Diagnostic Tools for Occult Hepatitis B

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Outline

- Diagnosis of OBI
  - HBV DNA
    - Methods
    - Challenges
  - Anti-HBc
    - Prevalence
- Novel markers/ tests for OBI
  - Linearized HBsAg
  - Hepatitis B core-related antigen (HBcrAg)
    - HBV reactivation

OBI: Definition and Gold Standard of Diagnosis

- Definition: Detection of HBV DNA in blood or liver of HBsAg negative persons

- Detection:
  - Gold standard: Episomal HBV DNA in the liver
  - Commonly used: HBV DNA in the blood
  - Surrogate often used: anti-HBc in the blood

Raimondo G et al., J Hepatol 2019 (in press)

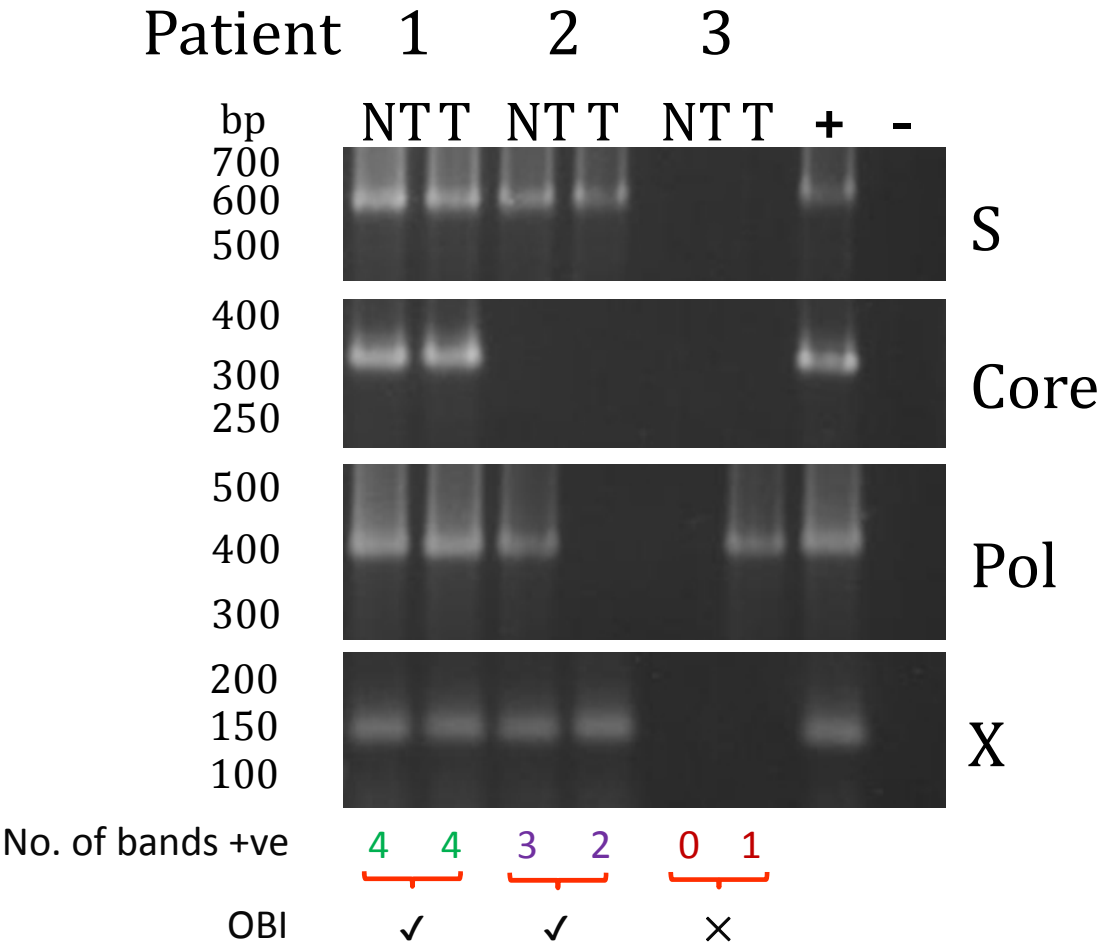
Diagnosis of OBI

- Detection:
  - Gold standard: Episomal HBV DNA in the liver
  - Commonly used: HBV DNA in the blood
  - Surrogate often used: anti-HBc in the blood

- Nested PCR:
  - More definite diagnosis of OBI
  - Positive for $\geq$ 2 different genomic regions e.g. S, Precore-core, Polymerase, X regions

Raimondo G et al., J Hepatol 2007;46:160-170

HBV DNA Detection by nested PCR



Wong DKH…Yuen MF. Hepatology 2011;54:829-36

Detection Rate using Different Genomic Regions

No. of samples with detectable PCR	NT (n = 29)	*P**	T (n = 30)	*P**
X	27 (93%)		22 (73%)	
S	18 (62%)	0.013	10 (33%)	0.006
Core	13 (45%)	<0.001	14 (47%)	0.020
Pol	19 (66%)	0.026	11 (37%)	0.011

* Compared to X region

Wong DKH…Yuen MF. Hepatology 2011;54:829-36

Detection of HBV DNA in Blood

- Liver biopsy for HBV DNA measurement: gold standard
  - Invasive
  - inconvenient

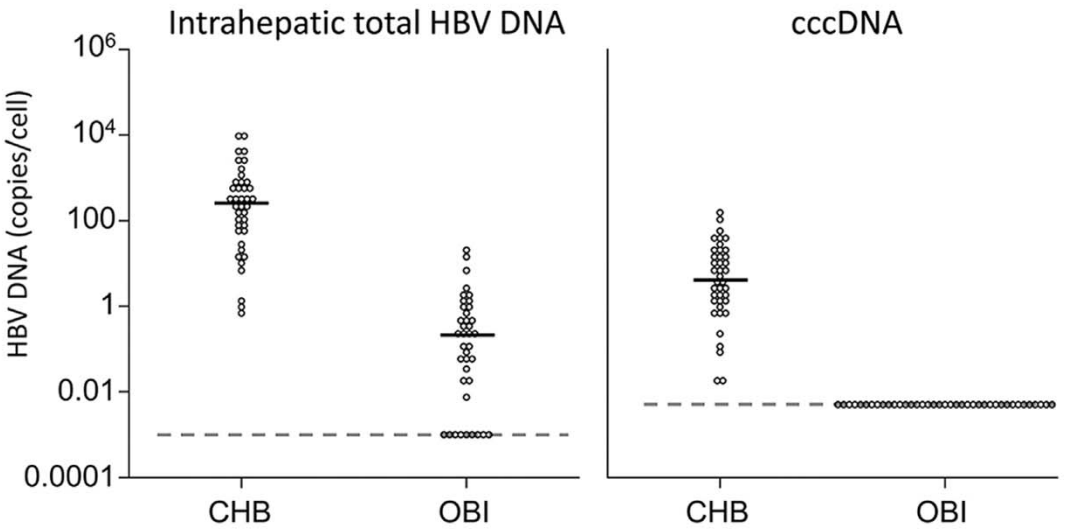
- Detection of HBV DNA in blood
  - In blood screening setting
    - Mini-pool NAT
    - Confirmatory test
  - Individual setting
    - Sensitive commercial assays

