New Biomarkers for Chronic Hepatitis B: ready for clinical use?

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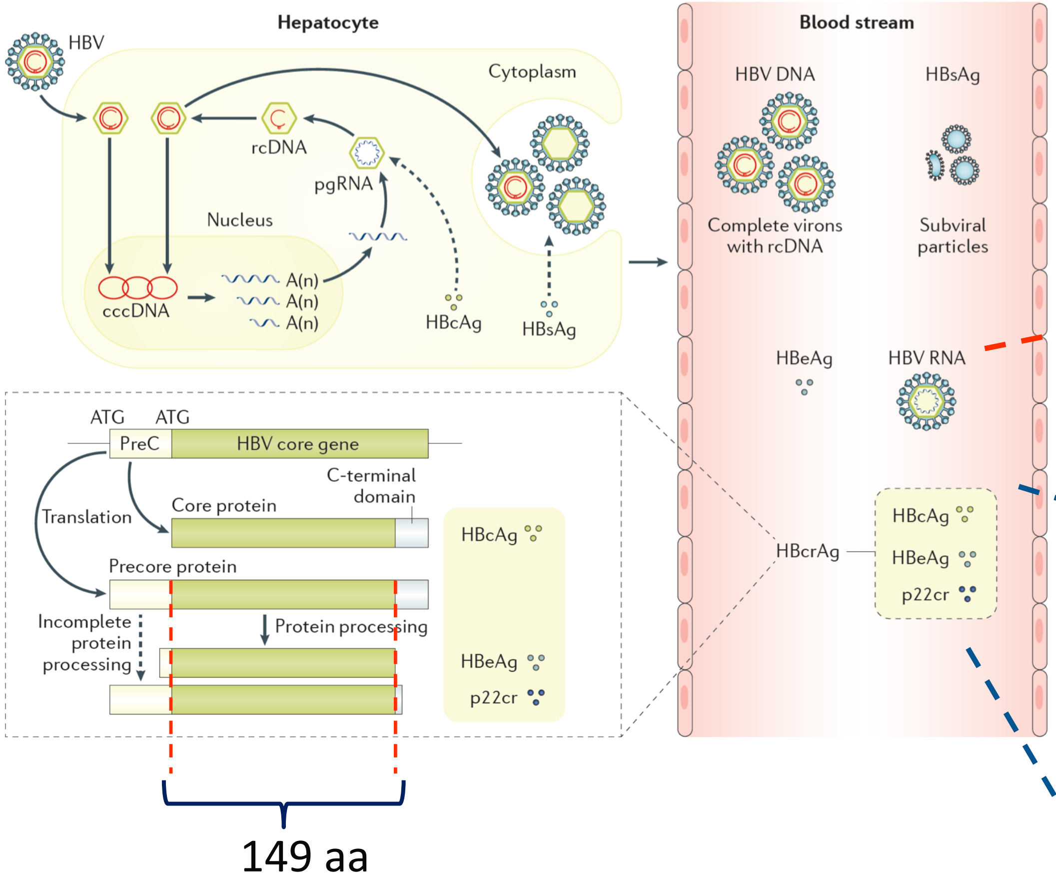
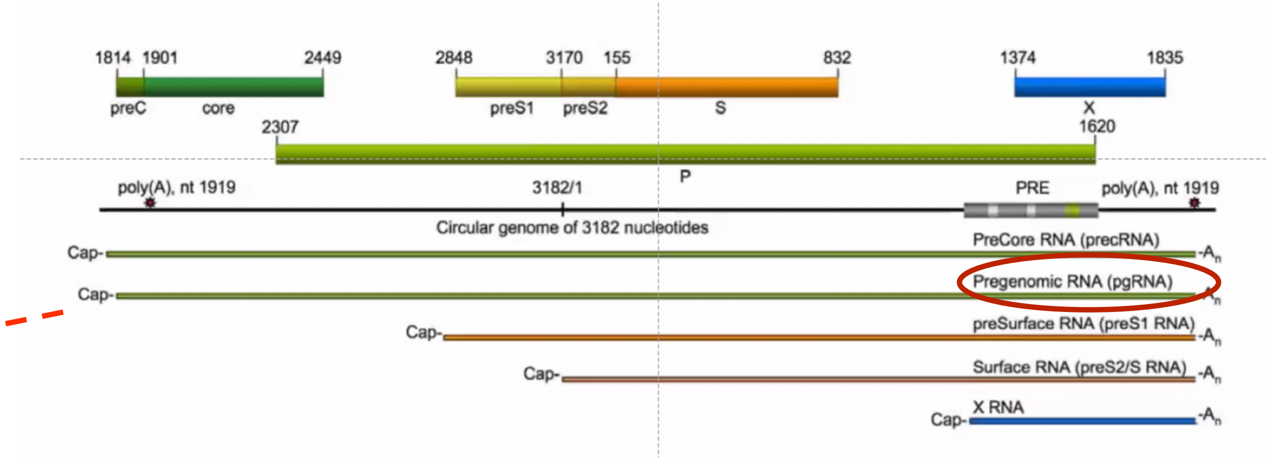
What are ideal biomarkers ?

- Non-invasive
- Able to stratify disease stages and risk of complications
- Reflect intrahepatic virologic status e.g. cccDNA content
- Predictive of cure including functional and complete cure
- Assist identification of treatment response including new agents
  - Proof of concept of modes of action
- Assist patient management including
  - Treatment initiation
  - Combination of treatment
  - Treatment cessation

Conventional and new serum biomarkers

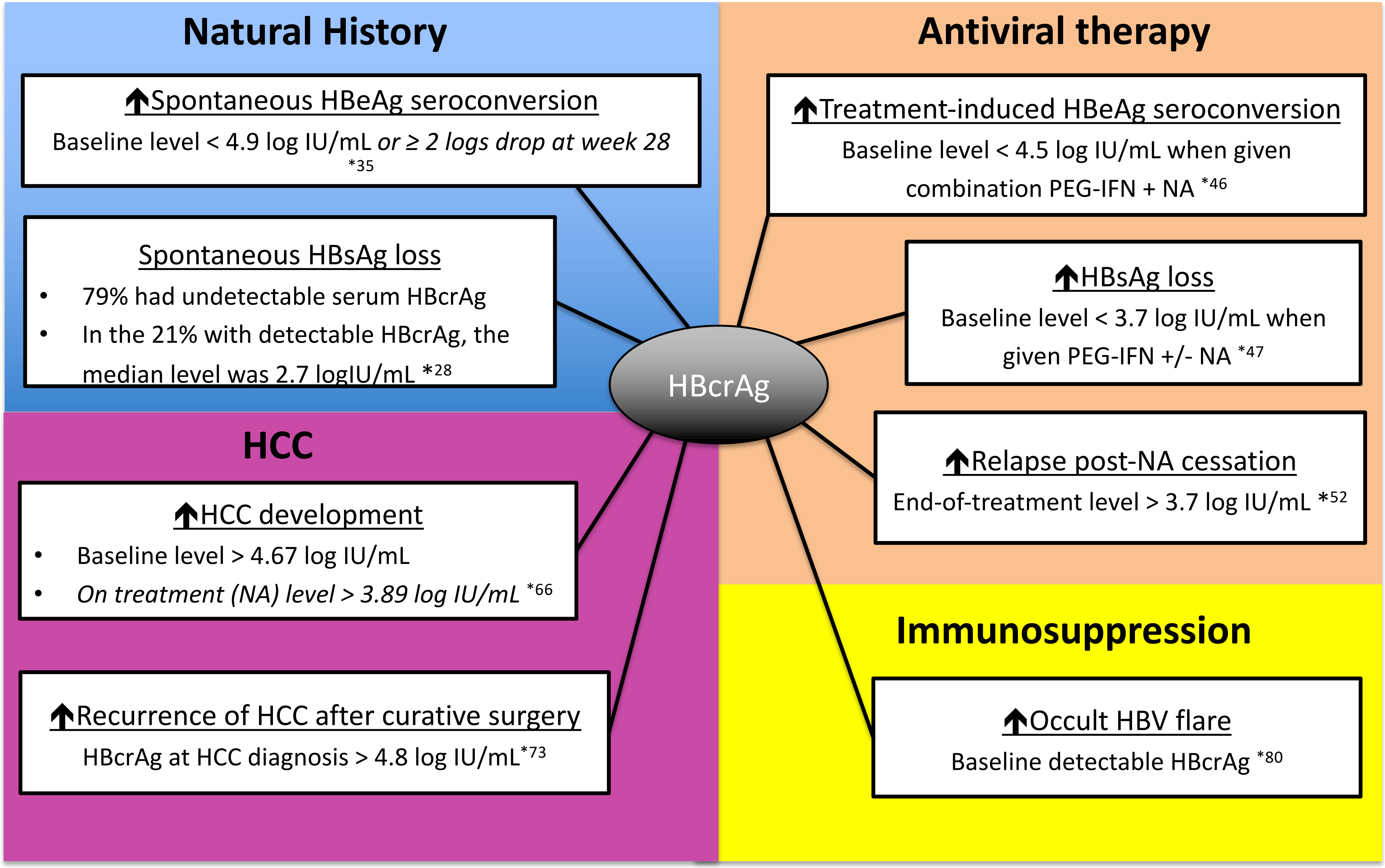


Yuen MF et al., Nat Rev Dis Primers. 2018;4:18035



Sommer G & Heise T. Front Biosci 2008;13:5533-47

Multifunctional roles of HBcrAg in chronic hepatitis B



Natural History

↑Spontaneous HBeAg seroconversion

Baseline level < 4.9 log IU/mL *or ≥ 2 logs drop at week 28* [*35]

Spontaneous HBsAg loss

- 79% had undetectable serum HBcrAg
- In the 21% with detectable HBcrAg, the median level was 2.7 logIU/mL [*28]

Antiviral therapy

↑Treatment-induced HBeAg seroconversion

Baseline level < 4.5 log IU/mL when given combination PEG-IFN + NA [*46]

↑HBsAg loss

Baseline level < 3.7 log IU/mL when given PEG-IFN +/- NA [*47]

↑Relapse post-NA cessation

End-of-treatment level > 3.7 log IU/mL [*52]

HBcrAg

HCC

↑HCC development

- Baseline level > 4.67 log IU/mL
- *On treatment (NA) level > 3.89 log IU/mL* [*66]

↑Recurrence of HCC after curative surgery

HBcrAg at HCC diagnosis > 4.8 log IU/mL[*73]

Immunosuppression

↑Occult HBV flare

Baseline detectable HBcrAg [*80]

Mak LY… Yuen MF. Aliment Pharmacol Ther 2018;47(1):43-54

HBcrAg in chronic hepatitis B disease

- Natural history of CHB
- Correlations with other established markers
- Relationship with HBeAg seroconversion and long-term complications
- Treatment effect
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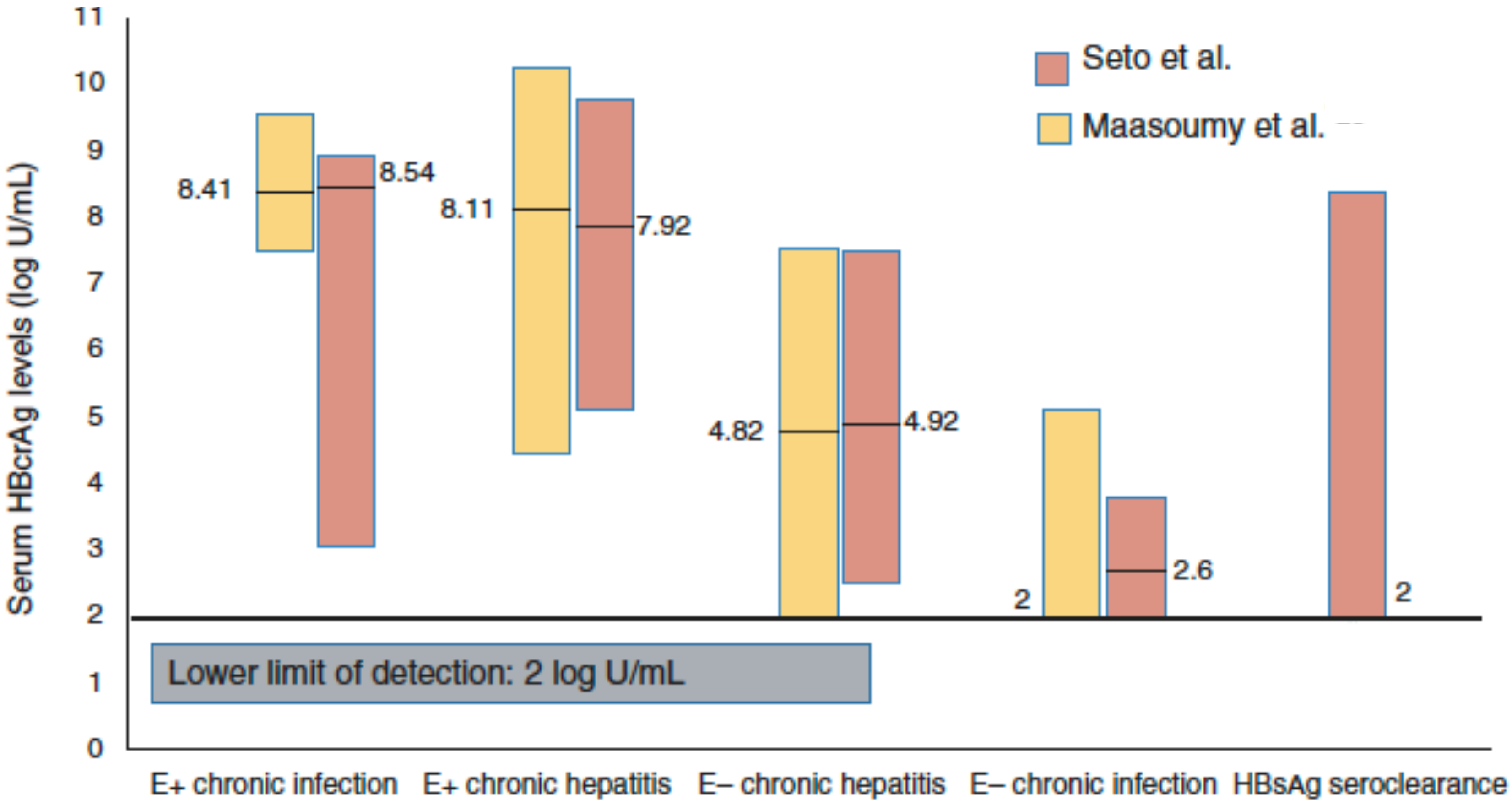
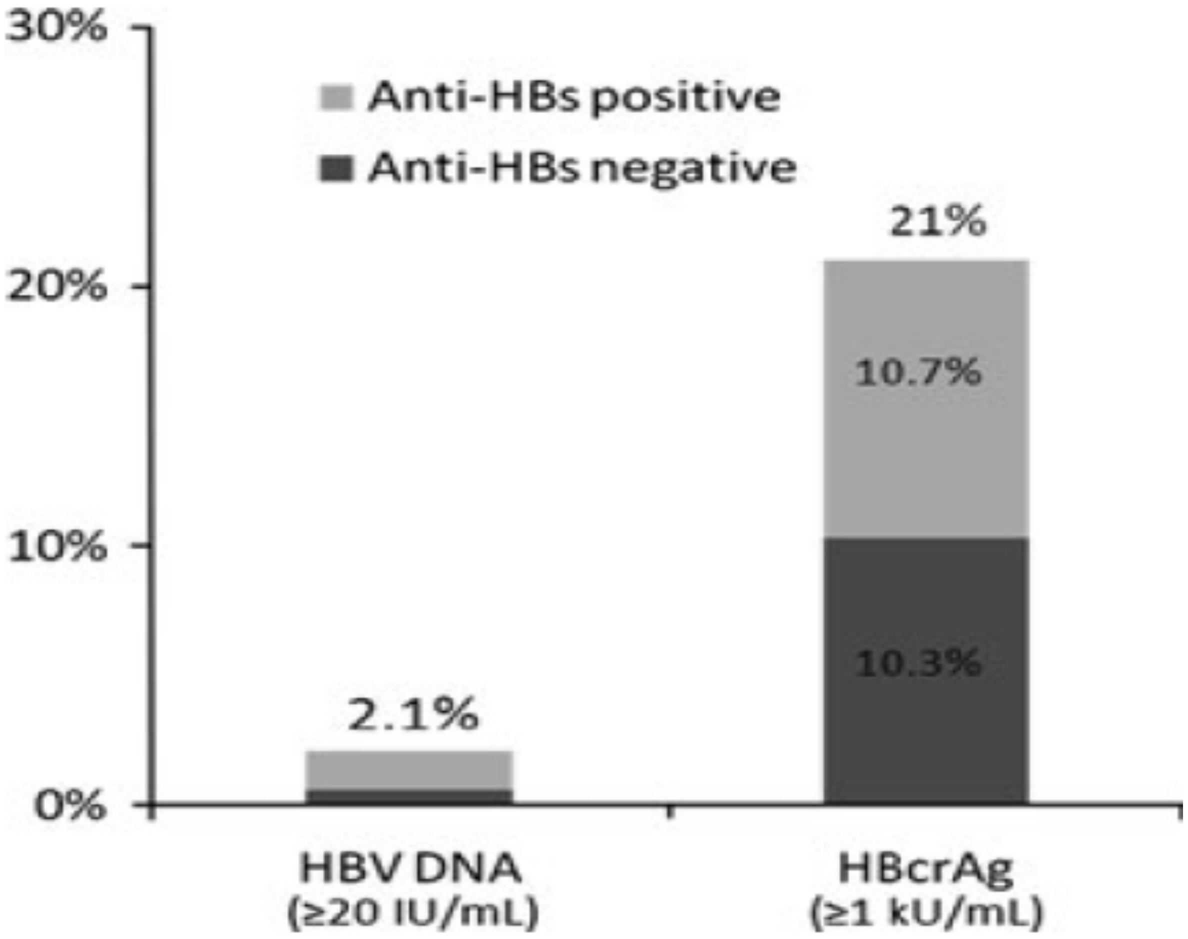
HBcrAg in natural history of chronic hepatitis B



