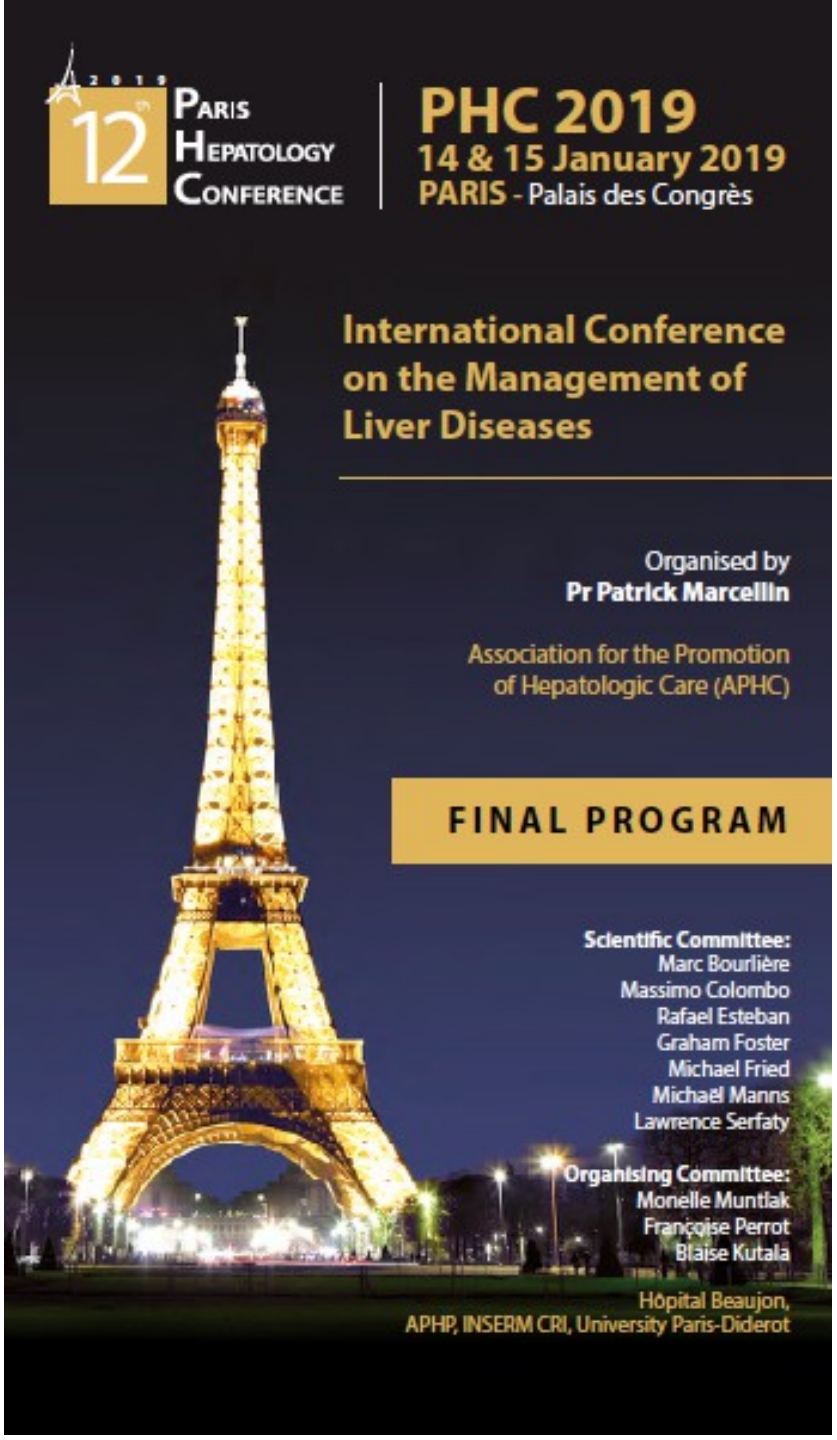

When and how to use quantitative HBsAg?

Maurizia Rossana Brunetto

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UO Epatologia – Azienda Ospedaliero Universitaria Pisana - Centro Riferimento Regionale *"Diagnosi e trattamento delle epatopatie croniche e del tumore di fegato"*

Disclosures

Advisory board: AbbVie, Gilead, Roche

Speakers' bureau: AbbVie, BMS, Gilead, MSD, Janssen

Research grant: AbbVie, BMS, MSD

Quantitative on-therapy measurement of serum HBsAg may be useful to identify patients who are most likely to benefit from interferon-based therapy and may help to tailor and optimize treatment duration

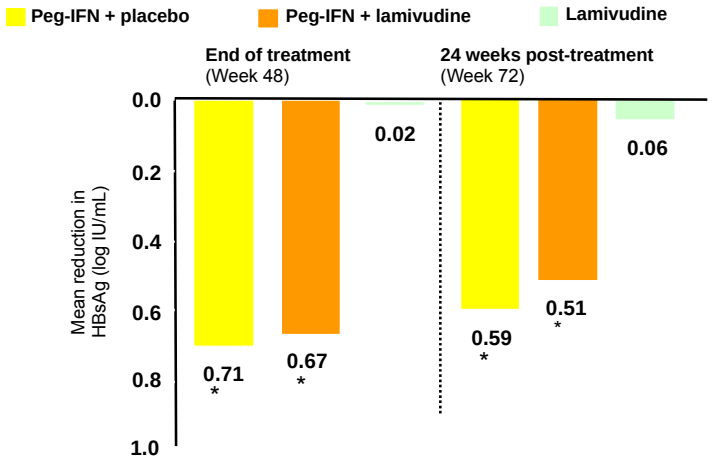
Brunetto MR et al. Hepatology 2009

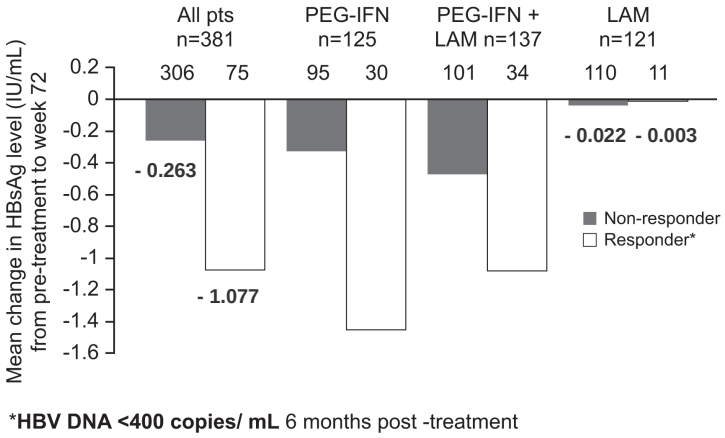
Serum quantitative HBsAg seems to be an excellent on-treatment marker predicting sustained off-treatment response, and identifying in the early phase of PEG-IFNα-2a therapy the patients who are most likely to benefit from this treatment