Challenge I: Detecting Blood HBV DNA

- Blood screening
  - NAT (lower limit of detection 2-4 IU/mL for individual sample)
  - Mini pool testing: dilution factor (usually 6 – 20 donations/ pool): decrease sensitivity significantly

- Individual testing
  - Approved standardized HBV DNA assays: LLOD 10 – 20 IU/mL
  - Challenges
    - Serum HBV DNA usually present in low concentrations and fluctuates with time
    - Intermittently detectable in same patients
    - Sensitivity of assays
  - Improve detection rate
    - Larger volume of sample for DNA extraction e.g. > 1 ml of serum/ plasma

Challenge II: Low Intrahepatic HBV Viral Load in OBI



Wong DKH…Yuen MF. Clin Microbiol Infect 2016;22:e1-3

- Viral load extremely low even in the liver
  - Technical difficulty: requires highly sensitive assays
  - Detection of HBV DNA in blood even more difficult

- **OBI detection rate: always underestimation**

Challenge III: HBV DNA Detectability Decreases with after Time of HBsAg Seroclearance (OBI)

- Measurement of HBV DNA (LLOD 1.1 IU/mL)
  - Detectability rate upon follow-up after HBsAg seroclearance
    - Within 1 year: 19 out of 142 patients (13.4%)
    - 5 – 10 years: 6 out of 99 patients (6.1%)
    - > 10 years: 1 out of 27 patients (3.7%)

Yuen MF et al. Gastroenterology 2008;135:1192-99

Droplet Digital PCR Assays for OBI Detection?

- Technically, improves assay sensitivity

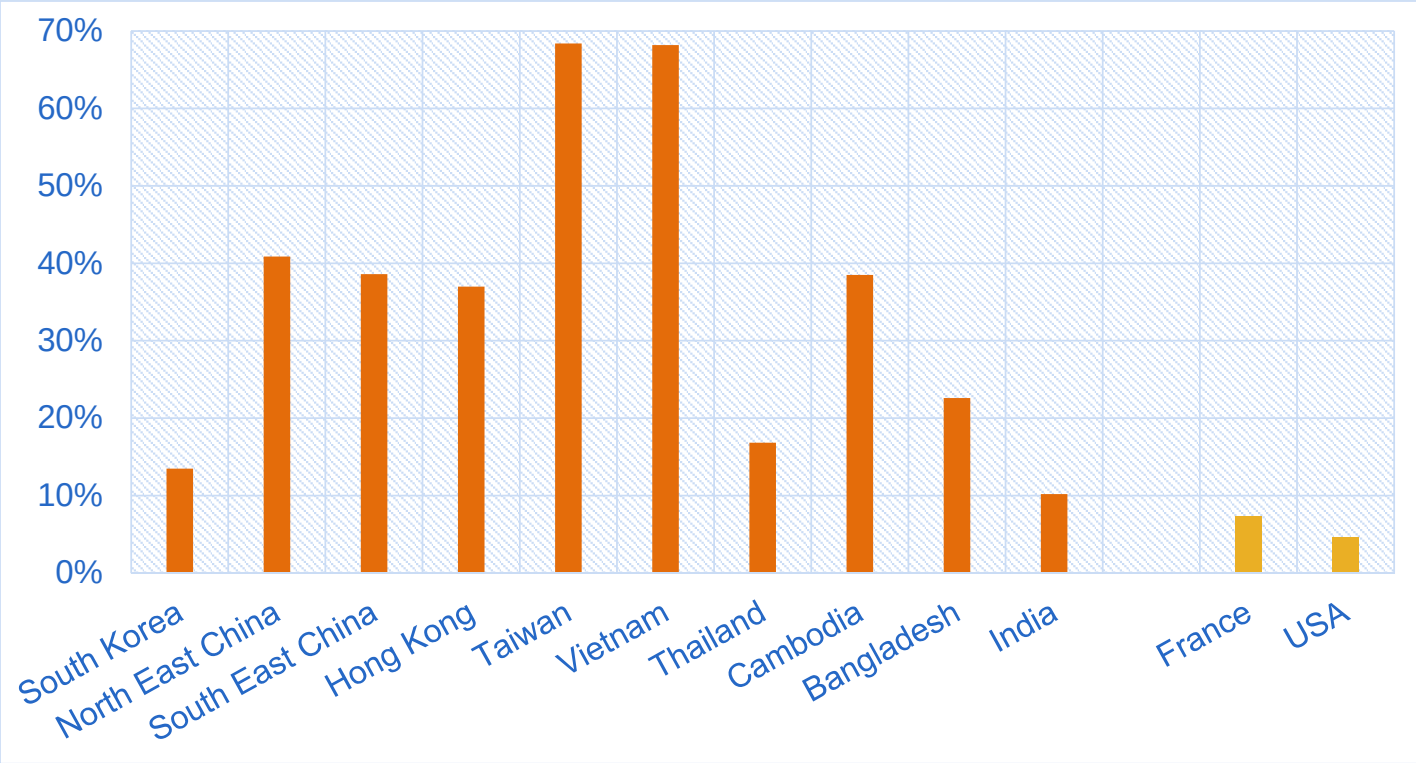
- Example
  - Intrahepatic cccDNA LLOD
    - Digital PCR vs. real time PCR
      - 10 – 100-fold more sensitive