Mak LY… Yuen MF. Aliment Pharmacol Ther 2018;47(1):43-54

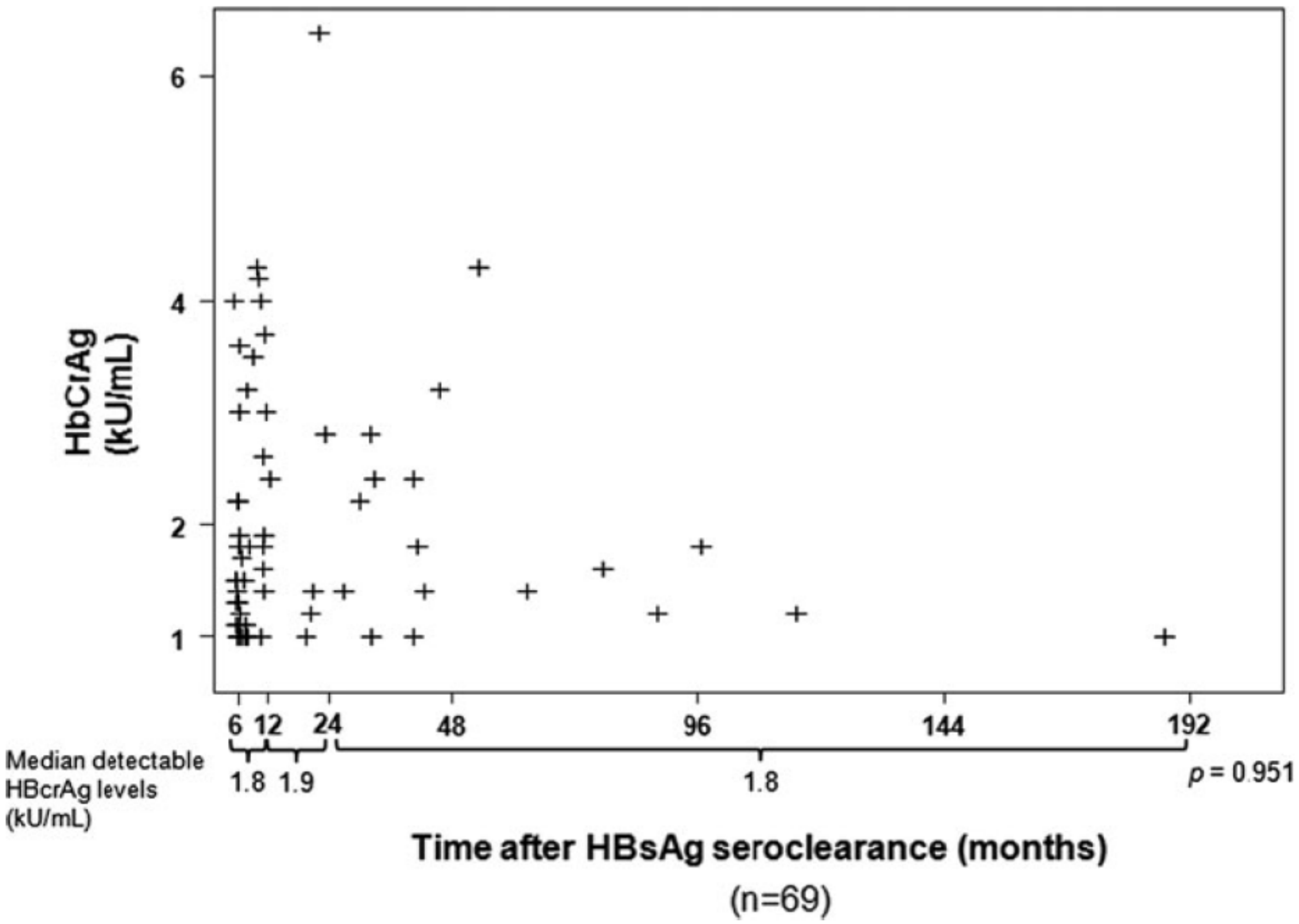
HBcrAg in chronic hepatitis B with HBsAg seroclearance

Patient population: 329 patients with HBsAg seroclearance



Seto WK… Yuen MF. Hepatol Int 2013;7(1):98-105

Detectable HBcrAg over long time after HBsAg seroclearance



Seto WK… Yuen MF. Hepatol Int 2013;7(1):98-105

HBcrAg in chronic hepatitis B disease

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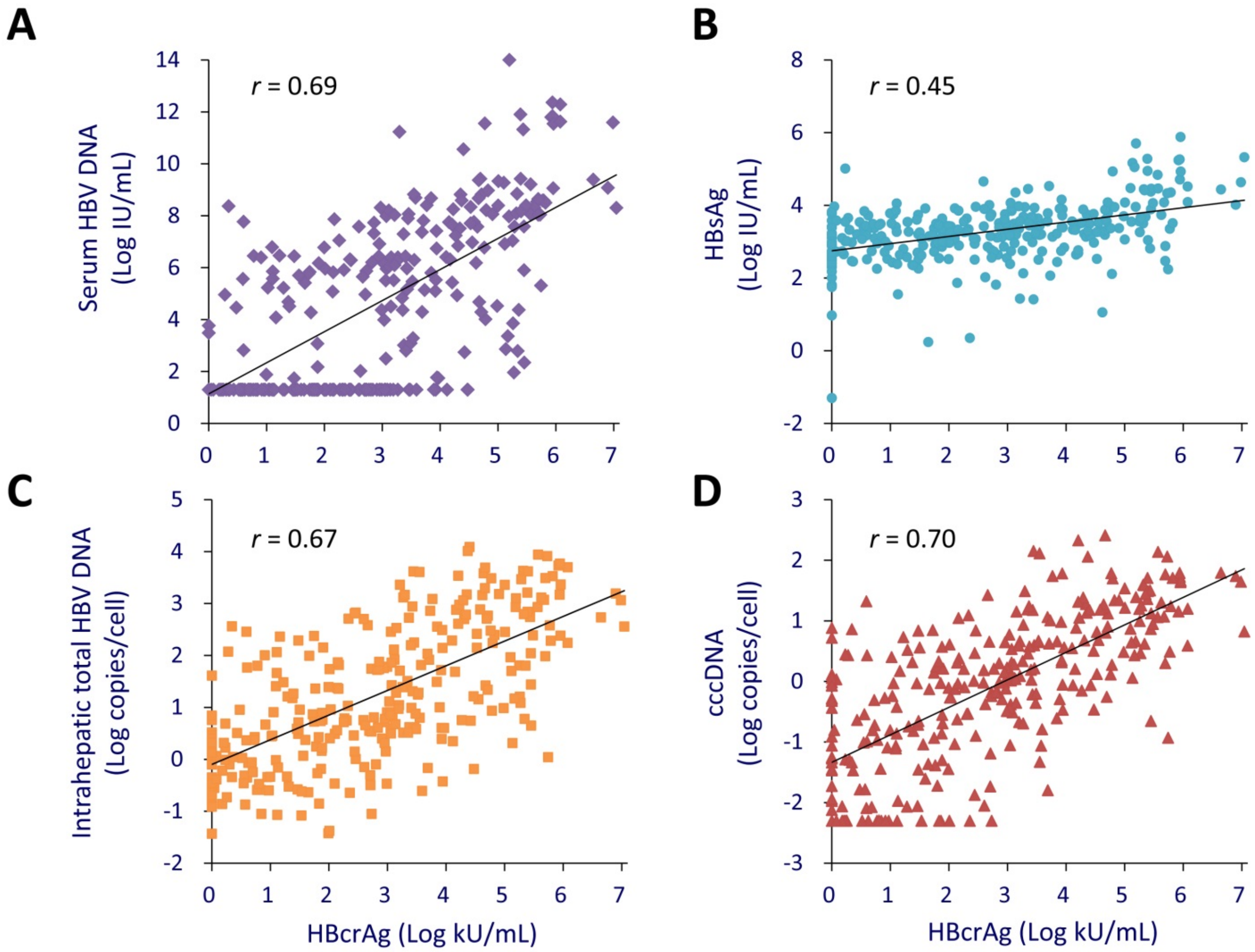
Correlation between HBcrAg and serum HBV DNA, HBsAg, intrahepatic total HBV DNA, cccDNA

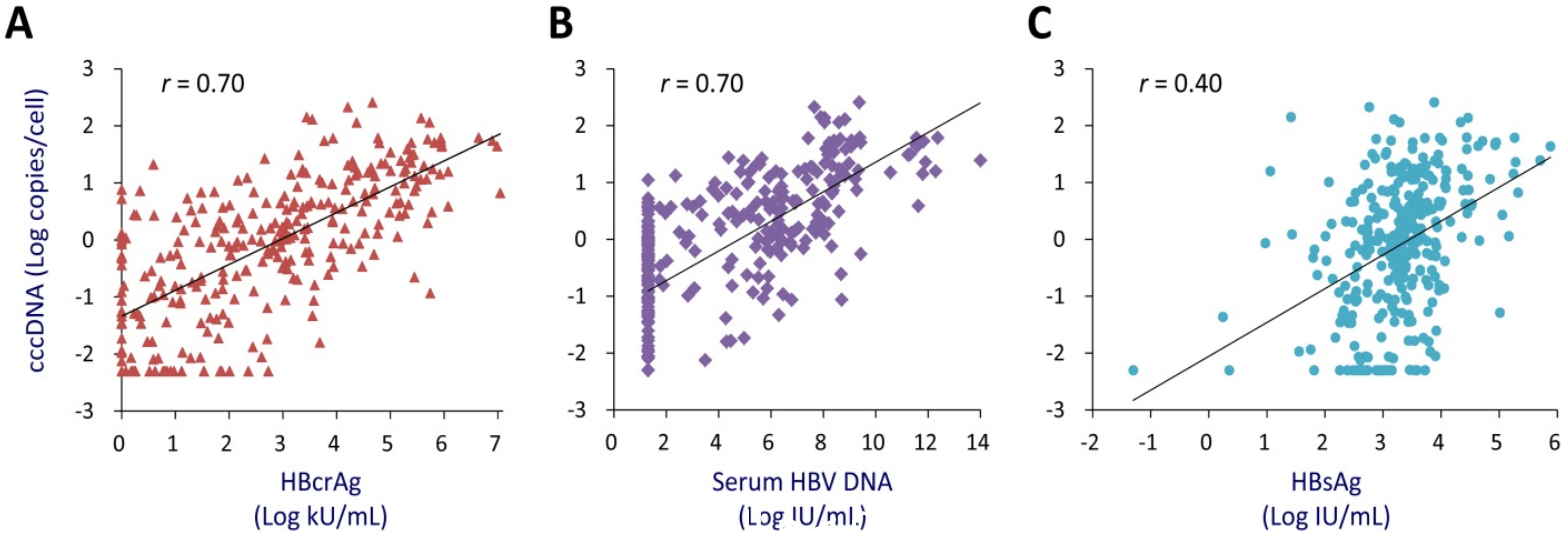
138 patients: 56% HBeAg +ve

- 305 liver samples with corresponding serum samples



Wong DK…Yuen MF. Liver Int 2017;37(7):995-1001

Correlation between cccDNA and serum HBcrAg, HBV DNA, HBsAg



Samples with undetectable serum HBV DNA
- 78% still have detectable HBcrAg
  - HBcrAg still correlated with cccDNA in the liver (r=0.42, p<0.0001)

