香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Parallel Session 4: Advanced Technologies

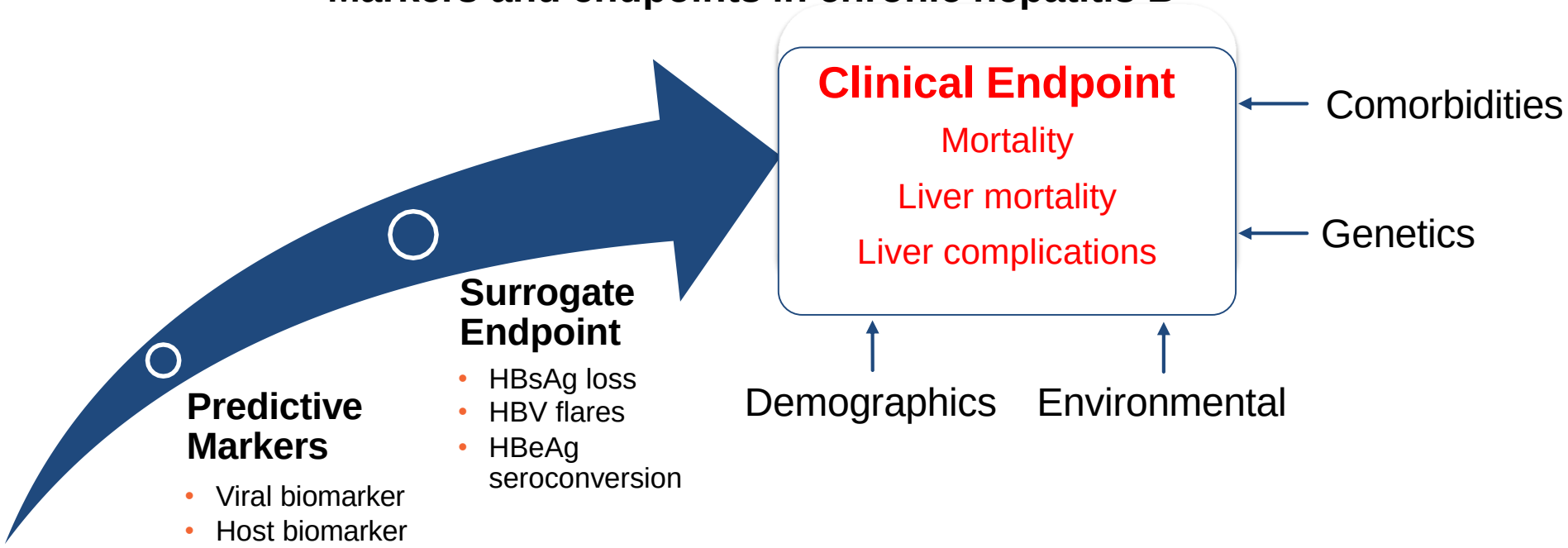
Risk of hepatocellular carcinoma in patients with chronic hepatitis B achieved complete viral suppression – role of on-treatment hepatitis B surface/core-related antigen (HBsAg/HBcrAg) levels

Applicants: *Wong Lai Hung Grace (PA), Henry Chan (Co-PA), Lilian Liang (Co-PA), Department of Medicine and Therapeutics, The Chinese University of Hong Kong (*HMRF 15160551)

Why we need novel viral markers?

As surrogates for clinically important endpoints

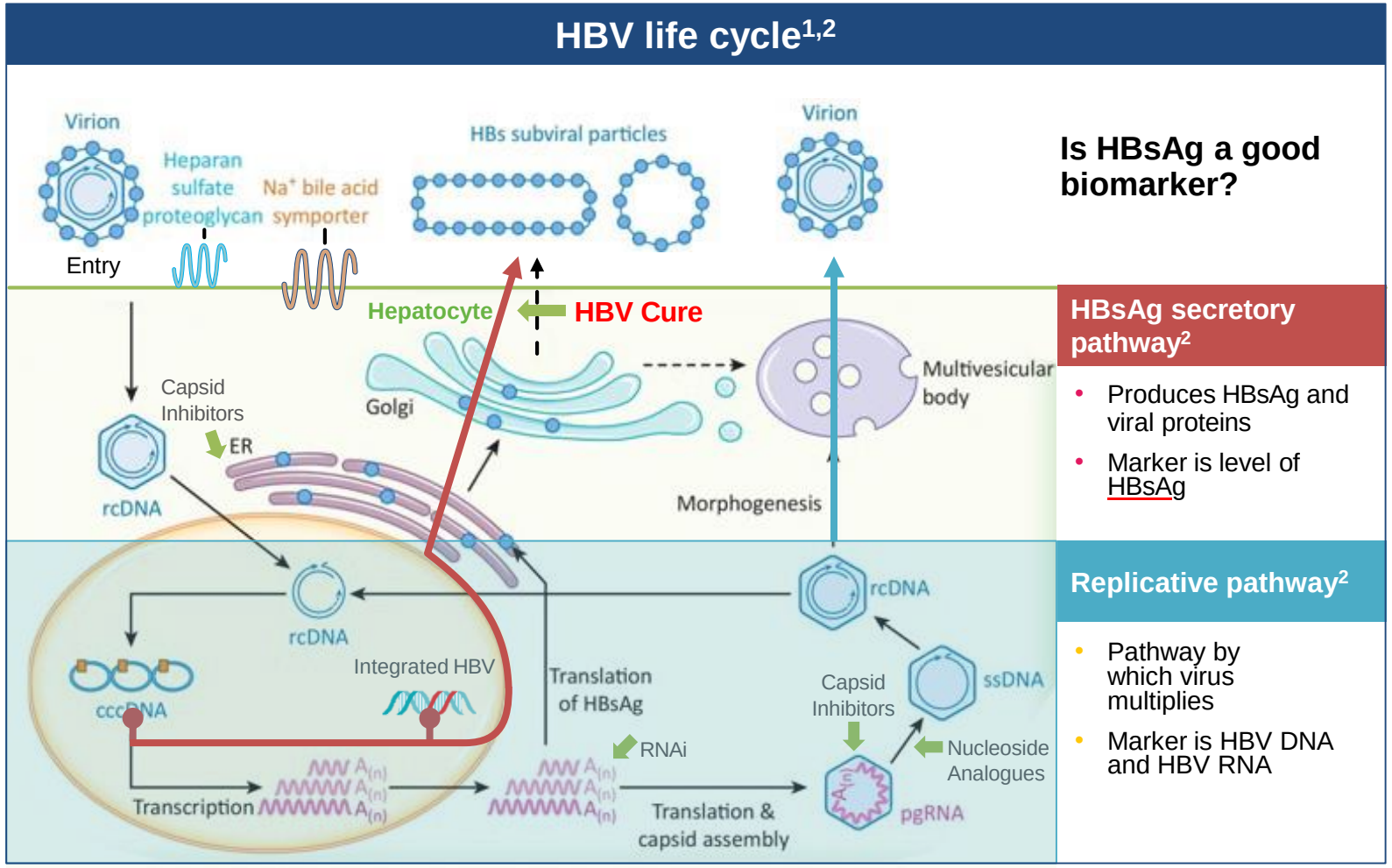
Markers and endpoints in chronic hepatitis B



HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

1. Lin CL, Kao JH. *Clin Mol Hepatol.* 2023;29:605–622
2. Tong H, et al. *Mutat Res Rev Mutat Res.* 2014;762:65–75

HBV life-cycle and viral markers

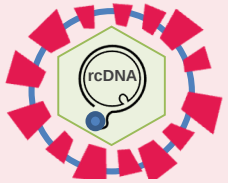
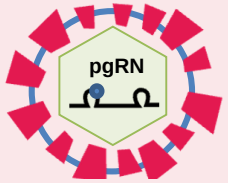
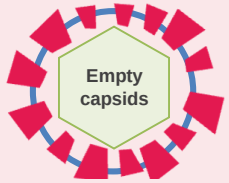
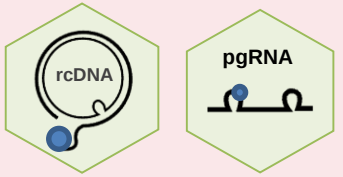


cccDNA, covalently closed circular DNA; ER, endoplasmic reticulum; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pre-genomic RNA; rcDNA, relaxed circular DNA; RNAi, RNA interference; ssDNA, single-stranded DNA.
Figures were recreated with permission from Elsevier.

香港中文大學
The Chinese University of Hong Kong
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

1. Shih C, et al. *Trends Microbiol.* 2018;26:386–387
2. Lim SG, et al. *Nat Rev Gastroenterol Hepatol.* 2023;20:238–253

Different HBV products generates different biomarkers

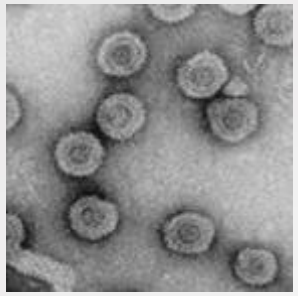
	Complete virions	RNA virions	Empty virions	Naked capsids	Subviral particles
Composition					
HBsAg	✓	✓	✓	X	✓
HBcrAg	✓	✓	✓	✓	X
HBV DNA	✓	X	X	✓	X
HBV RNA	X	✓	X	✓	X
Concentration	10^9/mL	10^6/mL	10^{11}/mL	?	10^{14}/mL
<u>Main function</u> Infectious Immunological	 ✓ ✓	 X ?	 X ?	 ? ?	 X ✓

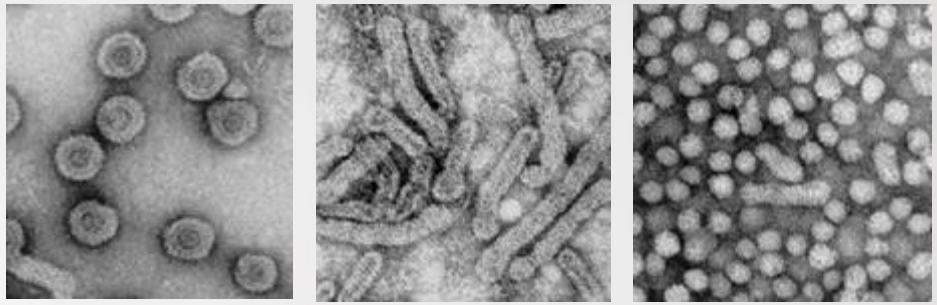
香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Buti M, et al. Semin Liver Dis 2020;40: 49-60.

HBV DNA *vs.* HBsAg





HBV-DNA (virions)

qHBsAg
virions + defective particles

HBV replication

HBV replication **cccDNA transcription/ mRNA translation**

Serum HBV DNA: a marker of HBV replication

Serum HBsAg: a marker of transcriptionally active cccDNA*

Wong GL, Chan HL. Clinical Liv Dis 2013;2:8-10.

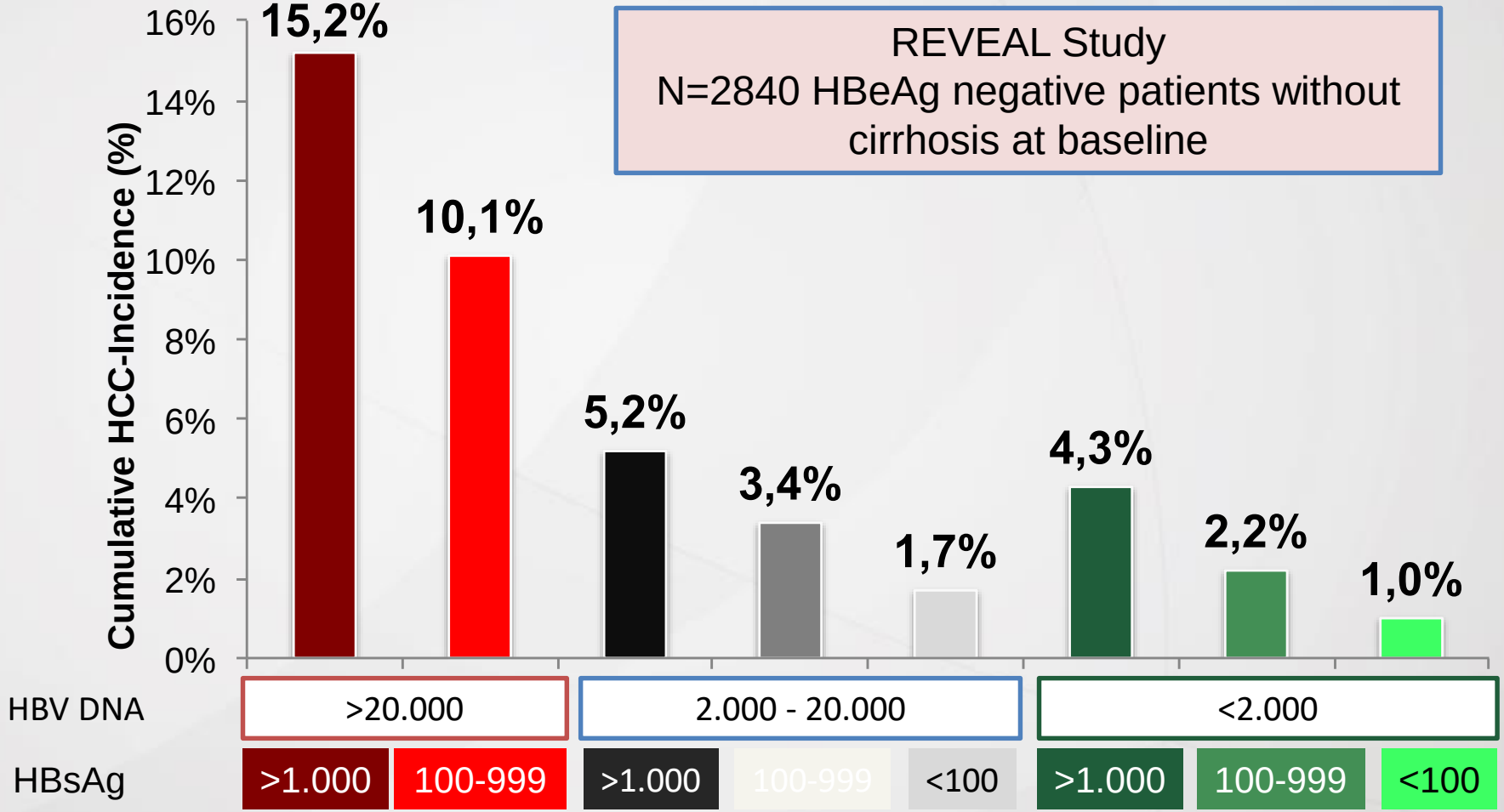
Low HBsAg is a marker for immune activation