Caviglai CP et al. J Hepatol 2018;69:301-7

Anti-HCV as Surrogate for OBI

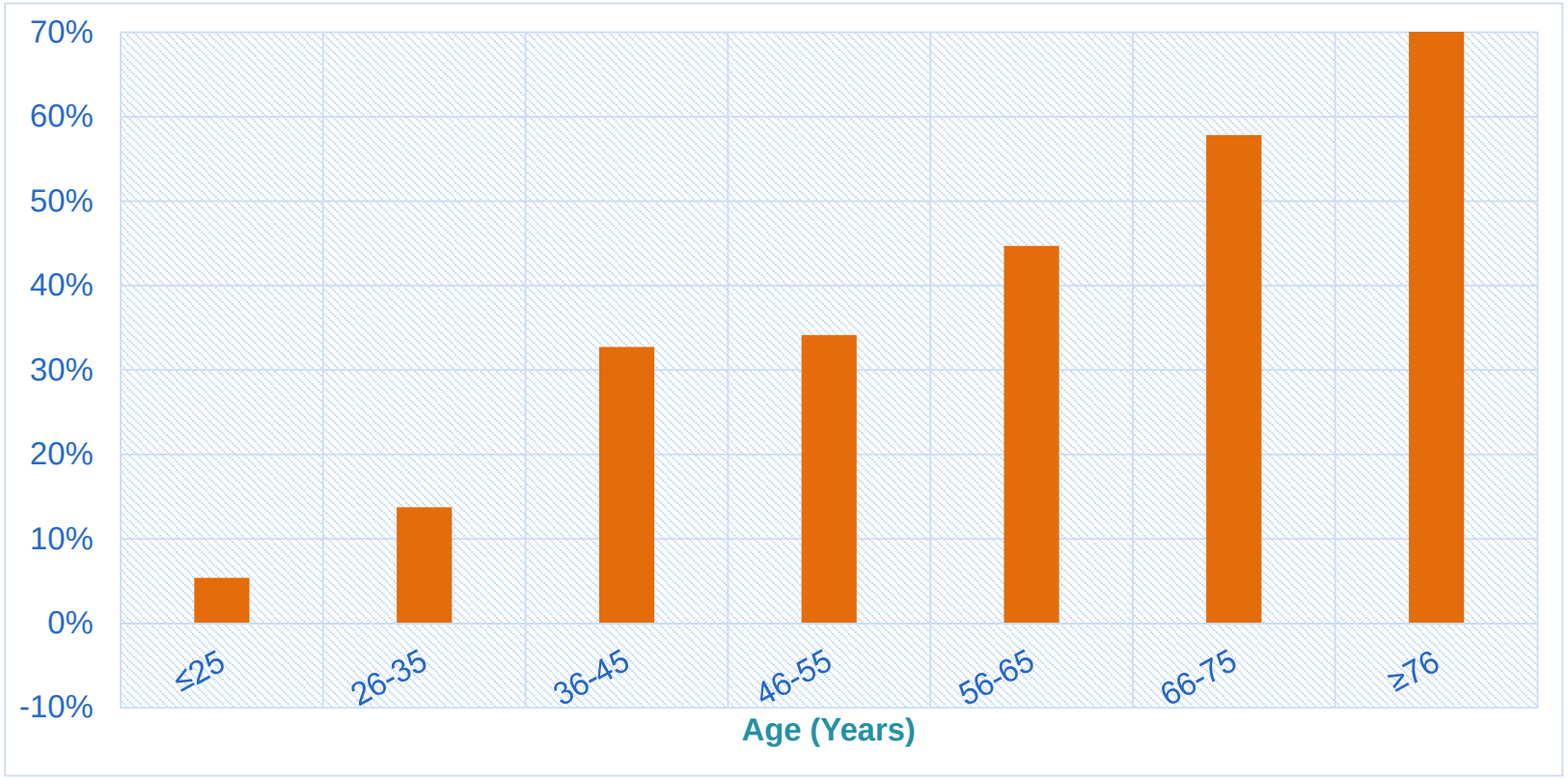
- Real OBI vs. past infection
  - Low rate of HBV DNA detectability in anti-HBc positive samples
  - Usually < 1%
  - In blood screening setting
    - Cannot exclude seronegative OBI and ”window period” HBV infection
    - Exclusion of blood products in areas with high prevalence of anti-HBc e.g. > 10%
      - Pressure on blood product availability
      - ? Infectivity

Prevalence of HBsAg –ve/ anti-HBc +ve in Different Countries



Seto WK, Yuen MF. In Immunosuppressives: Advances, Applications and Analyses (2017)

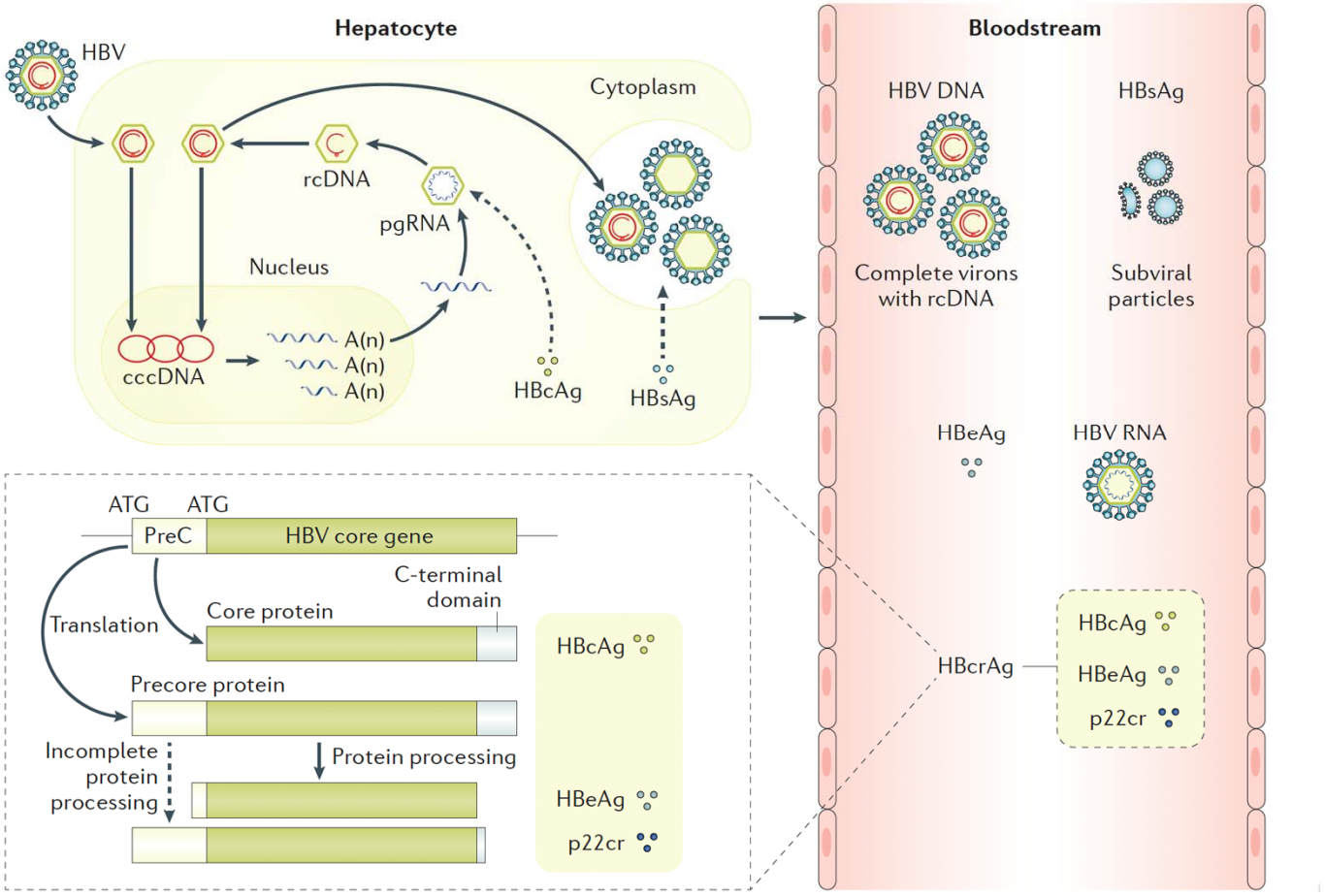
Anti-HBc Positivity Rate Increases with Age