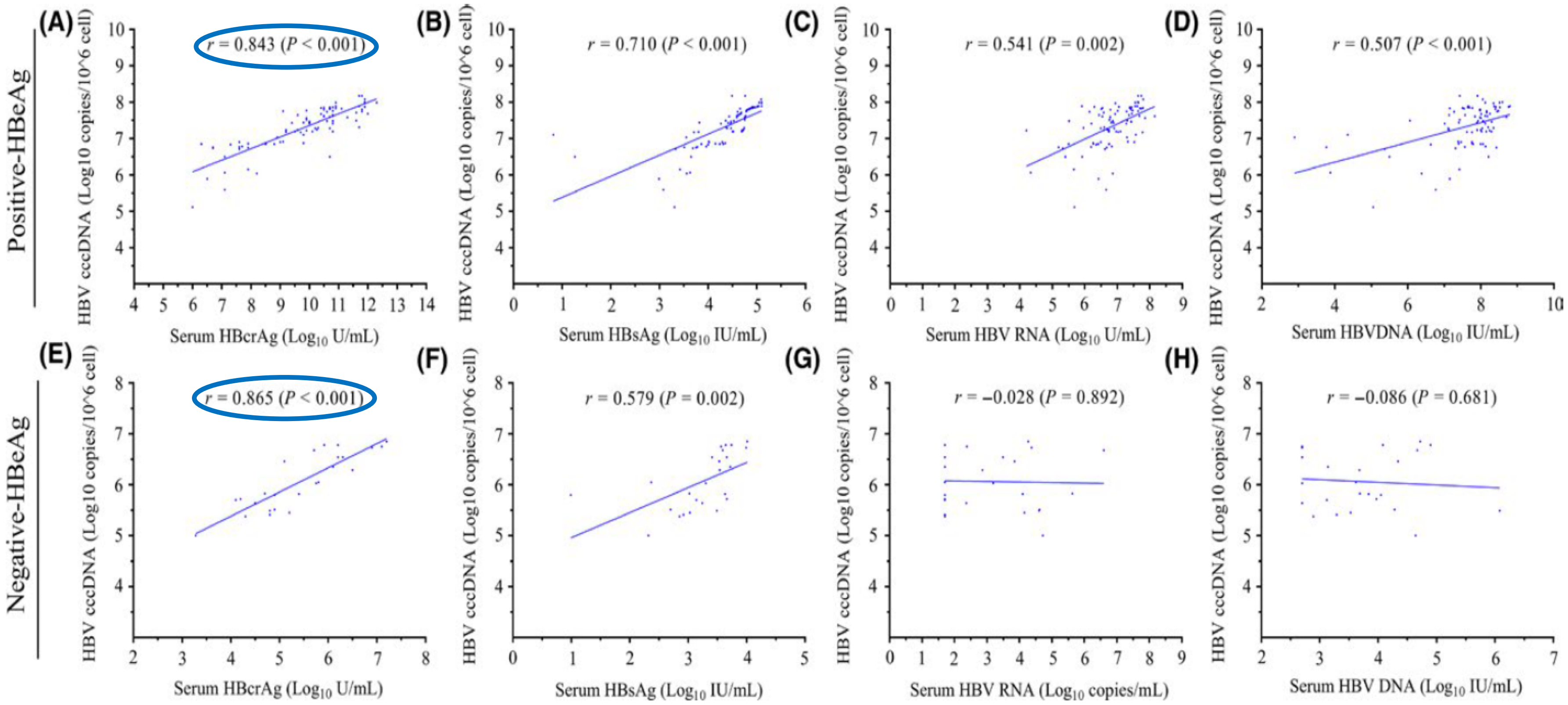
Wong DK…Yuen MF. Liver Int 2017;37(7):995-1001

Correlation between cccDNA and HBcrAg, HBV DNA, HBsAg & HBV RNA

110 treatment naïve patients

- 85 HBeAg +ve
- 25 HBeAg –ve

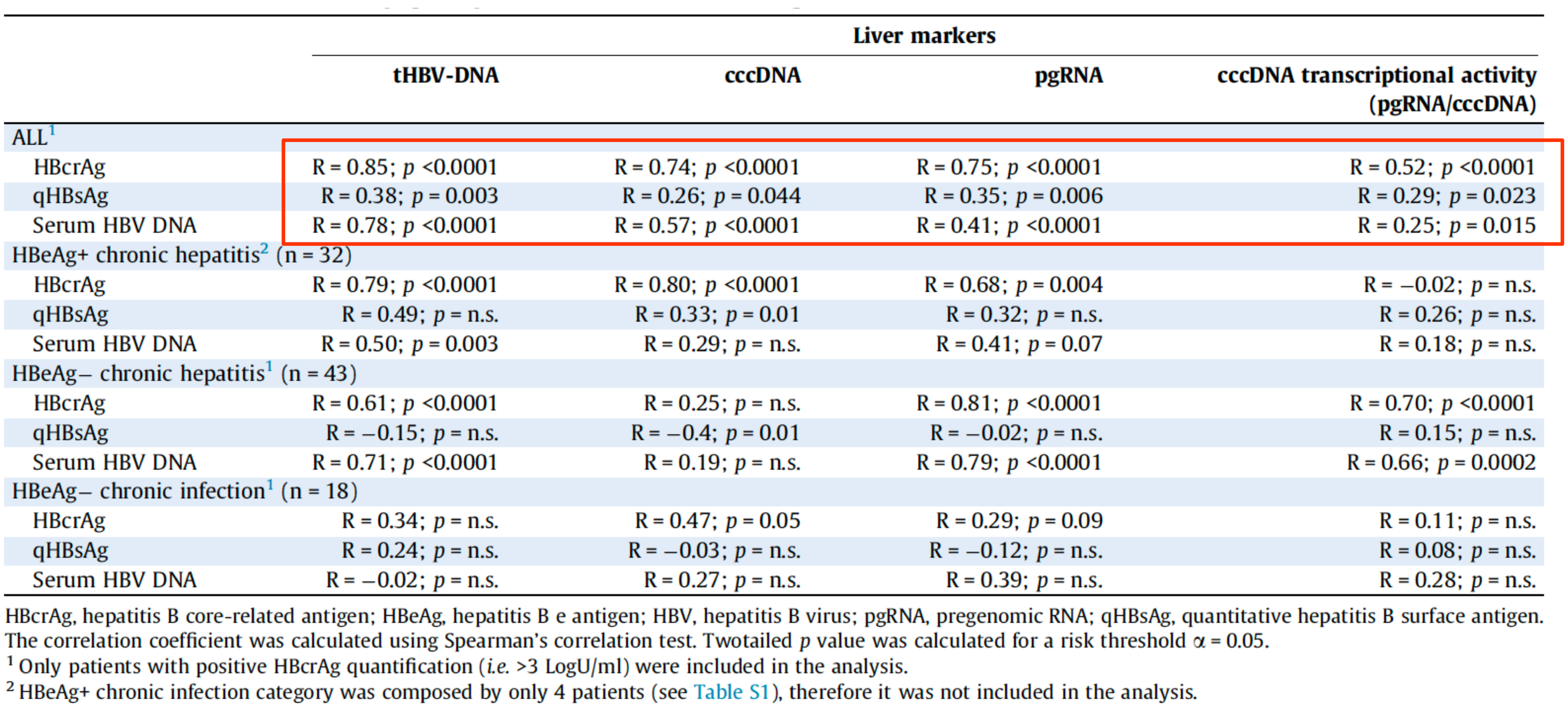
HBcrAg HBsAg HBV RNA HBV DNA



Chen EQ et al., J Viral Hepat 2019;26:586-95

Correlation between serum HBcrAg, HBsAg, HBV DNA and intrahepatic viral markers

Study population: 130 patients (36 HBeAg +ve; 94 HBeAg –ve)



	Liver markers			
	tHBV-DNA	**cccDNA**	**pgRNA**	**cccDNA transcriptional activity (pgRNA/cccDNA)**
ALL[1]				
HBcrAg	R = 0.85; $p < 0.0001$	R = 0.74; $p < 0.0001$	R = 0.75; $p < 0.0001$	R = 0.52; $p < 0.0001$
qHBsAg	R = 0.38; $p = 0.003$	R = 0.26; $p = 0.044$	R = 0.35; $p = 0.006$	R = 0.29; $p = 0.023$
Serum HBV DNA	R = 0.78; $p < 0.0001$	R = 0.57; $p < 0.0001$	R = 0.41; $p < 0.0001$	R = 0.25; $p = 0.015$
HBeAg+ chronic hepatitis[2] (n = 32)				
HBcrAg	R = 0.79; $p < 0.0001$	R = 0.80; $p < 0.0001$	R = 0.68; $p = 0.004$	R = –0.02; p = n.s.
qHBsAg	R = 0.49; p = n.s.	R = 0.33; $p = 0.01$	R = 0.32; p = n.s.	R = 0.26; p = n.s.
Serum HBV DNA	R = 0.50; $p = 0.003$	R = 0.29; p = n.s.	R = 0.41; $p = 0.07$	R = 0.18; p = n.s.
HBeAg– chronic hepatitis[1] (n = 43)				
HBcrAg	R = 0.61; $p < 0.0001$	R = 0.25; p = n.s.	R = 0.81; $p < 0.0001$	R = 0.70; $p < 0.0001$
qHBsAg	R = –0.15; p = n.s.	R = –0.4; $p = 0.01$	R = –0.02; p = n.s.	R = 0.15; p = n.s.
Serum HBV DNA	R = 0.71; $p < 0.0001$	R = 0.19; p = n.s.	R = 0.79; $p < 0.0001$	R = 0.66; $p = 0.0002$
HBeAg– chronic infection[1] (n = 18)				
HBcrAg	R = 0.34; p = n.s.	R = 0.47; $p = 0.05$	R = 0.29; $p = 0.09$	R = 0.11; p = n.s.
qHBsAg	R = 0.24; p = n.s.	R = –0.03; p = n.s.	R = –0.12; p = n.s.	R = 0.08; p = n.s.
Serum HBV DNA	R = –0.02; p = n.s.	R = 0.27; p = n.s.	R = 0.39; p = n.s.	R = 0.28; p = n.s.

HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; pgRNA, pregenomic RNA; qHBsAg, quantitative hepatitis B surface antigen. The correlation coefficient was calculated using Spearman's correlation test. Twotailed p value was calculated for a risk threshold $\alpha = 0.05$.

[1] Only patients with positive HBcrAg quantification (*i.e.* >3 LogU/ml) were included in the analysis.

[2] HBeAg+ chronic infection category was composed by only 4 patients (see Table S1), therefore it was not included in the analysis.

Testoni B et al., J Hepatol 2019;70:615-25

HBcrAg in chronic hepatitis B disease

- Natural history of CHB
- Correlations with other established markers
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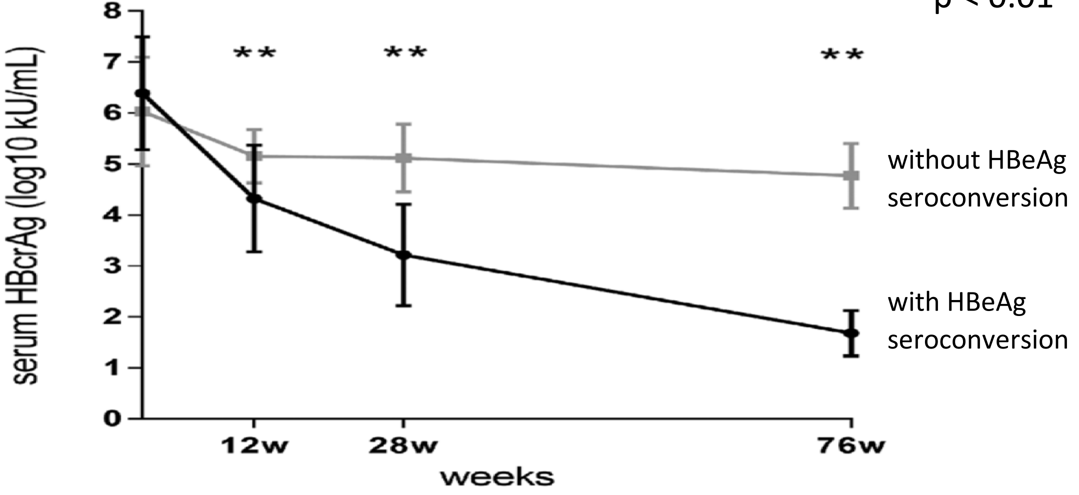
- Role in special populations

HBcrAg and spontaneous and Nuc-induced HBeAg seroconversion

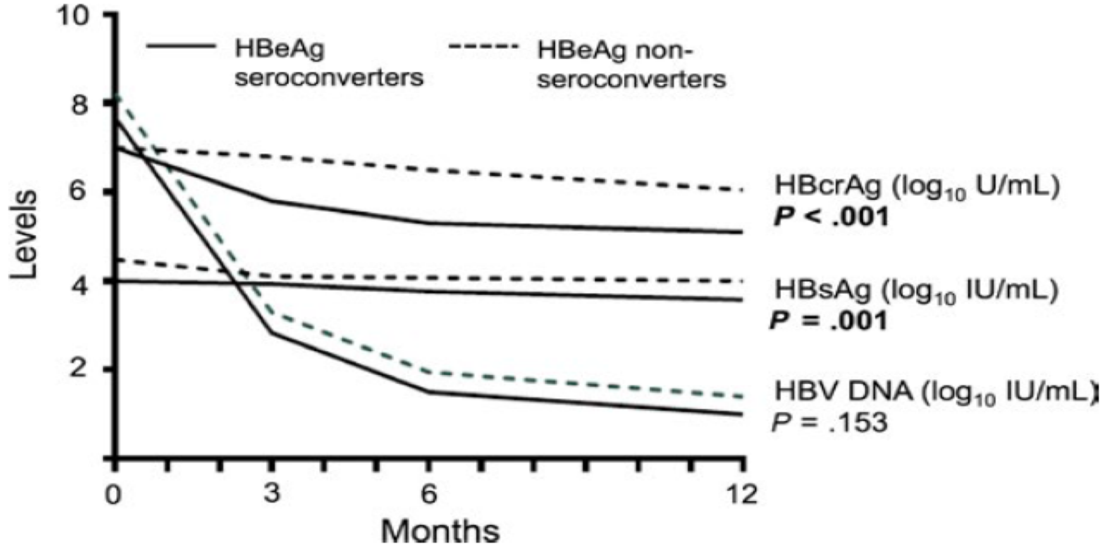
Patients without treatment



** $p < 0.01$

Song et al. J Med Virol 2017;89:463-468

Patients on nucleos(t)ide analogue

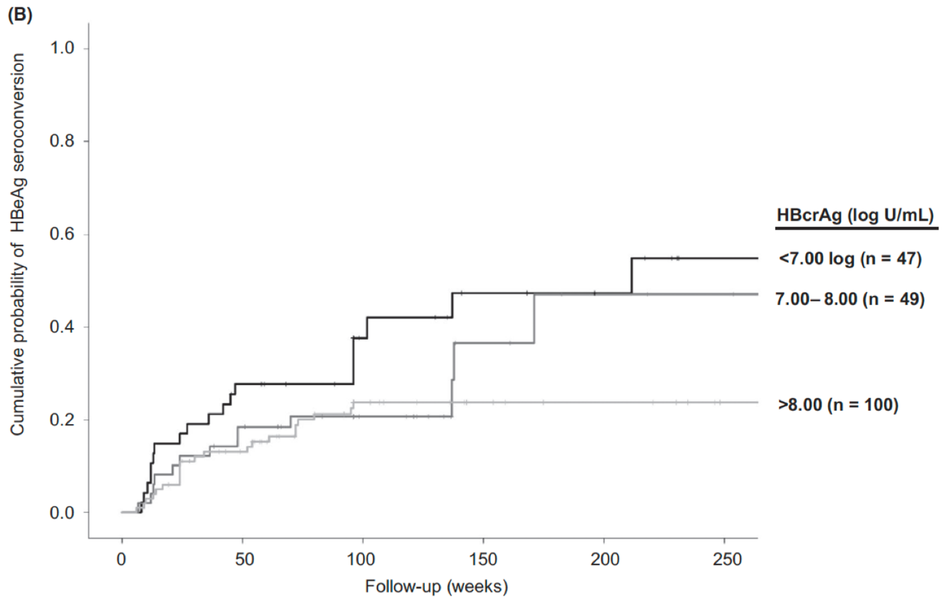


Wang B et al., J Viral Hepat 2018;25(8):886-893

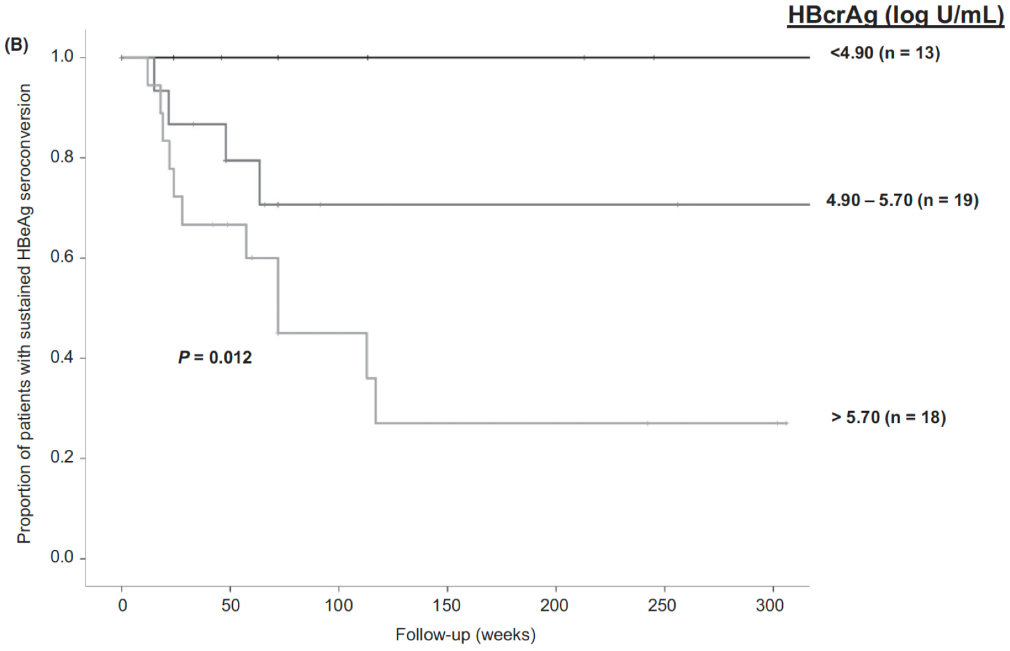
HBcrAg and Nuc-induced and sustained HBeAg seroconversion