Moucari R et al. Hepatology 2009

HBsAg levels: a guide to sustained response to peginterferon alfa-2a in HBeAg negative CHB

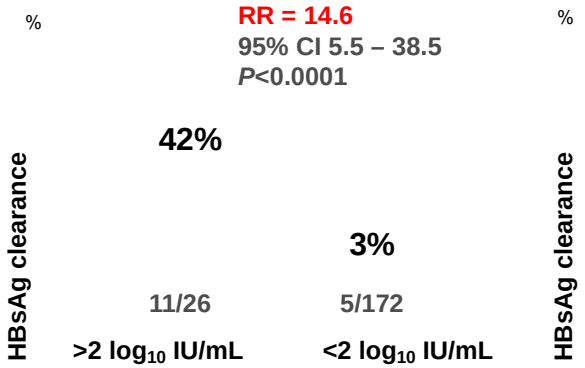
HBsAg decline according to treatment and response in 537 patients



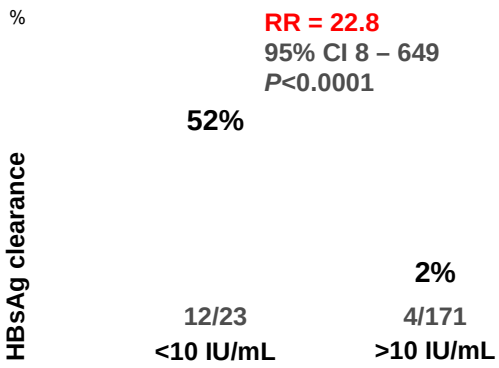


*HBV DNA <400 copies/ mL 6 months post -treatment

Predictive value of HBsAg reduction at week 48 for sustained HBsAg clearance by year 3



HBsAg reduction from BL to week 48



HBsAg levels at week 48

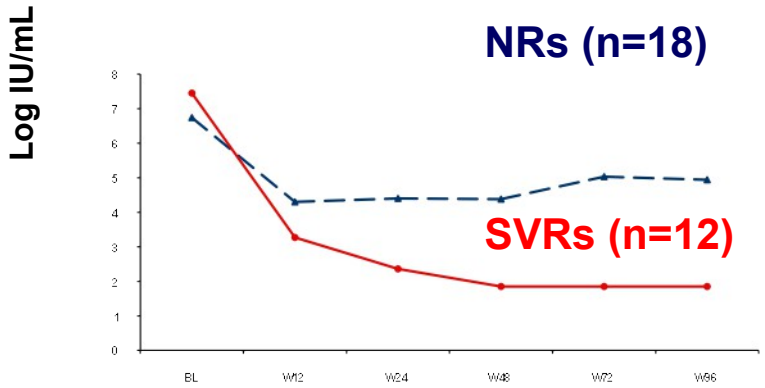
Brunetto MR et al, Hepatology 2009

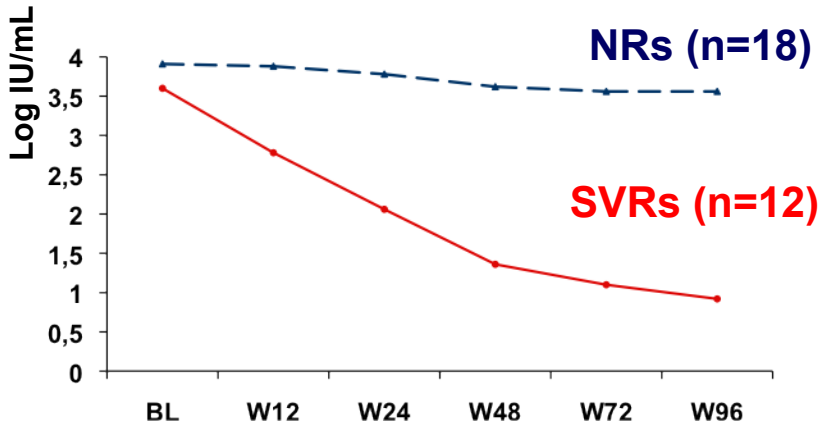
HBV-DNA and HBsAg Kinetics 48 HBeAg negative CHB patients treated with Peg-IFN

