)	US	No	No	2027
NCT05429190	Netherlands	Cirrhosis/ACLD	470	No (single arm)	US	Yes	No	2026
NCT04539717	USA	Cirrhosis	820	No (single arm)	US	Yes	No	2025
NCT05716620	India	High risk cirrhosis	320	No (single arm)	US	No	No	2025
NCT04288323	USA	Cirrhosis	150	No (single arm)	US	No	No	2023





Prospective, multi-centre study.

Enrolled patients with estimated annual risk of HCC >5%.

Alternating ultrasound with non contrast-AMRI could be a useful strategy for **high-risk patients**.

Abbreviated MRI may have superior performance over ultrasound (and acquisition time is less than conventional MRI).

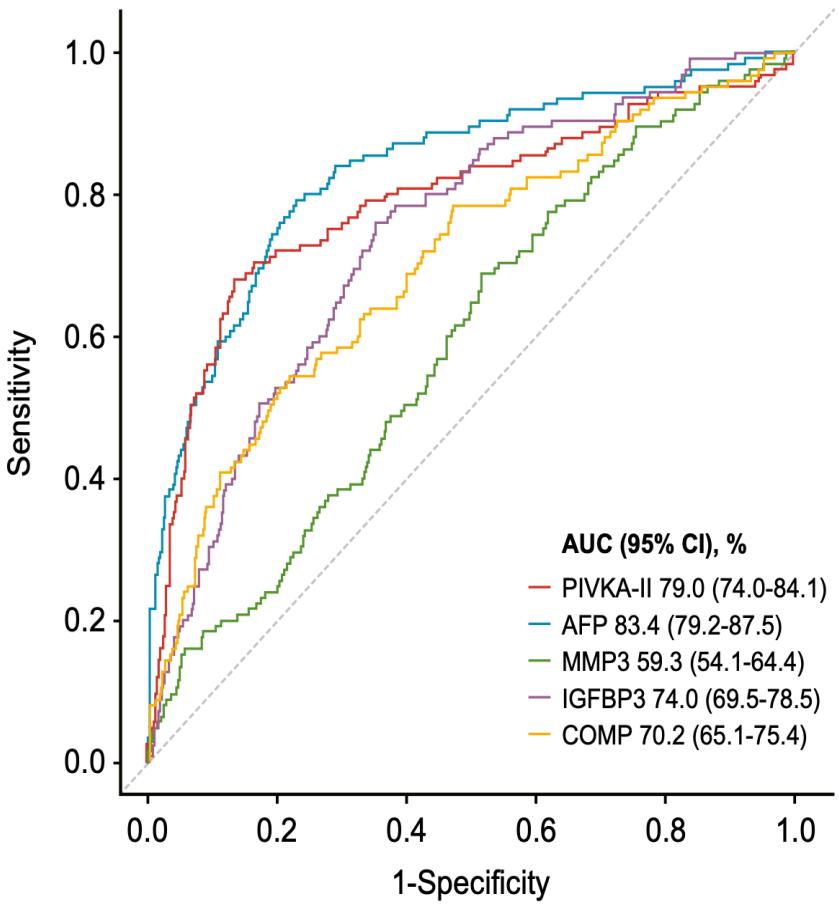
Numerous protocols: T1-weighted, T2-weighted, DWI, with or without contrast.

Kim et al. PMID 38636849

Biomarkers: AFP, AFP-L3 and DCP (PIVKA-II)