196 HBeAg +ve patients on NUCs
55 achieved HBeAg seroconversion over a median of 34 weeks

<u>Baseline HBcrAg level</u>
with HBeAg seroconversion (7.42 log U/mL) vs. without HBeAg seroconversion (7.86 log U/mL) p = 0.017



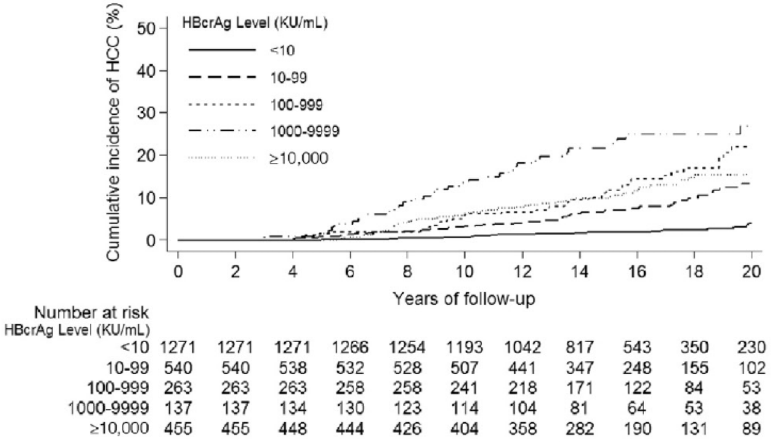
<u>Lower HBcrAg levels at HBeAg seroconversion</u>
high chance of sustained HBeAg seroconversion after stopping of NUCs



Sonneveld MJ et al., J Viral Hepat 2019;26:828-34

HBcrAg and HCC development

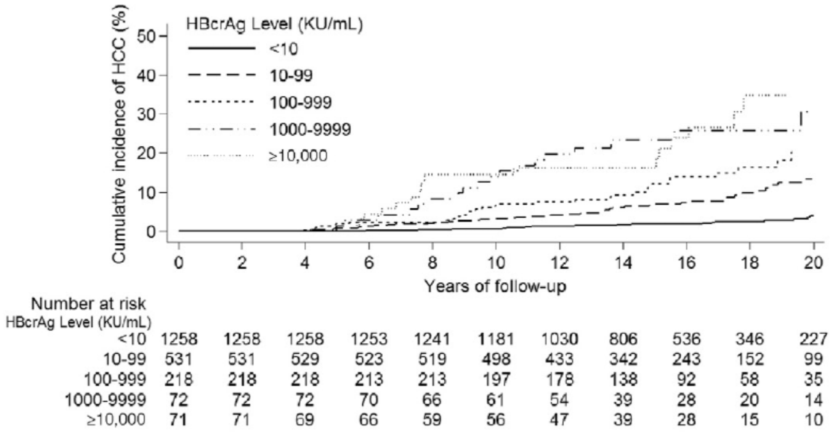
Whole population n=2,666



Number at risk

HBcrAg Level (KU/mL)	0	2	4	6	8	10	12	14	16	18	20
<10	1271	1271	1271	1266	1254	1193	1042	817	543	350	230
10-99	540	540	538	532	528	507	441	347	248	155	102
100-999	263	263	263	258	258	241	218	171	122	84	53
1000-9999	137	137	134	130	123	114	104	81	64	53	38
≥10,000	455	455	448	444	426	404	358	282	190	131	89

HBeAg –ve n=2,150

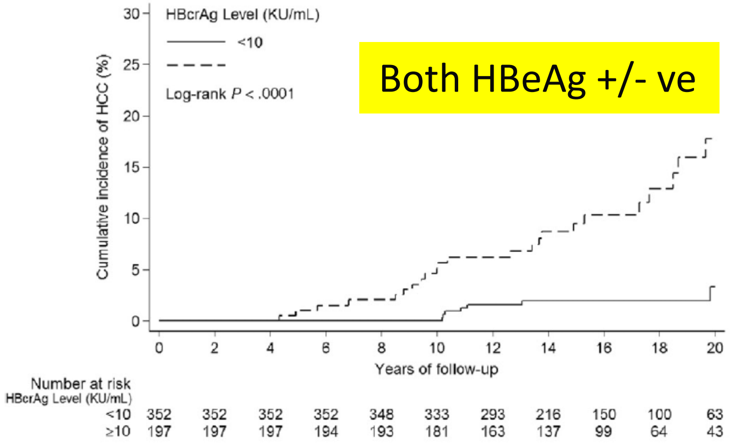


Number at risk

HBcrAg Level (KU/mL)	0	2	4	6	8	10	12	14	16	18	20
<10	1258	1258	1258	1253	1241	1181	1030	806	536	346	227
10-99	531	531	529	523	519	498	433	342	243	152	99
100-999	218	218	218	213	213	197	178	138	92	58	35
1000-9999	72	72	72	70	66	61	54	39	28	20	14
≥10,000	71	71	69	66	59	56	47	39	28	15	10

Overall, increasing levels of HBcrAg associated with higher risk of HCC

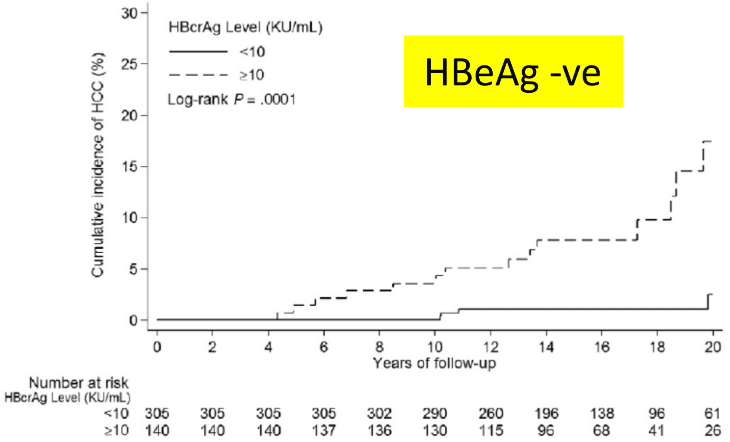
Patients with IVL (HBV DNA 2,000 – 19,999 IU/mL)



Both HBeAg +/- ve

Number at risk

HBcrAg Level (KU/mL)	0	2	4	6	8	10	12	14	16	18	20
<10	352	352	352	352	348	333	293	216	150	100	63
≥10	197	197	197	194	193	181	163	137	99	64	43



HBeAg -ve

Number at risk

HBcrAg Level (KU/mL)	0	2	4	6	8	10	12	14	16	18	20
<10	305	305	305	305	302	290	260	196	138	96	61
≥10	140	140	140	137	136	130	115	96	68	41	26

Among patients with IVL, HBcrAg > 10 KU/mL significantly associated with a higher risk of HCC

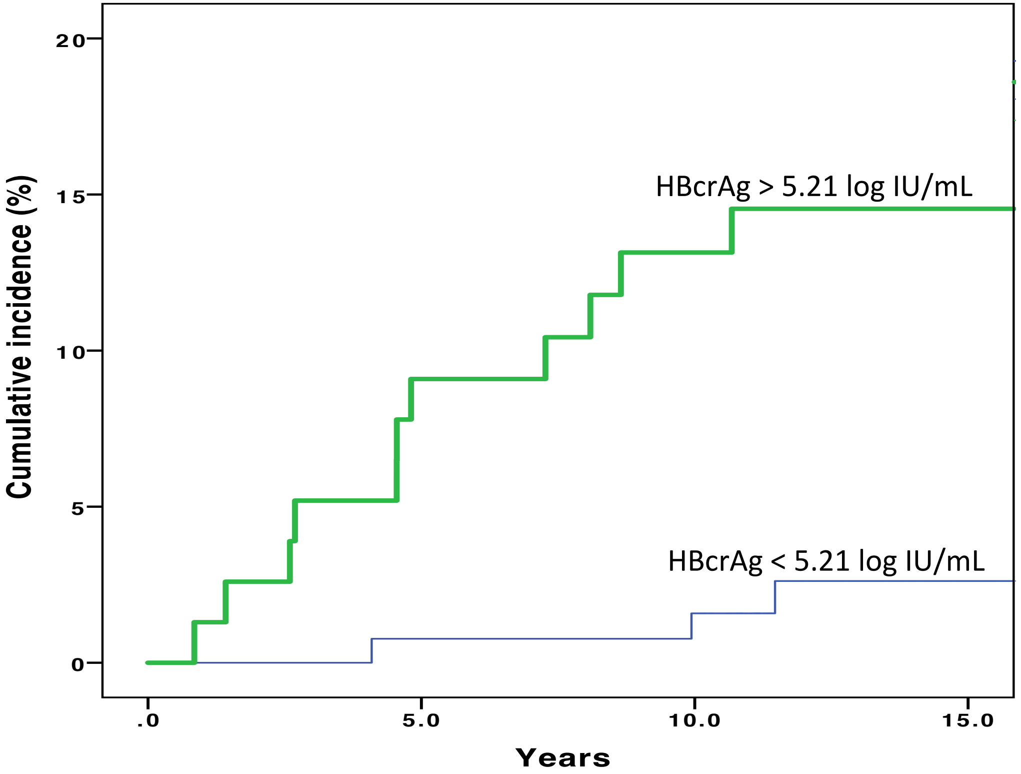
Tseng TC et al. Gastroenterology 2019;157:1518-29

HBcrAg after spontaneous HBeAg seroconversion: HCC development

- 207 CHB with documented time of HBeAg seroconversion
- HBcrAg and HBsAg measured within 3 years after HBeAg seroconversion

Multivariable analysis

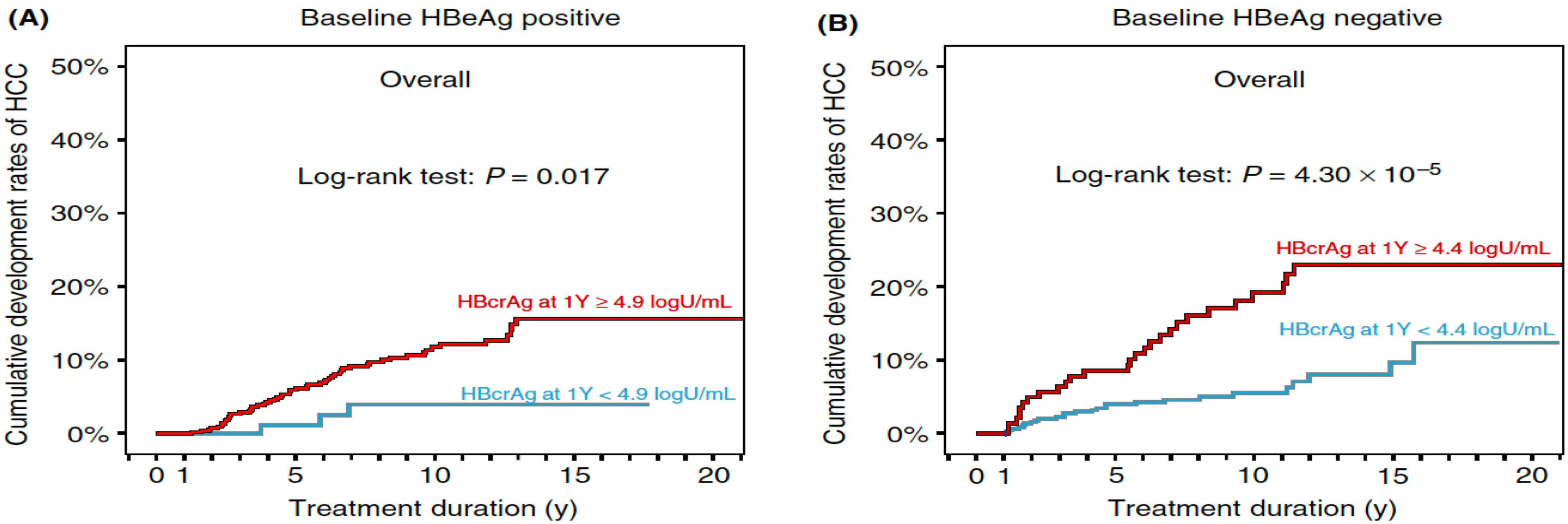
	Hazard ratio	95% CI	p value
Age (>40 years old)	4.60	1.00-21.04	0.049
Baseline cirrhosis	6.23	1.87-20.72	0.003
HBcrAg (log IU/ml)	1.75	1.06-2.90	0.032



To WP ... Yuen MF. J Viral Hepat 2019 26:1473-80

HBcrAg at year 1 of NUC treatment and HCC

Study population: 1,268 patients on Nucs (667 HBeAg +ve; 601 HBeAg –ve)