HBV DNA

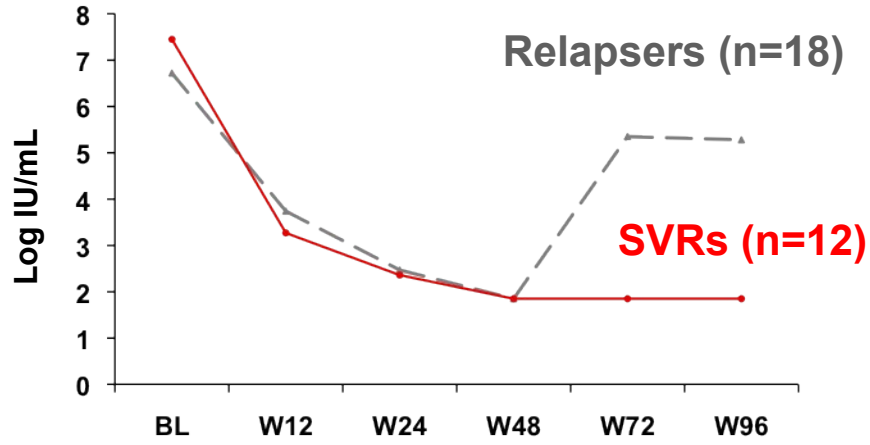
HBsAg

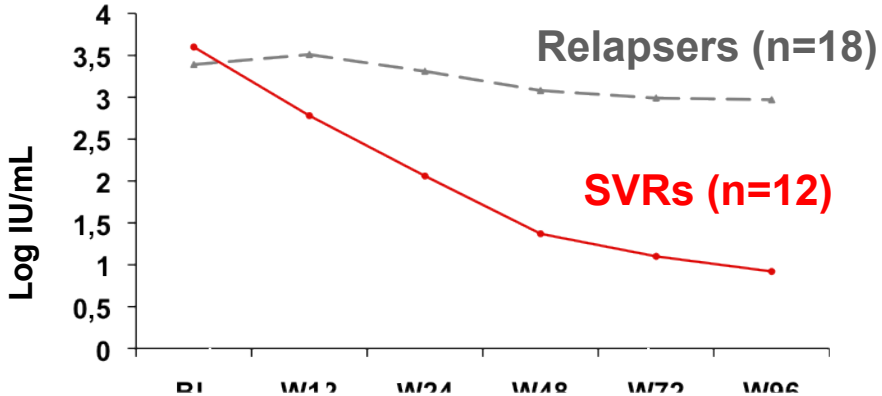
SVRs vs Non-Responders





SVRs vs Relapsers





Moucari R. et al , Hepatology 2009

HBsAg levels in the natural history of HBV-infection: A perspective on Asia and Europe

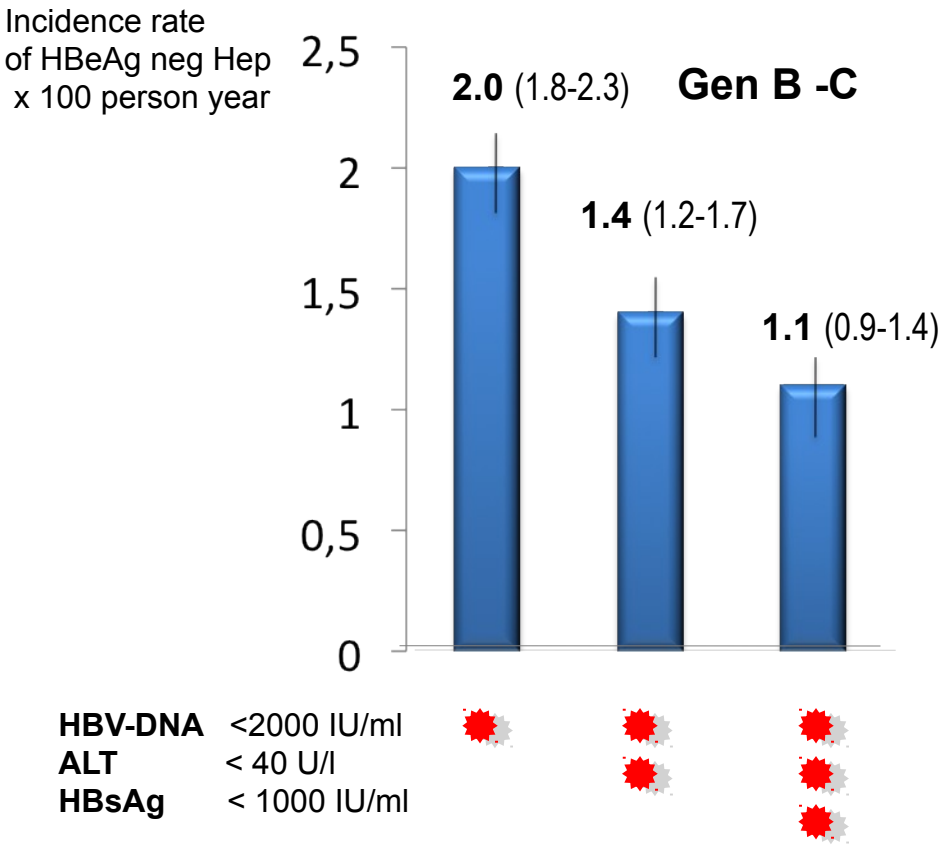
	HBeAg-positive		HBeAg-negative		P value
	Immune tolerance	Immune clearance	Immune clearance/ Reactivated	Immune control	
ASIA	**4.53**	**4.03**	**3.35**	**2.86**	**0.001**
N	32	55	83	50	
EUROPE	**4.96**	**4.37**	**3.89**	**3.09**	**<0.001**
N	30	48	68	68	

The higher is the control of HBV infection, the lower are the serum levels of HBsAg

Nguyen T et al; Jaroszewicz J et al, J Hepatol 2010

Use of HBsAg serum levels help to distinguish active from inactive HBV genotype D carriers

Prediction of:	Inactive infection
HBsAg levels HBV-DNA levels	***<1000 UI/mL plus <2000 IUI/mL***
Population	209
Sensitivity	**91.1%**
Specificity	**95.4%**
PPV	**87.9%**
NPV	**96.7%**
Diagnostic Accuracy	**94.5%**



HBsAg < 1000 IU/ml in combination with low levels of HBV-DNA and ALT helps to define minimal risk HBV carriers

Brunetto MR, Gastroenterology 2010; Tseng TC et al, Hepatology 2013

But, other factors may influence HBsAg production

Viral markers, such as HBV-DNA, HBsAg and HBeAg, are used in HBV carriers management because **their levels** and **kinetics** result from the **virus-host interplay**

However, **their levels** may be influenced by **constitutive features** of **the virus** and/or **viral infection**.

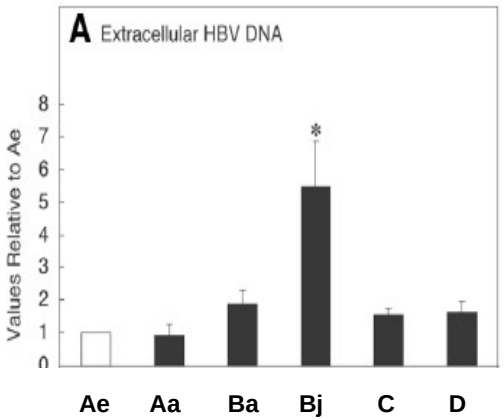
HBsAg serum levels may be influenced by:

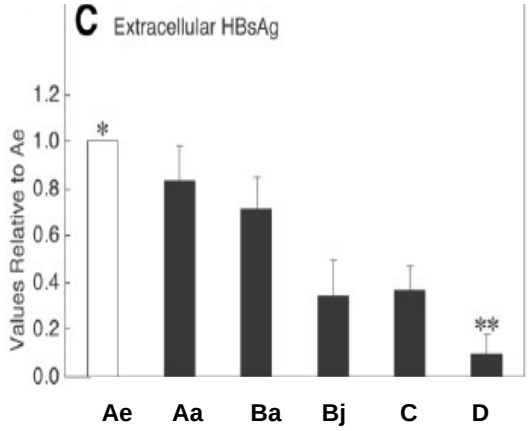
- HBV genotype
- Variability of the Pre-S/S region, by embalancing the Pre-S1/S ratio
- HBV DNA integration into the host genome