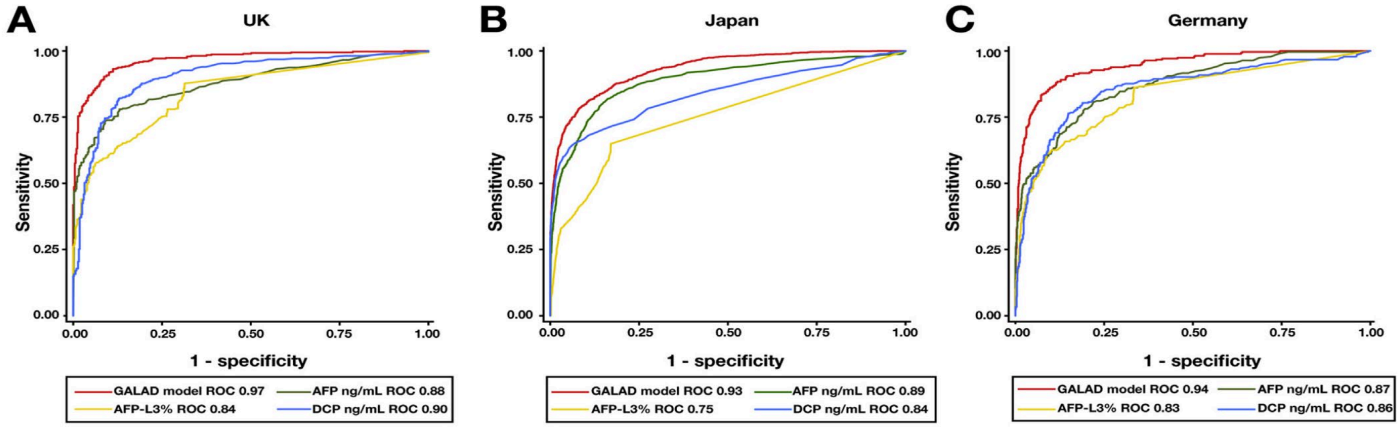
Screening tools for HCC: Poor performance if measured ALONE

Biomarker	AUC %	HCC sensitivity %*
AFP cobas e 601	83.4	53.6
PIVKA-II cobas e 601	79.0	56.0
GPC3-N	78.2	36.8
AFP-L3% Fujifilm Micro Total Analysis System Wako	77.8	47.2
AFP-L3 cobas e 601	76.5	48.8
HE4	76.5	34.4
IGFBP3	74.0	30.4
HGF	72.5	30.4
MMP2	72.4	26.4
TIMP1	71.1	30.4
Ang2	71.0	28.8
GGT2	70.9	28.0
COMP	70.2	36.0
GP73	70.1	31.2
sAxl	70.1	26.4
Cyfra 21-1	69.7	22.4
DKK1	68.9	21.6
CEA	68.8	25.6
IGF1	68.7	22.0
IL6	68.3	28.0
CA 19-9	68.1	32.0



Piratvisuth et al. PMID 34796691

GALAD score: Includes AFP, AFP-L3 and DCP



		All					Early HCC (within Milan criteria)				
Model/biomarker	Cut-off value	AUC (95% CI)	*P* value (GALAD vs biomarker)	Sensitivity, %	Specificity, %	Correctly classified, %	AUC (95% CI)	*P* value (GALAD vs biomarker)	Sensitivity, %	Specificity, %	Correctly classified, %
UK											
GALAD model	-0.63	0.97 (0.96–0.98)	-	91.6	89.7	90.6	0.93 (0.90–0.96)	—	80.2	89.7	87.9
AFP	20 ng/mL[a]	0.88 (0.85–0.90)	<.0001	60.7	96.4	79.5	0.84 (0.79–0.89)	<.0001	49.1	96.4	86.9
AFP-L3	7%[a]	0.84 (0.82–0.87)	<.0001	75.4	73.5	74.4	0.81 (0.76–0.85)	<.0001	71.7	73.5	73.2
DCP	0.48 ng/mL[a]	0.90 (0.88–0.93)	<.0001	62.4	93.8	79.2	0.81 (0.77–0.86)	<.0001	86.8	63.7	68.2
AFP + AFP-L3 + DCP[b]	Same as above	0.75 (0.72–0.77)	<.0001	99.2	50.0	72.9	0.75 (0.72–0.77)	<.0001	99.1	50.0	59.6
Japan											
GALAD model	-1.95[c]	0.93 (0.92–0.94)	-	81.4	89.1	86.5	0.91 (0.90–0.92)	—	82.1	81.6	81.7
AFP	20 ng/mL[a]	0.89 (0.88–0.90)	<.0001	51.3	97.3	81.8	0.87 (0.86–0.89)	<.0001	42.2	97.3	84.6
AFP-L3	7%[a]	0.75 (0.74–0.77)	<.0001	41.2	91.8	74.7	0.71 (0.70–0.73)	<.0001	30.0	91.8	77.5
DCP	0.48 ng/mL[a]	0.84 (0.83–0.85)	<.0001	57.3	97.4	83.8	0.78 (0.76–0.80)	<.0001	41.4	97.4	84.5
AFP + AFP-L3 + DCP[b]	Same as above	0.84 (0.83–0.85)	<.0001	79.3	88.3	85.3	0.80 (0.78–0.81)	<.0001	71.2	88.3	84.3
Germany											
GALAD model	-0.68[c]	0.94 (0.93–0.96)	-	88.4	88.2	88.3	NA	NA	NA	NA	NA
AFP	20 ng/mL[a]	0.87 (0.85–0.89)	<.0001	58.7	95.9	85.9	NA	NA	NA	NA	NA
AFP-L3	7%[a]	0.83 (0.80–0.86)	<.0001	71.3	79.7	77.9	NA	NA	NA	NA	NA
DCP	0.48 ng/mL[a]	0.86 (0.83–0.89)	<.0001	89.1	64.2	69.6	NA	NA	NA	NA	NA
AFP + AFP-L3 + DCP[b]	Same as above	0.73 (0.71–0.75)	<.0001	95.3	54.3	63.2	NA	NA	NA	NA	NA