Different meanings in different HBeAg status

e+ Low HBsAg → Immune pathology

e- Low HBsAg → Immune control

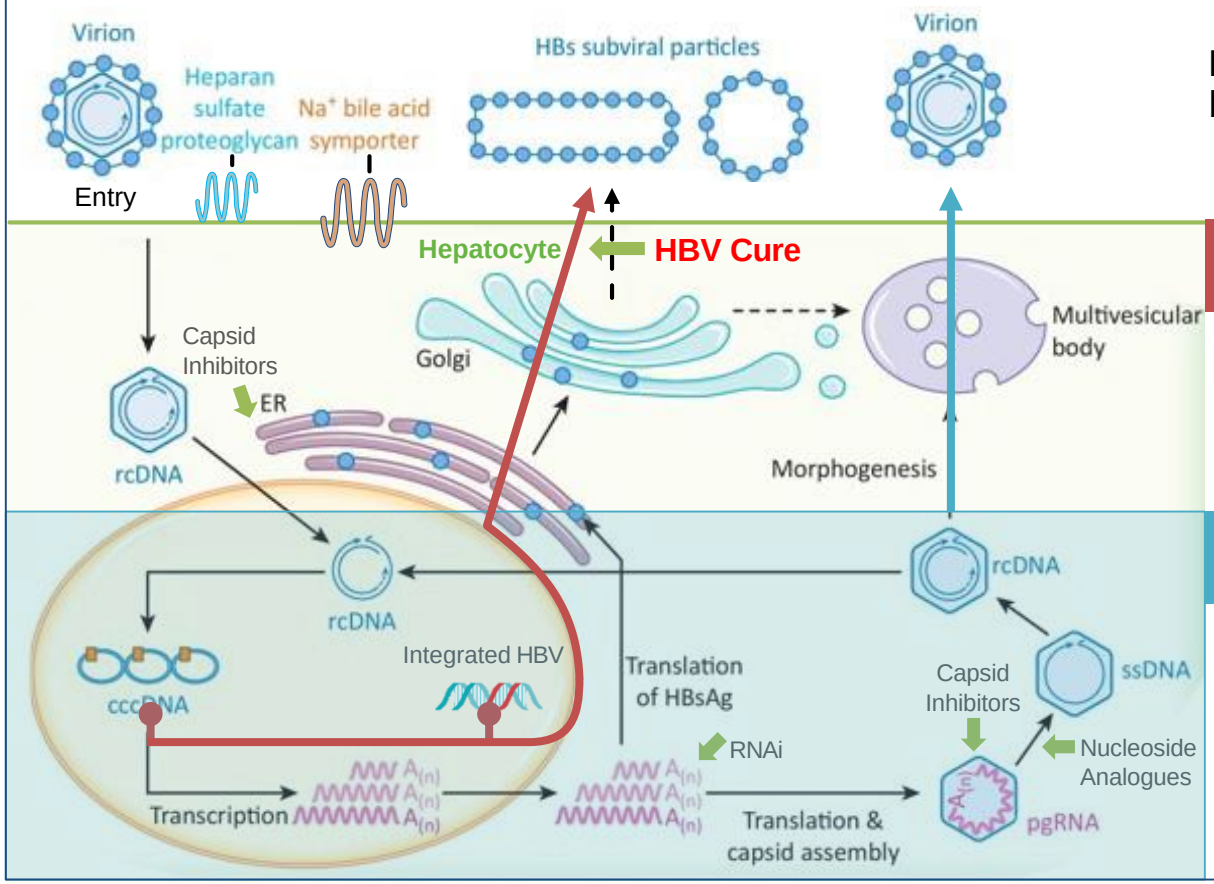
Quantification of HBsAg is useful in patients with low HBV DNA levels <20,000 IU/ml



Chen CJ, Lee MH, Liu J, et al. Hepatology 2011;54(Suppl):881A

HBV life-cycle and viral markers

HBV life cycle[1,2]



Is HBsAg a good biomarker?

HBsAg secretory pathway[2]

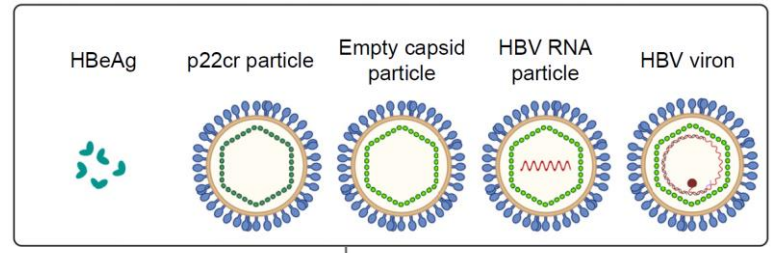
- Produces HBsAg and viral proteins
- Marker is level of HBsAg

Replicative pathway[2]

- Pathway by which virus multiplies
- Marker is HBV DNA and HBV RNA

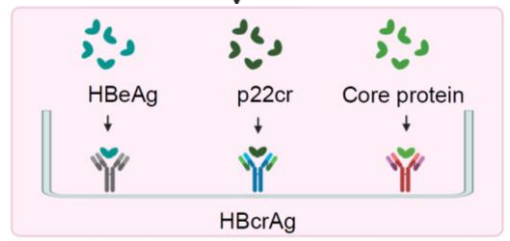
What is HBcrAg?[3]

In serum



In test tube

Denaturisation and sample preparation



Does the production of HBcrAg belong to the secretory or replicative pathway?[4]