Magritte R,

Influence of HBV Genotypes on the *in vitro* Extracellular Expression of Viral DNA and Antigens and *in vivo* HBsAg serum levels

- ✓ Huh7 cells were transfected with 1.24 HBV isolated from 14 patients
- ✓ HBV-DNA and HBsAg were analysed in the medium 3 days after the transfection

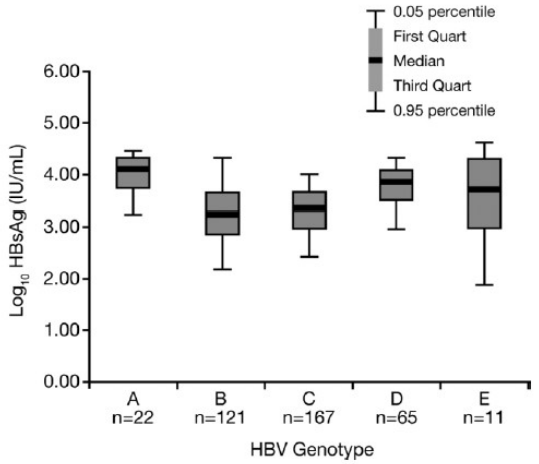




The highest levels of HBsAg secretion were observed by GT-A , the lowest by GT-D

Sugiyama M et al, Hepatology 2006

- ✓ Baseline HBsAg serum levels in 537 HBeAg negative CHB patients

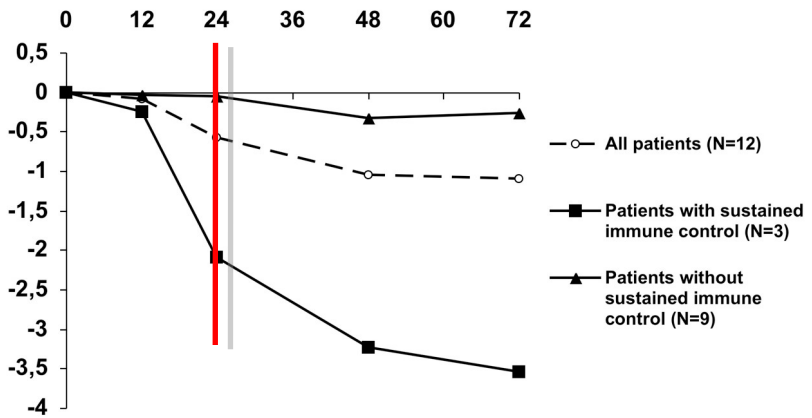


HBsAg levels varied between genotypes: the highest median levels in patients infected with HBV genotypes A (4.11 $\log^{10}$ IU/mL) and D (3.85 $\log^{10}$ IU/mL).

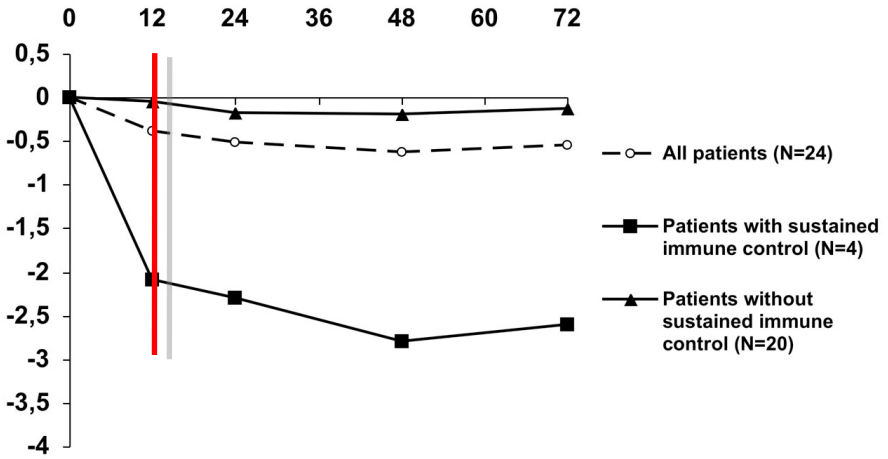
Brunetto MR et al. Hepatology 2009

HBeAg negative CHB: HBsAg decline on-treatment according to response 5 years post-treatment