GALAD score: Gender, Age, AFP-L3, AFP and DCP

Sensitivity (81.4-91.6%), Specificity (88.2-89.7%)

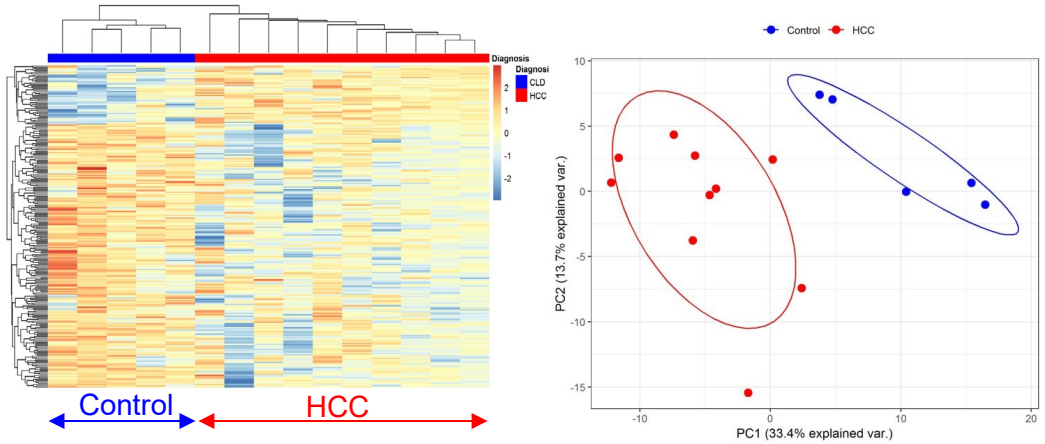
Excellent performance in multi-centre validation study (UK, Japan, Germany)

Etiology of liver disease had no effect on model performance (European cohort)

Berhane et al. PMID 26775025

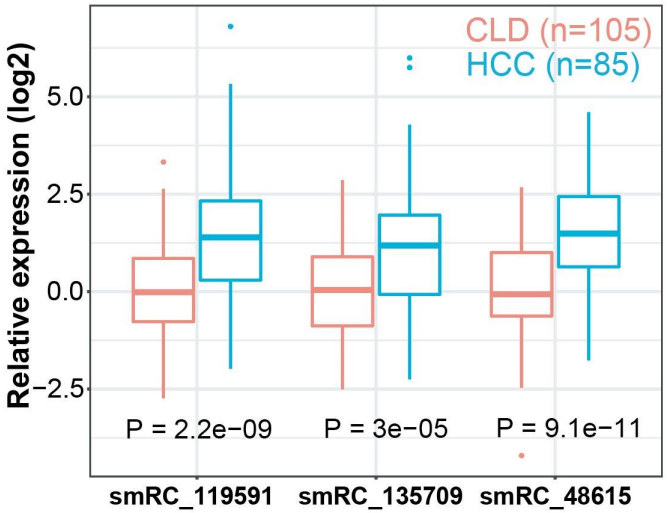
Liquid biopsy
Small RNA clusters – Extracellular vesicles