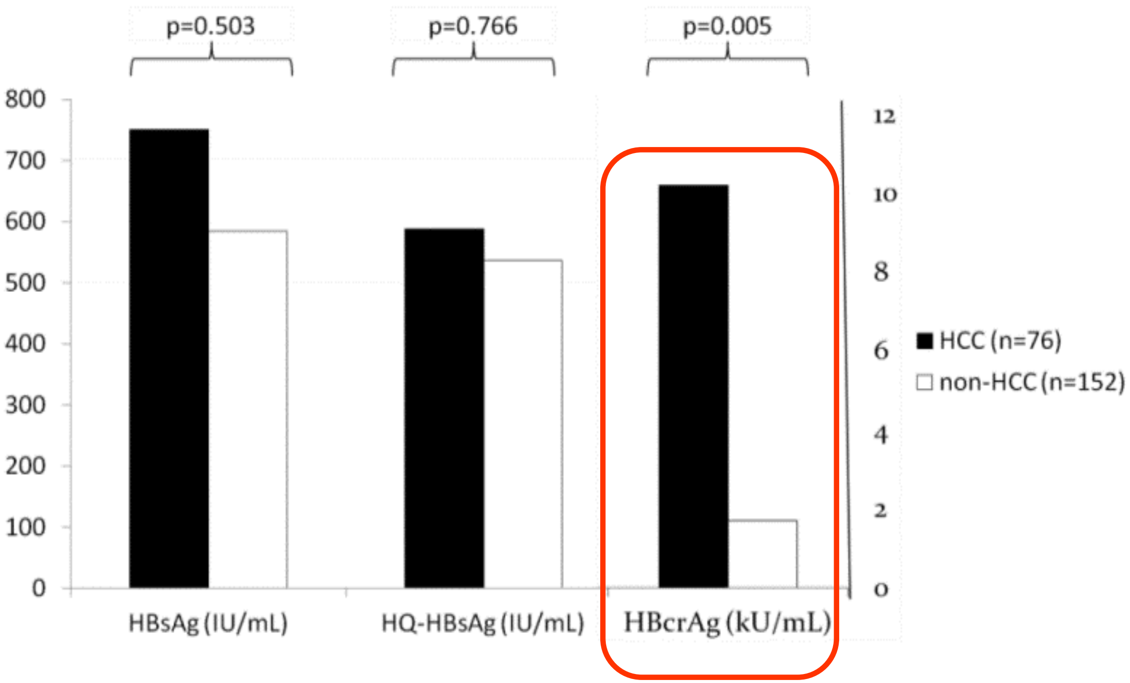


Hosaka et al., Aliment Pharmacol Ther 2019;49:457-71

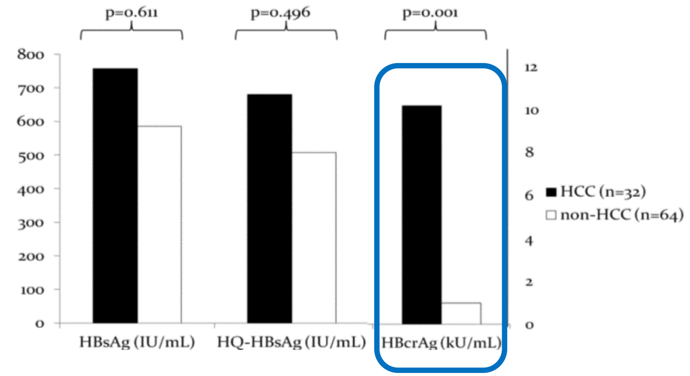
HBcrAg and HCC in NUC-treated patients with undetectable HBV DNA

Study population: 228 patients on Nucs

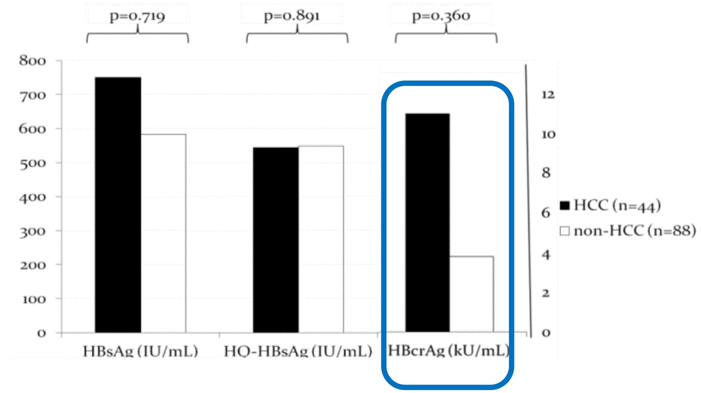
- 76 patients with HCC vs. 152 without HCC



Patients without cirrhosis



Patients with cirrhosis



Cheung KS…Yuen MF J Viral Hepat 2017:24(8):654-61

HBcrAg in chronic hepatitis B disease

- Natural history of CHB
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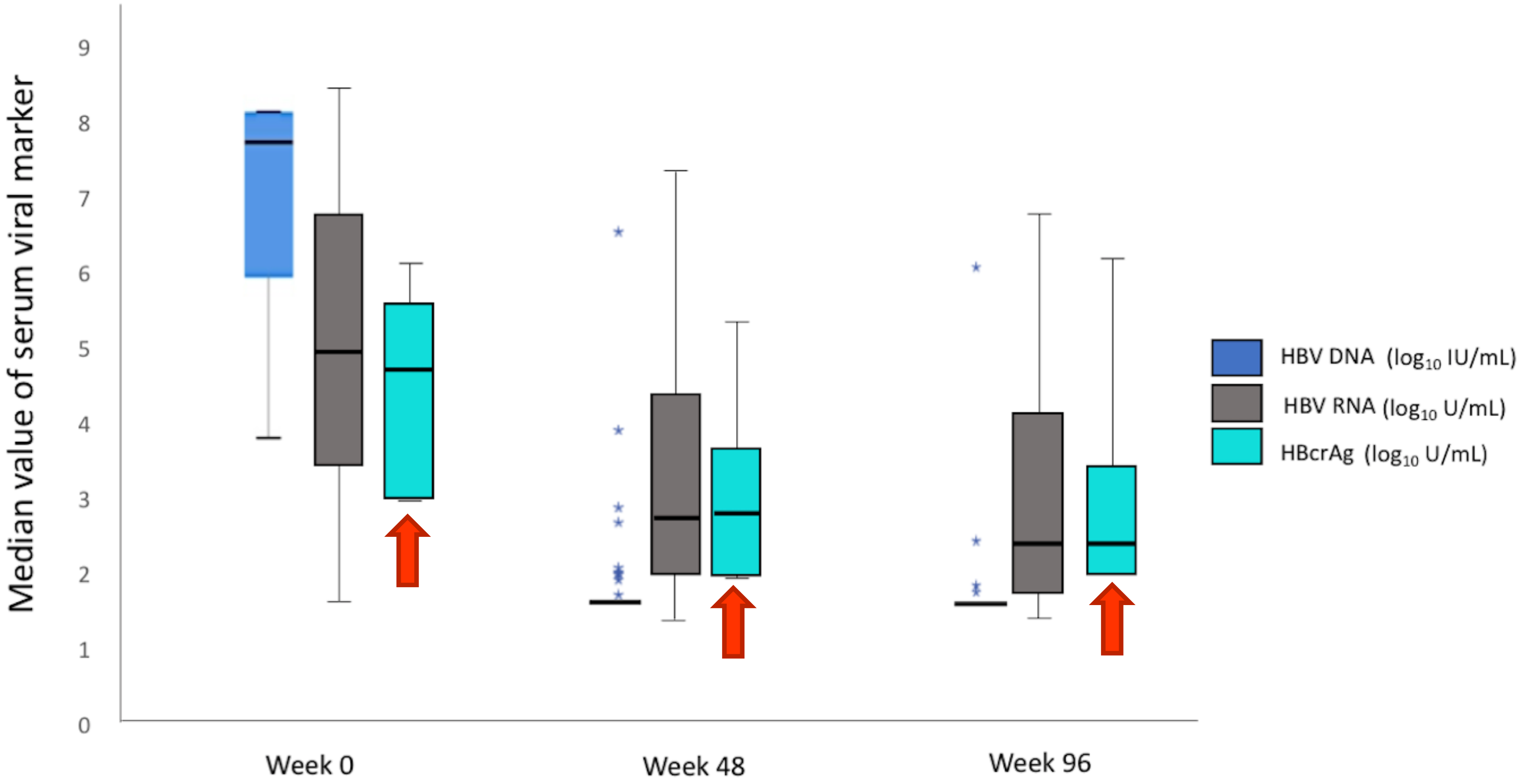
HBcrAg levels on 2-year TDF/ TAF/ ETV treatment



Mak LY…Yuen MF, Hepatology (in press)

HBcrAg levels on long-term entecavir treatment

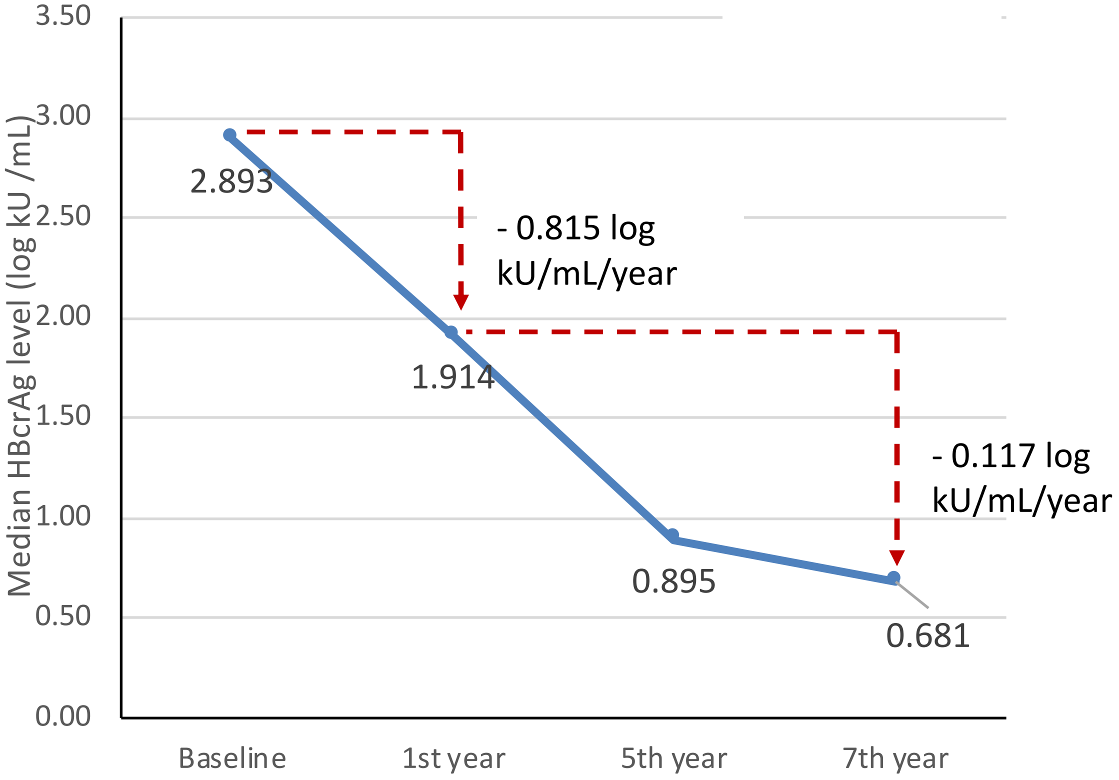
Treatment - naïve Chinese CHB patients treated for entecavir in the real world setting for up to 7 years

HBcrAg measurement at baseline, year 1, 5 and 7

Annual decline: 0.244 log KU/mL/year (p = 0.001)

Median levels:

baseline 2.8 Ku/mL
1st year 1.9 Ku/mL
5th year 0.9 Ku/mL
7th year 0.7 Ku/mL



Lam YF… Yuen MF. Clin Transl Gastroenterol 2017;8:e125

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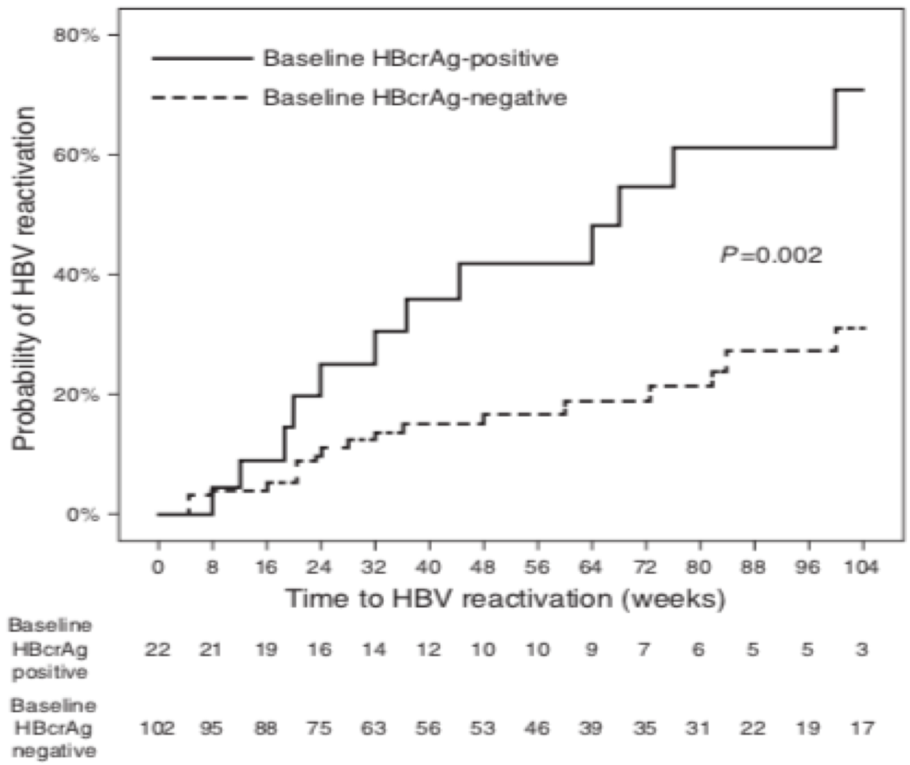
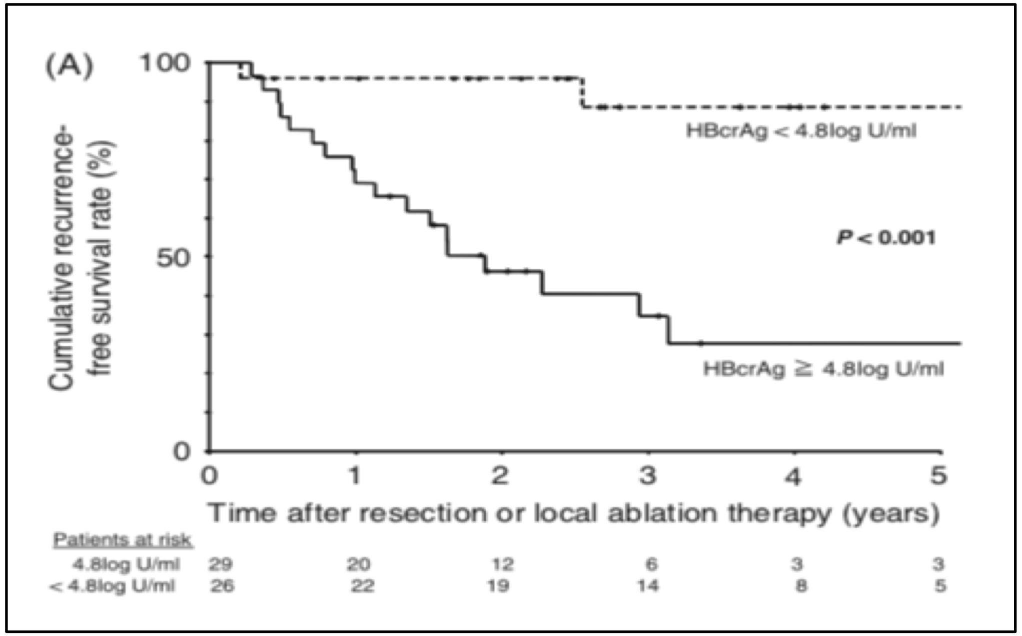
Role of HBcrAg in special populations

Predicts risk of HBV reactivation in patients receiving rituximab or allogeneic hematopoietic stem cell transplantation



Seto WK et al. Am J Gastroenterol 2016; 111:1788-95

Predicts recurrence free survival after curative treatment of HCC



Hosaka T et al. Liver International 2010; 30:1461-70

Clinical Utility of HBcrAg

- Monitor tool for different phases of CHB
- Detect occult hepatitis B/ CHB with HBsAg seroclearance
- Correlate with the gold standard of viral replication in serum: HBV DNA
- Highly reflect the intrahepatic HBV DNA and cccDNA contents
- Assess treatment efficacy
- Associate with the risk of HCC
- Predict disease outcome for immunosuppressive patients and HCC patients