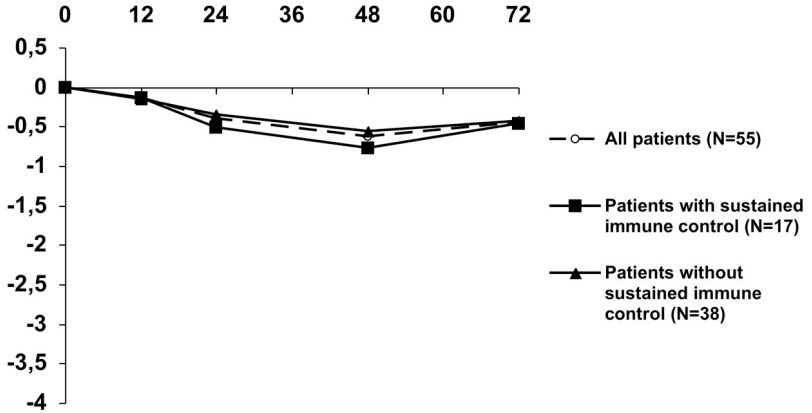
GENOTYPE A



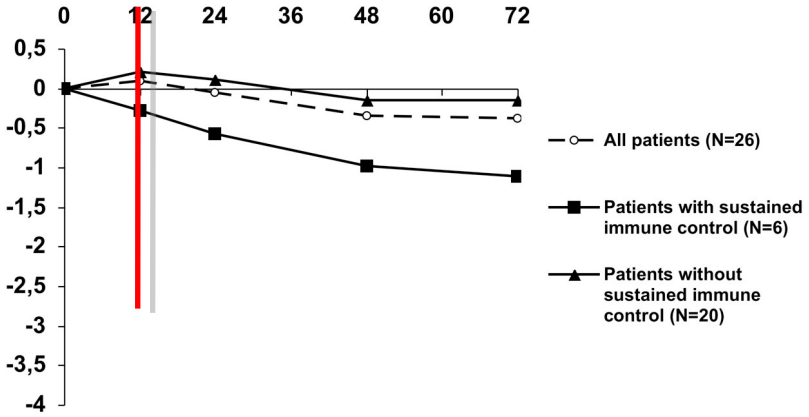
GENOTYPE B



GENOTYPE C

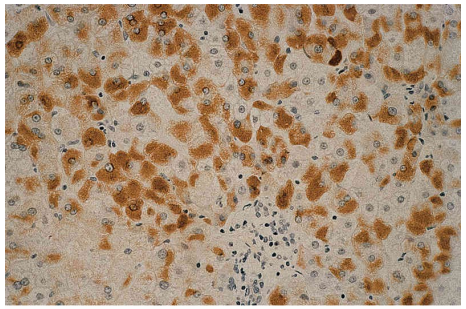


GENOTYPE D



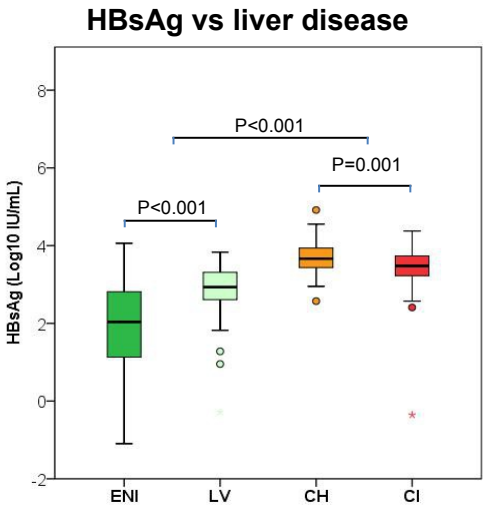
Brunetto MR, AASLD 2011; J Hep 2013

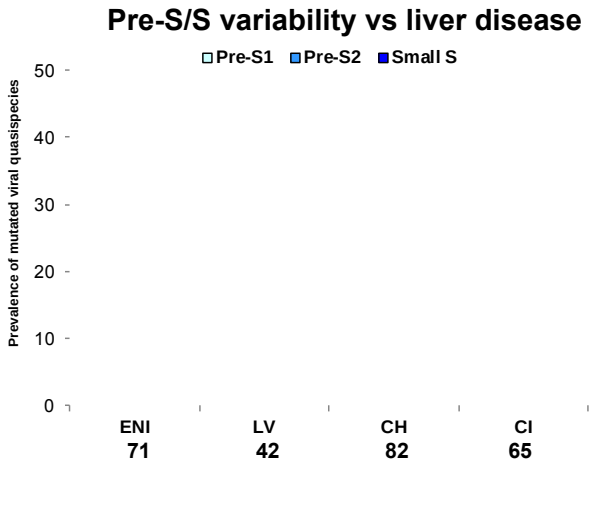
Surface Antigen Family: Regulation of HBsAg production

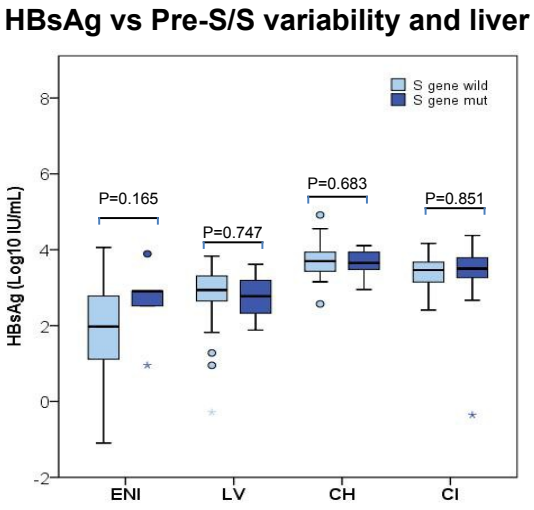


- specific stoichiometry between L and S proteins is required for secretion of HBsAg
- overexpression of L-HBs can cause intracellular storage of HBsAg
- Infection with preS/S mutants may be associated with significantly lower HBsAg serum levels

Pre-S/S viral quasispecies and HBsAg serum levels according to liver disease in 260 HBeAg negative carriers



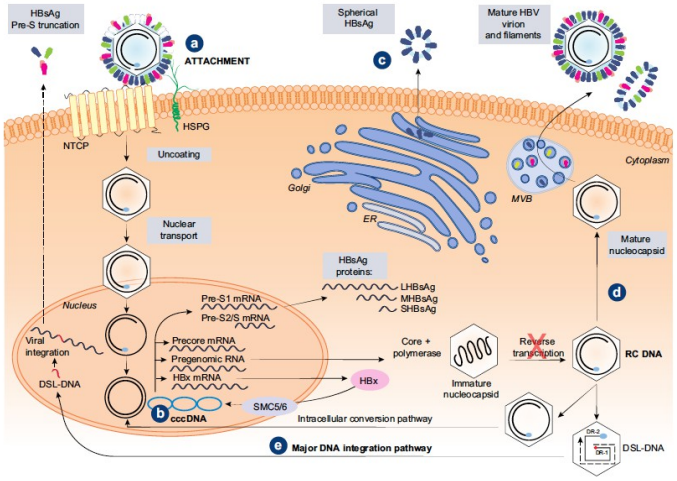




The evidence that patients with circulating Pre-S-S mutant quasispecies able to alterate HBsAg production maintain high serum HBsAg levels suggests different HBsAg genetic origins.