Exosome-based disease classifier (n=15 patients)

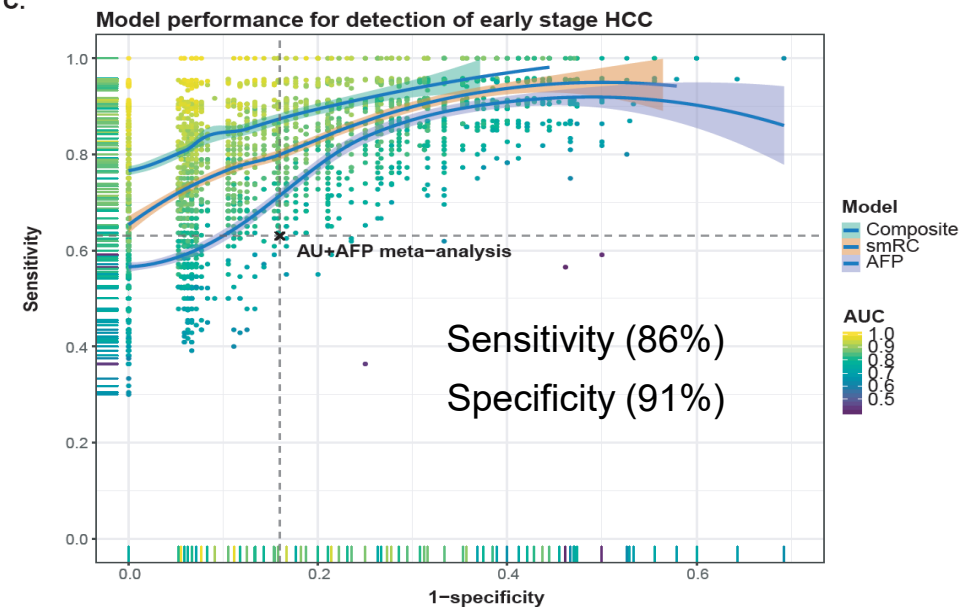


Exosome-based disease classifier (n=209 patients)

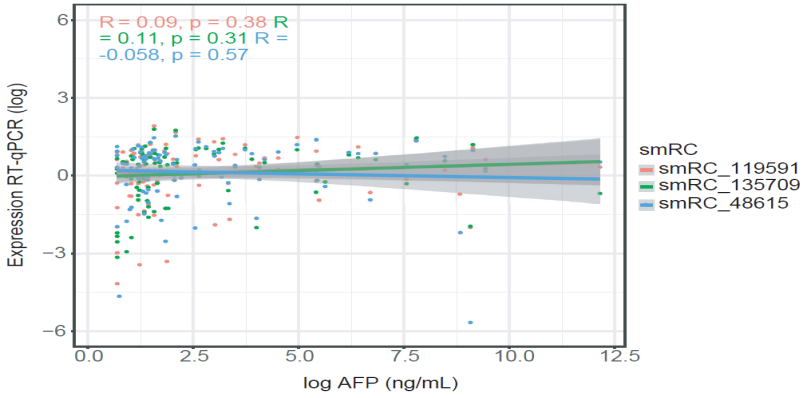
Small RNA in exosomes (n=190)



c.