Different HBV virions and HBV RNA

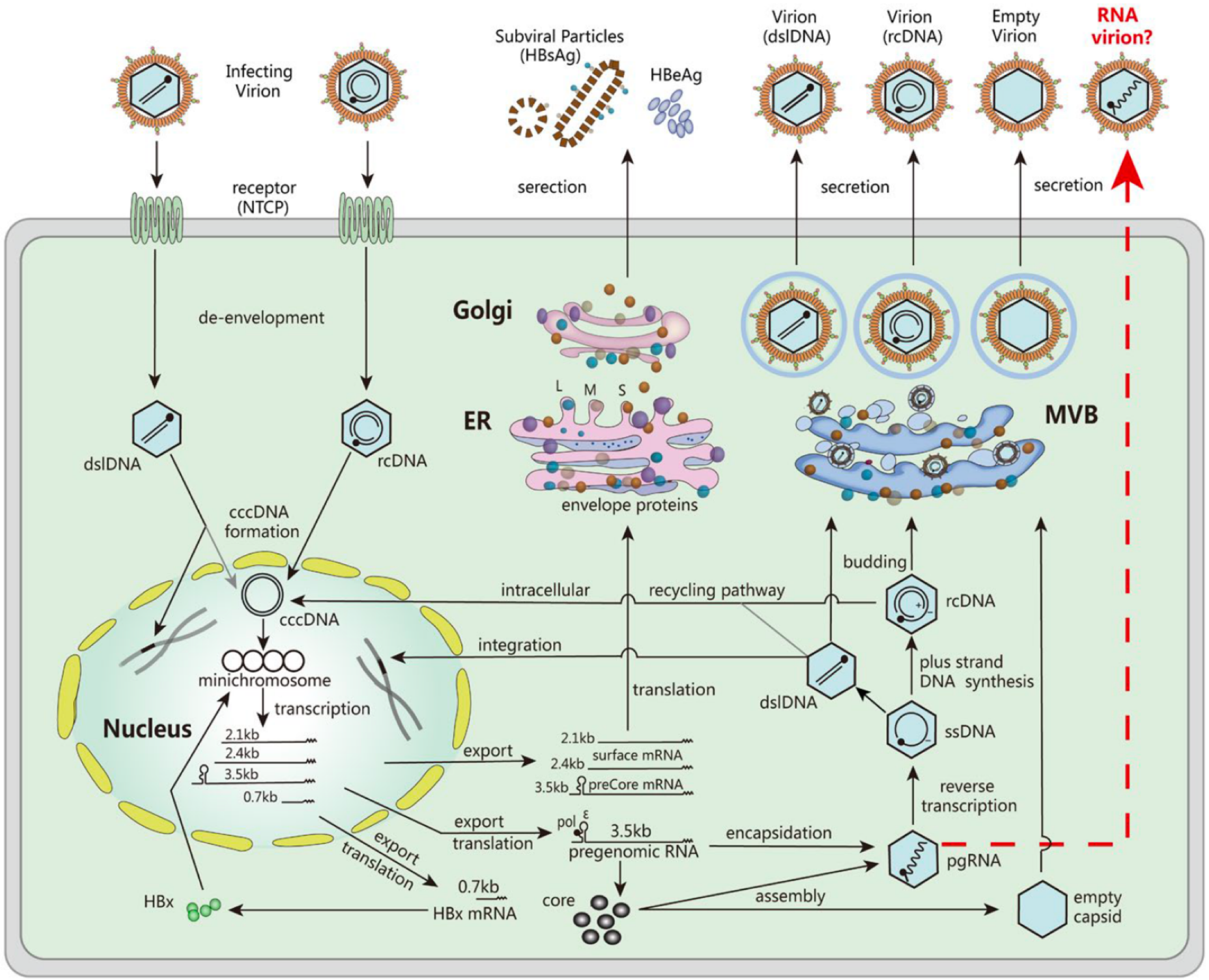


FIG. 1. HBV life cycle. The major steps in the HBV life cycle including entry, de-envelopment, cccDNA formation, mRNA transcription, protein translation, pgRNA encapsidation, DNA replication, viral particle assembly, and secretion are shown. See text for details. Abbreviations: ER, endoplasmic reticulum; L, large surface protein; M, middle surface protein; MVB, multivesicular body; NTCP, sodium taurocholate cotransporting polypeptide; pol, polymerase; S, small surface protein; ssDNA, single-stranded DNA.

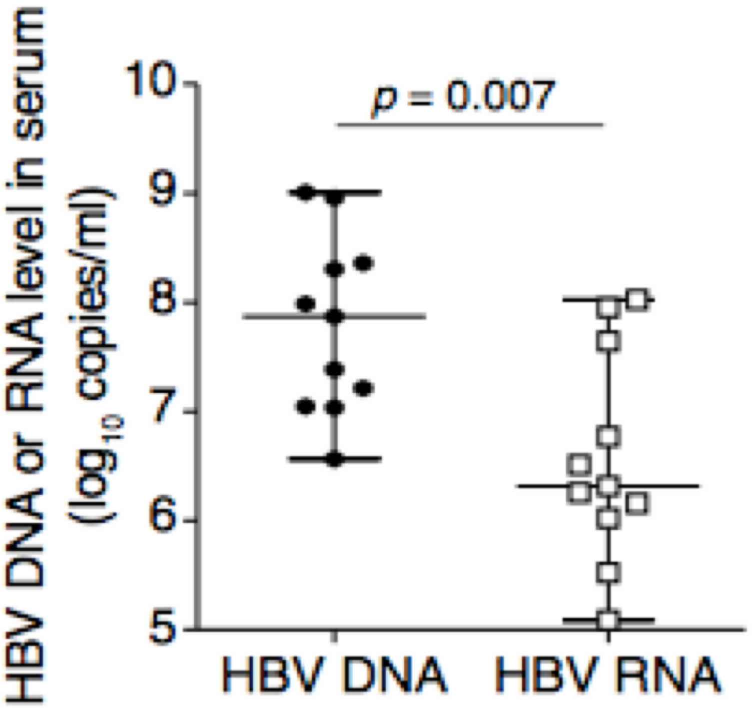
HBV RNAs

- Only produced from cccDNA by transcription.
- pgRNA 3.5 kb (species detected in serum)
- pcRNA 3.5 kb
- surface mRNAs 2.4/2.1 kB
- X mRNA 0.7 kb

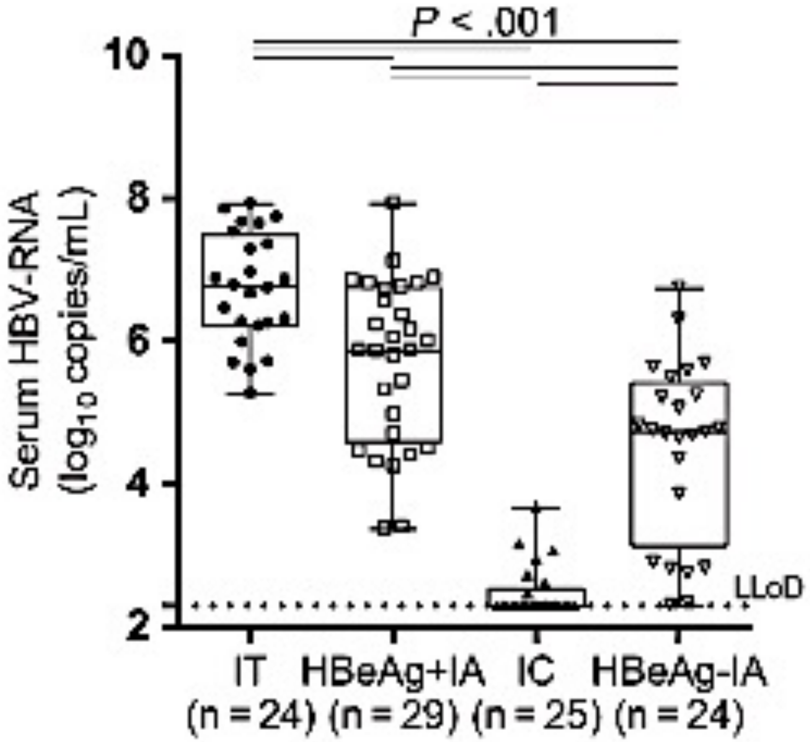
Liu S et al., Hepatology 2019:69:1816-27

EK Butler, et al. Hepatology 2018;68:2106-17

HBV RNA in natural history of HBV

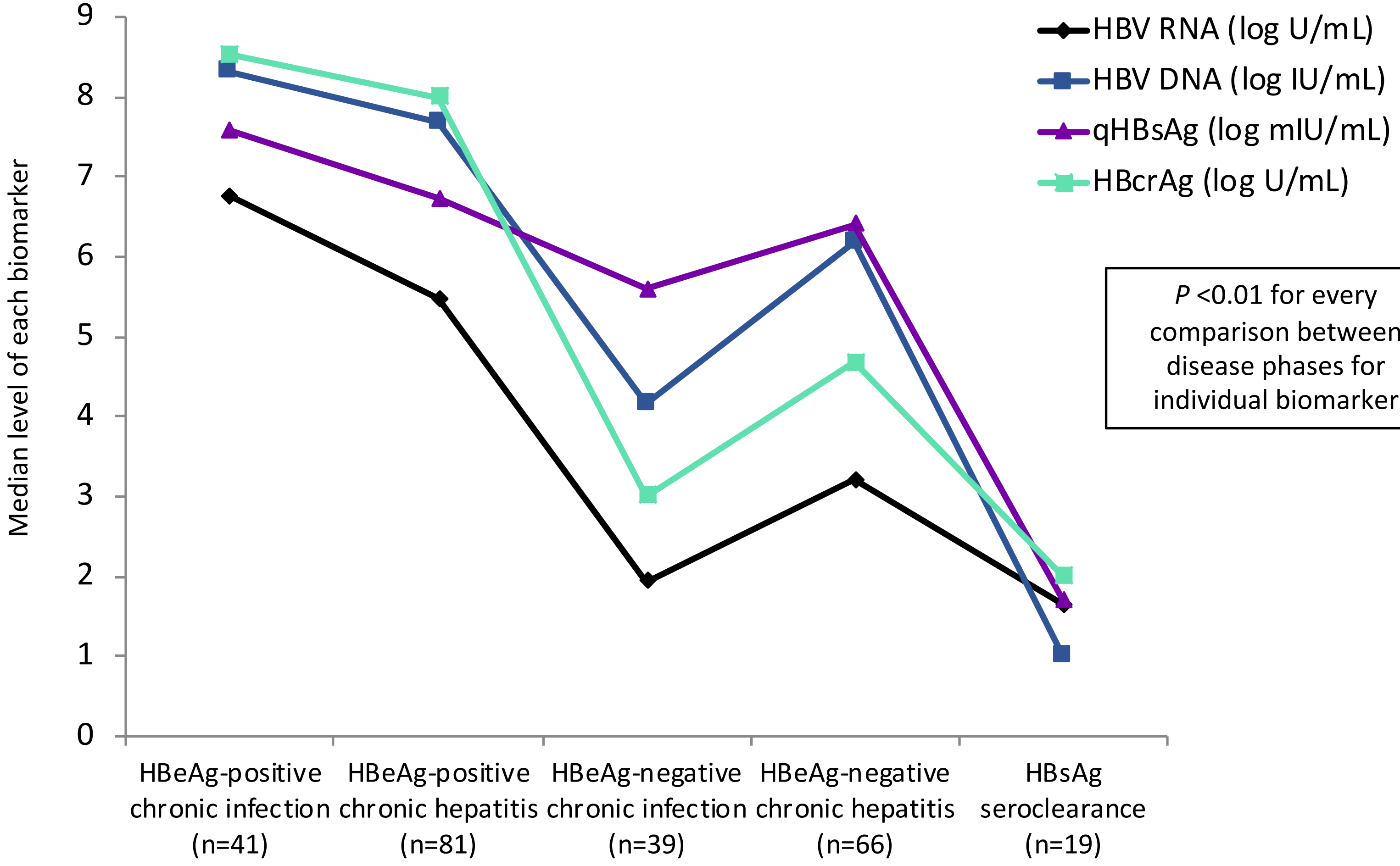


Wang J, et al. J Hepatol 2016; 65:700-710



Wang J et al, J Viral Hepat 2018;25(9):1038-47

HBV RNA in natural history of HBV



P <0.01 for every comparison between disease phases for individual biomarker

Mak LY … Yuen MF. Hepatology (in press)

Serum HBV RNA

HBeAg-positive chronic HBV infection (immune-tolerance phase)

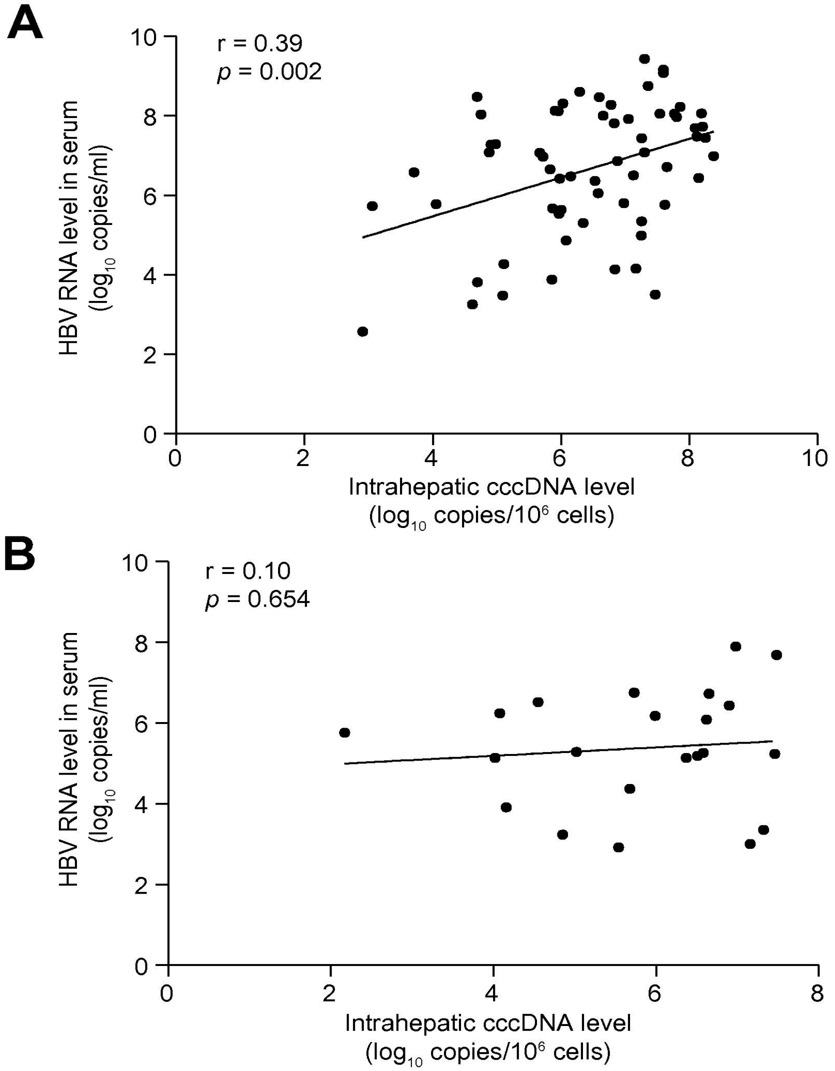
HBeAg-positive chronic hepatitis (immune-clearance phase)