Wang H-C , Am J Pathol 2003; Khan N, J Virol 2004; Pollicino T et al Hepatology 2012; Cavallone D et al, HBV Molecular Biology 2018

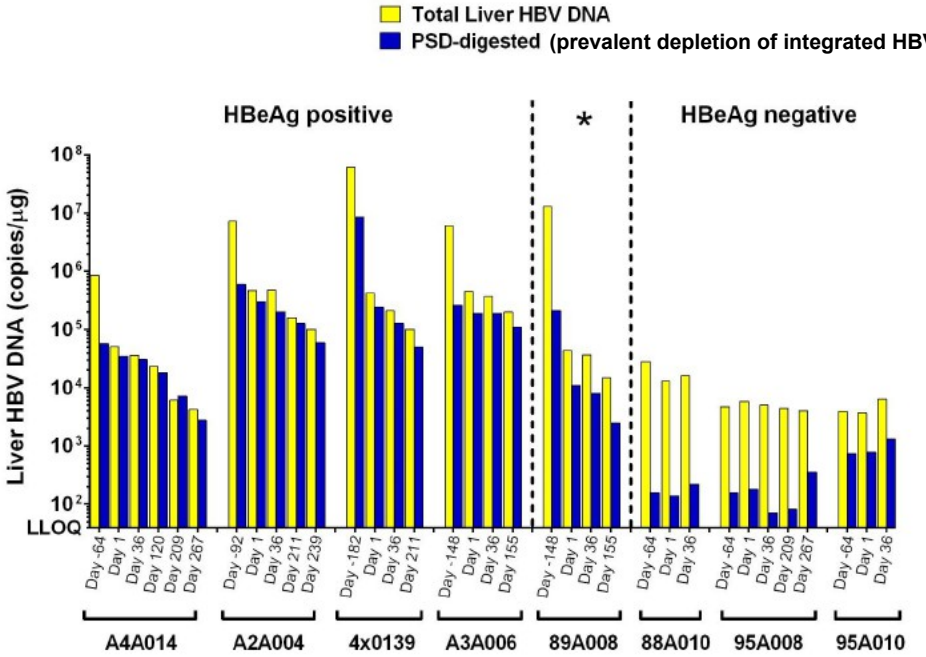
HBV-DNA integration



- Double stranded linear (DSL) DNA, that are produced during viral replication for a failure to translocate the RNS primer to prime the plus-stranded DNA synthesis, may be integrated into the host genome through recombination mechanisms by host enzymes

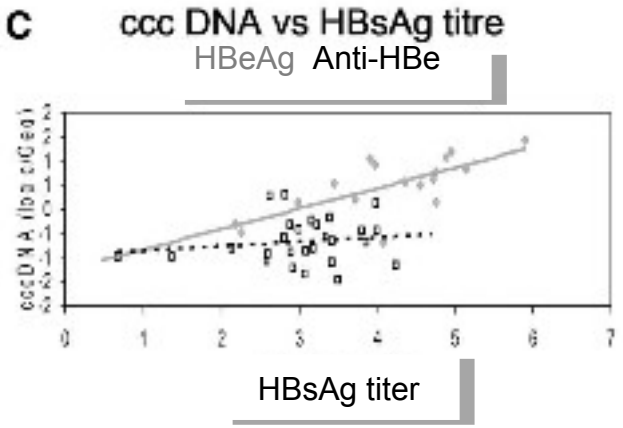
- HBV-DNA integration seems to occur early during infection, but at very low levels in the HBeAg positive phase.
- Recent studies in chimps suggest a dramatic increase of the integration in the HBeAg negative phase.
- >90% of the mRNAs deriving from integrated HBV-DNA
- A significant proprotion of HBsAg in HBeAg negative patients could derive from integrated HBV sequences

Quantification of liver HBV-DNA in ARC-520 treated chimps



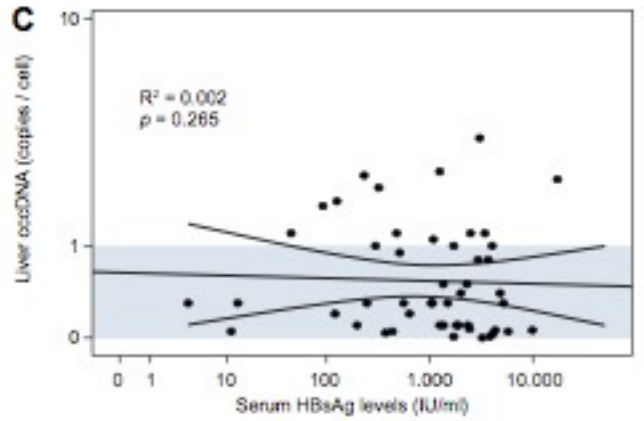
Cornberg M at al, J Hep 2017, Wooddell C et al Sci Transl Med 2017

HBsAg and cccDNA



HBeAg pos r=0.71, p<.001
Anti-HBe pos r=0.15, p=.45

Thompson AJV, Hepatology 2010



Manesis EK, J Hepatology 2011

- Positive correlation has been noted between HBsAg titer and serum HBV DNA and liver cccDNA in most studies of HBeAg pos patients.
- However, the regulation of HBsAg production and secretion appears to be disconnected from that of virions in HBeAg negative state.
- Additional studies are needed to determine the utility of HBsAg measurement in HBeAg negative patients

René Magritte: Controllo in bianco (1965)

Review

JOURNAL OF HEPATOLOGY

The role of quantitative hepatitis B surface antigen revisited

Markus Cornberg[1], Vincent Wai-Sun Wong[2], Stephen Locarnini[3], Maurizia Brunetto[4], Harry L.A. Janssen[5], Henry Lik-Yuen Chan[2,*]

HBsAg serum levels are useful to optimize the management of the HBsAg carriers provided that they are combined with a careful evaluation of the virologic and clinical settings:

- **High, stable HBsAg** (5 $\log^{10}$ IU/ml) and **HBV DNA** (>8 $\log^{10}$ IU/ml) serum levels are the **hallmark of HBeAg positive infection phase** (immune-tolerant phase).
- In the HBeAg negative phase, **the lower the best in low viral replication (≤2000 IU/ml), but the lower the worst in long lasting florid replication**.
- Thus, **HBsAg serum levels contribute to better define the "virologic phase of the infection", without providing direct information on liver disease**, that has to be staged by biochemestry and imaging.

What about qtHBsAg in Peg-IFN treatment management?