AFP and small RNAs not correlated



von Felden, Gut 2021

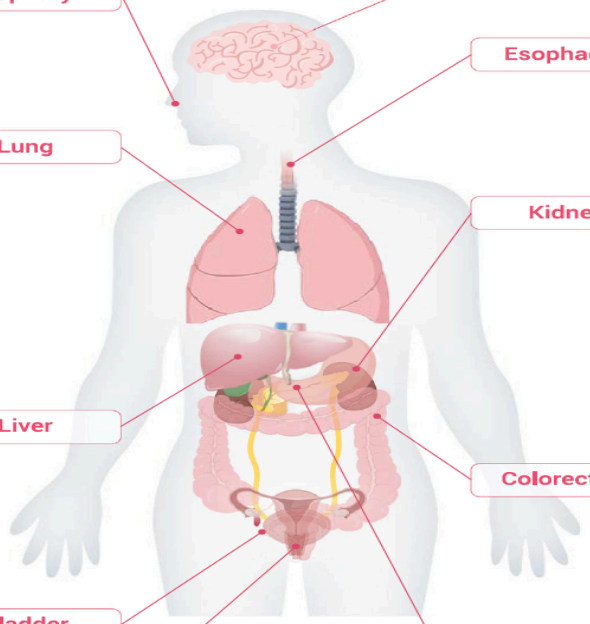
Permission Augusto Villanueva

Cell free DNA: Carries genetic and epigenetic footprint from cells of origin

Potential for early detection of cancer

- **LR score (methylation signatures) (stomach, esophageal, colorectal, lung and liver cancers)**
  Sensitivity: Post-diagnosis 87.6%/Pre diagnosis 94.9%; S pecificity: 96.1%;
  TOO accuracy: NA
- **cfDNA fragmentation patterns (b reast, colorectal, stomach, lung, ovarian, pancreatic and biliary tract cancers)**
  Sensitivities: Stage I, 68.0%; Stage II, 72.0%; Stage III, 79.0%; Stage IV, 77.0%;
  Specificity : 98.0%;
  TOO accuracy: 61.0% (Top prediction); 75.0% (top two predictions)
- **CancerSEEK (Circulating proteins and gene ovary, liver, stomach, pancreatic, esophageal, colorectal, lung and breast cancers)**
  Sensitivity: 33.0%–98.0%; Specificity: 99.1%;
  TOO accuracy: 39.0%–84.0% (Top prediction); 63.0%–100.0% (Top 2 predictions)

- **CCGA2 (Methylation based assay) (multiple cancers)**
  Sensitivity: Stage I, 18.0%; Stage II, 43.0%; Stage III, 81.0%; Stage IV, 93.0%;
  Specificity: 99.0%;
  TOO accuracy: 93%
- **CCGA3 (Methylation based assay)(multiple cancers)**
  Sensitivity: Stage I, 16.8%; Stage II, 40.4%; Stage III, 77.0%; Stage IV, 90.1%;
  Specificity: 99.5%;
  TOO accuracy: 93%
- **DETECT A (Circulating proteins and gene mutations + PET-CT/imaging) (multiple cancers)**
  Blood test alone
  Sensitivity: 27.1%; Specificity: 98.9%;
  TOO accuracy: NA
  Blood test plus diagnostic imaging
  Sensitivity: 27.1%; Specificity: 99.6%;
  TOO accuracy: NA



Nasopharynx

- **EBV DNA**
  Sensitivity: 97.1%
  Specificity: 98.6%

Lung

- **Promoter methylation of 6 genes (*SOX17, TAC1, HOXA7, CDO1, HOXA9* and *ZFP42*)**
  Sensitivity: 93.0%; Specificity: 62.0%
- **Lung Clip score (CNVs plus SNVs)**
  Sensitivities:
  Stage I, 63.0%
  Stage II, 69.0%
  Stage III, 75.0%
  Specificity: 80.0%

Liver

- **Cd score (10 methylation markers)**
  Sensitivity: 83.3%; Specificity: 90.5%
- **Wd score (32 5-hmC markers)**
  Sensitivity: 82.7%; Specificity: 76.4%
- **HCC screen score (*TP53, CTNNB1, AXIN1* and the promoter region of *TERT*, HBV sequence and protein markers, AFP and DCP)**
  Sensitivity: 100.0%; Specificity: 94.0%
- **HIFI score (5-hmC , motif, fragmentation, nucleosome footprint)**
  Sensitivity: 95.8%; Specificity: 95.0%
- **cfDNA fragmentation**
  Sensitivity: 96.8%; Specificity: 98.8%