Liu K…Yuen MF. J Infect Dis 2019 (in press)

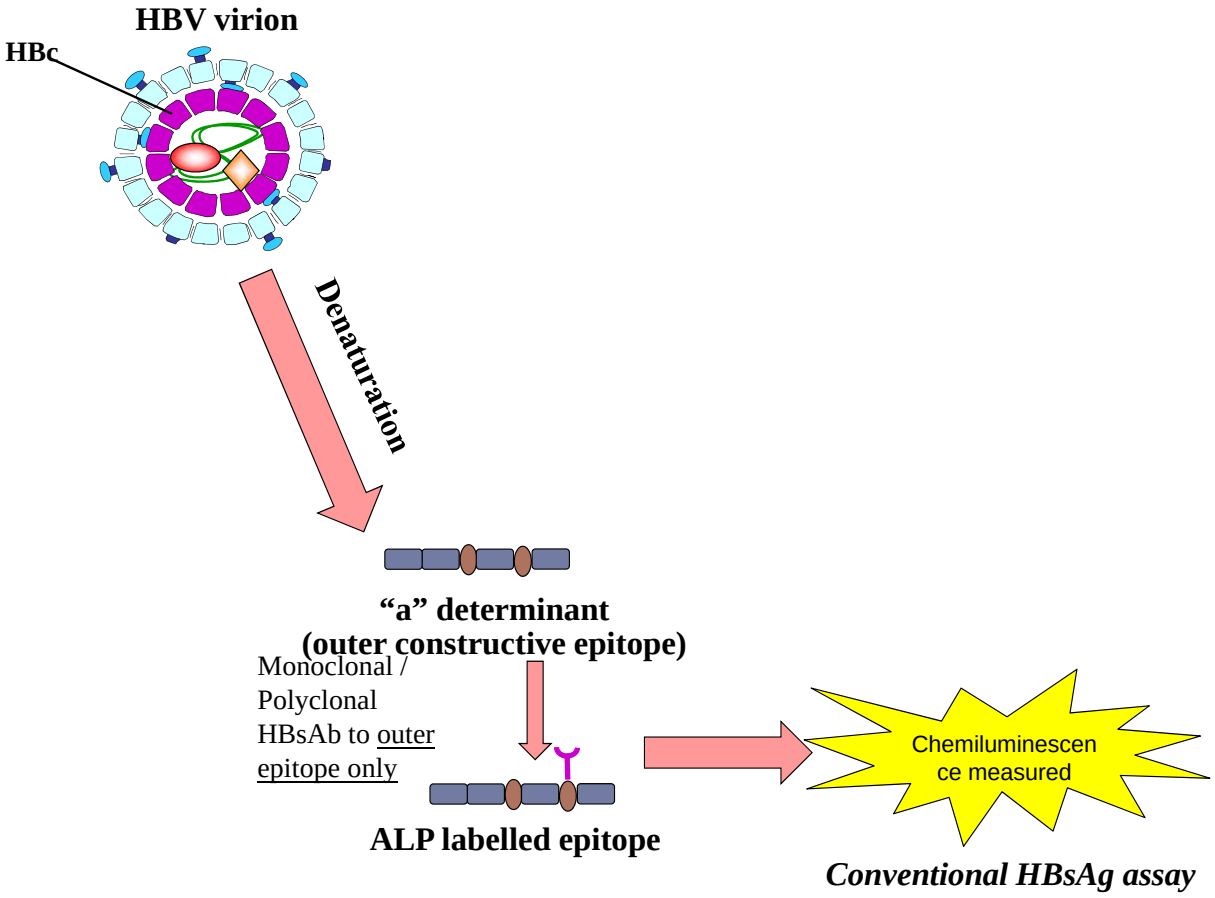
Novel Tests to Diagnose OBI

Circulating Viral Particles in HBV



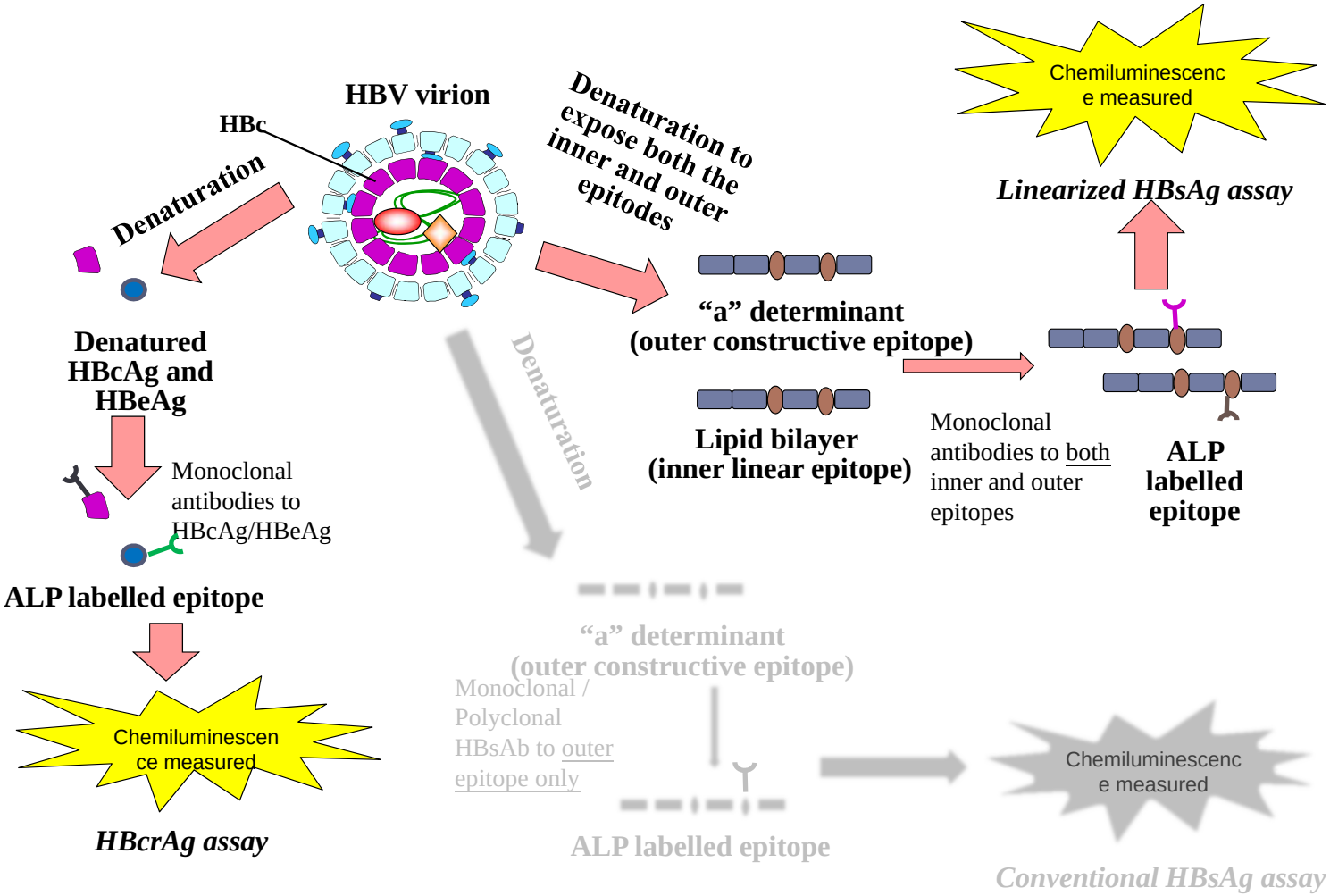
Yuen MF et al. Nat Rev Dis Primers 2018;4:18035