- Identification at the end of Peg-IFN treatment of patients with a high probablity of HBsAg clearance
- Identification of patients with high probability of sustained response

  HBeAg positive patients: HBsAg levels ≤ 1500 IU/ml at 12 or 24 week;

  HBeAg negative patients: EOT HBsAg levels < 400 IU/ml (GT A), < 50 IU/ml (GT B), < 75 IU/ml (GT C), <1000 IU/ml (GT D) associated with 75,47,71 and 75% PPV
- Early identification of non responders to Peg-IFN, using specific algorithms in the HBeAg positive or negative phase

Cornberg M at al, J Hep 2017

Response-guided Peg-IFN therapy in HBeAg-pos CHB using serum HBsAg levels: a pooled analysis of 803 patients

- **803** HBeAg-positive pts, treated with Peg-IFN ± LMV for 1 y in 3 global randomized studies (Peg-IFN phase 3, Neptune and HBV 99-01)
- HBV genotypes A 13%, B 25%, C (48%), D 14%
- HBsAg level <1,500 IU/mL at week 12 or 24: 45% resp. 46% response
- No HBsAg decline at week 12: 14% response
- HBsAg >20,000IU/mL at week 12: 6% response

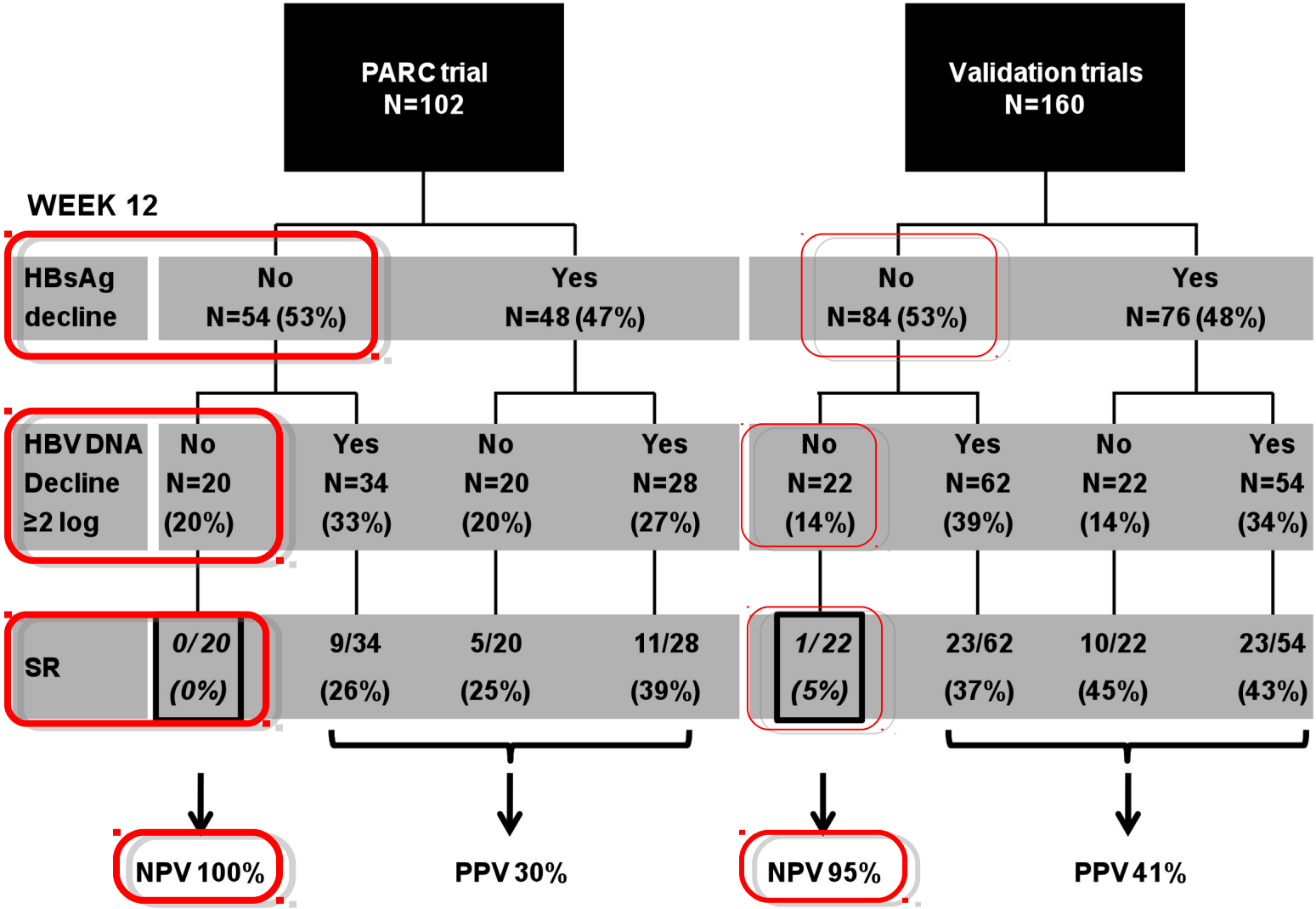
Stopping-rule based on **absence of any decline at week 12** was **superior** for genotypes **A** (NPV 88%) or **D** (NPV 98%)

HBsAg level >20,000 IU/mL at week 12 better identified **non-responders** genotype **B** (NPV 92%) or **C** (NPV 99%)

HBsAg >20,000 IU/mL at week 24: nearly all patients failed to achieve response, irrespective of genotype (**NPV 98%**)

Sonneveld M J et al, AASLD 2012; Hepatology 2013

Validation of a stopping rule at week 12 using HBsAg and HBV-DNA for HBeAg negative pts treated with Peg-IFN



The only patient who met the stopping rule but did achieve SVR was a Caucasian gen. A pt

Rijckborst V et al, J Hepatol 2012

What about qtHBsAg during NUCs treatment?

- Most patients treated with NAs will probably need decades of therapy to achieve HBsAg loss

Modelling HBsAg decline and clearance in 75 CHB pts (43 HBeAg negative) treated with ETV or TDF for a median period of 28 (22-35) months

	HBeAg-positive			HBeAg-negative		
	Total (n = 32)	High ALT [a] (n = 14)	Low ALT [b] (n = 18)	Total (n = 43)	High ALT [a] (n = 18)	Low ALT [b] (n = 25)
Baseline HBsAg (log IU) [c]	4.1 (0.6)	4.2 (0.7)	4.0 (0.6)	3.4 (0.7)	3.4 (0.9)	3.4 (0.9)
HBsAg decline (log/y) [d]	0.11 (0.04; 0.34)	0.30 (0.06; 0.82) [e]	0.07 (0.00; 0.13) [e]	0.07 (0.01; 0.18)	0.07 (0.04; 0.18)	0.07 (0.0; 0.18)
Years to 1 log decline [d]	6.6 (1.7; 17.5)	3.6 (1.3; 16.7)	8.1 (0.0; 18.9)	8.0 (0.5; 14.9)	8.4 (2.1; 15.8)	5.7 (0.0; 14.9)
Years to HBsAg loss [d]	36.4 (9.6; 98.3)	19.5 (7.3; 99.9)	44.8 (1.2; 100.0)	38.9 (1.3; 80.5)	43.2 (10.3; 85.1)	29.7 (0.0; 75.1)