Bladder

- **BCa-specific methylation markers**
  Sensitivity: 90.0%; Specificity: 83.1%

Cervix uteri

- **Methylation signatures: cytologically nonmalignant vs CIN2 + smear**
  AUC: 0.69–0.87

Glioma

- **DNA methylation profiles**
  IDH mutated gliomas AUC: 0.99
  IDH wide-type gliomas AUC: 0.99

Esophagus

- **5 methylation markers**
  Sensitivity: Stage I/II/III/IV, 43.0%/64.0%/77.0%/92.0%;
  Specificity: 91.0%

Kidney

- ***VHL* methylation**
  Sensitivity: 0%–50.3%;
  Specificity: 90.7%–100.0%
- ***RASSF1A* methylation**
  Sensitivity: 45.9%–62.9%;
  Specificity: 93.0%–93.3%
- **cfDNA methylation signatures**
  Plasma: AUC, 0.97–0.99;
  Urine: AUC, 0.85

Colorectum

- ***SEPT9* methylation**
  Sensitivity: 48.0%–90.0%;
  Specificity: 73.0%–97.0%
- ***VIM* methylation**
  Sensitivity: 59.0%;
  Specificity:93.0
- ***SFRP2* methylation**
  Sensitivity: 89.2%; Specificity:73.0%
- ***NDRG4 BMP3* methylation plus *KRAS* mutation and Hgb immunohistochemical**
  Sensitivity: 92.3%; Specificity: 73.0%
- **Cd-score (9 methylation markers)**
  Sensitivity: 87.9%; Specificity: 89.6%

Pancreas

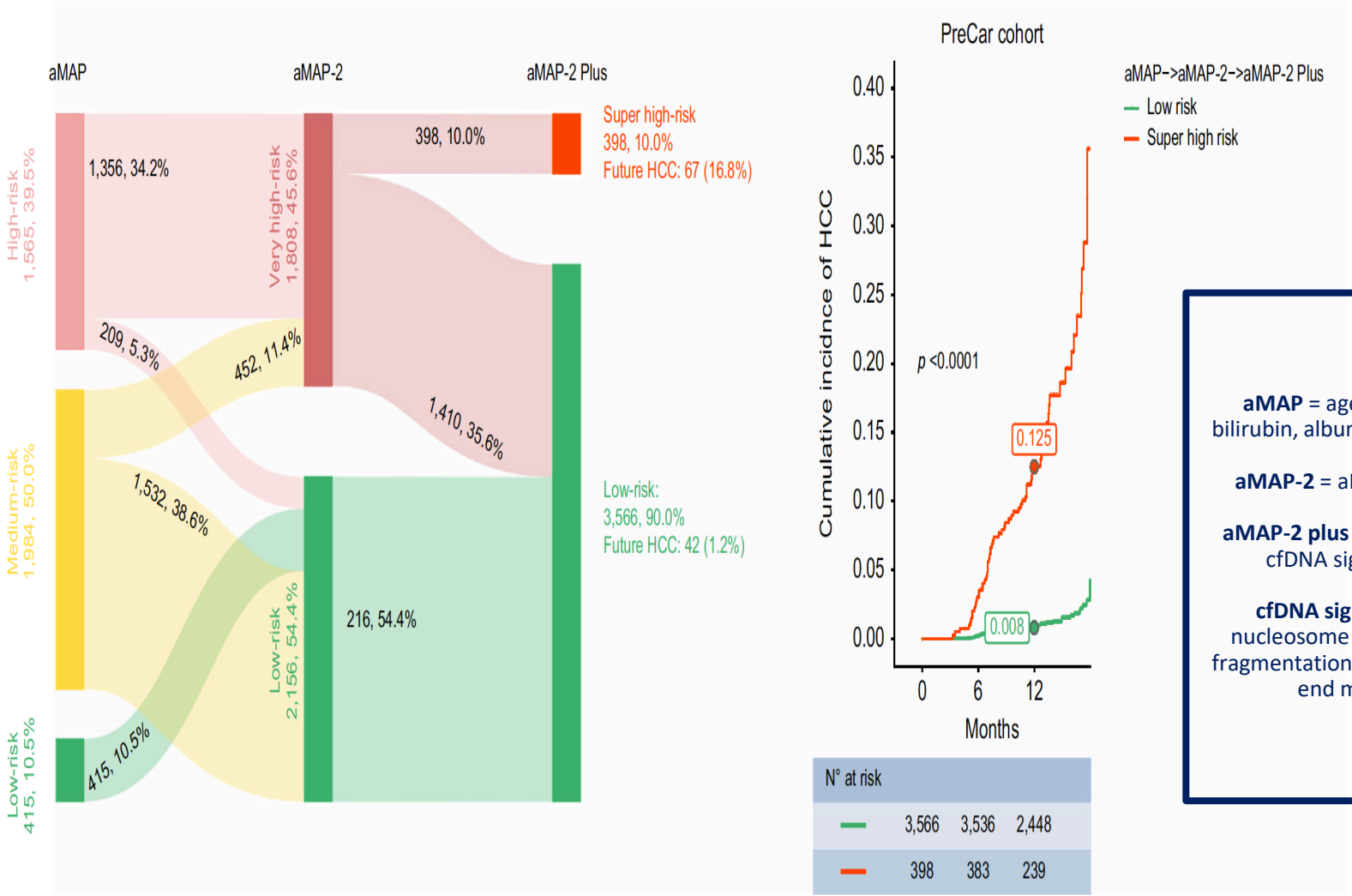
- ***KRAS* mutation plus protein biomarkers (CA19-9,TIMP1 and LRG1)**
  Sensitivity: 64.0%;
  Specificity: 99.5%
- **5-hmC markers**
  AUC : 0.74–0.97