Conventional HBsAg Test (LLOD: 0.05 IU/mL)



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

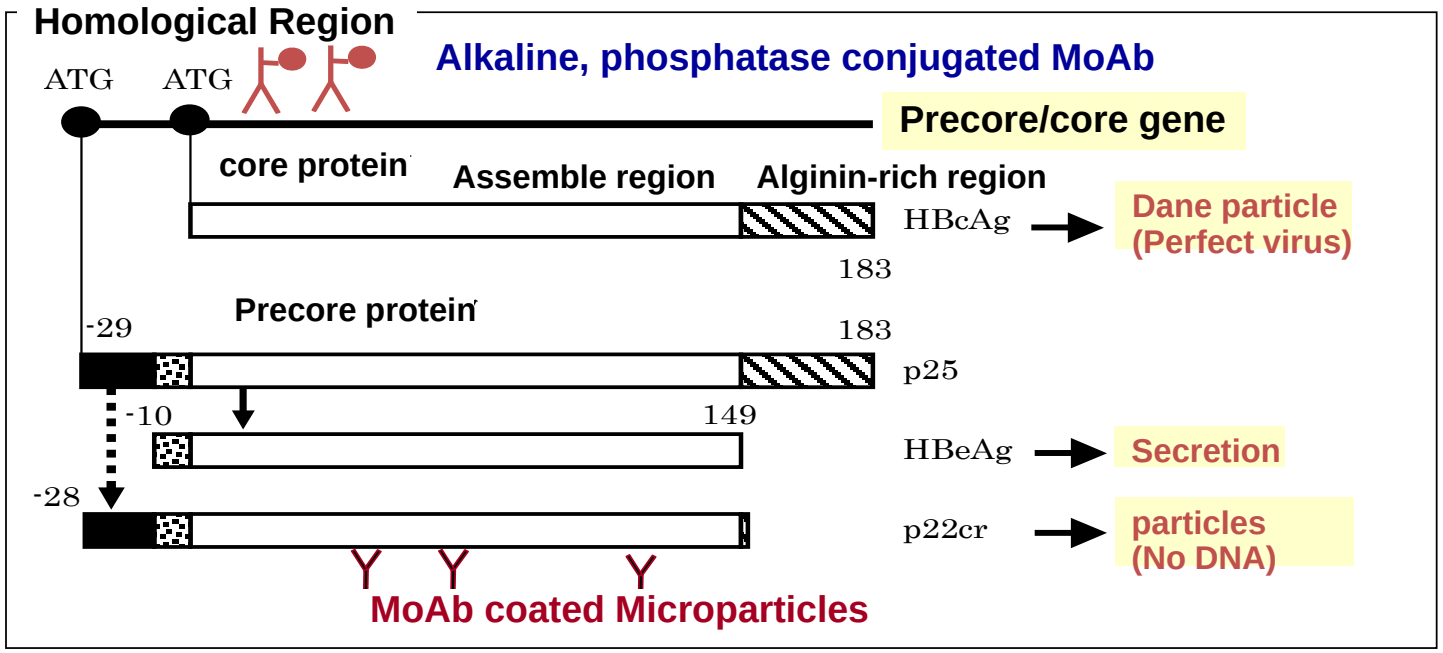
Linearized HBsAg (LLOD: 0.005 IU/mL) & HBcrAg



