La diagnostica dell'infezione da HBV e HDV

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XIII Workshop Nazionale

TERAPIE INNOVATIVE DELLE EPATITI CRONICHE VIRALI E DELLE INFEZIONI VIRALI

New anti-HBV
to achieve functional cure

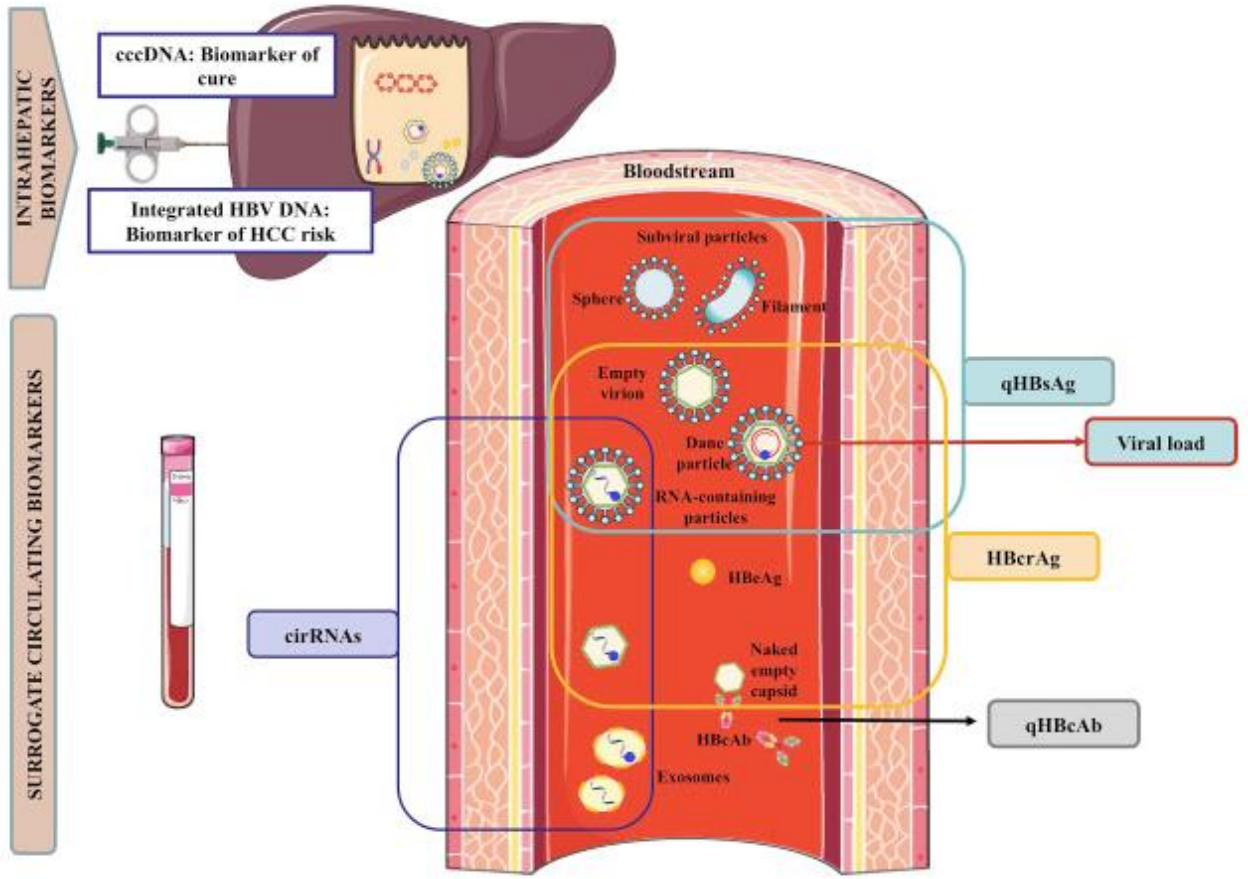
Novel strategies based on
NUC-suspension

**New challenges on
HBV and HDV clinical research**

New anti-HDV drugs for finite
or long term therapy

Novel concepts on
HBV occult infection

- **The success of all these apects is strictly related to the availablity of advanced diagnostics based on reliable and accurate biomarkers for a proper characterization of HBV intrahepatic reservoir**

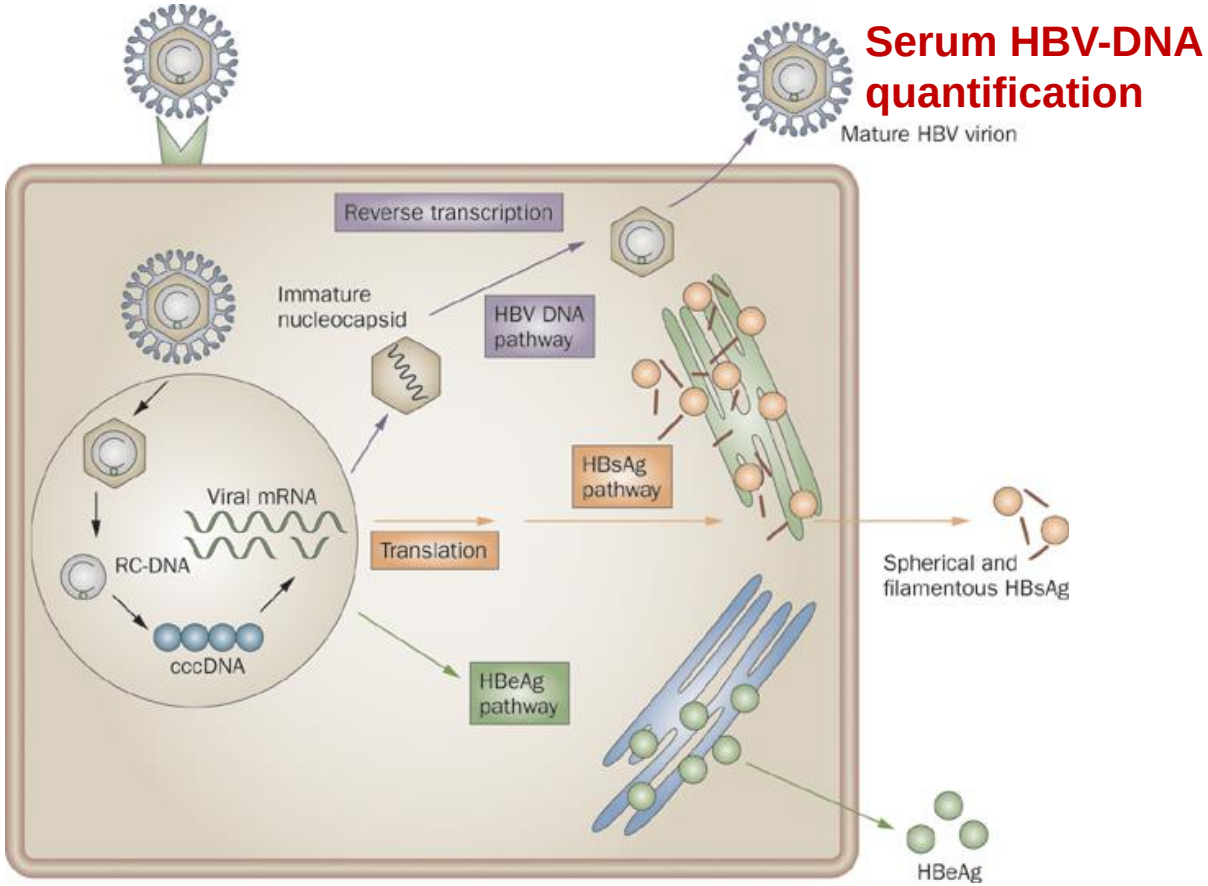


Charre et al., AVR 2019

Overview of classical and novel HBV biomarkers so far available

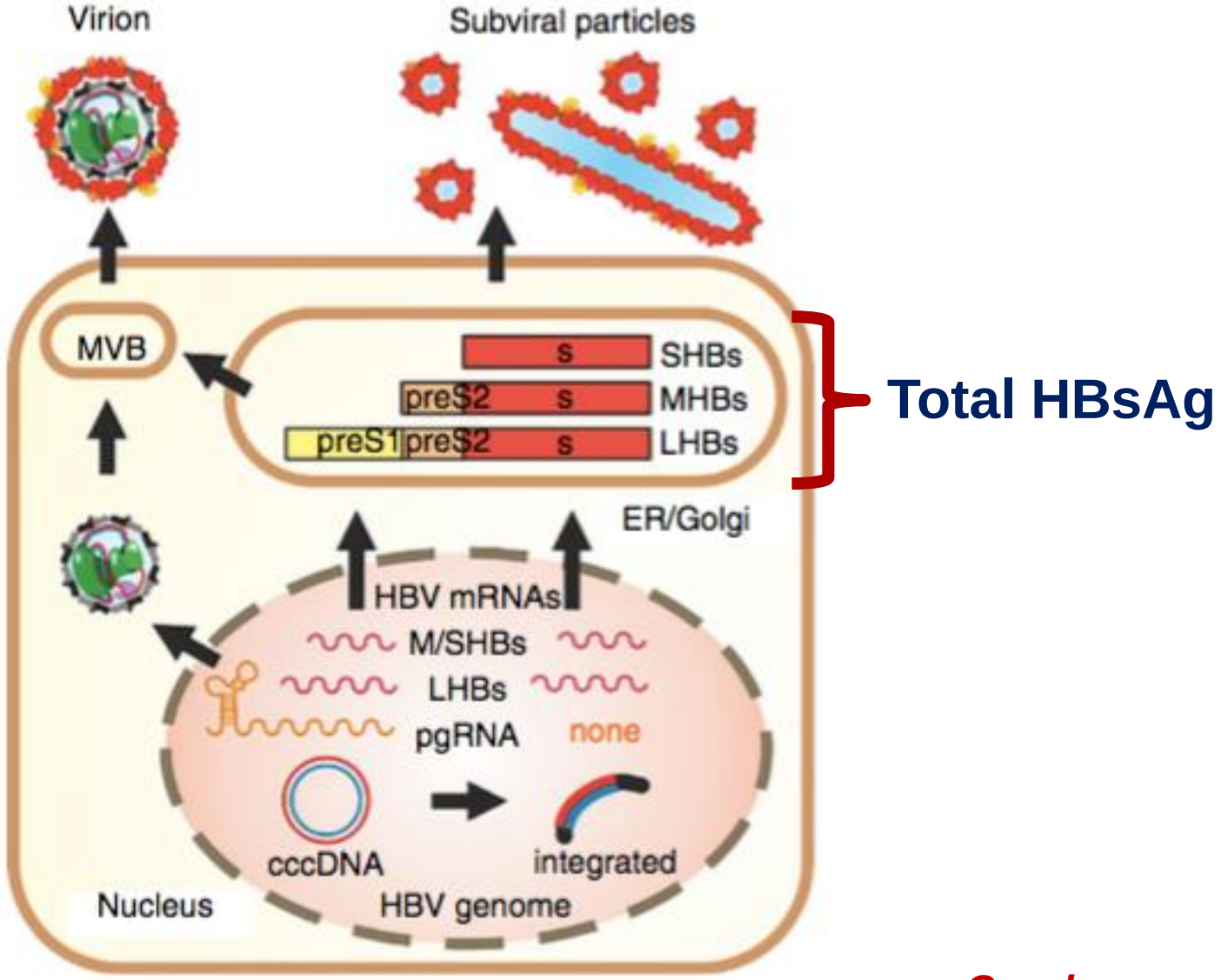
Classical biomarkers	Classical biomarkers optimized	Novel biomarkers
HBV-DNA	Three forms of HBsAg	HBcrAg
Quantitative HBsAg	Ultra-sensitive HBsAg	Serum HBV-RNA
Qualitative HBeAg/anti-HBe	Quantitative HBeAg	
Qualitative anti-HBc	anti-HBc titer	
Anti-HBs titer		