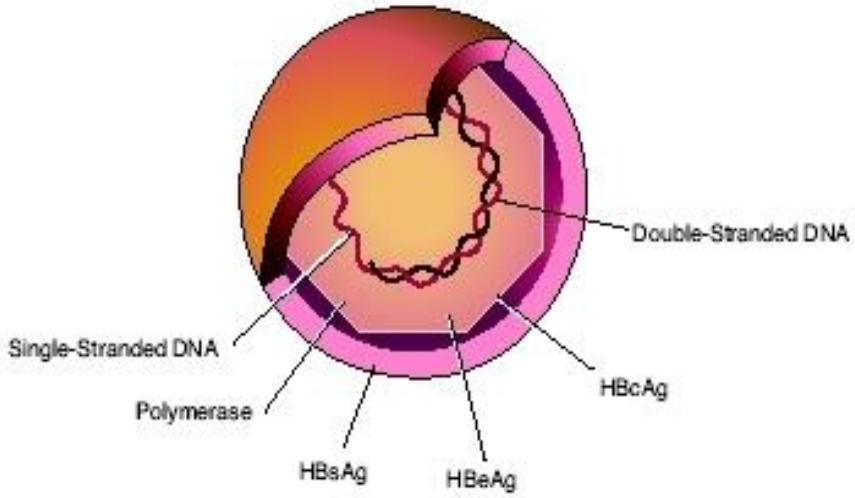
HBeAg-positive patients	HBeAg-negative patients:
HBeAg ~ 70% of HBcrAg	components of HBcrAg not quantifiable

cccDNA, covalently closed circular DNA; ER, endoplasmic reticulum; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBs, hepatitis B surface; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; pgRNA, pre-genomic RNA; rcDNA, relaxed circular DNA; RNAi, RNA interference; ssDNA, single-stranded DNA.
Figures were recreated with permission from Elsevier.

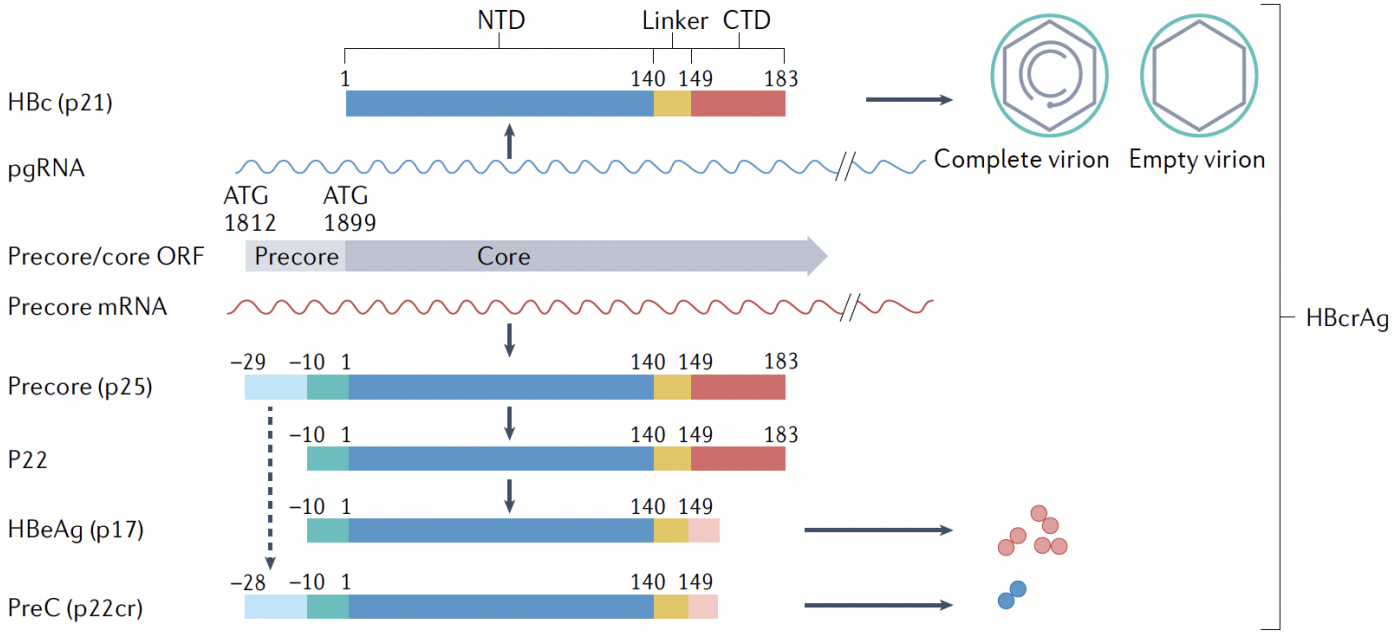
香港中文大學
The Chinese University of Hong Kong
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

1. Shih C, et al. *Trends Microbiol.* 2018;26:386–387;
2. Lim SG, et al. *Nat Rev Gastroenterol Hepatol.* 2023;20:238–253;
3. Adraneda C, et al. *J Hepatol.* 2023;; 4. Hong X, et al. *J Virol.* 2021

Hepatitis B virus core-related antigen (HBcrAg) – a new biomarker reflecting intrahepatic transcriptional activity