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

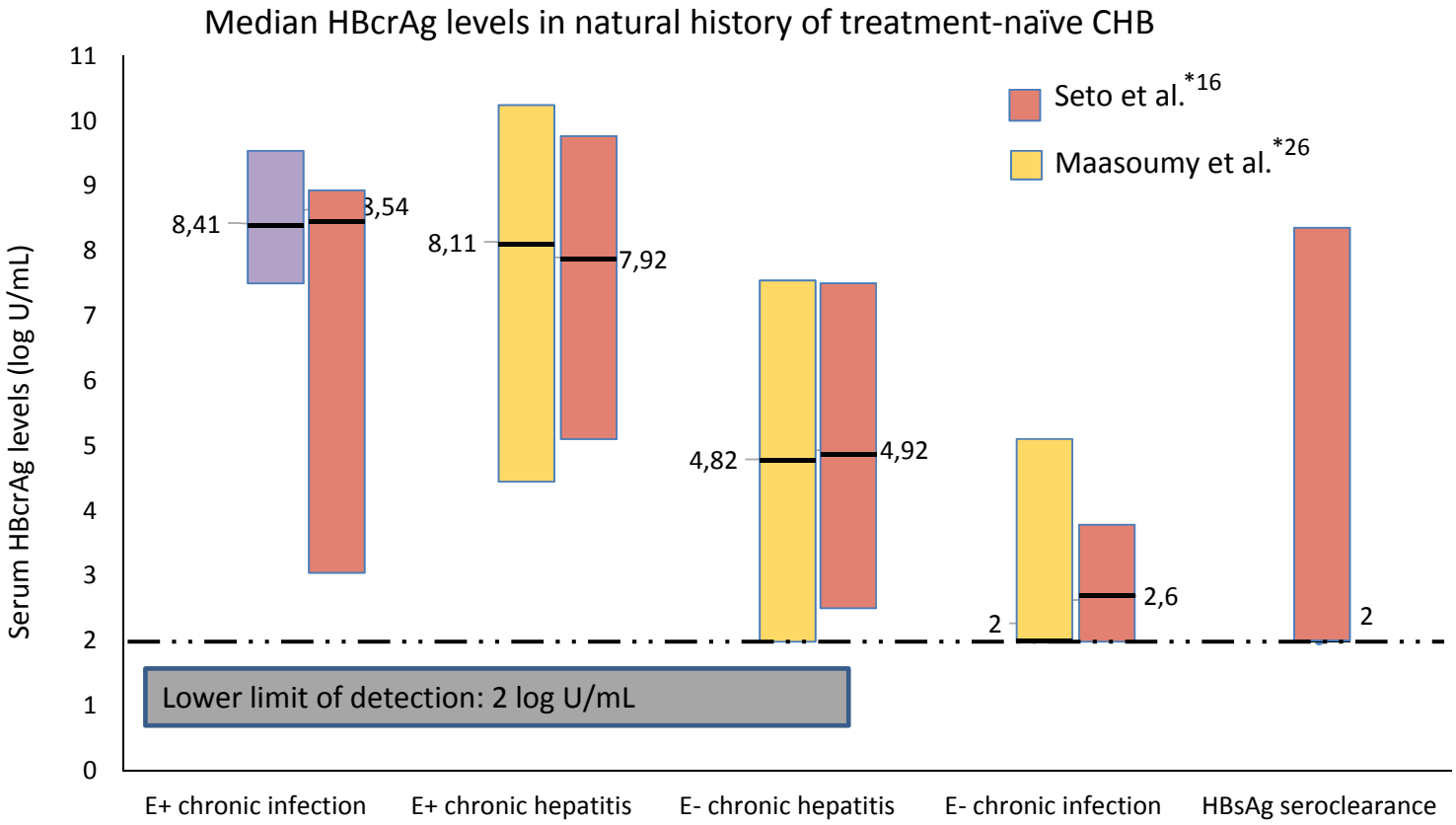
Characteristics of HBcrAg:
HB core-related Antigen Reagent

- HBeAg, HBcAg, and p22cr are HB core-related proteins having homological region.
- Antibodies for homological region are used to detect the three proteins.
- **Solid phase: Three kinds of anti HBcr MoAbs**
- **Conjugate: Two kinds of anti HBcr MoAbs**



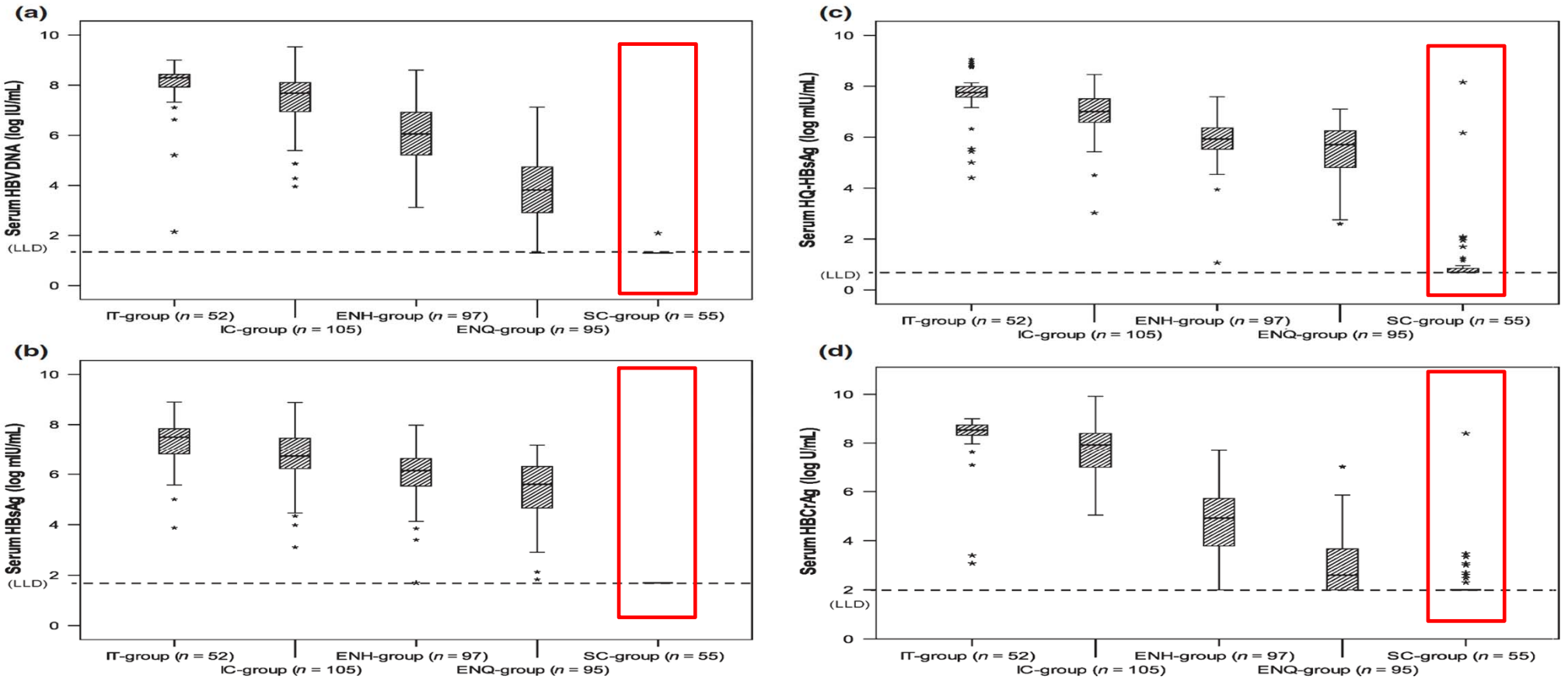
- **Detection limit: 2 log U/mL**
- **Measurement range: 3 – 7 Log U/mL**

HBcrAg in CHB Patients and Patients with HBsAg Seroclearance (OBI)



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

Linearized HBsAg & HBcrAg in Patients with HBsAg Seroclearance (OBI)