HBeAg-negative chronic hepatitis

HBeAg-negative infection

HBsAg seroclearance

Serum HBV RNA correlations with intra-hepatic cccDNA

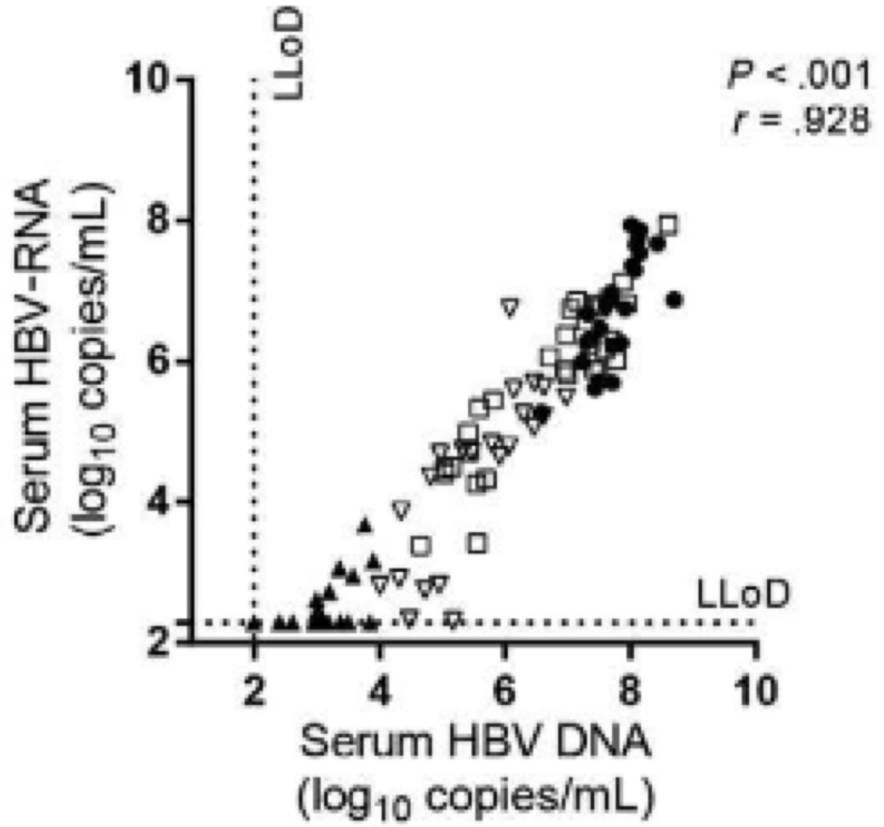


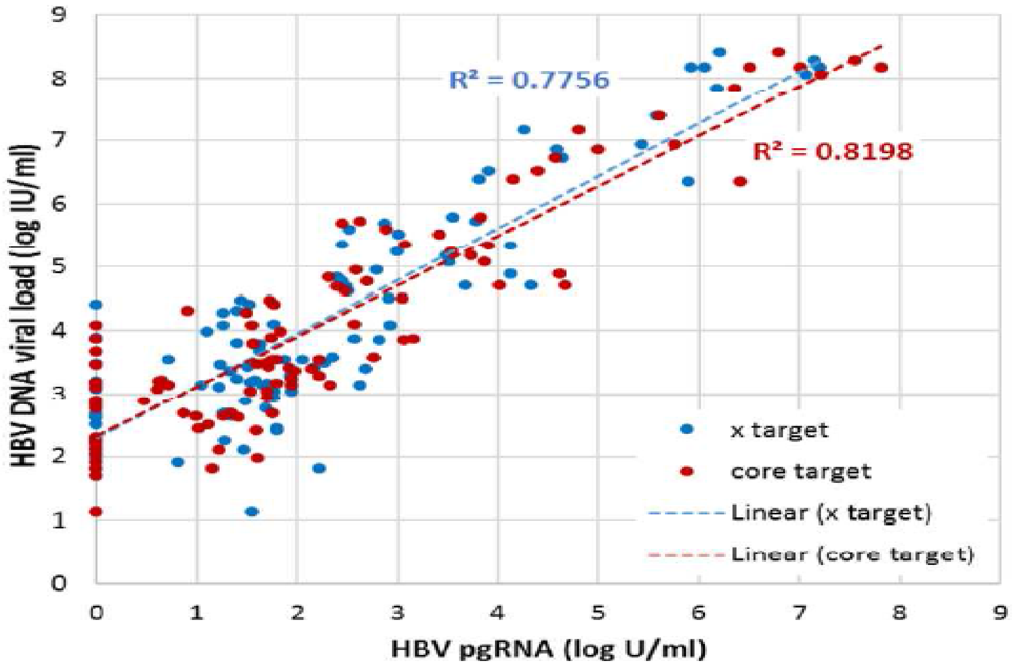
HBeAg positive patients (n=62)

HBeAg negative patients (n=22)

Wang J, et al. J Hepatol 2017; 2: 462-463

Serum HBV RNA and serum HBV DNA correlation



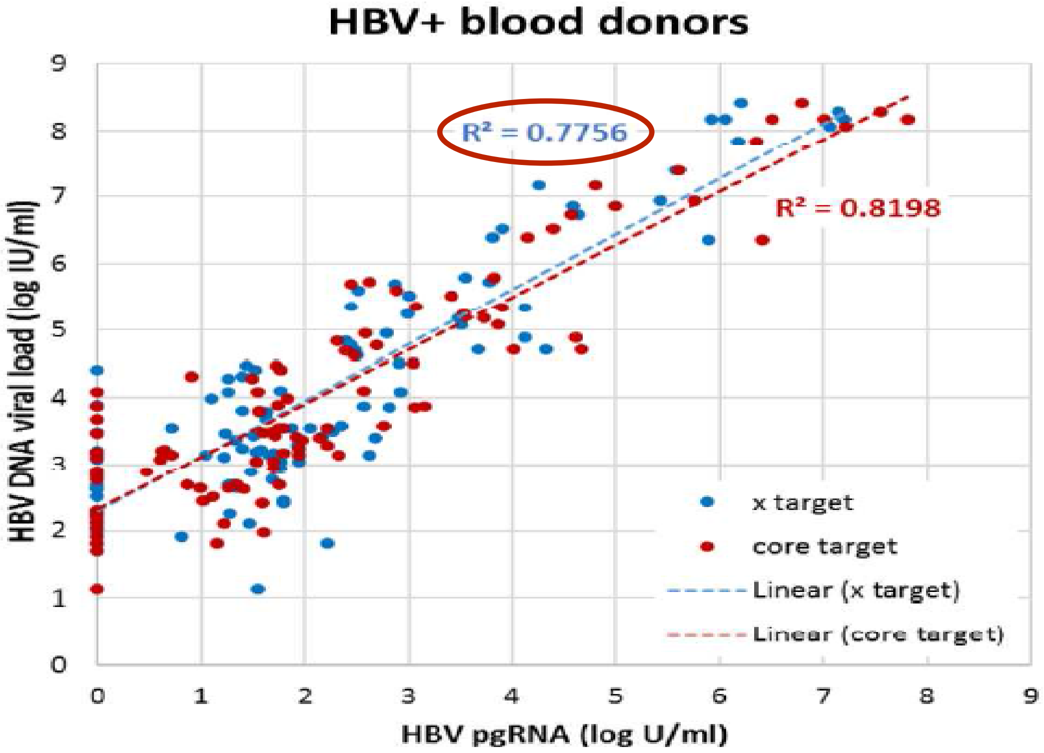


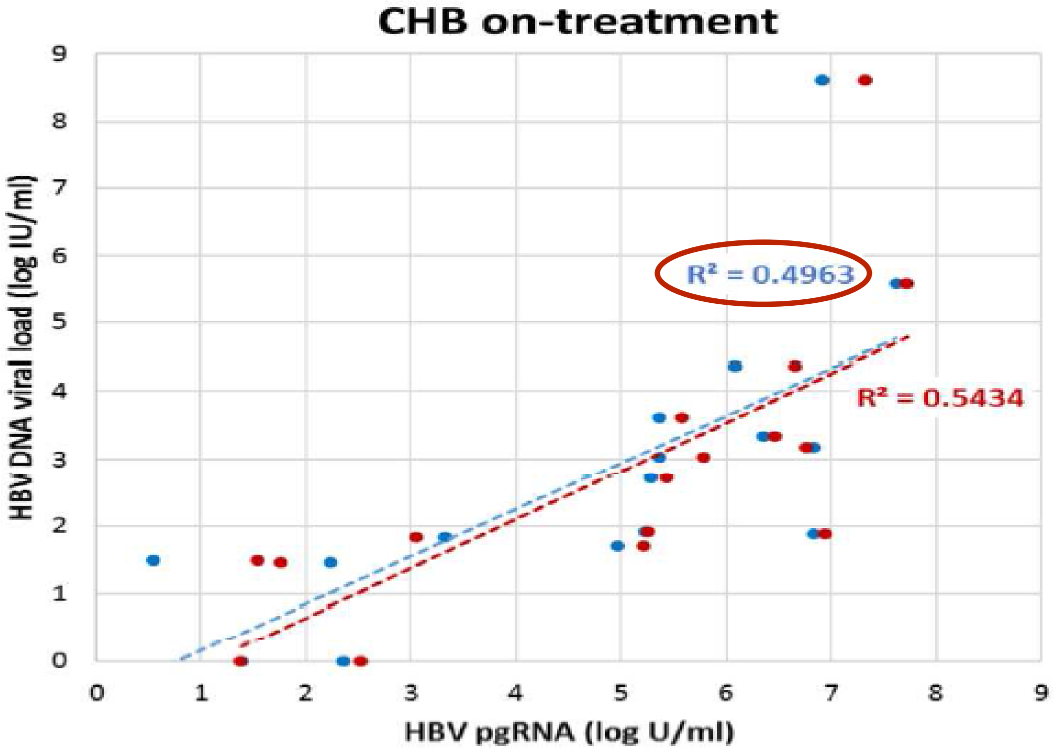
- 102 HBsAg+, HBV DNA+ samples (92 had detectable pgRNA)
- HBV DNA > pgRNA by 1.7 ± 0.71 log

Wang J et al, J Viral Hepat 2018;25(9):1038-47

EK Butler, et al. Hepatology 2018;68:2106-17

Correlations between serum HBV RNA and HBV DNA in treatment naive and experienced patients



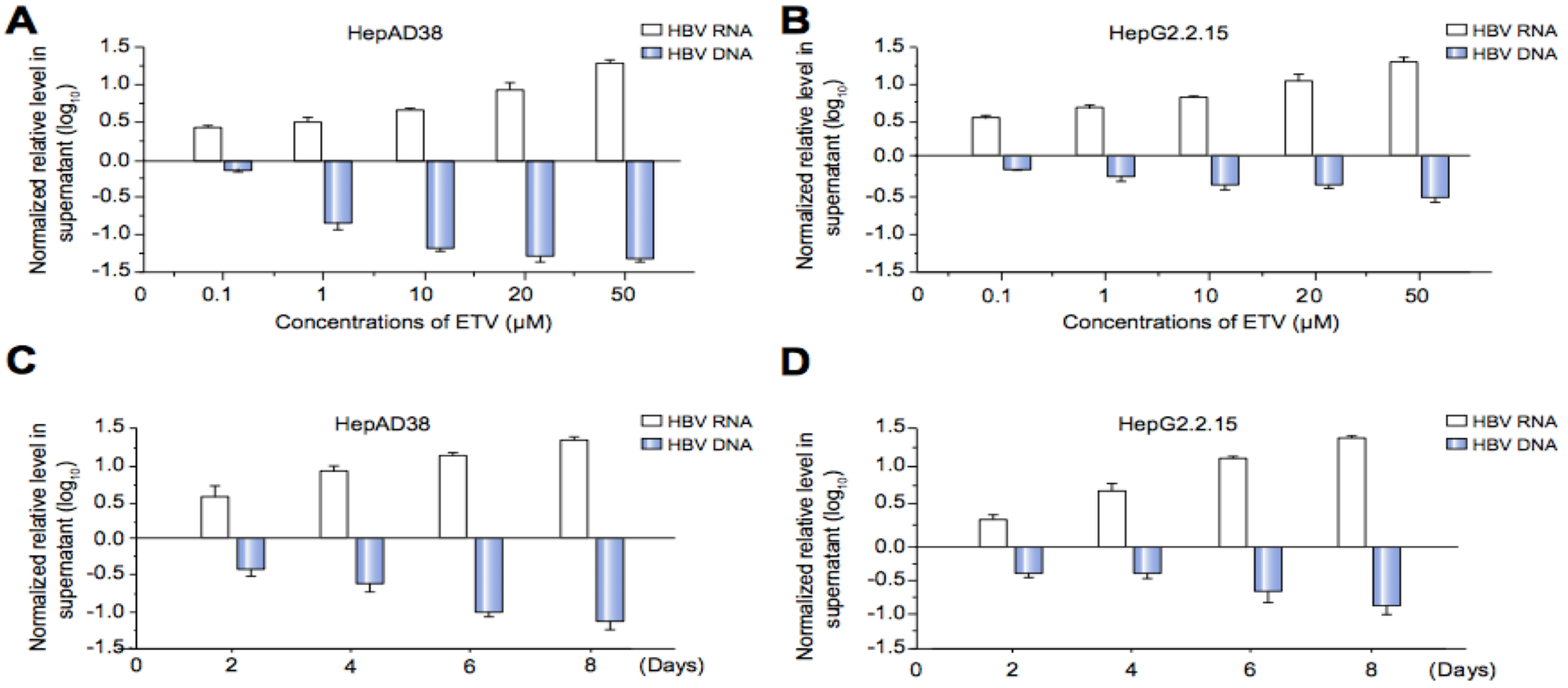


- Correlation between HBV DNA & pgRNA in NA-treated patients lower than in untreated patients
- NA-treated patients: pgRNA > HBV DNA

EK Butler, et al. Hepatology 2018;68:2106-17

HBV RNA profiles under HBV treatment

In vitro studies showed that suppression of the reverse transcription activity of HBV DNA polymerase increases the level of HBV pgRNA virions.