HBeAg and HBcAg share a 149-amino-acid sequence identity

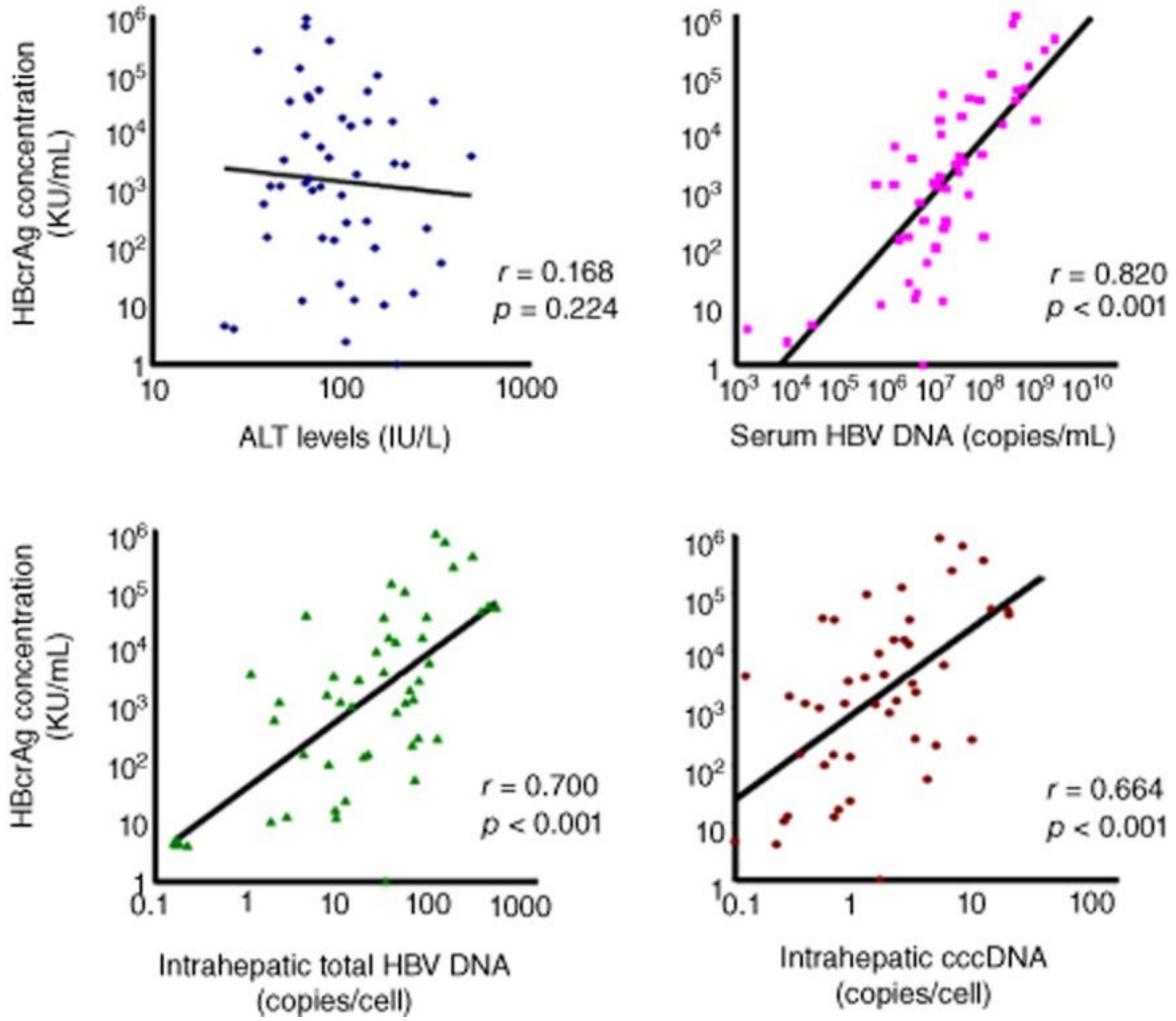


HbcrAg = a composite of 3 related proteins that share an identical 149 amino acid sequence:

- HBcAg
- HBeAg
- a truncated 22 kDa precore protein (p22cr)

Kramvis A et al. Nat Rev Gastroenterol Hepatol 2022;19:727-745

HBcrAg correlates well with HBV DNA, total hepatic HBV DNA and cccDNA



JSH Guidelines for the Management of Hepatitis B Virus Infection, Volume: 44, Issue: S1, Pages: 1-58, First published: 07 January 2014, DOI: (10.1111/hepr.12269)

Background & Aim

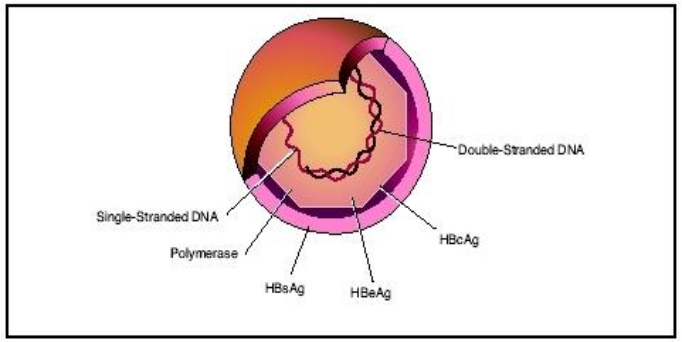
- Previous studies suggested that high serum HBcrAg level is associated with the development of hepatocellular carcinoma (HCC) in untreated CHB patients.

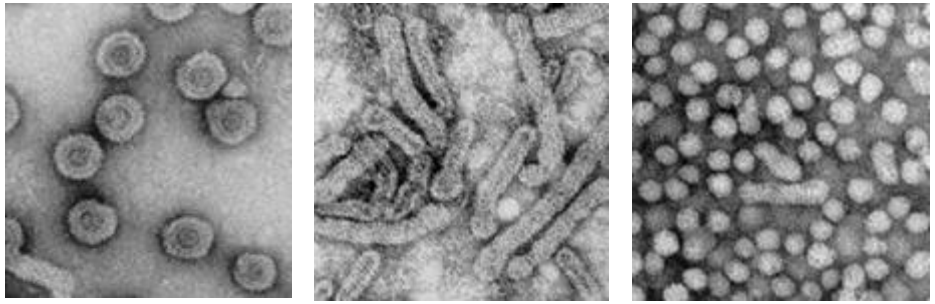
- To evaluate the role of serum HBcrAg levels to predict HCC in nucleos(t)ide analogues (NA) treated patients.

Methods

- NA-treated CHB patients with pre-treatment serum samples available were recruited.

- Pre-treatment serum HBsAg and HBcrAg and levels were measured. Primary endpoint was HCC.





Study design

- Retrospective-prospective cohort study
- Patients earliest stored serum samples were retrieved for HBsAg and HBcrAg assays
- Baseline was defined as the date of the retrieved serum samples
- Cut off values
  - HBsAg: 100 IU/mL
  - HBcrAg:
    - 2.9 log10 U/mL → HBeAg-negative patients
    - 4.9 log10 U/mL → HBeAg-positive patients
- The primary endpoint was HCC