Zoutendijk et al. J Infect Dis 2011

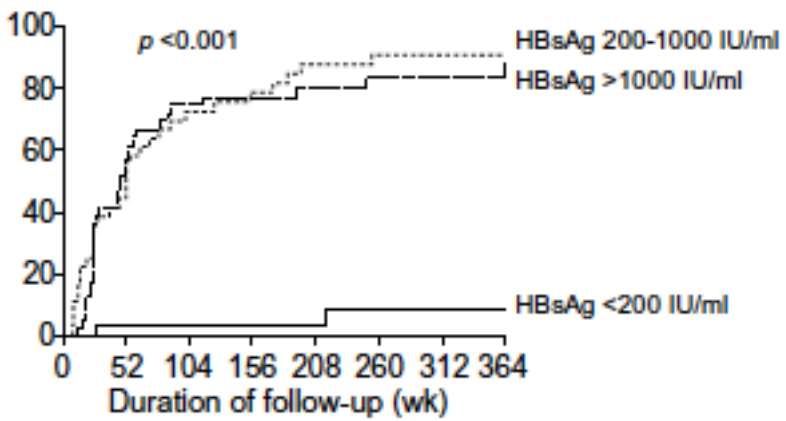
The role of HBsAg quantification in predicting HBsAg loss and HBV relapse after discontinuation of LMV treatment

- **188 CHB pts** (105 HBeAg negative), all infected by genotypes **B** and **C**, treated with LMV for a mean 89.3 ± 35.9 m, but who stopped LMV for at least 12 months
- Cumulative incidence at year 6 after stopping LMV of **HBsAg loss was 24%** and of **HBV relapse 65.9%**

- At **EOT prediction of HBsAg** loss was
  - 55.6% for HBsAg levels of **300 IU/m**l in **HBeAg pos** pts
  - 79.2% for HBsAg levels **<120 IU/ml** in **HBeAg neg** pts

- At **EOT** 93.3% **prediction of sustained virologic response** was achieved in HBeAg neg pts by HBsAg serum levels **< 200 IU/ml**

HBV relapse in HBeAg neg pts



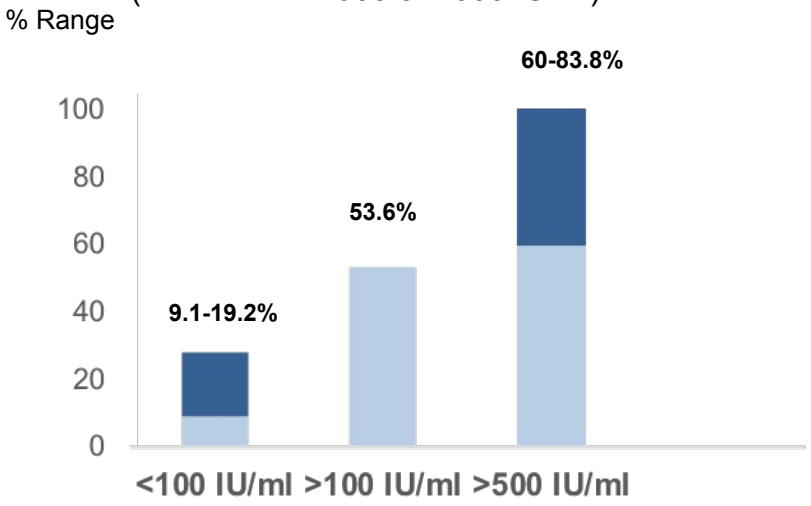
Chen C-H, J Hep 2014

The role of HBsAg in NUCs cessation among Asian CHB patients: a systematic review

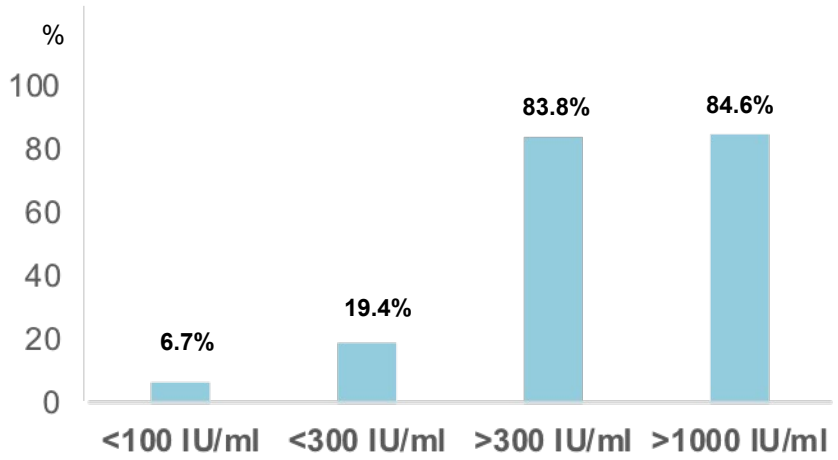
- 1761 papers were indentified on the topics and **11** were included in the review (randomized or observational studis, with ay least 10 pts enrolled; NUCs treatment duration of at least 24 months; treatment discontinuation for virological remissions; at least 12 months of post NUCs disontinuation; viralogical and clinical data available)

- **1716 patients**, median NUCs treatment ranged between 22.3-56.12 months, with a virologic remission before NUCs stopo of 12-36.7 months, duration of **post-treatment f.u. 12-157 months**
- **HBsAg loss** ranged **21.1-58-8%** in pts with HBsAg **< 100 IU/ml**, vs 3.3-7.4% in pts with HBsAg >100 IU/ml

Overall virologic relapse
(HBV-DNA > 1000 or 2000 IU/ml)



Virologic relapse in HBeAg neg pts
(HBV-DNA > 1000 or 2000 IU/ml)



Liu J et al Hepatology 2018

- qtHBsAg provides additional and complementary information to that of viral replication markers.
- its levels may be influenced by constitutive features of the virus and/or viral infection, therefore the results need to be interpreted according to the clinical setting
- qtHBsAg is a major diagnostic tool for the management of chronic HBV carriers.

Magritte R, La voix des airs 1931

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PERSONALIZED CARE

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Administrative Personel

Arianna Del Chicca

Bio-Physics

Luigi Civitano
Ranieri Bizzarri

Biologists

Daniela Cavallone
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