- **Serum HBV-DNA: an excellent biomarker to monitor the efficacy of NUC. Nevertheless, it cannot provide information on the intrahepatic HBV reservoir in virologically suppressed patients**



Nguyen, T. and Locarnini, S.
Nat. Rev. Gastroenterol. Hepatol. 2009

- **Quantification of total HBsAg: it allows to quantify all the forms of HBsAg, including Large-HBs, Medium-HBs and Small-HBs**



Cornberg and Glebe APT 2021

When to use HBsAg quantification:

- **Stopping rules to interferon-alpha**

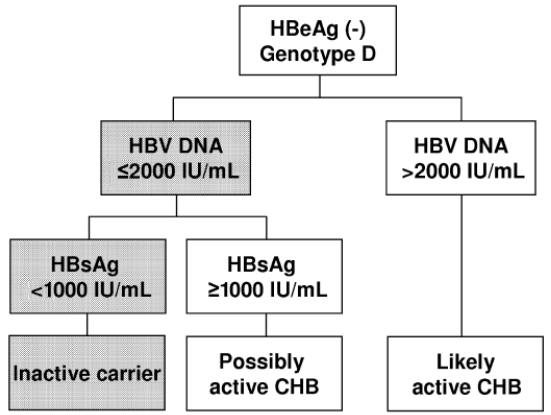
Week 12 Versus the Baseline			
HBsAg Decline	HBV DNA Decline ≥2 Log Copies/mL	Chance of SR	Recommendation to Continue
no	no	Absent	stop
no	yes	Intermediate	continue
yes	no	Intermediate	continue
yes	yes	High	strong recommendation for continuation

Rijckborst et al. Hepatology 2010, Rijckborst et al. J Hepatol 2012, Lampertico P, et al. EASL 2012

- **Prediction of vertical transmission**

HBsAg>4.1 log10IU/mL identified mothers who had transmitted the virus to their newborn with 100%sensitivity and 71% specificity (*EASL guidelines*)

- **Differential diagnosis between HBeAg-negative infection and hepatitis**



Brunetto et al., Hepatology 2010

Diagnostic objective

In chronic HBV infection, a category of individuals deserving clinical attention is represented by HBeAg-negative patients with low serum HBV-DNA: need to differentiate patients with

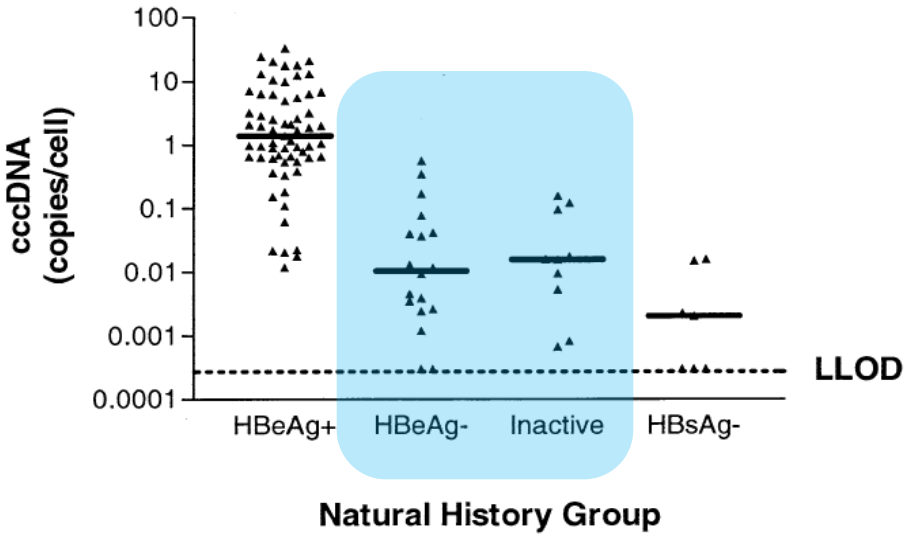
HBeAg-negative infection

(Lower risk of disease progression)

HBeAg-negative hepatitis (in remission phase)

(Higher risk of disease progression)

Common features between HBeAg-negative infection and HBeAg-negative hepatitis (in remission phase)



- HBsAg-positive
- HBeAg-negative
- HBV DNA <2000IU/ml
- Persistently normal transaminases
- Comparable cccDNA burden

Werle et al., Gastroenterology 2004

Quantitative HBsAg can help us in discriminating these 2 conditions

HBsAg quantification plays a pivotal role in the differential diagnosis between HBeAg-negative infection and hepatitis

Hepatitis B Surface Antigen Serum Levels Help to Distinguish Active From Inactive Hepatitis B Virus Genotype D Carriers

Normal ALT levels combined with HBV DNA levels ≤2,000 IU/ml and qHBsAg levels <1,000 IU/ml at a single time point had a positive predictive value (PPV) of 88% and a negative predictive value (NPV) of 97% for the iden- tification of inactive carriers in those with genotype D chronic HBV infection

Brunetto et al., Gastroenterology 2010

Serum levels of hepatitis B surface antigen and DNA can predict inactive carriers with low risk of disease progression

In HBeAg-negative patients with genotype B or C HBV infection, these three combined criteria had a PPV of 83% and a NPV of 74% for identifying inactive carriers

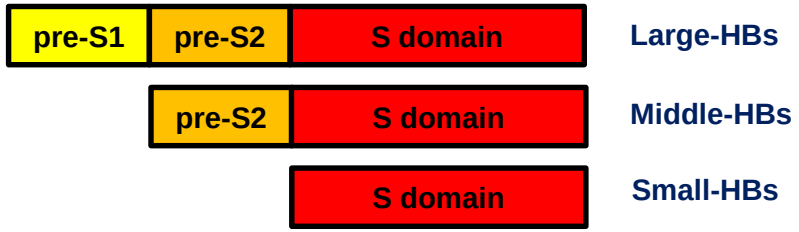
Liu et al., Hepatology 2016

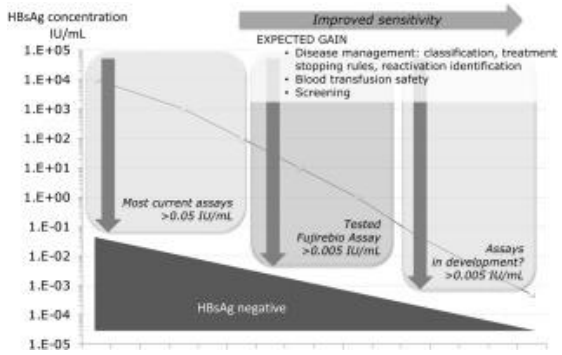
Going towards an optimization of HBsAg

Quantification of 3 HBsAg forms

Ultra-sensitive HBsAg

Quantification of HBsAg immunocomplexes





An Increase in the Levels of Middle Surface Antigen Characterizes Patients Developing HBV-Driven Liver Cancer Despite Prolonged Virological Suppression

Brancaccio, Salpini et al., Microorganisms 2021

- In conclusion, an increase in M-HBs levels characterizes a significant fraction of HCC-patients while under prolonged HBV suppression and stable/reduced total-HBs. The role of M-HBs kinetics in identifying patients at higher HCC risk deserves further investigation.

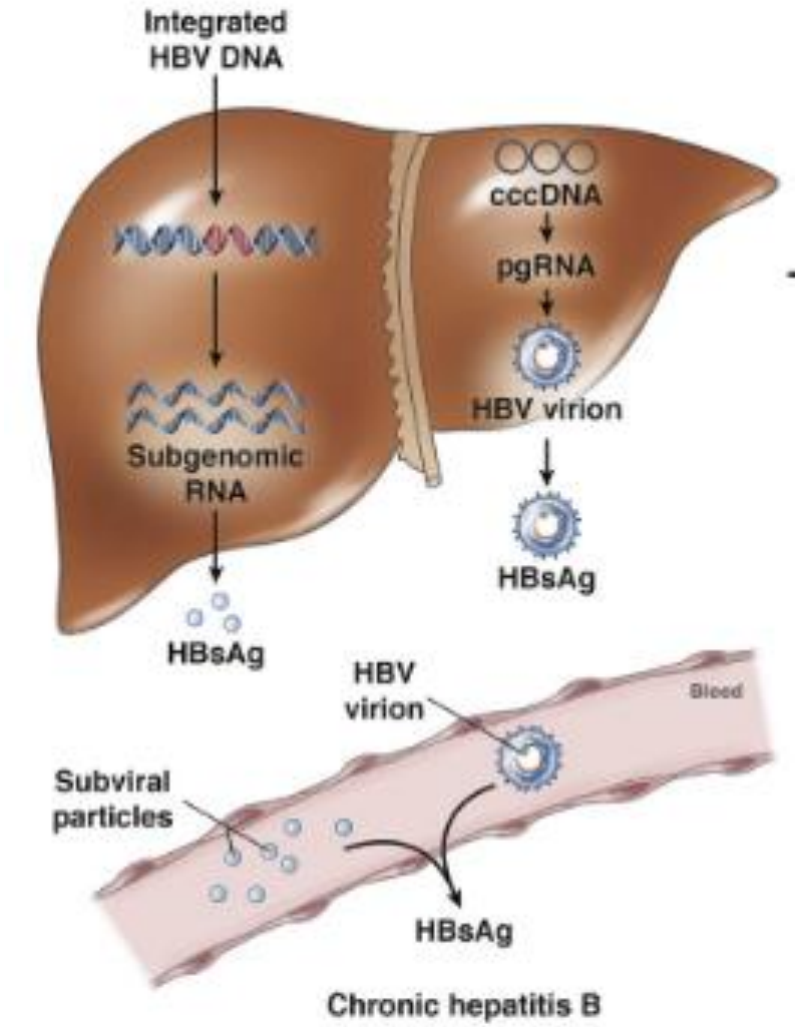
Serum PreS1 and HBsAg ratio reflects liver fibrosis and predicts the development of hepatocellular carcinoma in chronic hepatitis B patients

- Multivariate analysis identified age ≥53 years and preS1/HBsAg ratio ≥0.12 as significant and independent factors for HCC development in CHB patients. The preS1/HBsAg ratio directly reflects liver fibrosis, and the ratio might be a predictive marker for HCC development in CHB patients.