Gao et al. PMID 35647572

HCC surveillance – potential of cfDNA

	No. of participants	Sequencing approach	Sensitivity	Specificity
Cd-score	HCC (n=1098) Healthy controls (n=835)	Targeted bisulphite sequencing (10 methylation markers)	Training: 85.7% Validation: 83.3%	Training: 94.3% Validation: 90.5%
Wd-score	HCC (n=1204) CHB or cirrhosis (n=392) Benign liver lesions (n=388) Healthy controls (n=570)	Genome wide 5-hmC profiles (32 5-hmC markers)	Training: 89.6% Validation-1: 82.7%	Training: 78.9% Validation-1: 76.4% *Xu et al. PMID 29035356*
cfDNA fragmentation	HCC (n=159), ICC (n=26), cHCC-ICC (n=7), CHB or liver cirrhosis (n=53), health controls (n=117)	Whole genome sequencing	Test: 96.8%	Test: 98.8% *Cai et al. PMID 31358576*
HIFI score	HCC (n=508) Cirrhosis (n=2250) Healthy controls (n=476)	Low pass WGS (5-hmC, motif, fragmentation, nucleosome footprint)	Test: 95.4% Validation: 95.8%	Test: 97.8% Validation: 95.0% *Zhang et al. PMID 34954829* *Chen et al. PMID 33589745*
HCC screen score	HCC (n=56) CHB (n=70) AFP/US-negative CHB (n=331)	PCR (TP53, CTNNB1, AXIN1, TERT, HBV sequence, AFP, DCP)	Training: 85.0% Validation: 100%	Training: 93.5% Validation: 94.0% *Qu et al. PMID 30858324*

Step-wise application of risk scores embedded into clinical practice



aMAP = age, gender, bilirubin, albumin, platelets

aMAP-2 = aMAP + AFP

aMAP-2 plus = aMAP-2 + cfDNA signature

cfDNA signature = nucleosome footprint + fragmentation pattern + 5'-end motif

Stratifies patients into 2 cohorts (Annual incidence of HCC 0.8% vs 12.5%)

Fan et al. PMID 37302583

This is how surveillance should look?