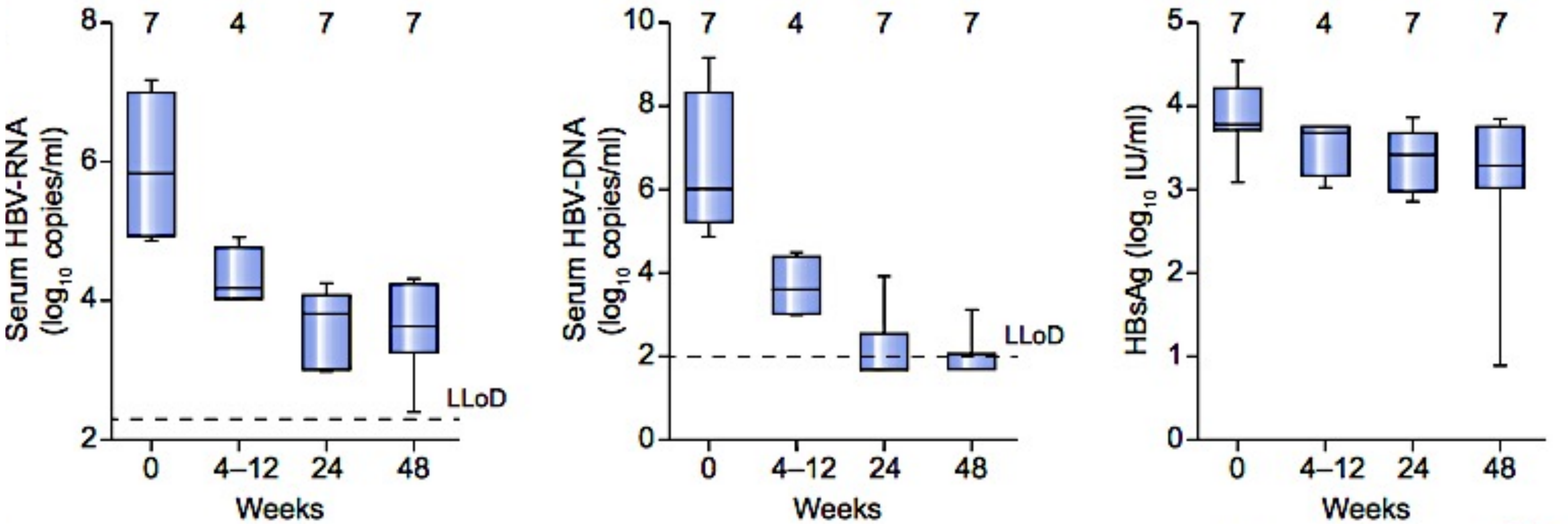


↑ ETV concentration: ↓HBV DNA levels ↑HBV RNA levels

↑ ETV duration (up to 8 days): ↓ HBV DNA levels ↑HBV RNA levels

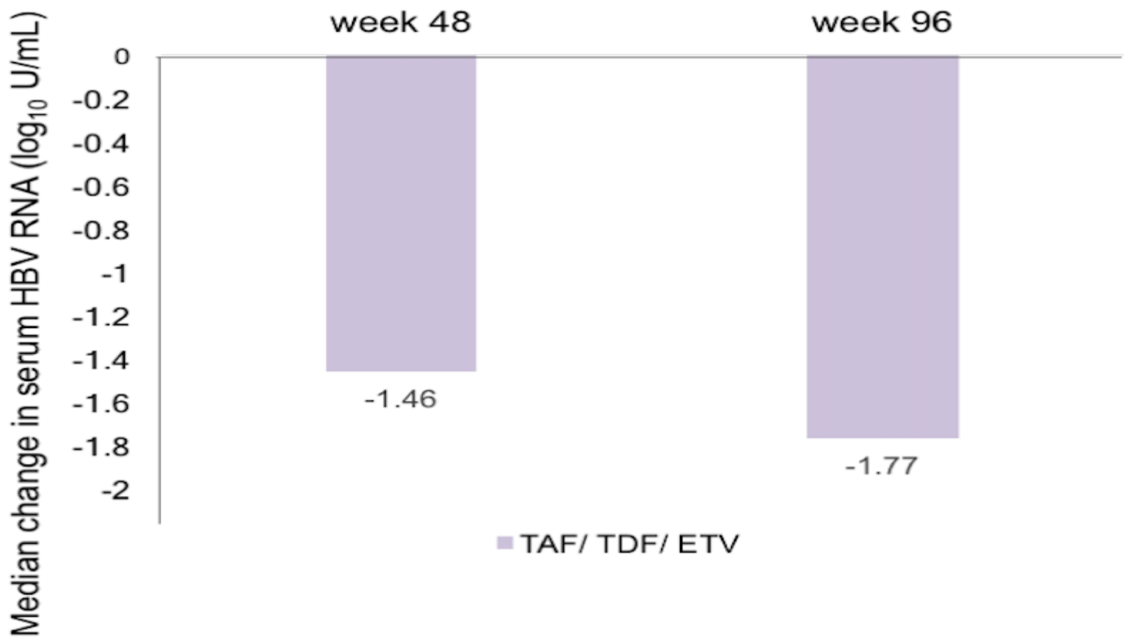
Wang J, et al. J Hepatol 2016; 65:700-710

In vivo dynamic changes of serum HBV RNA with entecavir therapy

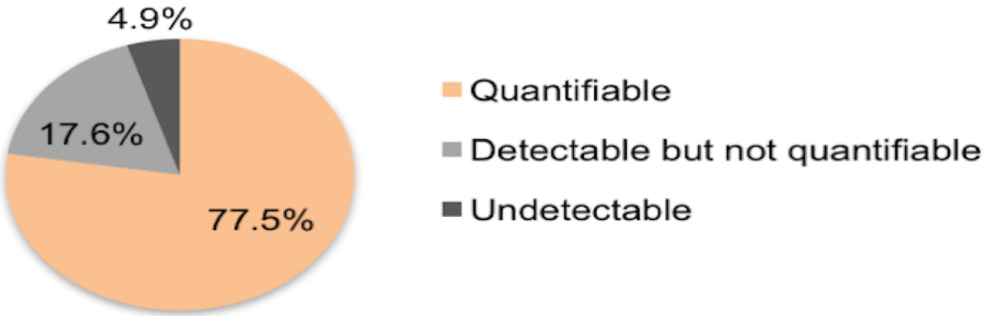


Wang J, et al. J Hepatol 2018;68:16-24

Changes of HBV RNA after 2 years of antiviral treatment



Serum HBV RNA at week 96 of NA



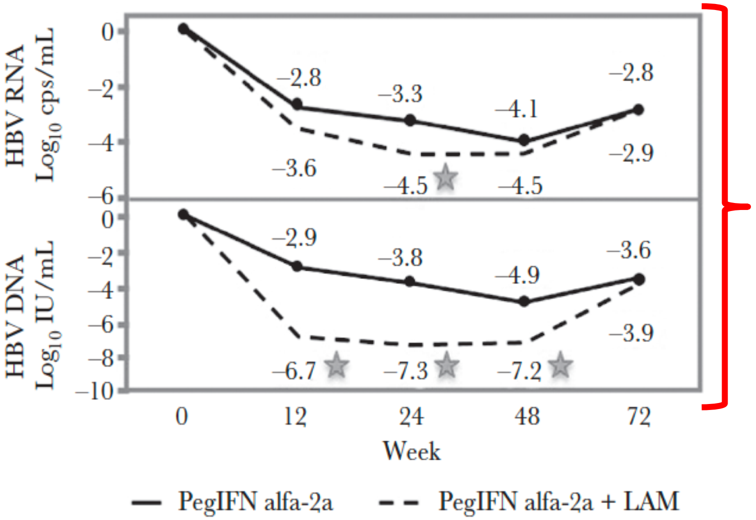
Mak LY… Yuen MF. Hepatology (in press)

HBV RNA levels under PegIFN +/- lamivudine

Study population 131

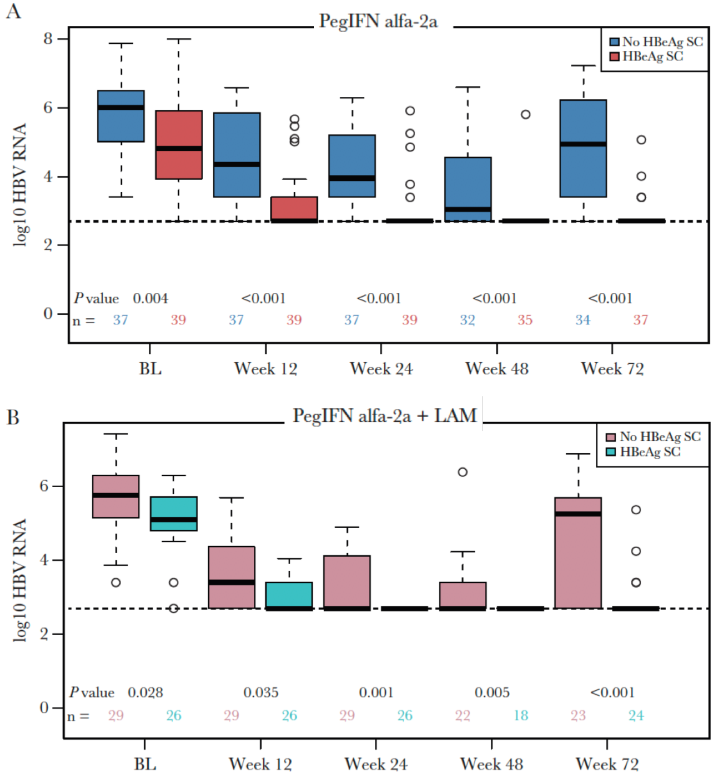
- HBeAg +, ALT (> 1 – 10 x ULN)
- 48-week treatment
- PegIFN alpha 2a: 76
- PegIFN alpha 2a + LAM: 55

HBV DNA and RNA during treatment



1) Both DNA and RNA decreased in both groups
2) Reduction of DNA & RNA more pronounced in PegIFN + Lam vs. PegIFN alone

HBeAg seroconversion at 96 weeks

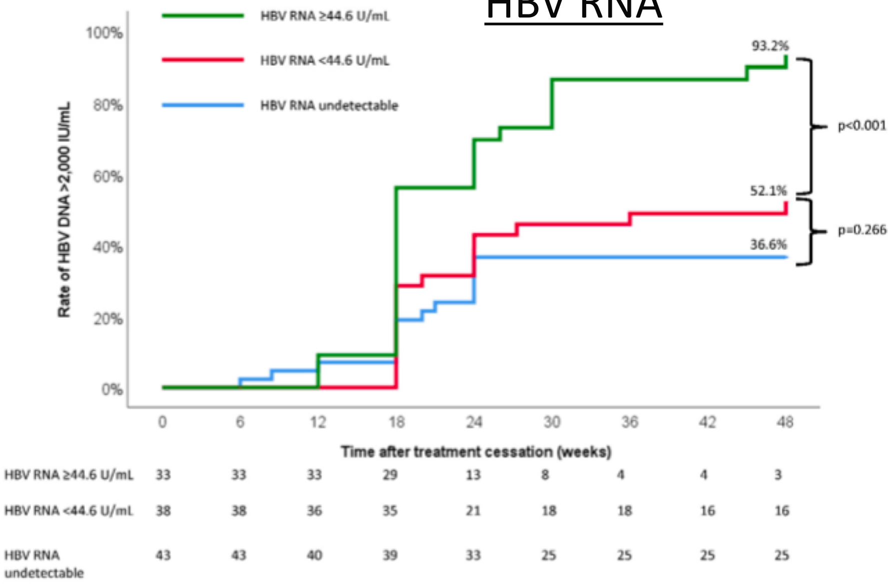


HBV RNA levels were lower at all time points during treatment in patients achieving HBeAg seroconversion

Bommel FV et al., J Infect Dis 2018:218:1066-74

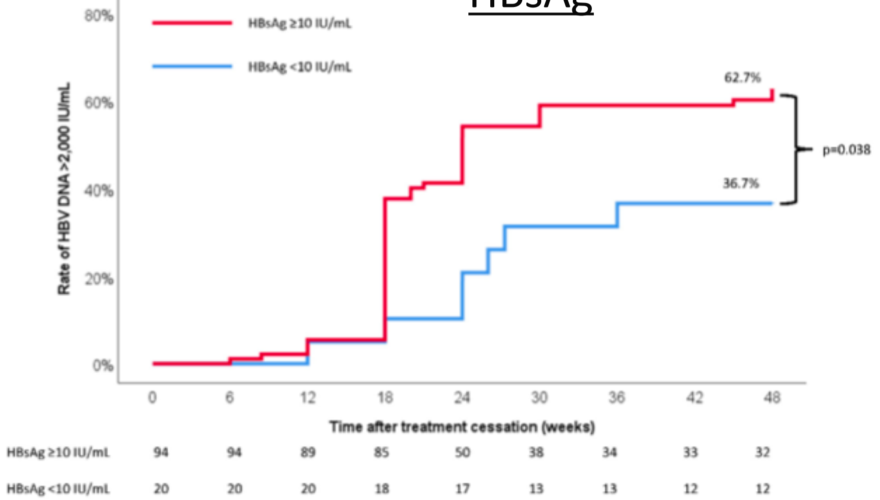
Role of HBV RNA/ HBsAg to predict disease relapse upon treatment cessation

HBV RNA



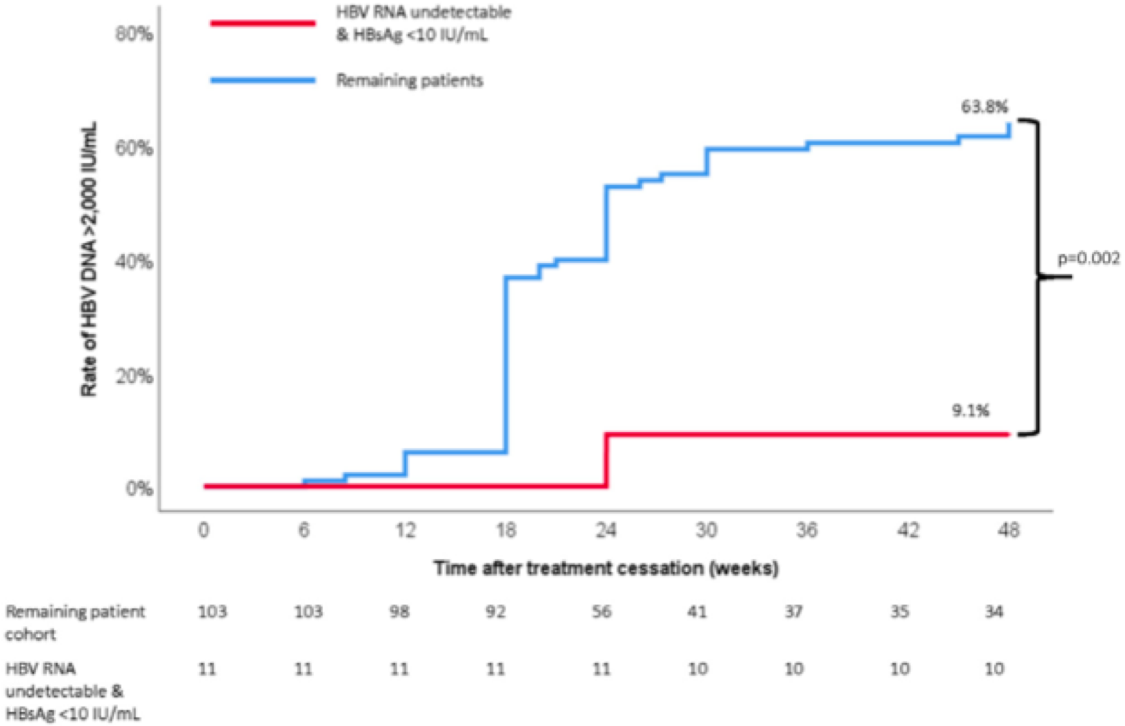
	0	6	12	18	24	30	36	42	48
HBV RNA ≥44.6 U/mL	33	33	33	29	13	8	4	4	3
HBV RNA <44.6 U/mL	38	38	36	35	21	18	18	16	16
HBV RNA undetectable	43	43	40	39	33	25	25	25	25

HBsAg



	0	6	12	18	24	30	36	42	48
HBsAg ≥10 IU/mL	94	94	89	85	50	38	34	33	32
HBsAg <10 IU/mL	20	20	20	18	17	13	13	12	12

HBV RNA + HBsAg



	0	6	12	18	24	30	36	42	48
Remaining patient cohort	103	103	98	92	56	41	37	35	34
HBV RNA undetectable & HBsAg <10 IU/mL	11	11	11	11	11	10	10	10	10

Seto WK … Yuen MF. Gut 2020 (online)

Clinical Utility ofHBV RNA

- Detected in serum as virus-like particles with encapsidated RNA.
- RNA levels differ in different disease phases of CHB
- Present in serum of chronic hepatitis B patients, albeit in lower levels, compared to serum HBV DNA.
- Correlated with serum HBV DNA (correlation became weaker during NA treatment)
- Correlated with intra-hepatic cccDNA (HBeAg-positive mainly) in treatment-naïve patients
- Long term effects of NUCs on HBV RNA remains to be defined.
- May predict response to HBeAg seroconversion (IFN based therapy + NUCs) and disease relapse in patients after NA cessation

Thank you