Nishida et al., JVH 2021

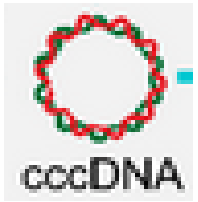
An important concept to be taken into account on HBsAg……

- **In HBeAg-negative hepatitis, integrated HBV DNA may be important source of HBsAg**



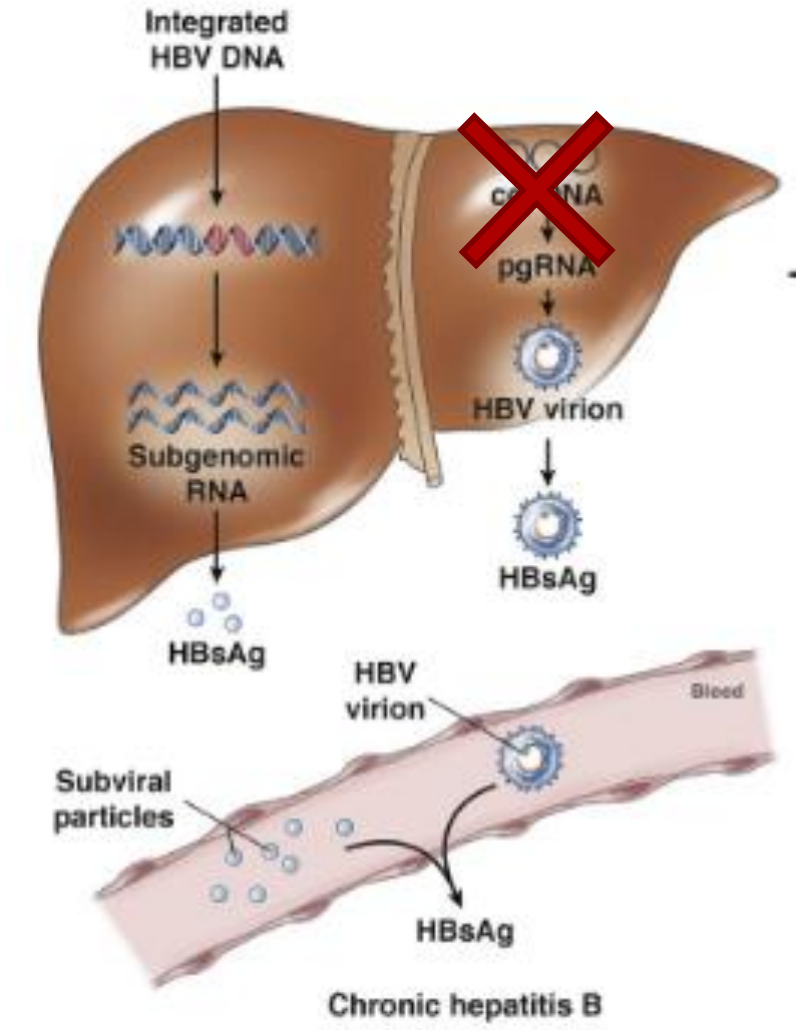
HBsAg amount can derive from

cccDNA **Integrated HBV DNA**

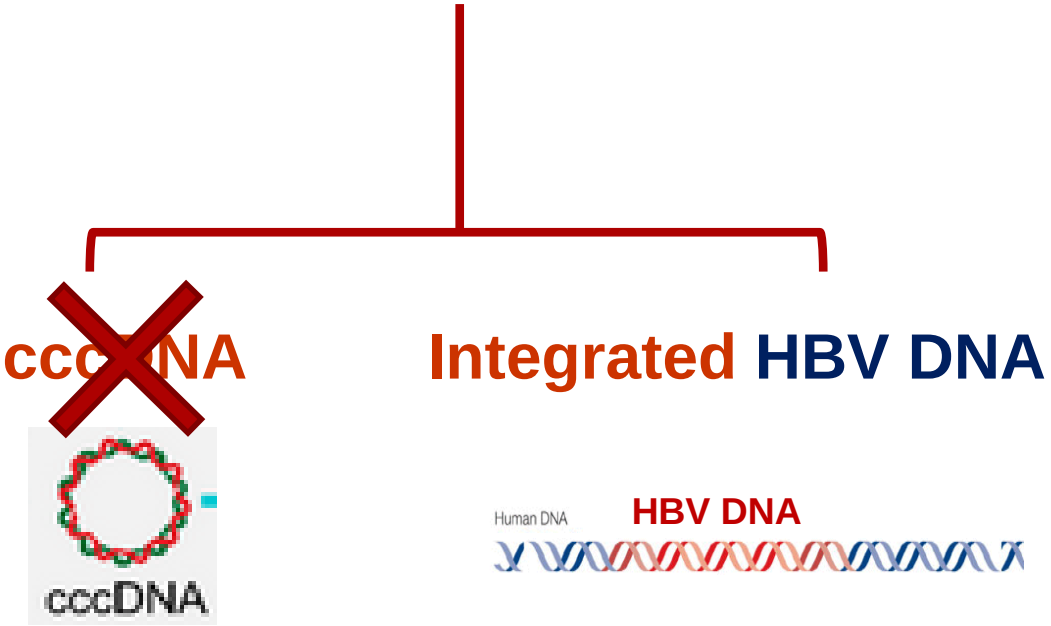


Wang and Duscheiko, Gastroenterol 2019
Liaw, Nature Reviews 2019
Meier et J Hepatol 2021

- **In HBeAg-negative hepatitis, integrated HBV DNA may be important source of HBsAg even in presence of completely silenced cccDNA**



HBsAg amount can derive from



Wang and Duscheiko, Gastroenterol 2019
Liaw, Nature Reviews 2019
Meier et J Hepatol 2021

Overall findings support the importance to integrate classical and novel HBV biomarkers

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Towards the optimization of classical biomarkers

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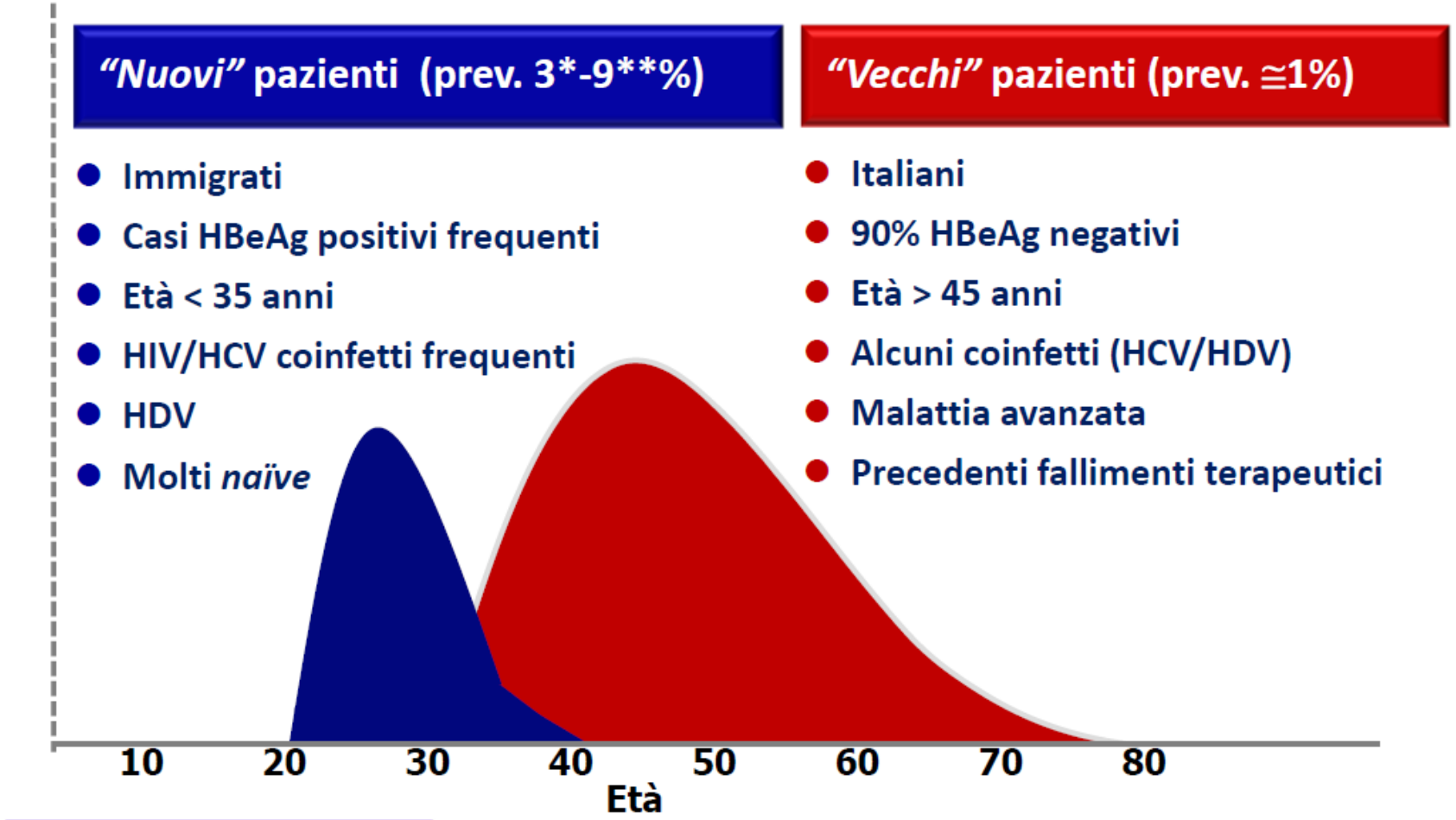
Anti-HBs titer		