Patients

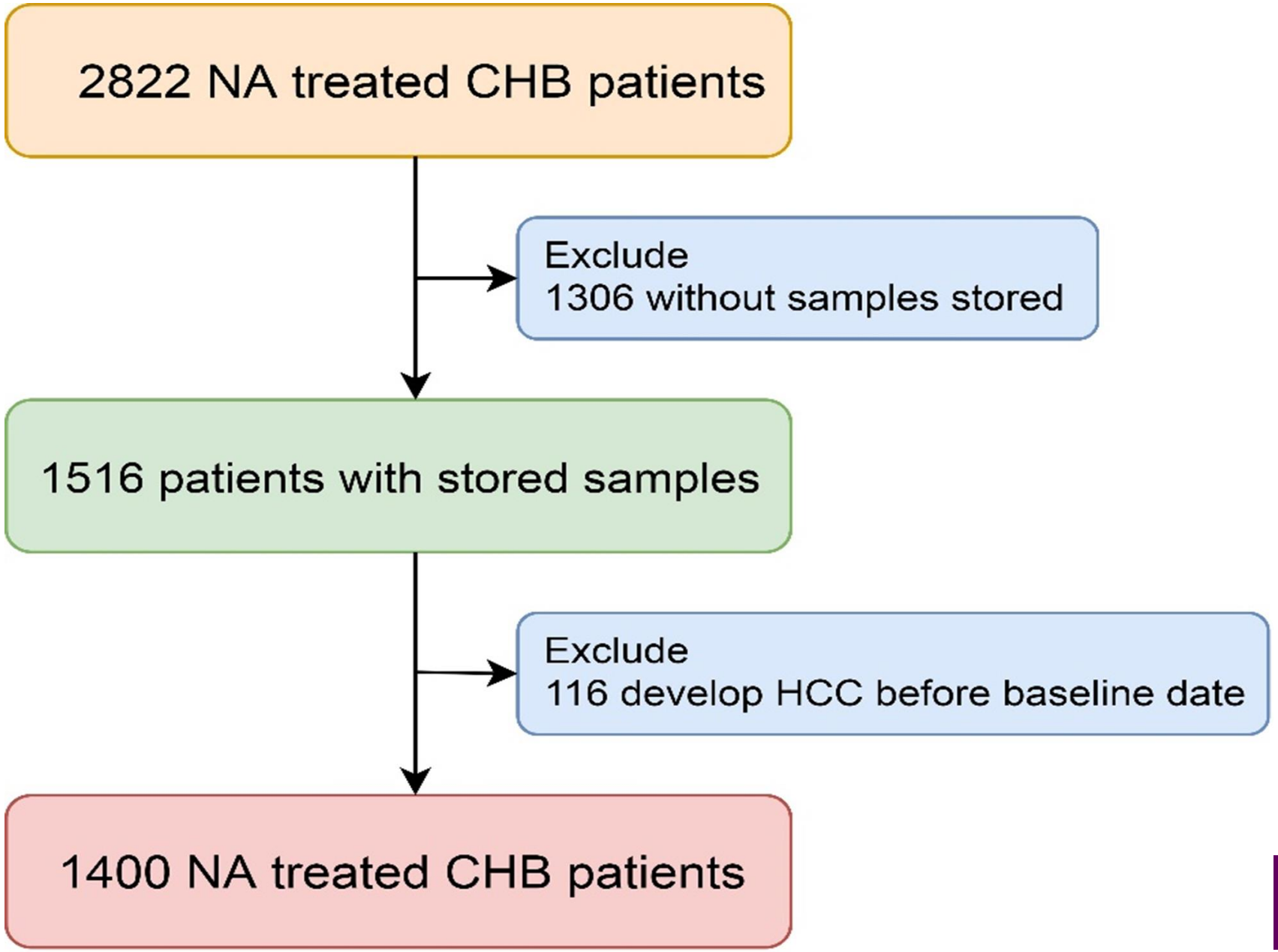
Inclusion

- Adult CHB patients who had received NA at the Hepatitis clinics of the Prince of Wales Hospital since December 2005
- With available stored serum samples

Exclusion

- History of HCC, portal vein thrombosis, previous liver surgery, liver transplantation
- With serious concurrent illness leading to a life expectancy shorter than 12 months
- Major diseases
- Refused to consent

Patient flow chart



Baseline clinical characteristics

	HBeAg-positive	HBeAg-negative
Number of patients	358	1042
Male gender (n, %)	251 (70.1)	762 (73.1)
Age (years)	46.6 ± 12.2	55.8 ± 10.8
Hemoglobin (g/dL)	14.1 ± 1.6	14.1 ± 1.6
Missing (%)	28.2	27.4
White cell count($x10^9$/L)	6.1 ± 2.6	5.8 ± 1.8
Missing (%)	28.2	27.4
Platelet ($x10^9$/L)	182.8 ± 60.1	167.0 ± 64.2
Missing (%)	28.2	27.4
Albumin (g/L)	43.5 ± 3.8	43.6 ± 3.7
Total bilirubin (μmol/L)	16.9 ± 41.8	15.1 ± 22.8
ALT (IU/L)	32.0 [22.0-52.5]	28.0 [21.0-40.0]
HBV DNA (log_{10} IU/mL)	3.84 ± 2.53	3.41 ± 2.19
HBsAg (log_{10} IU/mL)	3.2 ± 0.9	2.8 ± 0.9
HBcrAg (log_{10} U/mL)	5.3 ± 1.3	3.9 ± 1.1
Cirrhosis (n, %)	70 (19.6)	300 (28.8)
Entecavir	239 (66.8)	832 (79.8)
Tenofovir disoproxil fumarate	125 (34.9)	206 (19.8)
Antiviral treatment duration (months)	102.6 ± 35.4	109.0 ± 47.9

Variables associated with HCC in all patients (n = 1,400)

	Univariate analysis			Multivariable analysis		
	Hazard ratio	95% CI	P values	Adjusted Hazard ratio	95% CI	**P values**
Male gender	1.48	0.84-2.61	0.176			
Age (years)	1.07	1.05-1.09	<0.001	1.06	1.03-1.08	**<0.001**
Platelet (*10^9/L)	0.99	0.98-0.99	<0.001			
Albumin (g/L)	0.89	0.85-0.93	<0.001	0.95	0.91-0.99	**0.028**
Total bilirubin (LN µmol/L)	1.29	0.95-1.76	0.103			
ALT (x upper limit of normal)	0.95	0.87-1.05	0.350			
Positive hepatitis B e antigen	0.53	0.29-0.96	0.037			
Baseline HBV DNA (log_{10} IU/mL)	1.01	0.91-1.12	0.897			
1st HBsAg (log_{10} IU/mL)	0.93	0.73-1.17	0.513			
1st HBcrAg >2.9&4.9 (log_{10} U/mL)	2.04	1.12-3.71	0.020	2.10	1.09-4.06	**0.027**
Cirrhosis	9.72	5.65-16.72	<0.001	6.28	3.36-11.73	**<0.001**
Antiviral treatment at baseline ≥ 3 years	0.97	0.61-1.54	0.897			
History of interferon treatment	**0.30**	**0.04-2.17**	**0.233**			

In HBeAg-negative patients (n = 1,042)

	Univariate analysis			Multivariable analysis		
	Hazard ratio	95% CI	P values	Adjusted Hazard ratio	95% CI	**P values**
Male gender	1.45	0.77-2.73	0.248			
Age (years)	1.07	1.04-1.10	<0.001	1.06	1.03-1.10	**<0.001**
Platelet (*10^9/L)	0.99	0.98-0.99	<0.001			
Albumin (g/L)	0.92	0.87-0.96	0.001			
Total bilirubin (µmol/L)	1.28	0.89-1.84	0.184			
ALT (x upper limit of normal)	0.97	0.88-1.08	0.616			
Baseline HBV DNA (log_{10} IU/mL)	1.02	0.90-1.15	0.786			
1st HBsAg (log_{10} IU/mL)	1.02	0.77-1.36	0.886			
1st HBcrAg >2.9 (log_{10} U/mL)	1.94	1.01-3.73	0.047	2.20	1.06-4.57	**0.034**
Cirrhosis	7.01	3.95-12.42	<0.001	5.60	2.92-10.74	**<0.001**
Antiviral treatment at baseline ≥ 3 years	0.93	0.56-1.54	0.763			
History of interferon treatment	**0.05**	**0.00-14.42**	**0.295**			

In HBeAg-positive patients (n = 358)