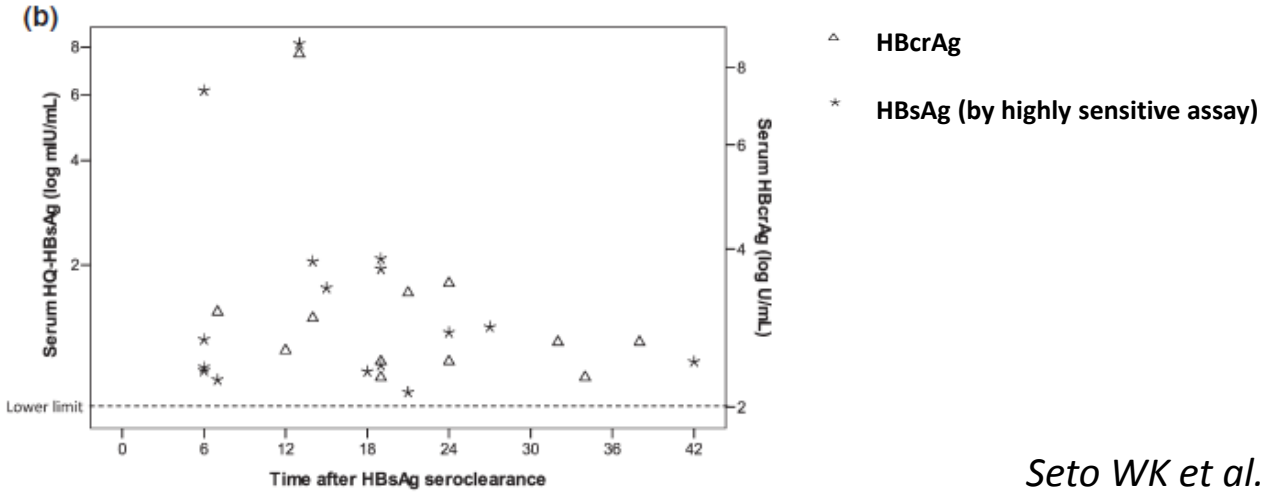


Seto WK… Yuen MF. Clin Microbiol Infect 2014;20: 1173-80

Detectable Linearized HBsAg & HBcrAg in HBsAg Seroclearance (OBI)

55 patients with HBsAg seroclearance

- 16 (29.1%) had detectable linearized HBsAg
- 12 (21.8%) had detectable HBcrAg
  - 22 (40%) had detectable in either linearized HBsAg or HBcrAg
- only 1 (1.8%) patient had detectable HBV DNA

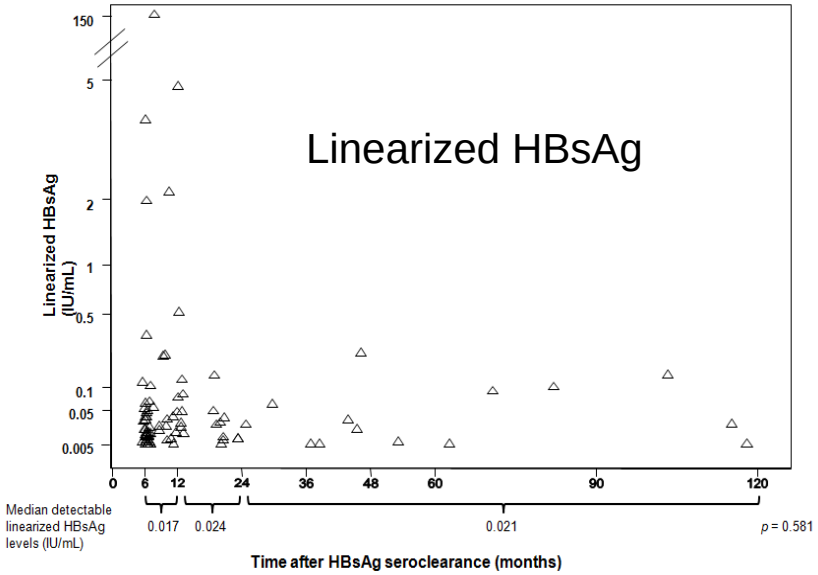


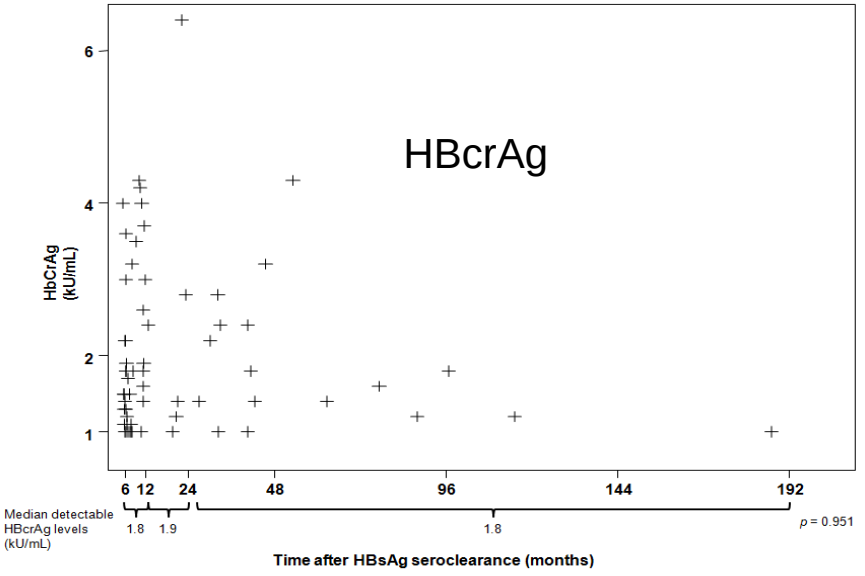
Seto WK et al. Clin Microbiol Infect 2014;20:1173-80

Detectable Linearized HBsAg & HBcrAg in HBsAg Seroclearance (OBI)

329 patients with HBsAg seroclearance

- 85 (25.8%) detectable linearized HBsAg
- 69(21%) detectable HBcrAg
  - 133 (40.4%) detectable of either one
  - 21 (6.4%) detectable both
- Only 7 (2.1%) detectable HBV DNA

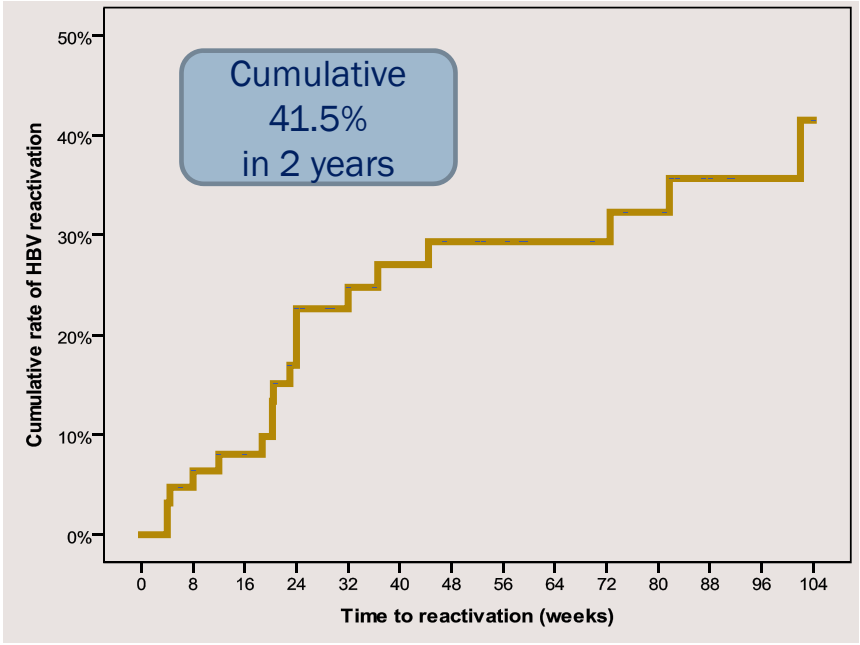




Seto WK… Yuen MF. Hepatol Int 2013;7:98-105

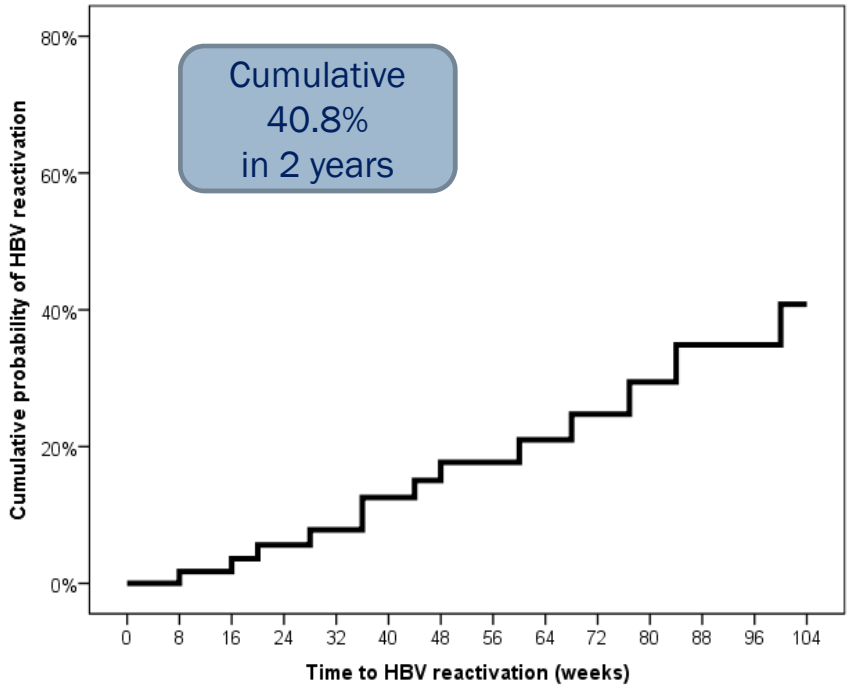
HBV Reactivation in HBsAg –ve, Anti-HBc +ve Patients Receiving Immunosuppressive Therapy

Patients receiving anti-CD 20



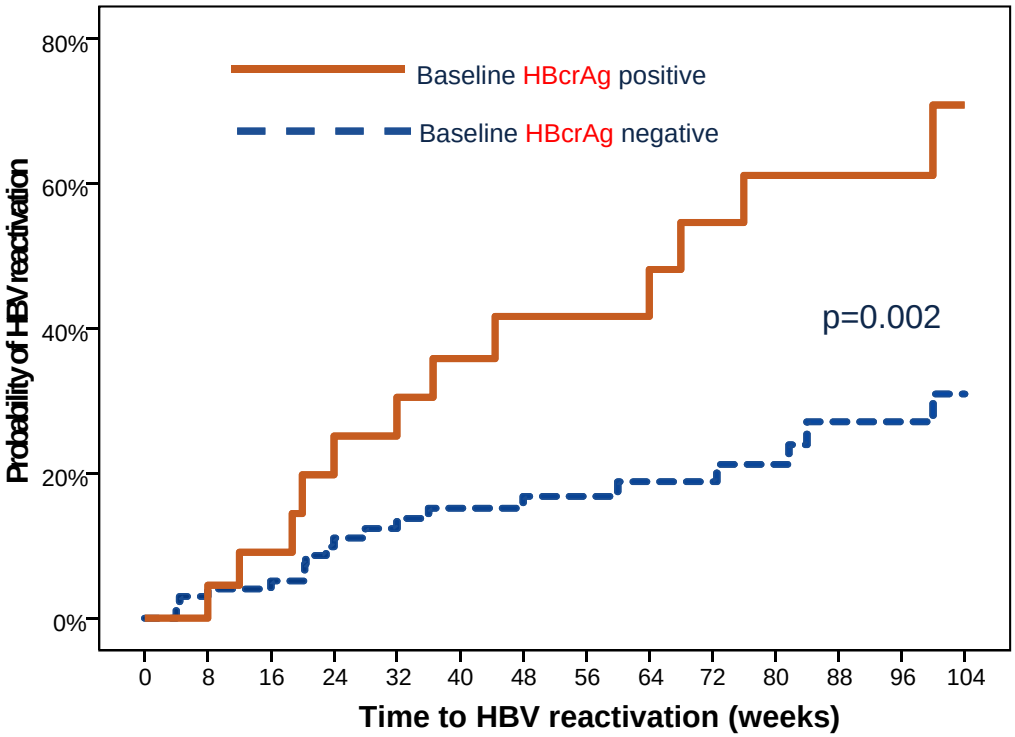
Seto WK…Yuen MF J Clin Oncol 2014;32933:3736-43

Patients receiving immunosuppressants after HSCT



Seto WK…Yuen MF. Hepatology 2017;65:1451-61

Detectable HBcrAg at Baseline Associated with the Risk of for HBV Reactivation in HBsAg-/ anti-HBc + patients Immunosuppressive Therapy



Seto WK…Yuen MF. Am J Gastroenterol 2016;111:1788-95

Conclusions

- Diagnosis of OBI
  - Always a challenging task because of
    - Working on extremely low HBV DNA levels
    - HBV DNA levels fluctuate: ? Need to repeat tests
    - No good and reliable surrogates to replace HBV DNA
  - Prevalence always underestimated
  - Improvement on detection
    - ? Digital PCR
    - Other serologic markers
      - Novel: HBcrAg
      - Improvement on the old: HBsAg (linearized)

Thank you