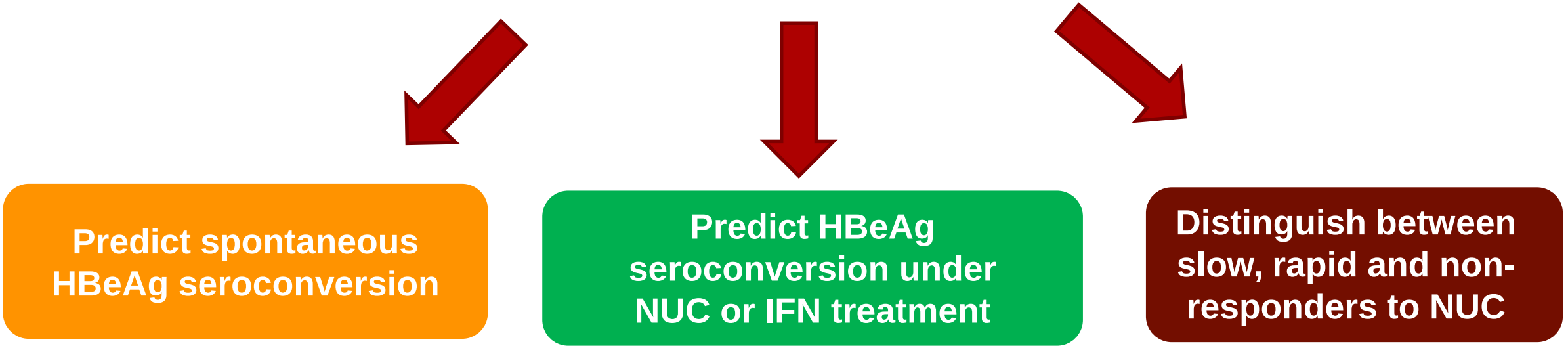
Increased proportion of HBeAg-positive young patients in Italy



* gravide; **migranti

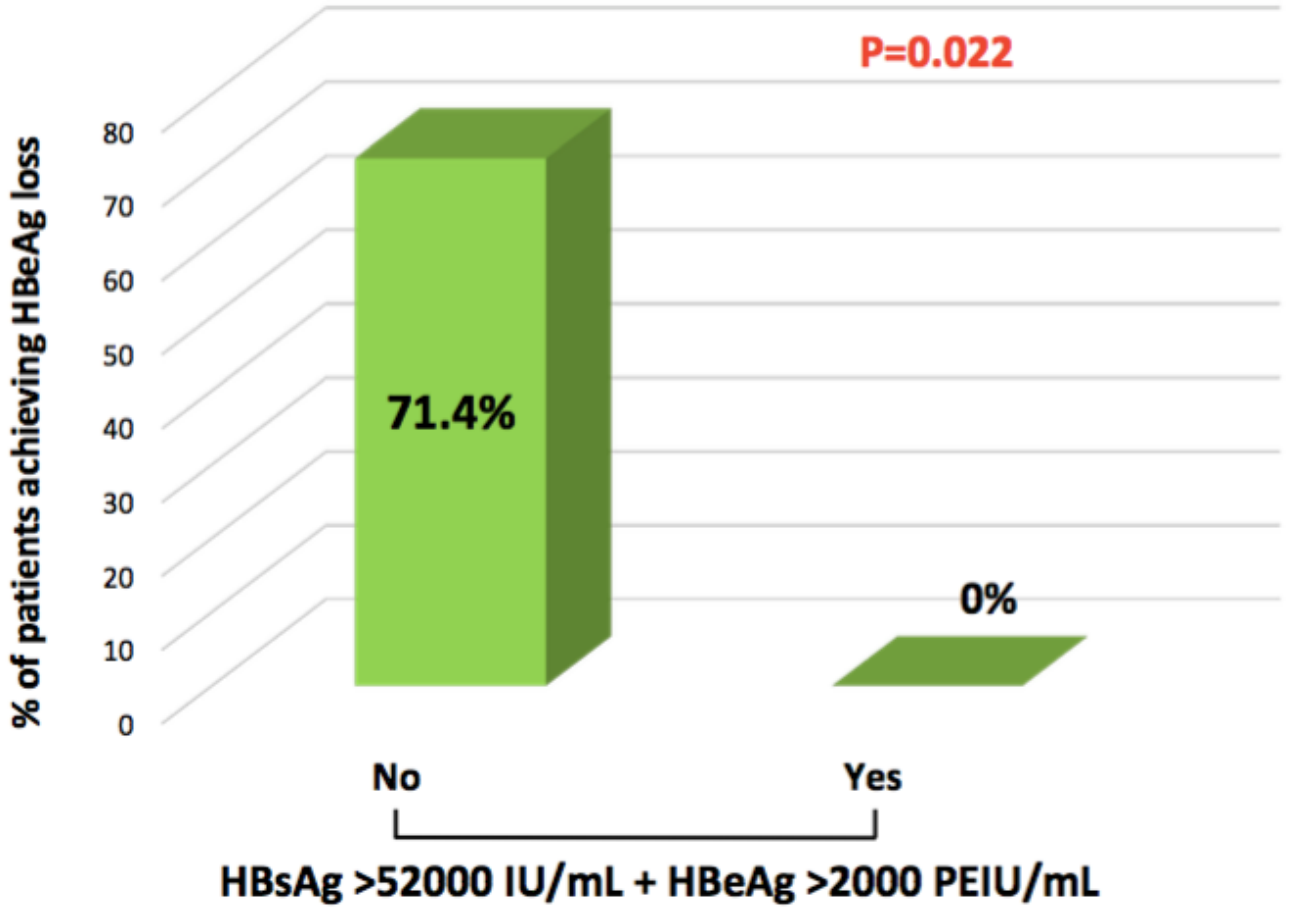
Modified from A. Marzano

Quantification of HBeAg can be useful to



Buster et al., Am J Gastroenterol 2009; Thompson et al., Hepatology 2010; Lee et al., Hepatology 2011; Matthews et al., Plos One 2013; Boyd et al., Liver Int 2015; Kramvis et al., Front Microbiol 2018; Lee et al., JVH 2021

In immunesuppressed patients with HBV-reactivation, the combination of high qHBeAg and high qHBsAg identifies patients not reaching HBeAg seroconversion during anti-HBV treatment

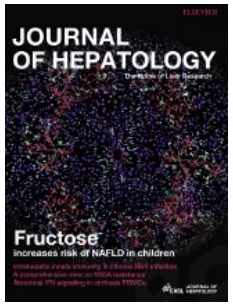


Median time of observation: 24 (min-max 12-36) months

Piermatteo et al., Biomedicines 2021

What about anti-HBc titer?

J Hepatol. 2018 Apr 2. pii: S0168-8278(18)31963-9. doi: 10.1016/j.jhep.2018.03.021. [Epub ahead of print]



Quantitation of HBV cccDNA in anti-HBc-positive liver donors by droplet digital PCR: a new tool to detect occult infection.

Caviglia GP[1], Abate ML[2], Tandoi F[3], Ciancio A[2], Amoroso A[4], Salizzoni M[3], Saracco GM[2], Rizzetto M[2], Romagnoli R[3], Smedile A[2].

Author information

Abstract

BACKGROUND & AIMS: The accurate diagnosis of occult HBV infection (OBI) requires the demonstration of HBV DNA in liver biopsies of HBsAg-negative subjects. However, in clinical practice a latent OBI is deduced by the finding of the antibody to the HB-core antigen (anti-HBc). We investigated the true prevalence of OBI and the molecular features of intrahepatic HBV in anti-HBc-positive subjects.

METHODS: The livers of 100 transplant donors (median age 68.2 years; 64 males, 36 females) positive for anti-HBc at standard serologic testing, were examined for total HBV DNA by nested-PCR and for the HBV covalently closed circular DNA (HBV cccDNA) with an in-house droplet digital PCR assay (ddPCR) (Linearity: R^2 = 0.9998; lower limit of quantitation and detection of 2.4 and 0.8 copies/10^5 cells, respectively).

RESULTS: A true OBI status was found in 52% (52/100) of the subjects and cccDNA was found in 52% (27/52) of the OBI-positive, with a median 13 copies/10^5 cells (95% confidence interval 5-25). Using an assay specific for anti-HBc of IgG class, the median antibody level was significantly higher in HBV cccDNA-positive than negative donors (5.7 [3.6-9.7] vs. 17.0 [7.0-39.2] COI, p = 0.007). By multivariate analysis, an anti-HBc IgG value above a 4.4 cut-off index (COI) was associated with the finding of intrahepatic HBV cccDNA (OR = 8.516, p = 0.009); a lower value ruled out its presence with a negative predictive value of 94.6%.

CONCLUSIONS: With a new in-house ddPCR-based method, intrahepatic HBV cccDNA was detectable in quantifiable levels in about half of the OBI cases examined. The titer of anti-HBc IgG may be a useful surrogate to predict the risk of OBI reactivation in immunosuppressed patients.

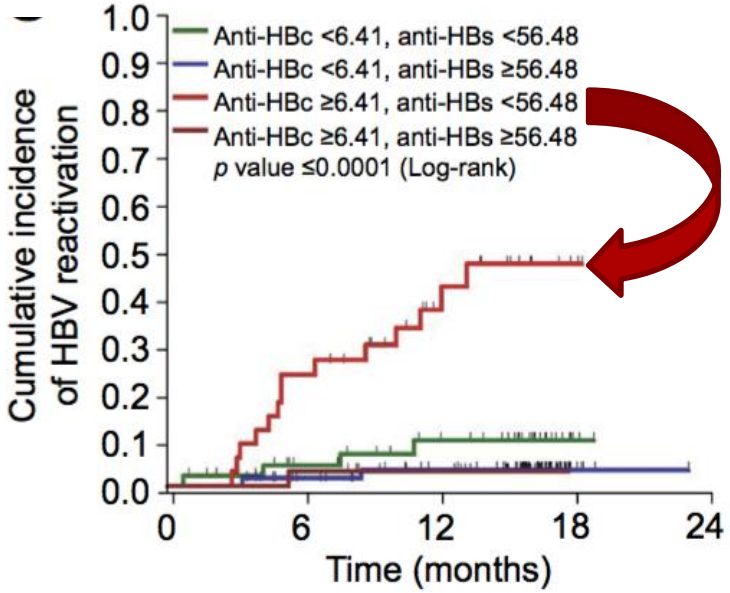
LAY SUMMARY: The covalently closed circular DNA (cccDNA) form of the Hepatitis B virus (HBV) sustains the persistence of the virus even after decades of resolution of the florid infection (Occult HBV infection=OBI). In the present study we developed an highly sensitive method based on droplet digital PCR technology for the detection and quantitation of HBV cccDNA in the liver of subjects with OBI .We observed that the amount of HBV cccDNA may be inferred from the titer in serum of the IgG class antibody to the hepatitis B core antigen (anti-HBc IgG). The quantitation of anti-HBc IgG may represent a surrogate to discriminate the patients at the highest risk of HBV reactivation following immunosuppressive therapies.