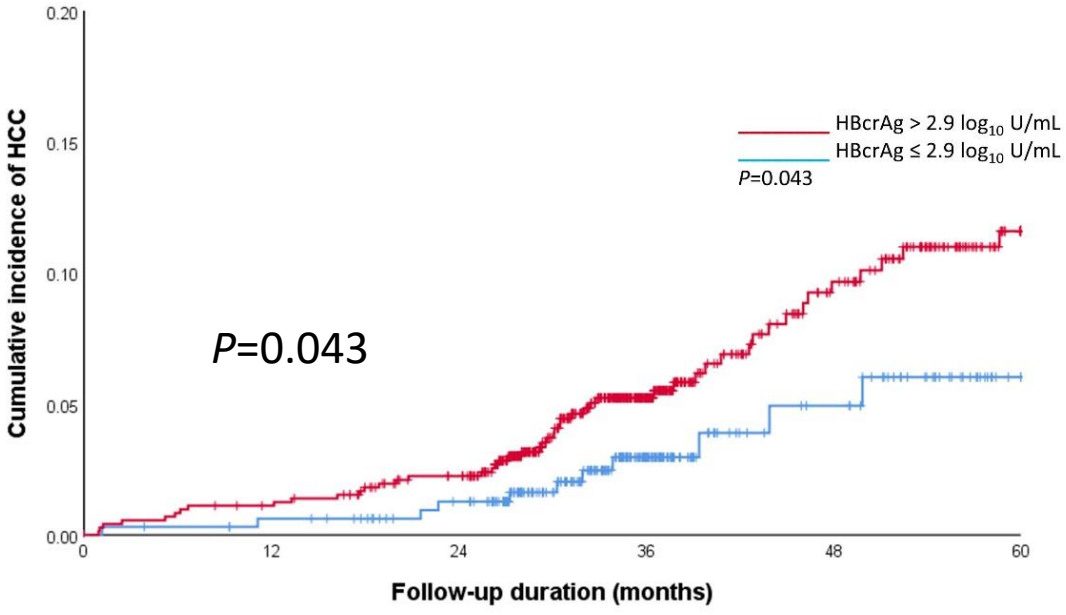
	Univariate analysis			Multivariable analysis		
	Hazard ratio	95% CI	P values	Adjusted Hazard ratio	95% CI	**P values**
Male gender	1.45	0.40-5.27	0.573			
Age (years)	1.08	1.04-1.13	<0.001			
Platelet (*10^9/L)	0.99	0.98-0.998	0.021			
Albumin (g/L)	0.81	0.76-0.88	<0.001	0.88	0.81-0.95	**0.002**
Total bilirubin (LN µmol/L)	1.38	0.74-2.56	0.315			
ALT (x upper limit of normal)	0.93	0.73-1.18	0.529			
Baseline HBV DNA (log_{10} IU/mL)	1.09	0.90-1.32	0.373			
1st HBsAg (log_{10} IU/mL)	0.82	0.51-1.31	0.398			
1st HBcrAg ≥4.9 (log_{10} U/mL)	2.46	0.54-11.10	0.242			
Cirrhosis	52.20	6.79-401.60	<0.001	24.95	3.13-199.05	**0.002**
Antiviral treatment at baseline ≥ 3 years	0.89	0.29-2.71	0.833			
History of interferon treatment	**1.53**	**0.20-11.77**	**0.684**			

Results

- 1,400 CHB patients (mean age 54 ± 12 years, 72% male, 81% entecavir-treated and 27% tenofovir-treated, 25% HBeAg positive) were included.
- The mean baseline serum HBV DNA, HBsAg and HBcrAg levels were 3.99 ± 2.30 log10 IU/mL, 2.9 ± 0.9 log10 IU/mL, and 4.2 ± 1.3 log10 U/mL, respectively.
- 88 patients developed HCC during a follow-up of 45 ± 20 months. Serum HBcrAg level above 2.9 log10 IU/mL was an independent risk factor of HCC (adjust hazard ratio 2.83, 95% CI 1.39-5.78, p=0.004), in addition to male gender, advanced age, low platelet count and low serum albumin level.
- In contrast, HBeAg and HBV DNA at baseline were not associated with HCC. HBcrAg level remained an independent risk factor in the subgroup of patients with negative HBeAg, or low serum HBV DNA < 2,000 IU/mL at baseline.

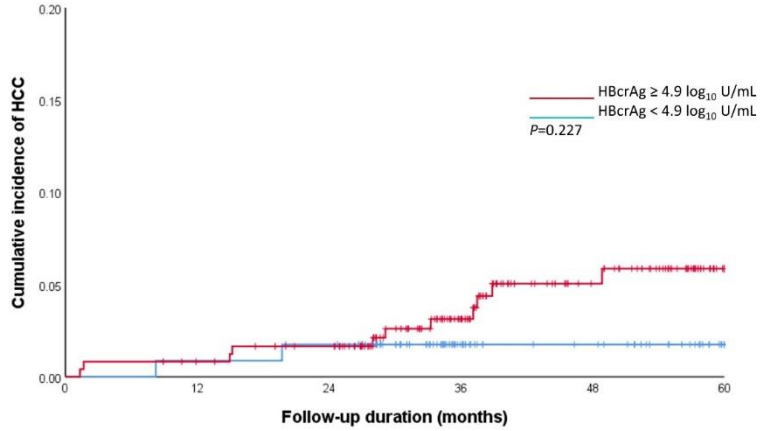
HBcrAg predicts HCC in HBeAg-negative patients
but not HBeAg-positive patients

HBeAg-negative patients



No. at risk						
HBcrAg > 2.9 $\log_{10}$ U/mL	719	707	678	370	218	140
HBcrAg ≤ 2.9 $\log_{10}$ U/mL	323	317	304	154	90	57