Anti-HBc titers can represent a valid surrogate marker for estimating HBV intrahepatic reservoir

The added value of combining anti-HBc and anti-HBs to predict HBV reactivation risk

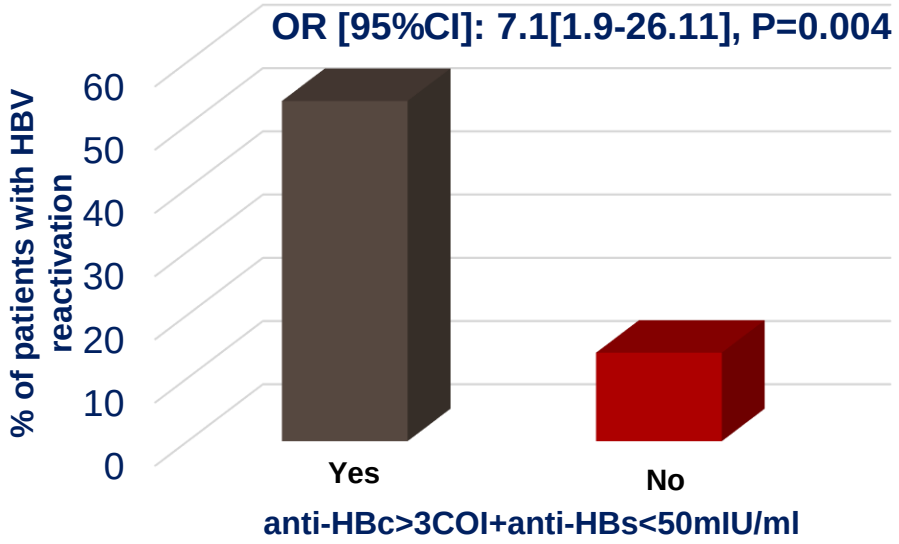
Baseline anti-HBc >6.4COI + anti-HBs<56.48mIU/ml may predict HBV reactivation in patients with lymphoma and help optimize prophylactic antiviral therapy for high-risk patients

Yang et al., J Hepatol 2018



The combination of anti-HBc>3COI and anti-HBs persistently or declining to <50mIU/ml correlates with a higher risk to develop HBV-reactivation after suspending antiviral prophylaxis in oncohematological patients

Cerva & Salpini et al., EASL 2019 and manuscript under submission



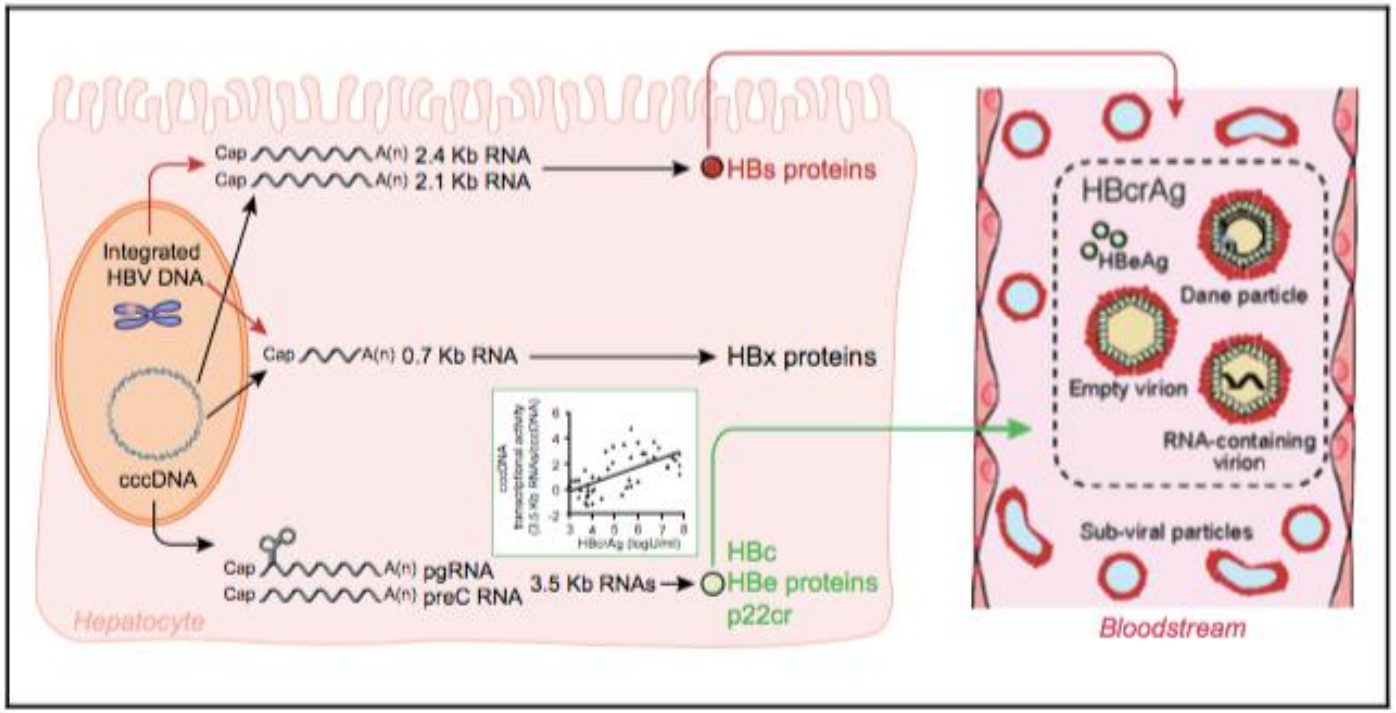
Overall findings support the importance to integrate classical and novel HBV biomarkers

Classical biomarkers	Classical biomarkers optimized	Novel biomarkers
HBV-DNA	Three forms of HBsAg	HBcrAg
Quantitative HBsAg	Ultra-sensitive HBsAg	Serum HBV-RNA
Qualitative HBeAg/anti-HBe	Quantitative HBeAg	
Qualitative anti-HBc 	anti-HBc titer 	
Anti-HBs titer 		

The quantification of HBcrAg reflects the capsid protein HBcAg, HBeAg and a precore protein (p22) coded with the precore/core region

- In **HBeAg positive patients,** HBcrAg mainly reflects the total amount of cccDNA. This is explained by the fact that HBcrAg is the measure of the translation of both pre-core and pgRNA cccDNA transcripts

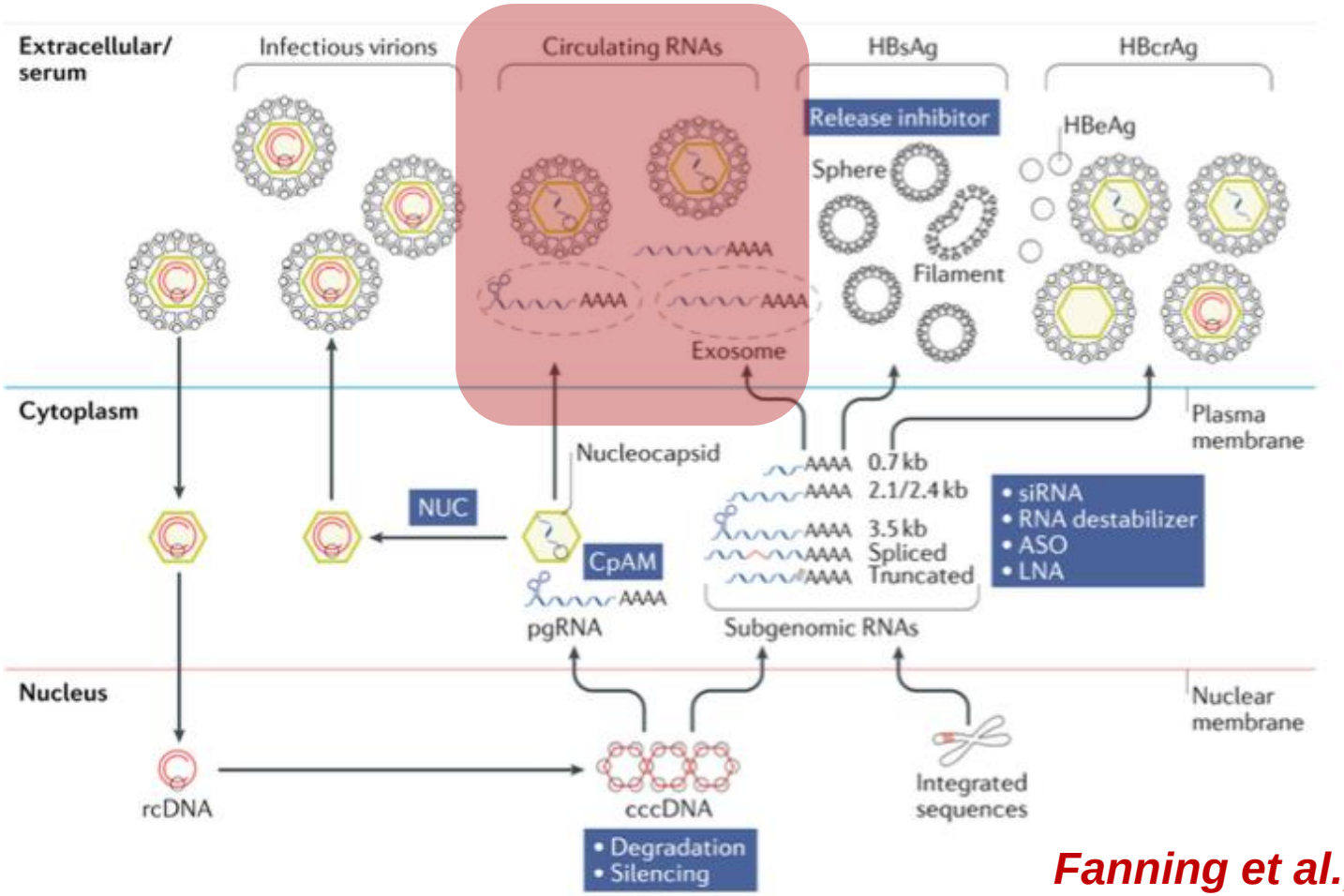
- In **HBeAg negative patiens,** HBcrAg correlates better with cccDNA trasncriptional activity (pgRNA and pgRNA/cccDNA) since HBcrAg is the measure of pgRNA translation



Testoni et al., J Hepatol 2018

HBV-infected hepatocytes can release:

- **viral particles containing HBV-DNA produced by RT**
- **viral particles containing pre-genomic RNA (not retrotranscribed)**
  - **Thus, serum HBV-RNA measures the release of viral particles containing pre-genomic RNA and thus it measures cccDNA transcriptional activity**



Fanning et al., Nature Review 2019

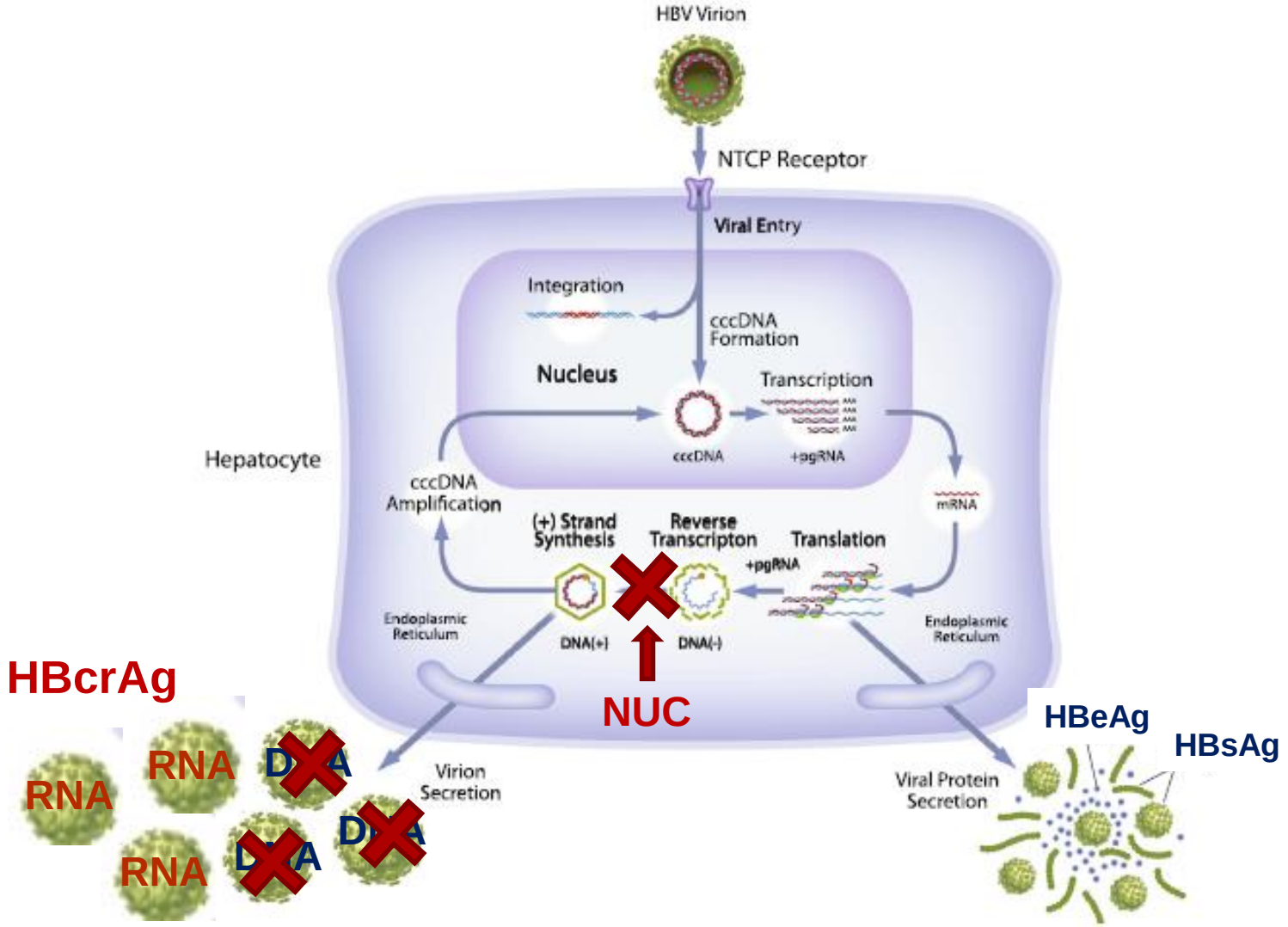
The role of HBcrAg and serum HBV-RNA has been investigated in different clinical clinical settings:

- **Differential diagnosis between HBeAg chronic infection and hepatitis** (***Wang et al., JVH 2018; Mak et al., Aliment Pharmacol Ther 2018; Brunetto et al., APT 2020)***

- ***Risk to develop HCC*** ***(Honda 2016; Tada 2016, Tseng 2019 Hosaka 2019)***

and also…..

- **NUCs cannot affect the production of HBcrAg and serum HBV-RNA**
- **The biomarkers provide the advantage to measure intrahepatic HBV reservoir in virologically suppressed patients**



- **HBcrAg and serum HBV-RNA progressively decrease during long term NUC treatment**
- **At 5 year of treatment, 14% and 27% of patients are still positive to serum HBV-RNA and HBcrAg**

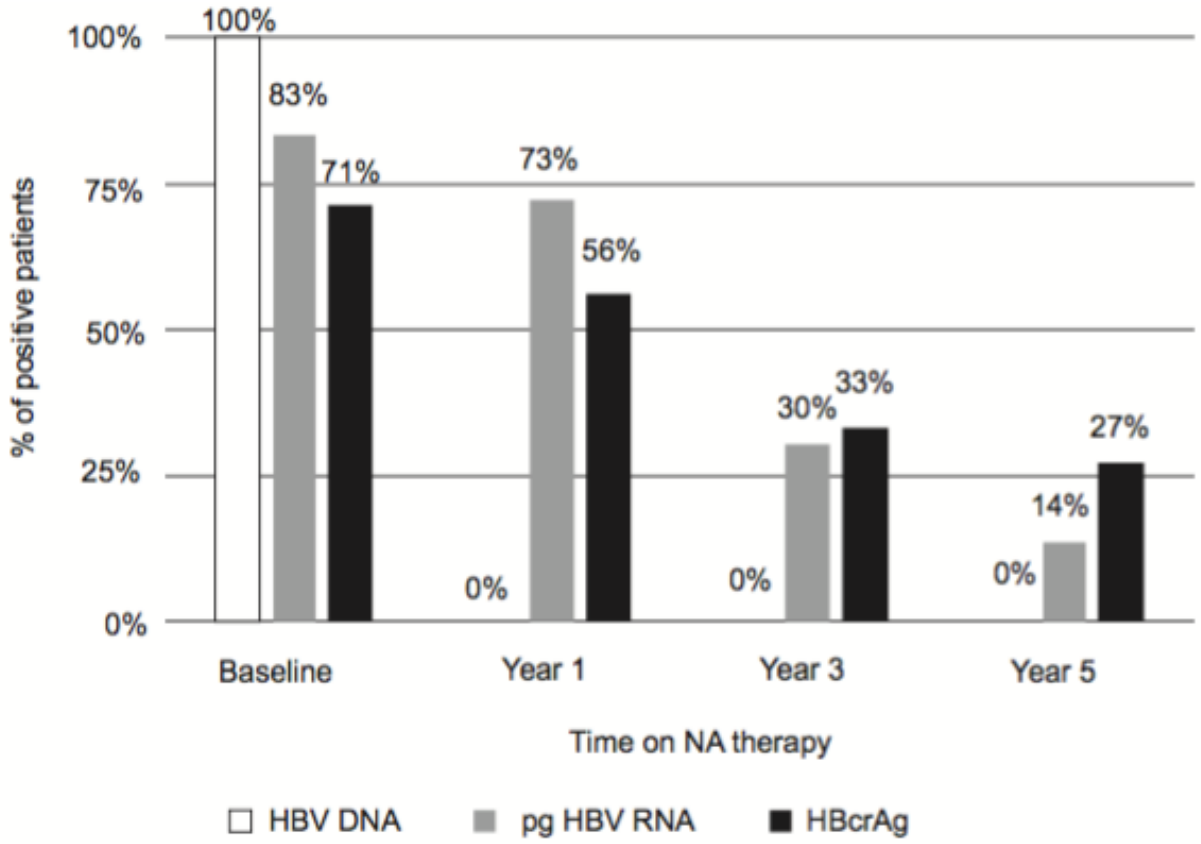


FIG. 1. Proportions of patients with detected HBV DNA, pg HBV RNA, and HBcrAg during NA therapy in cohort A.

Carey et al., Hepatology 2020

Factors Associated With the Biphasic Kinetics of Serum HBV RNA in Patients With HBeAg-positive Chronic Hepatitis B Treated With Nucles(t)ide Analogues

Liu et al., APT 2020

Methods: We enrolled 76 HBeAg-positive chronic hepatitis B patients receiving NAs from randomised controlled trials. Laboratory assays were undertaken every 3 months. Factors associated with serum HBV RNA kinetics were identified by generalised estimating equations.

Results: Baseline serum HBV RNA was 8.5 ± 1.0 log_{10} copies/mL. Decline in serum HBV RNA during NAs therapy was biphasic: the first phase (HBV DNA detectable) had a fast decrease (median slope, -0.207 log_{10} copies/mL/month) and was followed by a second phase (HBV DNA undetectable) with slow decrease (median slope, -0.071 log_{10} copies/mL/month). In the first phase, factors independently associated with lower initial serum HBV RNA were male sex (OR, 0.685, P = 0.044), low baseline HBsAg (OR, 0.525, P = 0.001) and rapid virological response (RVR) (OR, 0.624, P = 0.031). In the second phase, only RVR was independently associated with serum HBV RNA kinetics, including its lower initial level (OR, 0.694, P = 0.043) and greater decline (OR, 0.966, P = 0.002). Based on viral dynamics, time needed to achieve undetectable serum HBV RNA from baseline was 43.56 (IQR: 29.49-66.40) months.

Conclusion: RVR was a significant determinant for biphasic decline in serum HBV RNA during NAs treatment, which significantly influenced the treatment duration required to achieve undetectable serum HBV RNA.

The progressive decrease in HBcrAg and serum HBV-RNA levels can be explained:

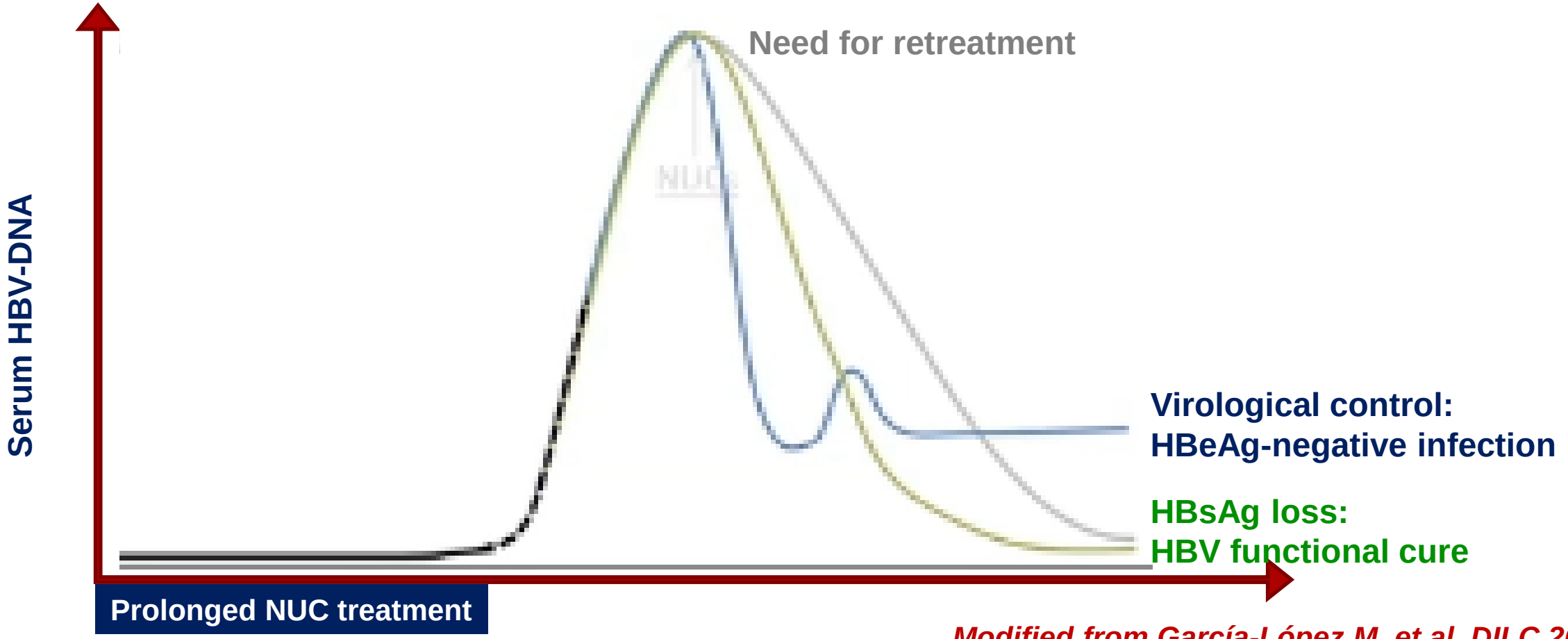
- **potential weakening of cccDNA transcriptional activity during NUC treatment*? (according to Balagopal et l., JCI Insight. 2020)***
- **reduced pool of infected hepatocytes? *(according to Huang et al., Hepatology 2021)***

Further studies are necessary to unravel this intriguing topic not perceived by using the classical HBV markers

HBV biomarkers & NUC suspension

Outcomes of NUC suspension:

- Achieving the status of HBeAg-negative chronic infection
- Achieving the status of HBsAg loss (functional cure)
- Need to restart treatment



Modified from García-López M, et al. DILC 2020

Viral and immune factors associated with successful treatment withdrawal in HBeAg-negative chronic hepatitis B patients

García-López M, et al. J Hepatol 2020

RESULTS

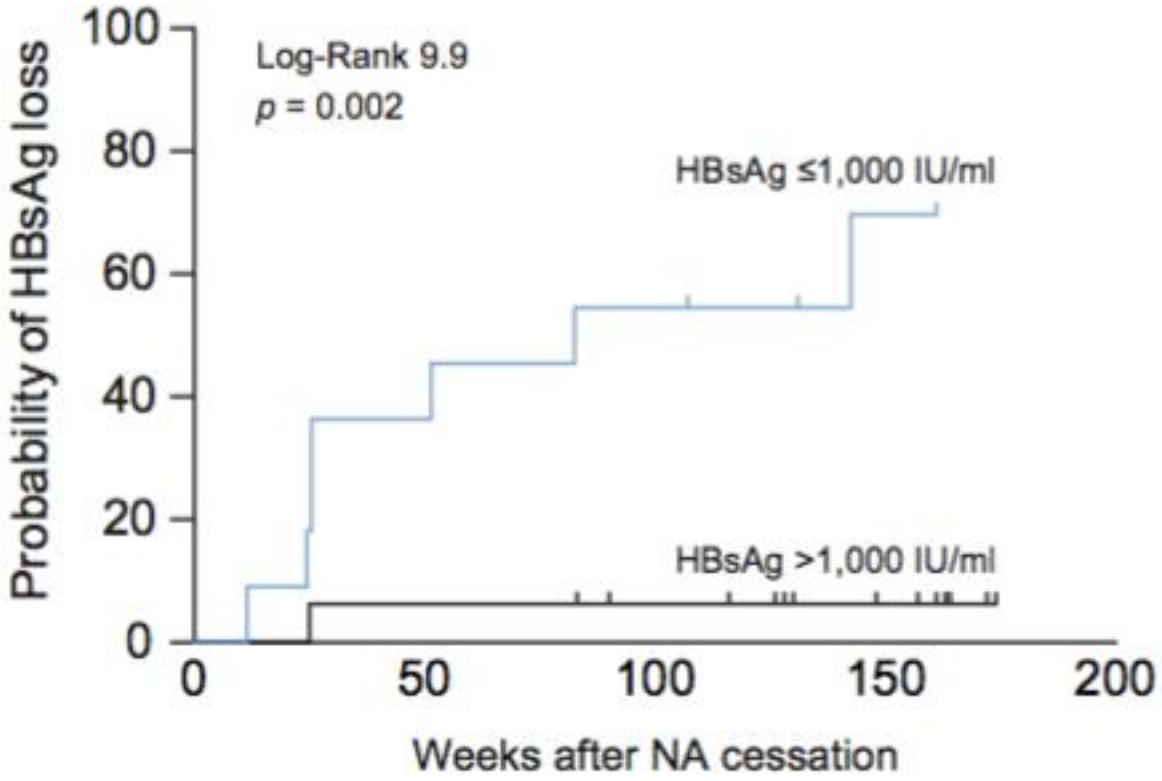
- At median FU 34 months (IQR 26−37):
  - 22 (81%) patients remain off therapy
    - **HBsAg loss in 8 (30% of total cohort)**
  - 5 (19%) required NA reintroduction due to relapse

- All patients had detectable cccDNA with similar levels regardless of clinical outcomes

- **Baseline HBsAg correlated significantly with iHBV DNA (rho = 0.65, p<0.001) and iHBV RNA (rho = 0.48, p<0.05), and these were lower in patients achieving HBsAg loss**

- **Decreased cccDNA transcription levels are associated with HBsAg loss**

A lower HBsAg (reflecting a limited HBV reservoir) at NUC suspension correlates with higher probability to achieve HBsAg loss



Se	Sp	PPV	NPV	LR+	LR-
88%	79%	64%	94%	4.16	0.16

García-López M, et al. J Hepatol 2020

Prediction of Sustained Response After Nucleo(s)tide Analogue Cessation Using HBsAg and HBcrAg Levels: A Multicenter Study (CREATE)

Sonneveld et al., Clin Gastroenterol Hepatol. 2020

Conclusions: In this multicenter study, off-treatment outcomes after NA cessation varied with ethnicity. **Lower levels of HBcrAg and HBsAg were associated with favorable outcomes.**

Role of serum HBV RNA and hepatitis B surface antigen levels in identifying Asian patients with chronic hepatitis B suitable for entecavir cessation

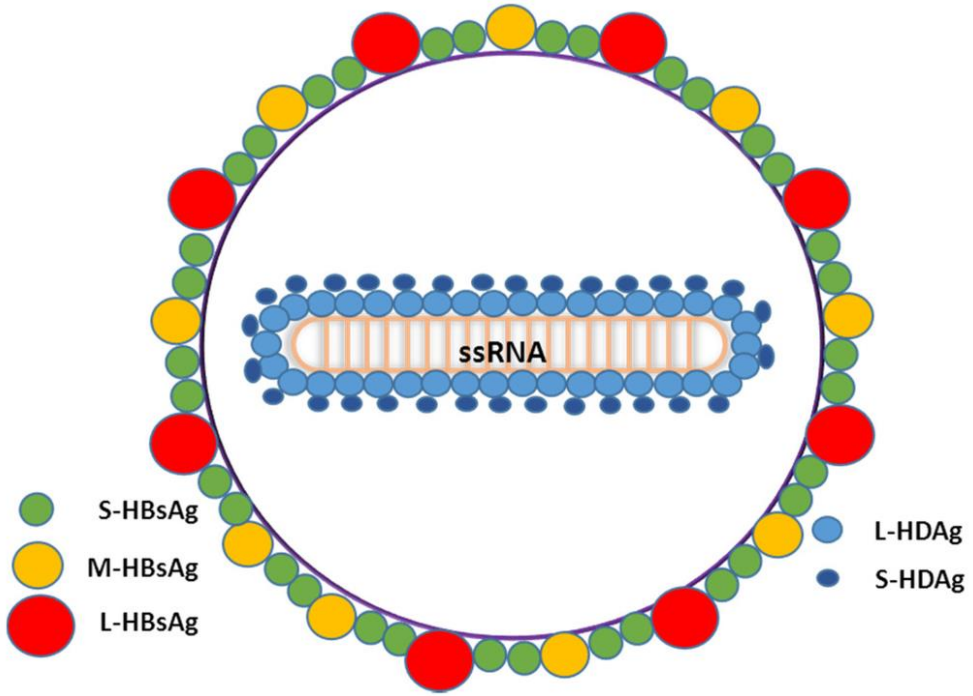
Seto et al., GUT 2020

Conclusion: Serum HBV RNA measurement is essential for deciding on entecavir cessation in patients with chronic HBV, especially with low HBsAg levels. Patients can be stratified on their risk of off-treatment relapse based on both viral determinants.

- **These findings highlight the importance of a limited intrahepatic HBV reservoir as a pre-requisite for the success of NUC suspension**

- **At the same time, these data reinforce the importance to properly integrate HBV biomarkers to optimize the management of HBV infected patients**

HDV: the smallest RNA virus, causing the most severe forms of hepatitis



Distinct Cytokine Profiles Correlate with Disease Severity and Outcome in Longitudinal Studies of Acute Hepatitis B Virus and Hepatitis D Virus Infection in Chimpanzees

Engle et al., mBio 2020

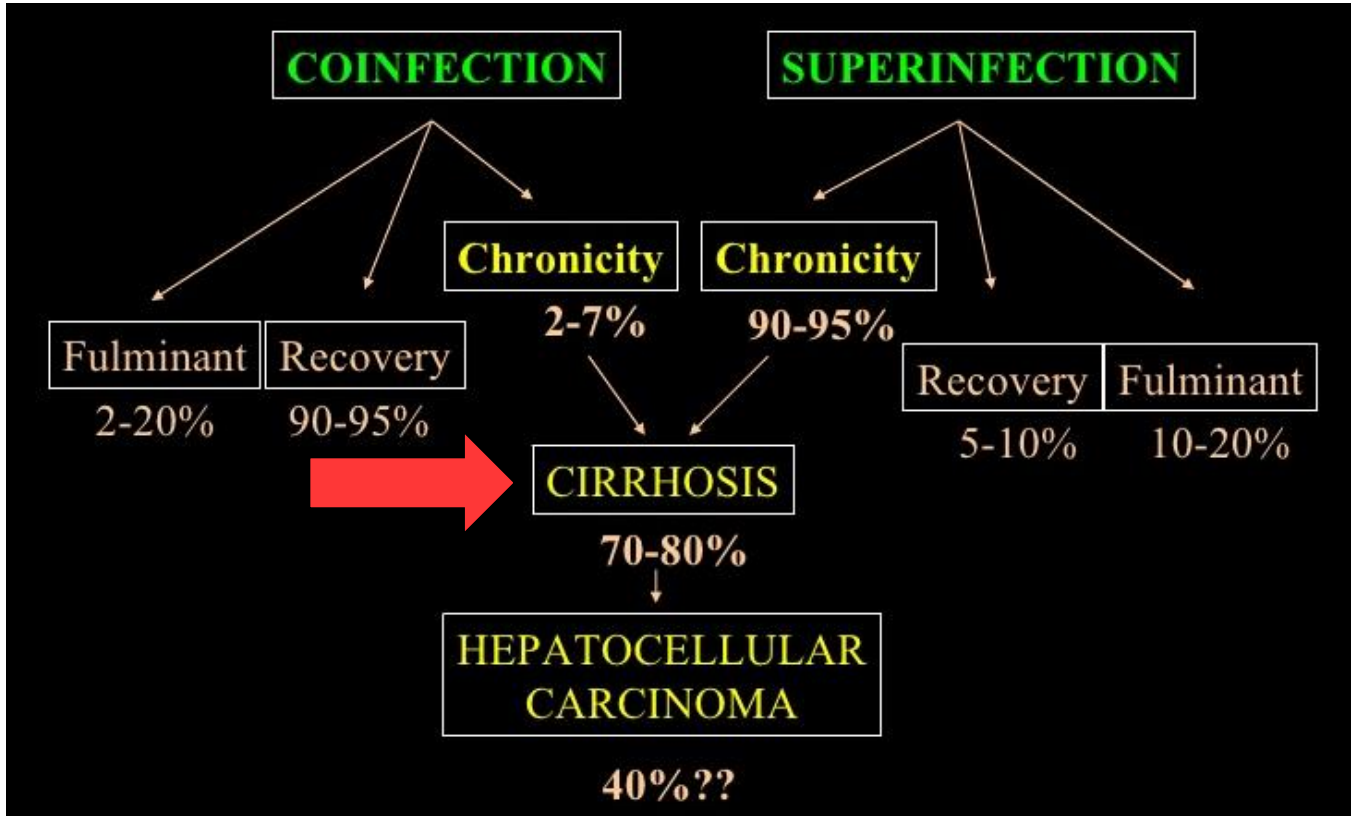
- We found that distinct cytokine profiles were associated with disease severity and clinical outcome. In particular, resolution of classic acute hepatitis B (AHB) correlated with a predominant Th1 response, whereas HBV/HDV coinfection showed a predominant proinflamma- tory response.

- Severe AHB and HDV superinfection showed a restricted cytokine pro- file and no evidence of Th1 response.

- **The lack of cytokines associated with adaptive T-cell responses toward the precore HBV mutant and HDV superinfection argues in favor of a direct cytopathic effect of these viruses.**

- Chronic HDV infection can evolge towards cirrhosis in 70-80% of patients within a decade within 5-10 years (Fattovich et al., J Hepatol 2008).

- The probability of survival at 5 and 10 years is of 49 e 40%, respectively (Sereau et al., J Hepatol 2016)



The pathogenic role of HDV strongly highlights the need to:

- **Sensitize clinicians to extend HDV testing**
- **Set up a systematic screening of HBsAg-positive patients**
- **Set up adequate prevention program**

In patients positive to anti-HDV, the quantification of serum HDV-RNA is crucial to

- **retrieve information on disease progression**
- **monitor virological response to new anti-HDV drugs**

Long-Term Study of Hepatitis Delta Virus Infection at Secondary Care Centers: The Impact of Viremia on Liver-Related Outcomes

Kamal et al., Hepatology 2020

- HDV RNA viremia is associated with a 3.8-fold higher risk for liver-related outcomes.
- The prognosis was rather poor for patients with HDV viremia without cirrhosis at baseline
- Our findings may be of importance when making decisions about treatment and evaluating potential outcomes of upcoming antivirals against HDV.

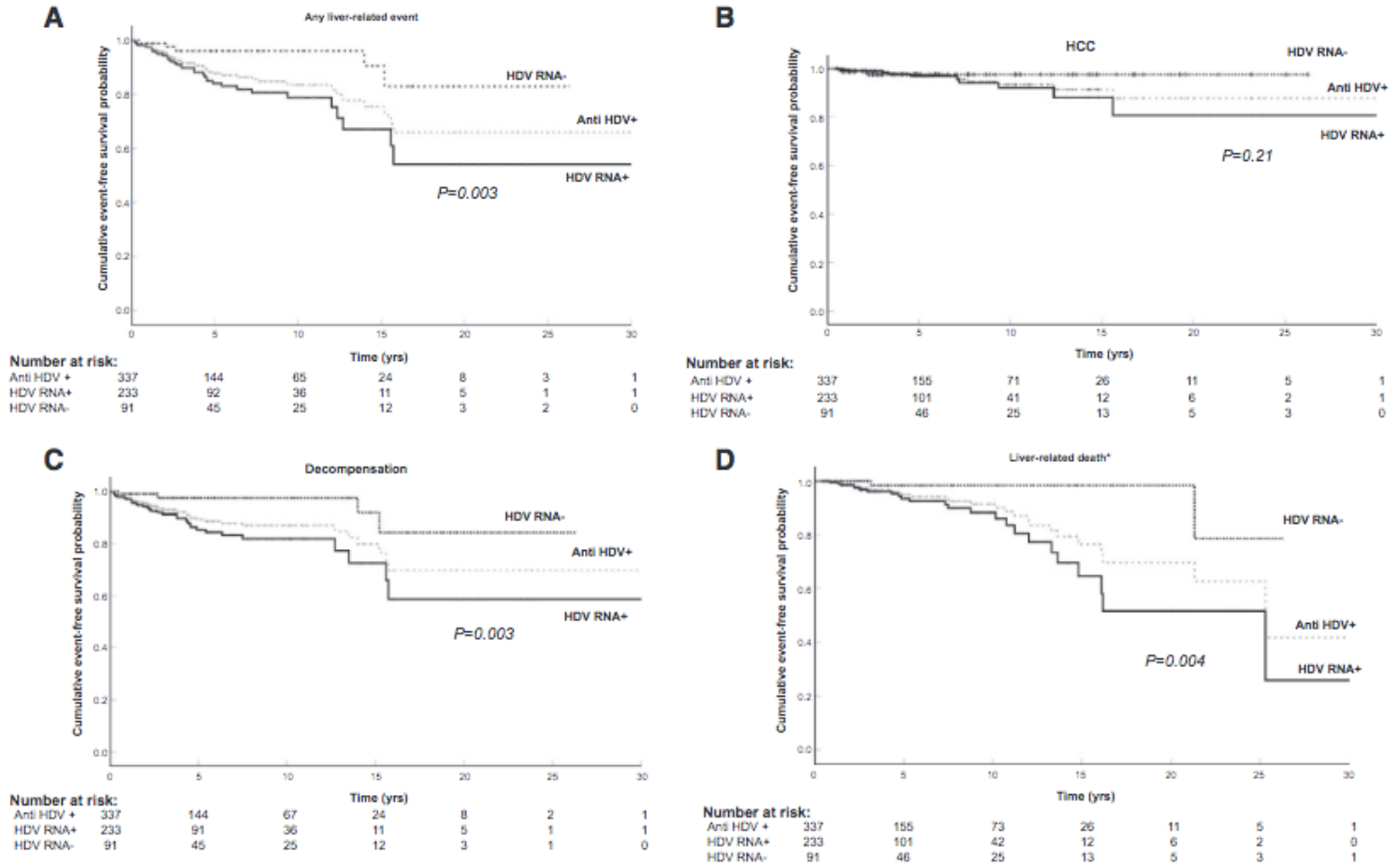


FIG. 2. Time to event-free survival by HDV RNA status for (A) any liver-related event, (B) HCC, (C) decompensation, and (D) liver-related death/liver transplantation. Abbreviations: HCC, hepatocellular carcinoma; HDV, hepatitis D virus.

In patients positive to anti-HDV, the quantification of serum HDV-RNA is crucial to

- **retrieve information on disease progression**
- **monitor virological response to new anti-HDV drugs**

Anti-HDV treatment : two possible approaches

- **Long-term monotherapy**
- **Finite treatment with IFN with the ultimate goal to achieve HDV functional cure**

Residual low HDV viraemia is associated HDV RNA relapse after PEG-IFNa-based antiviral treatment of hepatitis delta: Results from the HIDIT-II study

Bremer et al., Liver International 2020

- We found that detection of low HDV viraemia during treatment with PEG-IFNa was associated with a high risk for post-treatment virological relapse.
- Our findings confirm this association for the first time for interferon-based treatment of HDV infection as the risk of post-treatment relapse increased by about three-fold if HDV RNA was detectable in the highly sensitive assay.
- Our study highlights that reliable measurement of HDV viral load is crucial for an optimal patient management.

Conclusions

- **The spectrum of HBV biomarkers has increased:**
  - Current data highlight the importance to properly integrate and combine the classical and novel biomarkers

- **HDV diagnostics need to be further improved in term of enhanced screening and accurate assays for HDV-RNA quantification**

- **These issues are crucial for a precision medicine approach to HBsAg-positive patients with or without HDV coinfection**