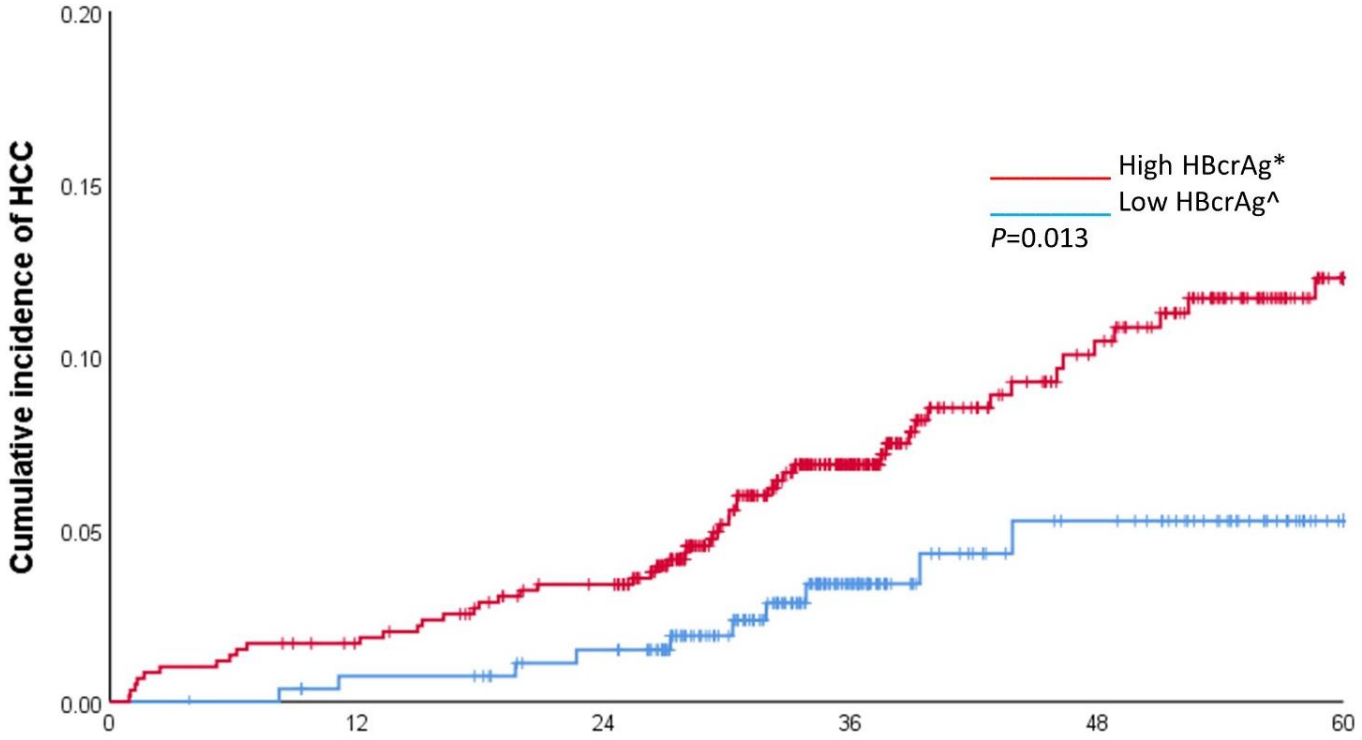
HBeAg-positive patients



No. at risk						
HBcrAg ≥ 4.9 $\log_{10}$ U/mL	244	239	232	166	117	74
HBcrAg < 4.9 $\log_{10}$ U/mL	114	113	111	66	52	30

Liang LY, …… Wong GL. J Gastroenterol. 2020;55:899

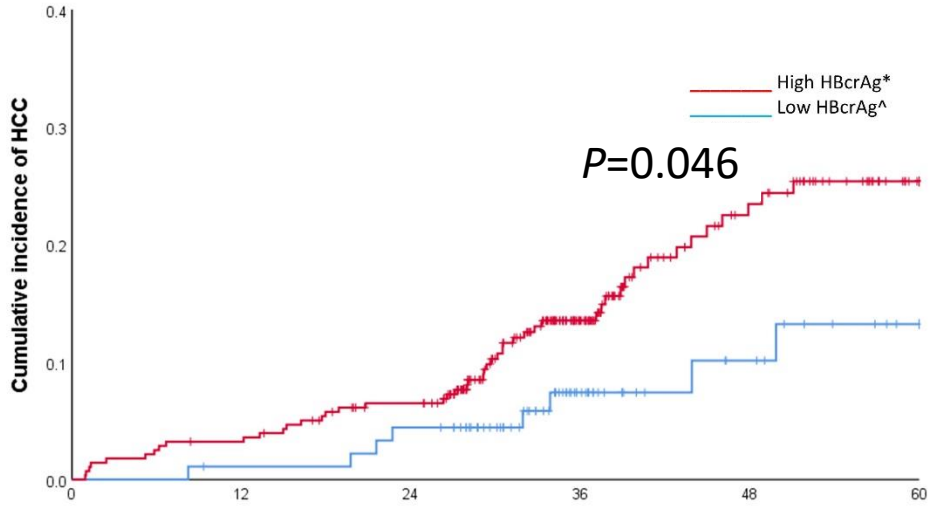
HBcrAg level predicts HCC risk in high-risk patients defined by PAGE-B score



No. at risk						
High HBcrAg	593	578	556	351	226	143
Low HBcrAg	272	267	259	143	98	65

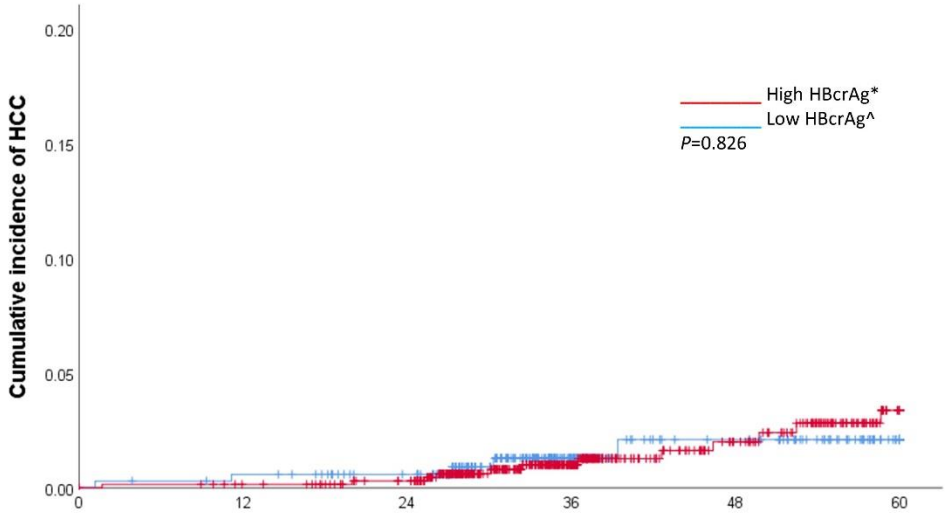
HBcrAg level predicts HCC risk in cirrhotic patients

Cirrhotic patients



No. at risk						
High HBcrAg	279	269	252	142	81	50
Low HBcrAg	91	89	86	47	31	22

Non-cirrhotic patients



No. at risk						
High HBcrAg	684	677	658	394	254	164
Low HBcrAg	346	341	329	173	111	65

Limitations of qHBsAg and HBcrAg as biomarkers

qHBsAg

- Tests cannot distinguish between HBsAg from integrated HBV DNA or cccDNA[1]
- Ultra-sensitive tests are required to measure HBsAg loss more effectively[2,3]

HBcrAg

- Potential for false positive and negative results during testing[4]
- Currently available tests have low sensitivity[5]
- Utility of biomarker in predicting outcomes requires clarification[6]

anti-HBe, anti-hepatitis B e antibody; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus; qHBsAg, quantitative hepatitis B surface antigen.

1. Pollicino T et al. *Viruses* 2021; 2. Kramvis A et al. *Nat Rev Gastroenterol Hepatol* 2022; 3. Loggi E et al. *Viruses* 2022; 4 Adraneda C et al. *J Hepatol* 2023; 5. Watanabe T et al. *Microorganisms* 2021. Vachon A, Osiowy C. *Viruses*. 2021

Conclusions

- The baseline HBcrAg level predicts the risk of HCC accurately in NA-treated HBeAg-negative CHB patients.

- HBcrAg level can be incorporated into HCC risk prediction model to further stratify HCC risk levels.

Impact of project outcomes

- The accuracy of predicting the risk of HCC after antiviral therapy may be further improved with this novel viral marker HBcrAg.

- Clinicians may use this viral markers to better differentiate HBV patients and give more specific surveillance method which may save medical resources and help to decrease excessive medical care.

Funding: HMRF 15160551 awarded to Grace Wong

CUHK

Hepatologists:

Prof. Henry Chan

Prof. Vincent Wong

Prof. Grace Wong

Data scientists

Dr. Terry Yip

Mr. Kit Tse

Miss Becky Yuen

PhD student / Post-doctoral fellow

Dr Lilian Liang

大垣市民病院

Ogaki Municipal Hospital

Gifu, Japan

Hidenori Toyoda

Toshifumi Tada

Takashi Kumada

香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong