HBsAg quantification:
is useful for staging liver disease?

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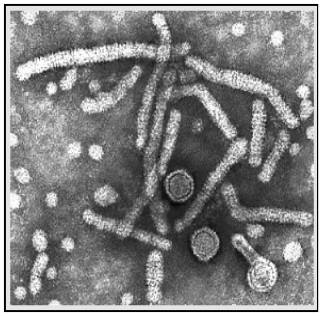
Serum HBsAg



Virions + defective particles
(exceeding virions by a factor of 102-105)

replication

cccDNA transcription/
mRNAs translation

HBsAg serum levels reflect:

- **in HBeAg positive patients, the overall amount of cccDNA**
- **in HBeAg negative carriers, the transcriptionally active cccDNA**

Adapted from Brunetto et al, J Hepatol 2010

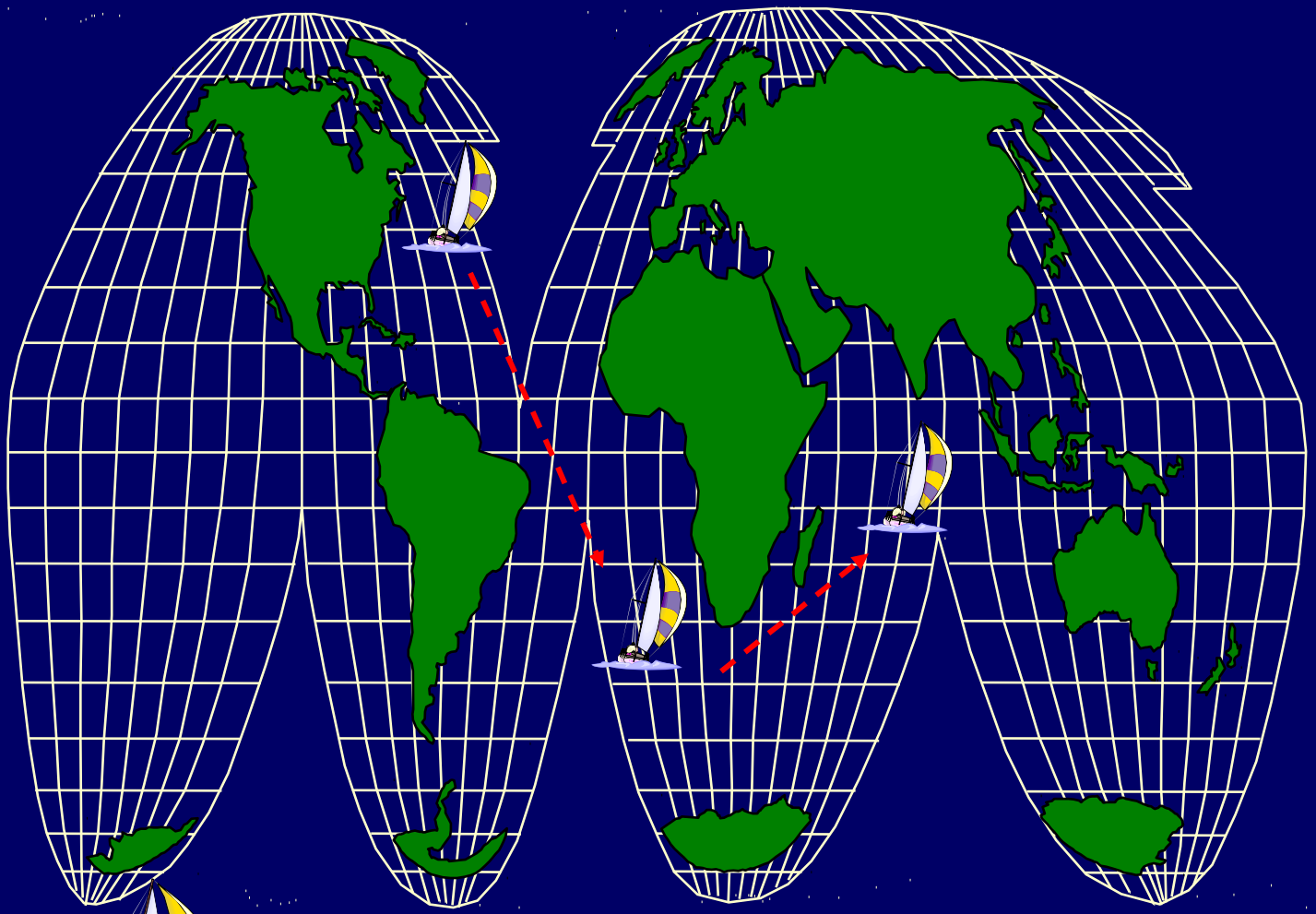
HBsAg levels in the natural history of HBV-infection: A perspective on Asia and Europe

	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

MANAGEMENT OF HEPATITIS B VIRUS CARRIERS



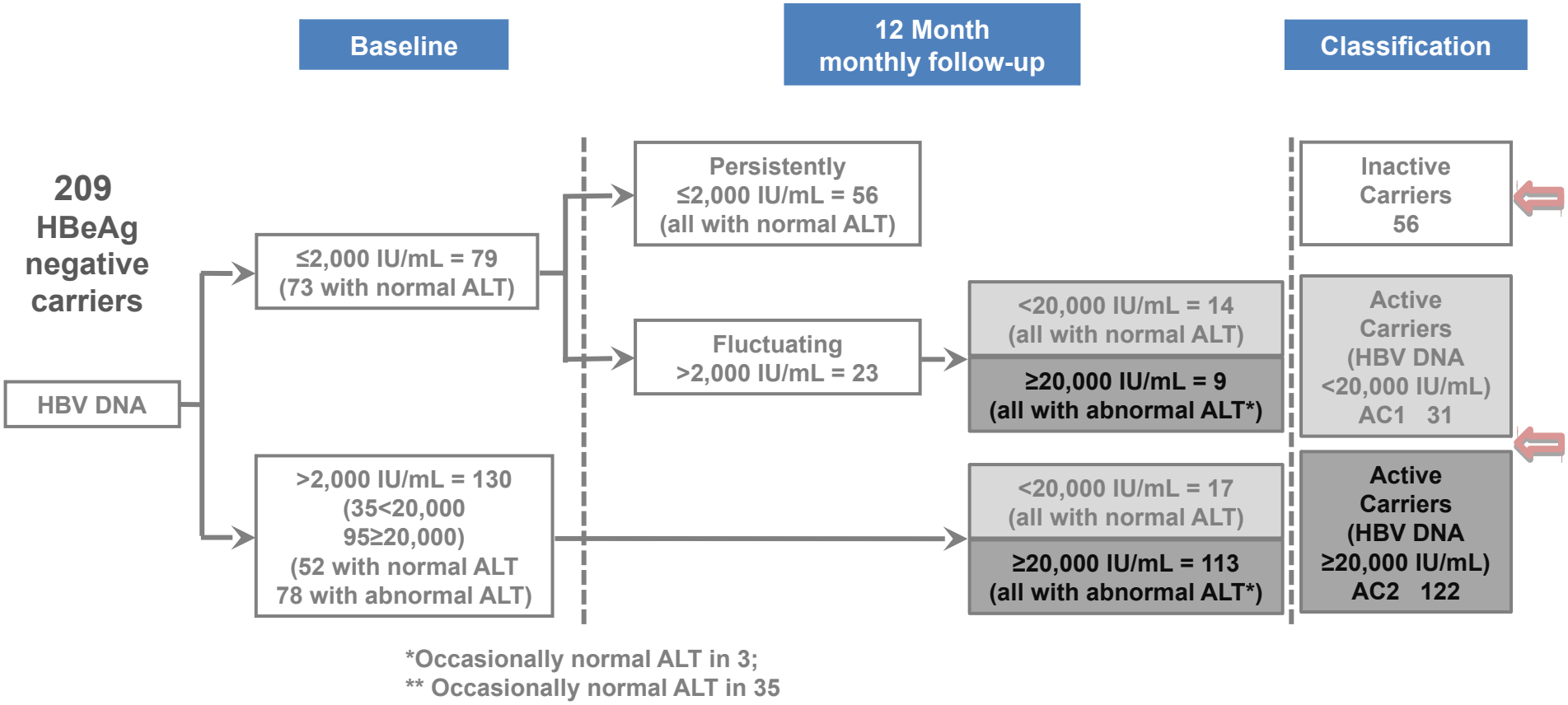
Latitude
Longitude
Time
Date

ALT
HBV-DNA
IgM anti-HBc
HBsAg

32°09'18"N, O 40°07'13"; 8.15 a.m., 19.01.01
29°00'S, E 24°00'; 8.15 a.m., 29.01.01
3°15'N, E 73°00' 8.15 a.m., 3.2.02

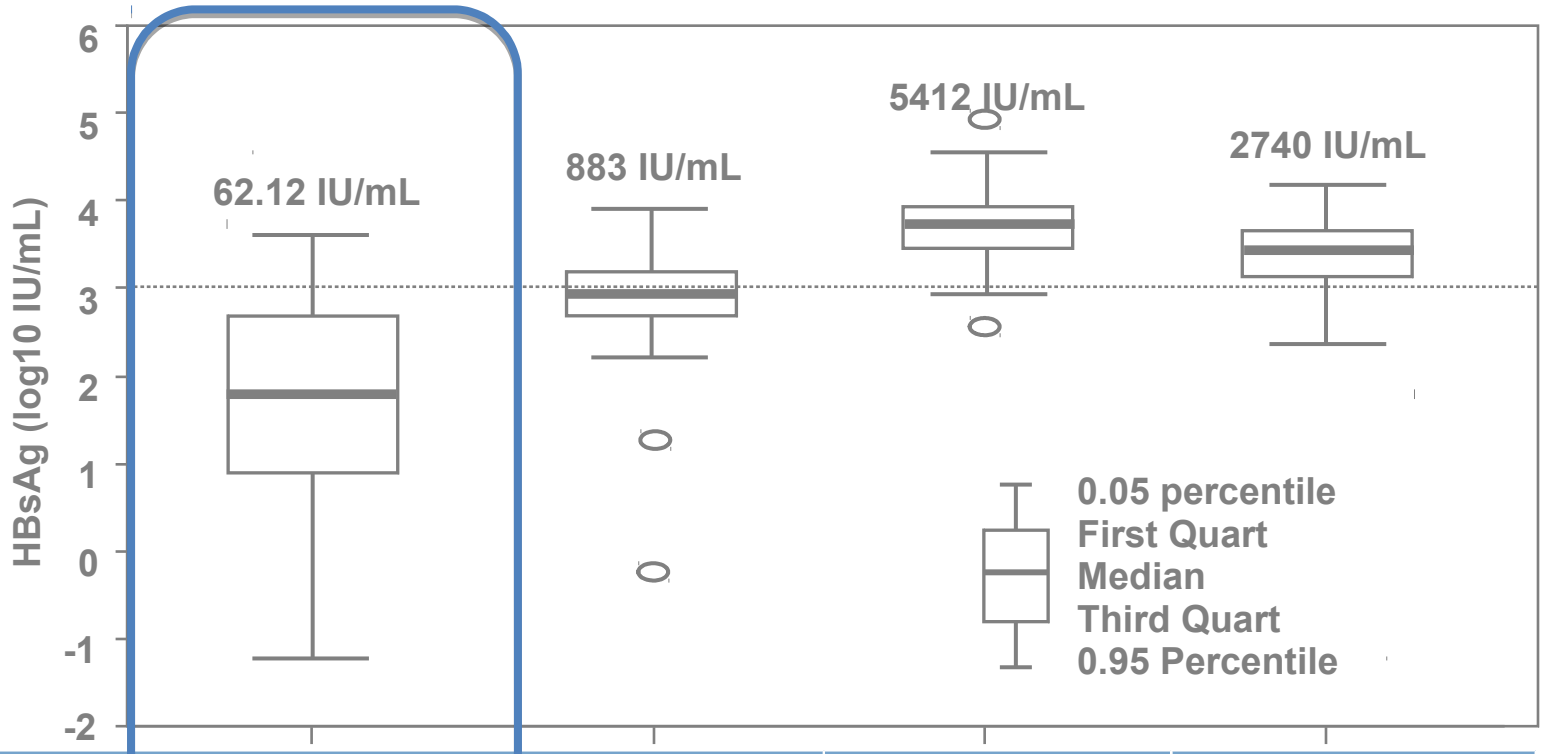
Adapted from Brunetto et al, J Hepatol 2010

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



Brunetto MR et al.Gastroenterology 2010

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



Subjects (N)	56	31	84	38
HBV DNA (IU/mL)	≤2,000	2,001 - 19,999	≥20,000	≥20,000
Liver disease	Absent	Absent	Chr Hep	Cirrhosis
Carriers	Inactive	Active	Active	Active

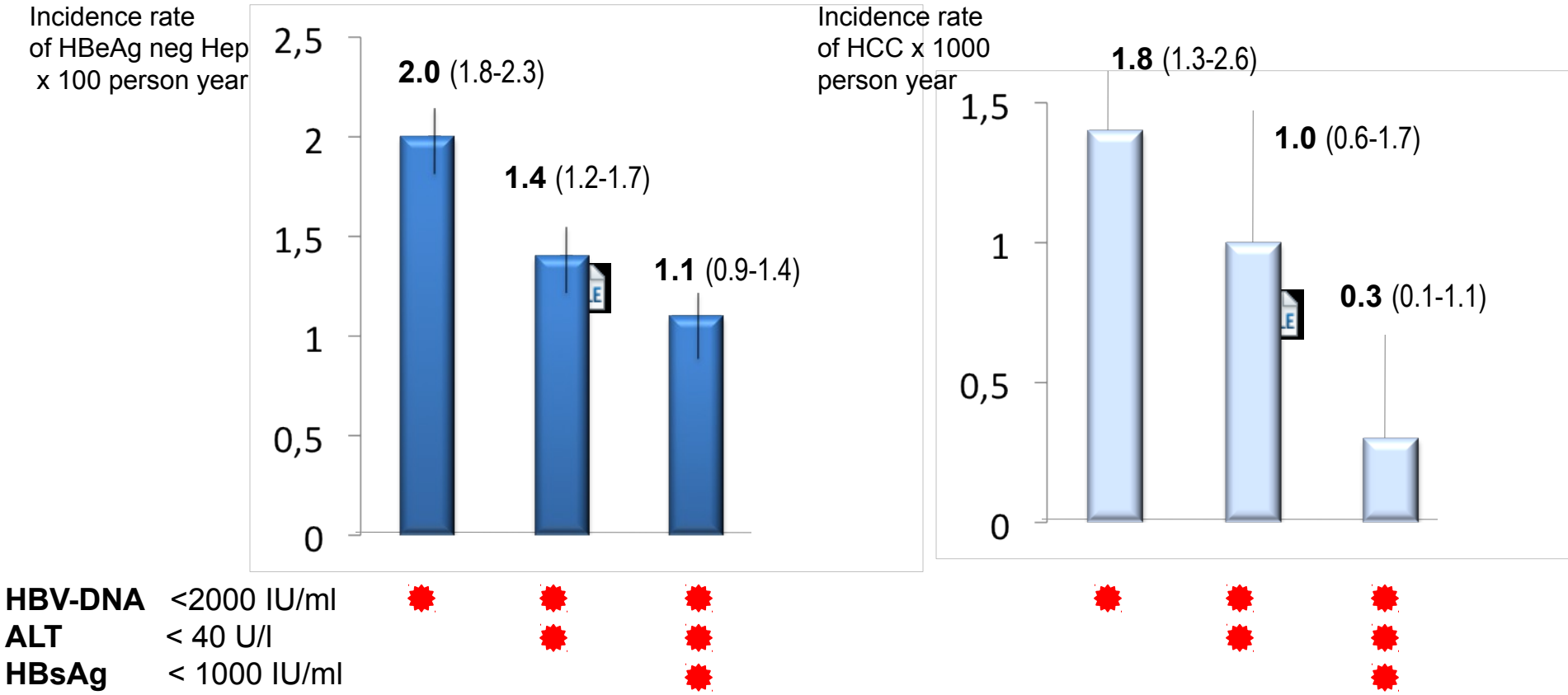
Brunetto MR et al.Gastroenterology 2010

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%

Brunetto MR, Gastroenterology 2010

Serum HBsAg levels helps predict disease progression in patients with low HBV loads

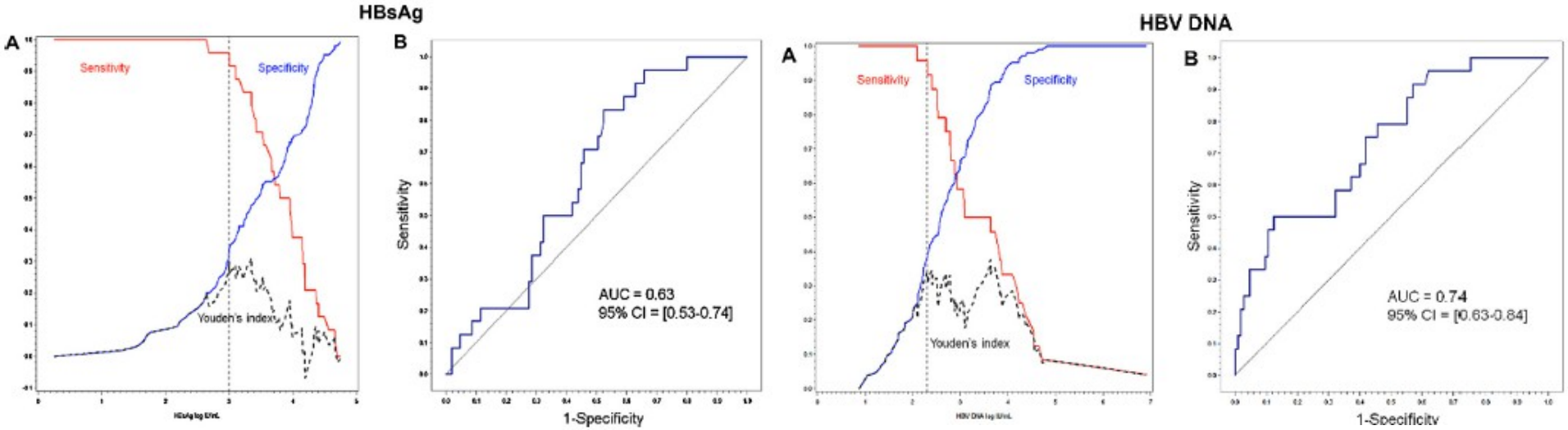


- In HBeAg negative pts with low viral load and gen B or C, a higher HBsAg level can predict disease progression.
- **HBsAg < 1000 IU/ml in combination with low levels of HBV-DNA and ALT help define minimal risk HBV carriers**

Tseng TC et al, Hepatology 2013

Prediction of disease reactivation in asymptomatic HBeAg negative CHB patients using baseline measuremens of HBsAg and HBV-DNA

- 129 HBeAg negative carriers (gen. A-E) with normal ALT at BL, followed-up for 1 year with every 3 months ALT and HBV-DNA measurements: 82 resulted to be Inactive Carriers, 23 Active Carriers,

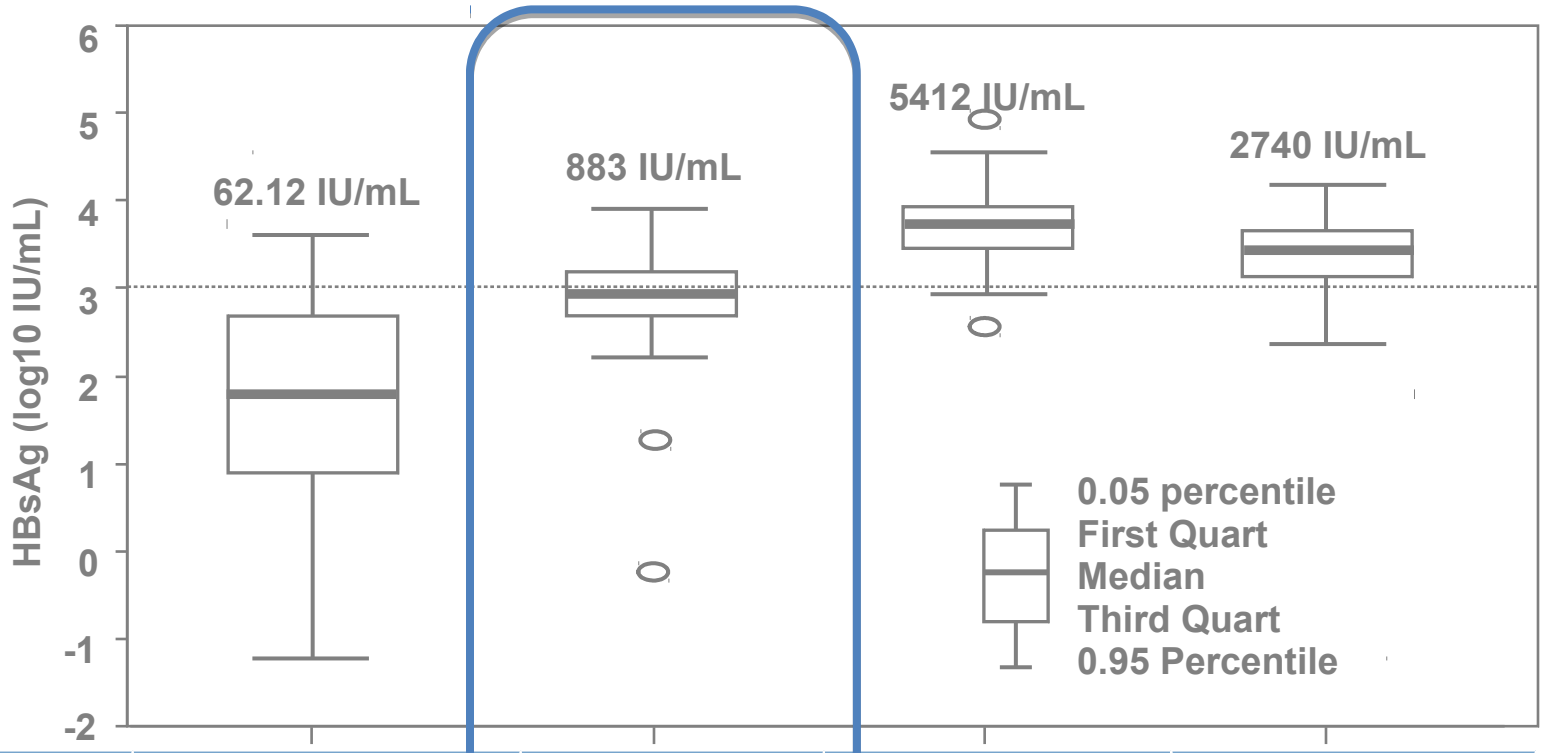


- The optimal cut offs to identify HBV carriers at risk of reactivation were: HBsAg >1000 IU/ml and HBV-DNA >200 IU/ml

- The combined used of the 2 differentiated CHB carriers with reactivation from those without (IC and AC) with 92% sensitivity, 51% specificity, 96% NPV and 30% PPV

Martinot-Peignoux M et al J Clin Virol 2013

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



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HBV DNA (IU/mL)	≤2,000	2,001 - 19,999	≥20,000	≥20,000
Liver disease	Absent	Absent	Chr Hep	Cirrhosis
Carriers	Inactive	Active	Active	Active

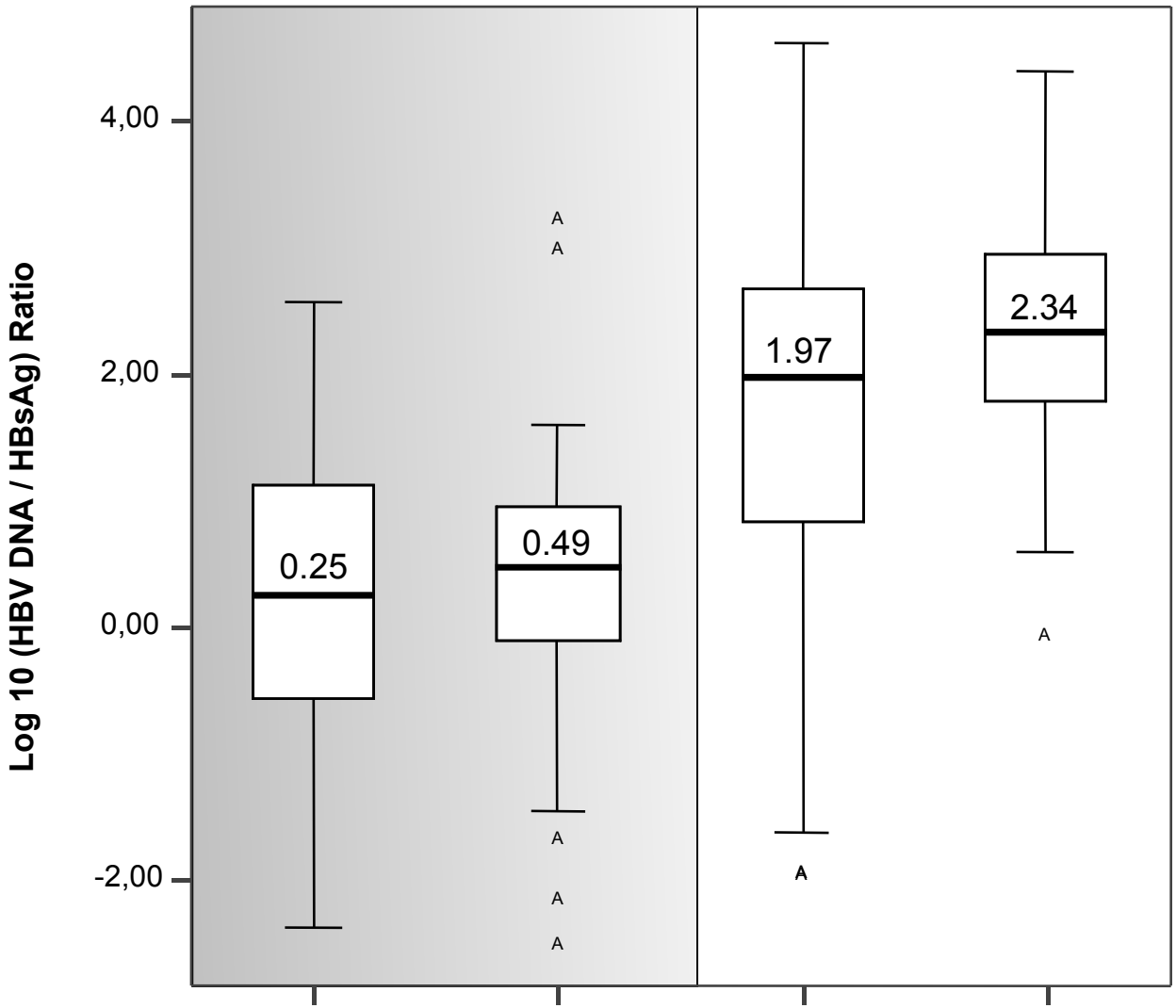
Brunetto MR et al.Gastroenterology 2010

	Inactive infection HBV DNA ≤2000 IU/mL (IC)	Active infection HBV DNA >2000 & <20000 IU/mL (AC1)	Active Infection HBV DNA ≥20000 IU/mL (AC2)
Carriers Number	56	31	122
Age (years)	49 (20 – 75)	43 (21 – 64)	47 (18 – 77)
Male/female	29 / 27	10 / 20	65 / 58
Follow-up (months)	38 (24 – 104)	33 (24 – 106)	33 (6 – 110)
Baseline HBsAg (IU/mL)	62.12 (0.1 – 4068)	883 (0.5 – 7838)	4233 (164 – 82480)
End of f.u HBsAg (IU/mL)	40.92 (n.d. – 4143)	613 (0.41 – 7754)	3887 (172 – 65160)
Baseline HBV DNA (IU/mL)	49 (n.d. – 1990)	2758 (n.d. – 19524)	389500 (98 – 166000000)
End of f.u HBV DNA (IU/mL)	30 (n.d. – 1114)	1483 (n.d. – 14532)	396450 (15 – 151000000)
Baseline ALT (U/L)	21 (10 – 35)	22 (11 – 39)	68 (11 – 722)
End of f.u ALT (U/L)	20 (13 – 38)	23 (12 – 40)	98 (15 – 2056)
Liver elastometry by Fibroscan (kPa)	4.3 (3.1 –5.6)	4.7 (3.2 – 5.8)	11.2 (3.2 – 59.8)

Liver biopsy in 10 patients: *Grading* 3/18 (6 pts); 4/18 (4 pts) // *Staging* 0/6 (8 pts); 1/6 (2 pts)

Brunetto MR et al.Gastroenterology 2010

Log 10 HBV-DNA/HBsAg ratio by phase of infection and disease stage



N. of subjects:	**56**	**31**	**84**	**38**
HBV DNA (IU/mL):	**≤2000**	**>2000 & <20000**	**20000**	**20000**
Liver disease:	**Absent**	**Absent**	**Chr. Hepatitis**	**Cirrhosis**
Carriers:	**IC**	**AC1**	**AC2**	**AC2**

IC vs AC
P<.001

IC vs AC1
P=NS

AC1 vs AC2
P=.001

CH vs CI
P=.023

EASL JOURNAL OF HEPATOLOGY

EASL Clinical Practice Guidelines: Management of chronic hepatitis B virus infection

European Association for the Study of the Liver*

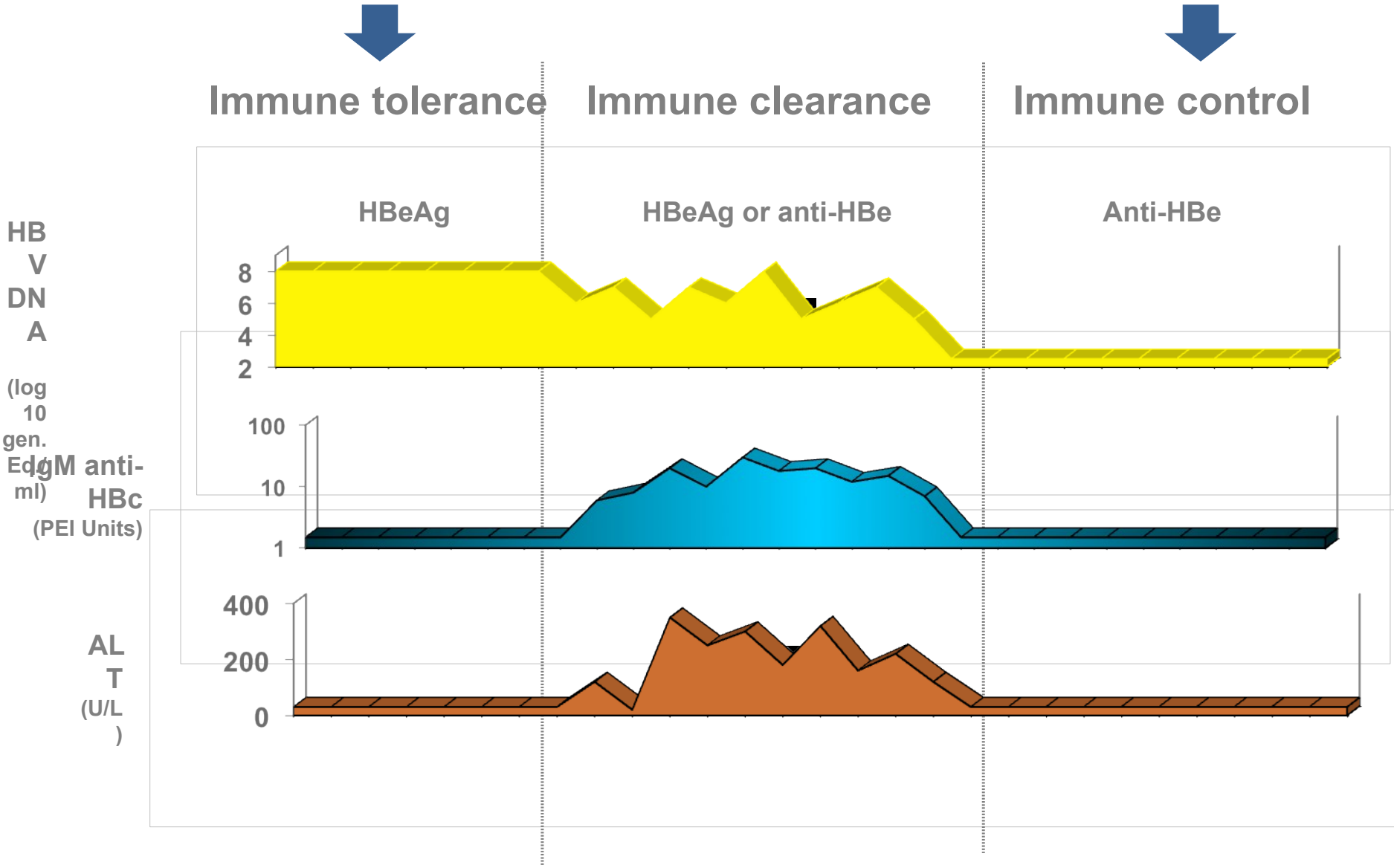
A minimum follow-up of 1 year with ALT levels at least every 3–4 months and serum HBV DNA levels is required before classifying a patient as inactive HBV carrier.

ALT levels should remain persistently within the normal range according to traditional cut-off values (approximately 40 IU/ml) and HBV DNA should be below 2000 IU/ml.

Some **inactive carriers**, however, may have **HBV DNA levels greater than 2000 IU/ml (usually below 20000 IU/ml)** accompanied by persistently normal ALT levels.

Patients with HBV-DNA <2000 IU/ml and elevated ALT should be advised to undergo liver biopsy for the evaluation of the cause of liver injury

Natural history of Chronic HBV Infection



A Longitudinal Study on the Natural History of serum HBsAg changes in CHB

HBsAg (log IU/ml)

117 chronic hepatitis B patients
FU for 99±16 months; number of visits 17 (8-49)

Immune tolerance (7)

e+ active CHB (25)

e seroconversion (17)

e- active CHB (46)

e- inactive CHB (22)

Chan HL, et al. Hepatology 2010

High HBsAg levels predict insignificant fibrosis in HBeAg positive CHB

Seto W-K ET al, PLOS ONE 2012

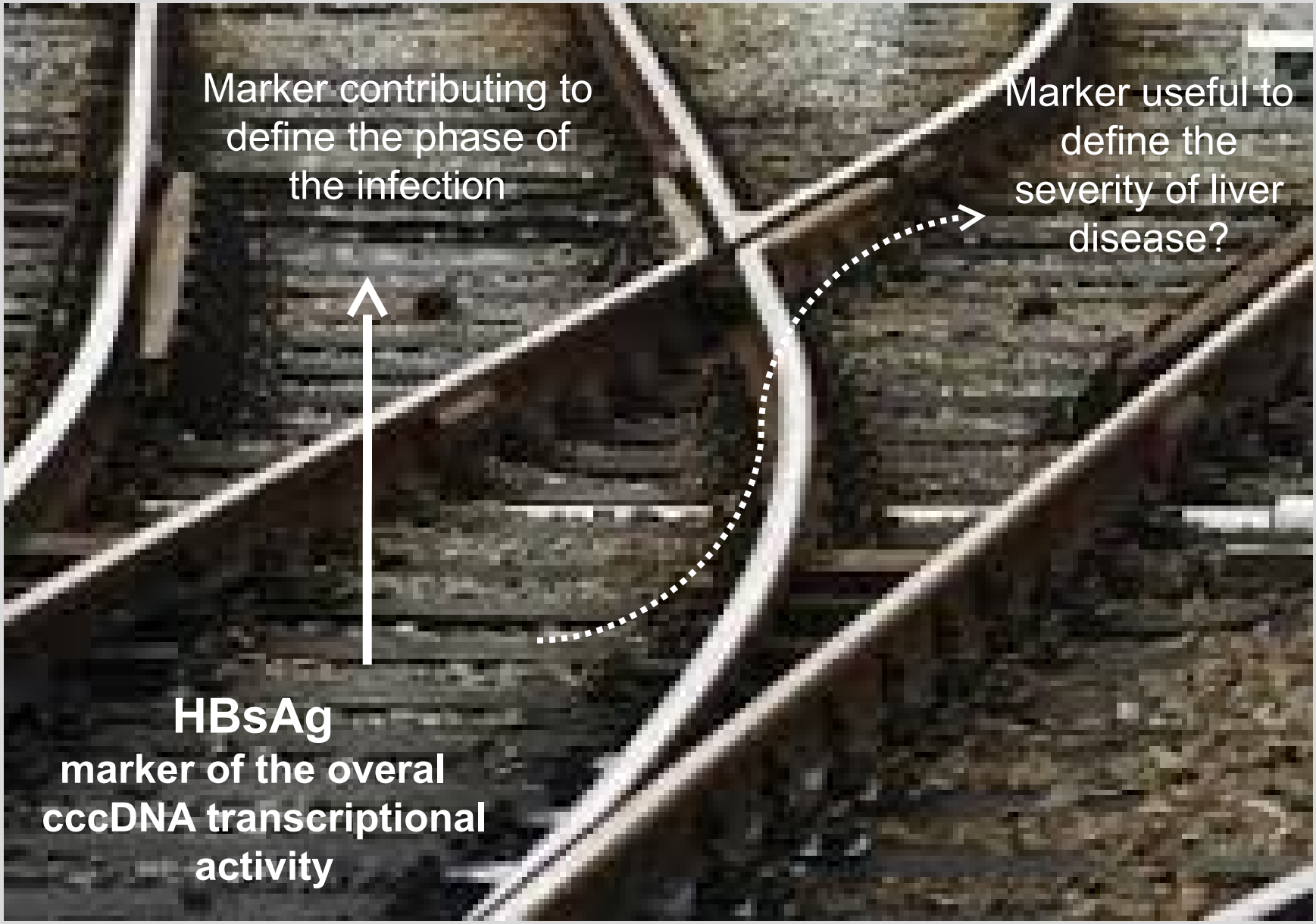
140 HBeAg positive patients, 56 (40%) of whom had ALT levels ≤ 2 x ULN, 72 (51.4%) had fibrosis score ≤ 1 and necro-inflammation grading ≤ 4

In pts with ALT ≤ 2 x ULN HBsAg levels achieved a ROC curve of 0.869 in prediciting fibrosis score ≤ 1

Diagnostic performance of different HBsAg levels for predicting insignificant fibrosis among patients with ALT ≤ 2 x ULN (n.56)

HBsAg IU/ml	N° pts	Sensitiv %	Specific %	PPV %	NPV %
≥ 10.000	45	90.9	58.3	88.9	63.6
≥ 25.000*	41	86.4	75	92.7	60.0
≥ 50.000	32	68.2	83.3	93.8	41.7
≥ 75.000	24	52.3	91.7	95.8	34.4
≥ 100.000	16	36.4	100	100	30.0

* The 7.3% (3 of 41) of patients with HBsAg serum levels ≥ 25000 IU/m, but fibrosis stage >1, had only stage 2


Marker contributing to define the phase of the infection
Marker useful to define the severity of liver disease?
HBsAg
marker of the overal cccDNA transcriptional activity

Clinical significance of serum HBsAg levels and association with liver histology in HBeAg positive chronic hepatitis B

198 HBeAg positive naive CHB patients (125 gen. B/ 73 gen. C)

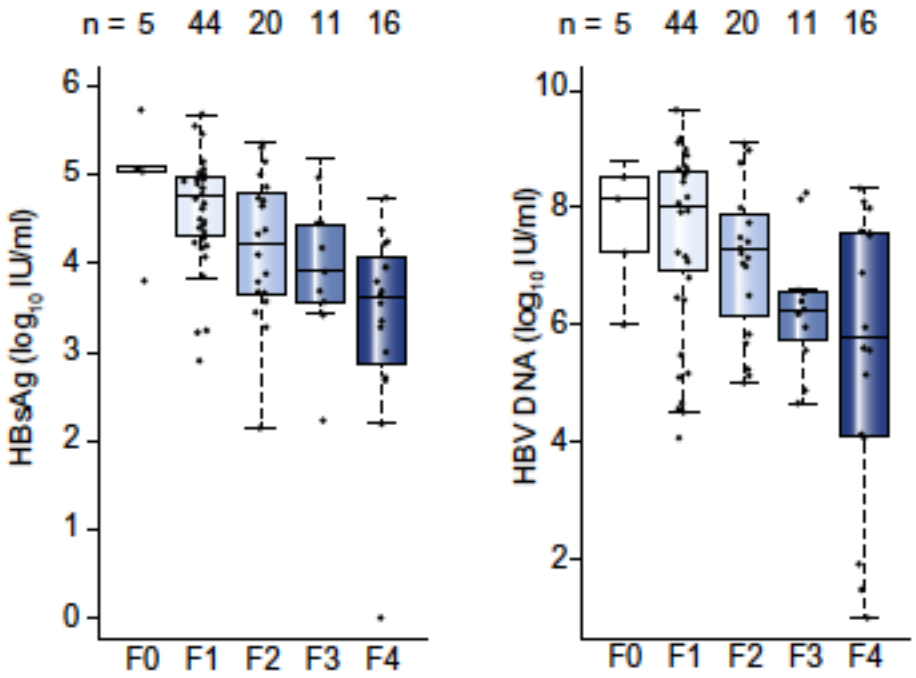
- lower HBsAg serum levels were associated with gen. C, pre-core mutations, Knodell necroinflammation grading ≥ 7 and advanced fibrosis (Ishak score 4-6)
- HBsAg serum levels were the only independent factor correlating with advanced fibrosis (coefficient: - 0.975, p=0.039, OR 0.377, CI 0.149-0.953)
- At ROC curve analysis 3.580 log IU/ml (3810 IU/ml) HBsAg serum levels identified advanced fibrosis with 82% sensitivity, 56% specificity (AUROC: 0.716, p 0.001)

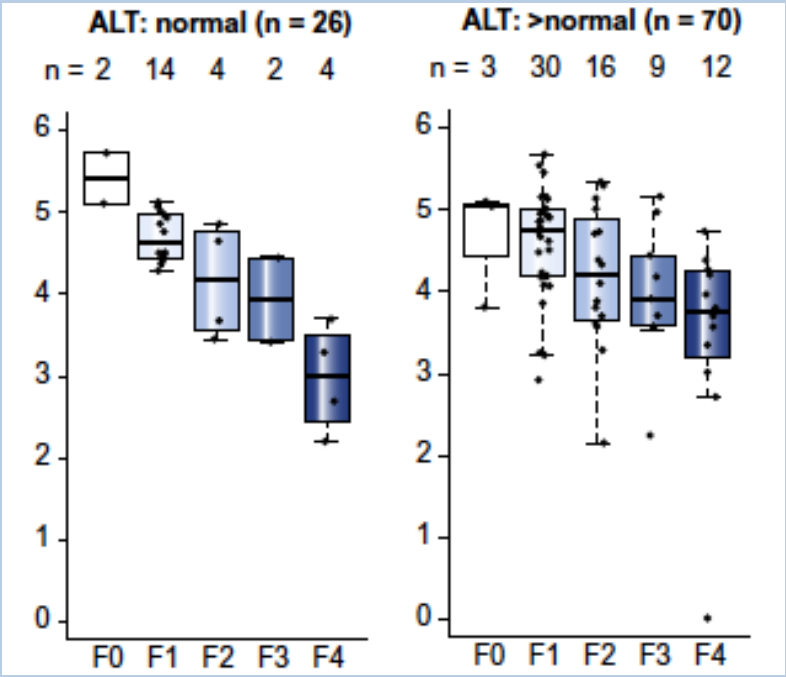
Cheng P-N et al J Clin Virol 2013 (57):323-330

HBsAg serum level is associated with fibrosis severity in treatment-naive, e antigen-positive patients

406 CHB patients, 101 HBeAg pos (38% gen. B-C)

- In HBeAg positive patients HBsAg serum levels strongly correlate with fibrosis *(r=0.43, p<0.0001)* with higher correlation in patients with normal ALT





Martinot-Peignoux M et al J Hep 2013 (58):1089-1095

HBsAg serum level is associated with fibrosis severity in treatment-naive, e antigen-positive patients

406 CHB patients, 101 HBeAg pos (38% gen. B-C)

- HBeAg positive patients with moderate to severe fibrosis showed lower HBsAg serum levels *(** p<0.0001, F0-1vs F2-F4, by METAVIR)*

Patient group	All	HBeAg(+)	HBeAg(-)
Serum HBsAg, log_{10} IU/ml (mean ± SD)			
Fibrosis*			
F0-F1	3.77 ± 0.95	4.63 ± 0.58	3.56 ± 0.89
F2-F4	3.61 ± 0.98	3.84 ± 1.01**	3.51 ± 0.95
Serum HBV DNA, log_{10} IU/ml (mean ± SD)			
Fibrosis*			
F0-F1	4.54 ± 2.16	7.62 ± 1.40	3.72 ± 1.49
F2-F4	5.34 ± 1.97†	6.47 ± 1.81†	4.82 ± 1.82**

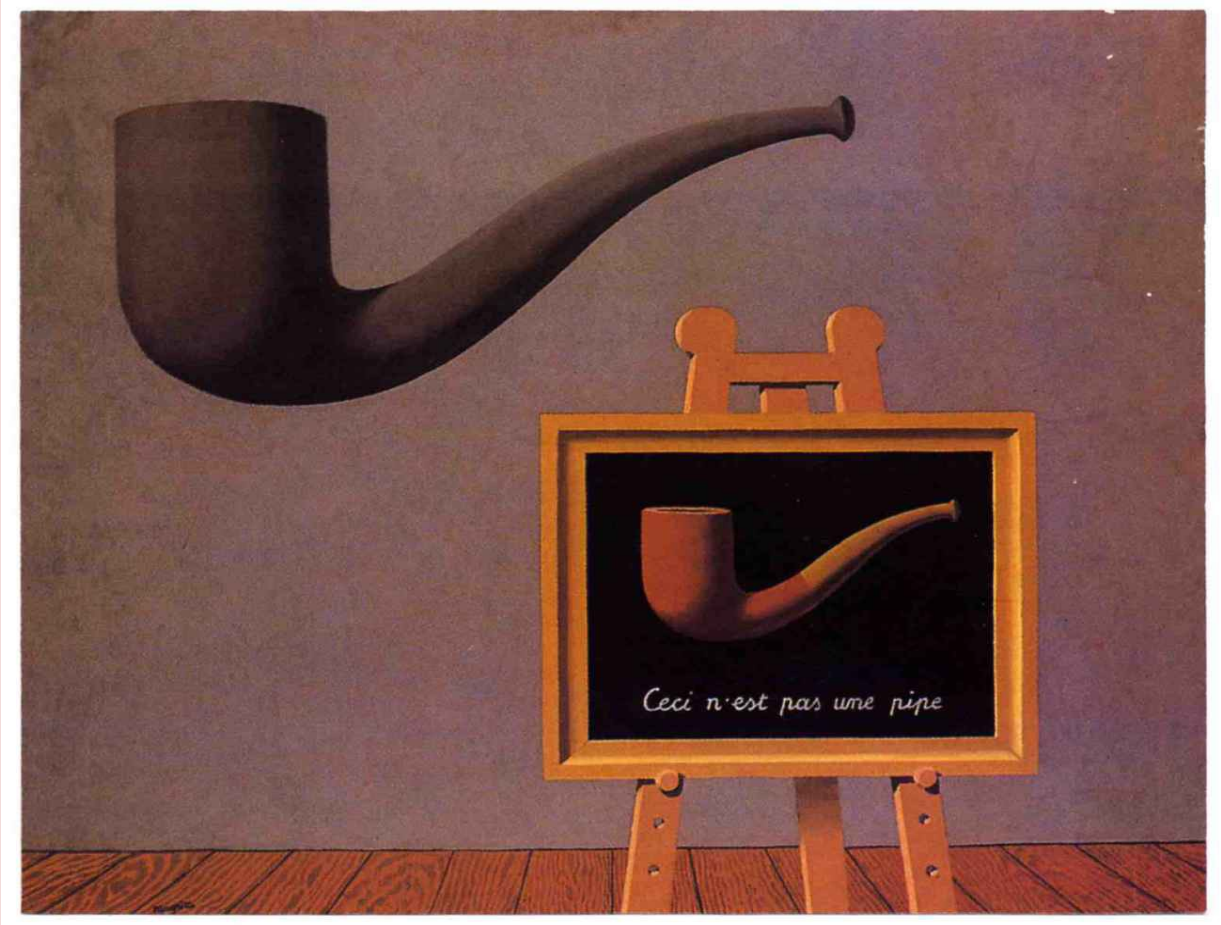
- In gen B-C patients at ROC curve 3.85 log IU/ml (7000 IU/ml) HBsAg serum levels identified advanced fibrosis with 100% sensitivity, 86% specificity (AUROC: 0.89)
- No correlation between HBsAg serum levels and severity of fibrosis was observed in HBeAg negative patients *(** HBV-DNA= p<0.001, F0-1vs F2-F4)*

Martinot-Peignoux M et al J Hep 2013 (58):1089-1095

A statistical association can be found between HBsAg serum levels and the stage of liver disease in specific clinical setting.

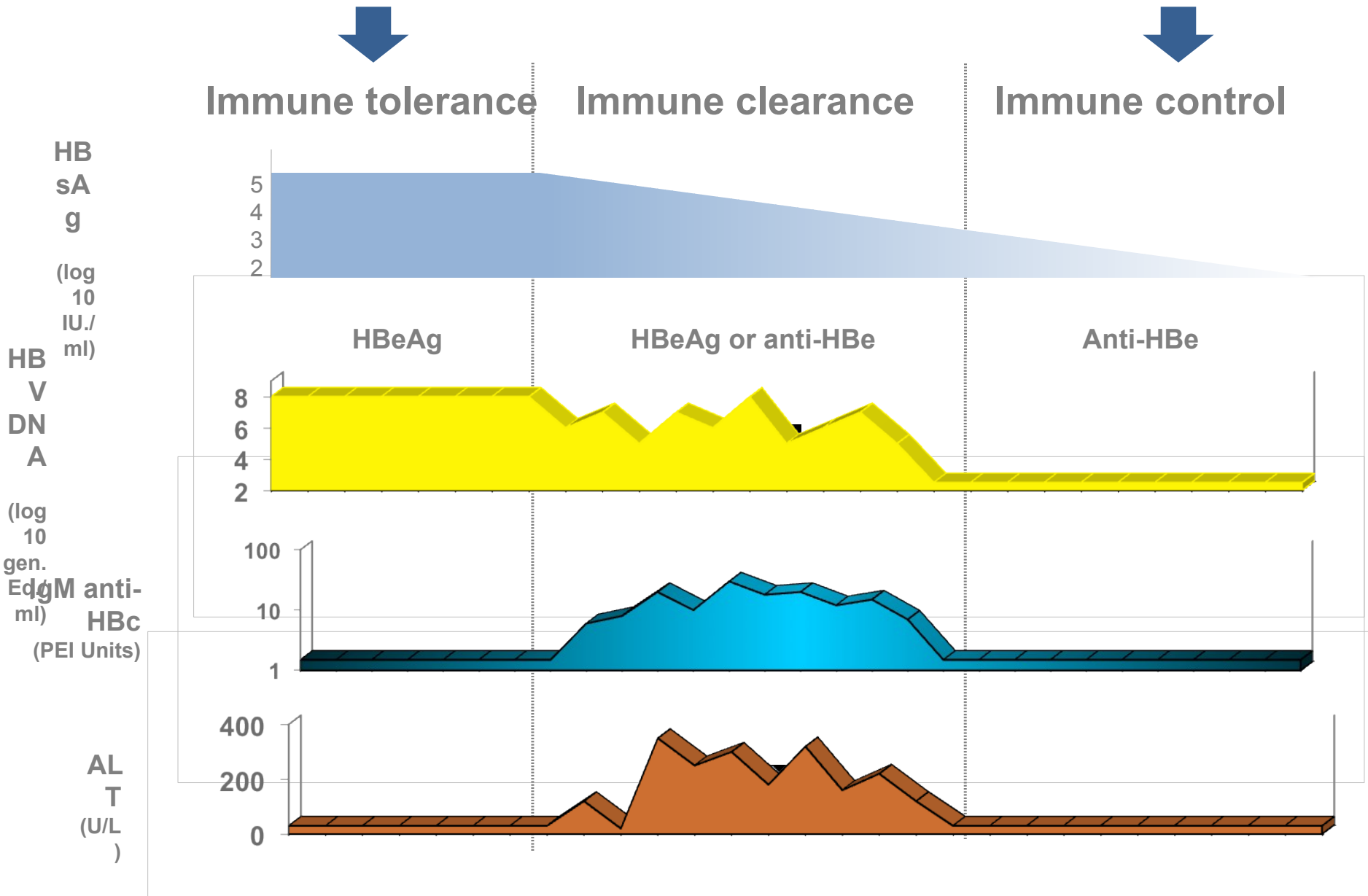
However, we must be aware that HBsAg serum levels reflect the virological status of the HBV carrier.

Whereas, the extent of liver damage results from multiple factors and not only from the virologic status.



Magritte, I due misteri 1966

Natural history of Chronic HBV Infection



Hepatology Unit

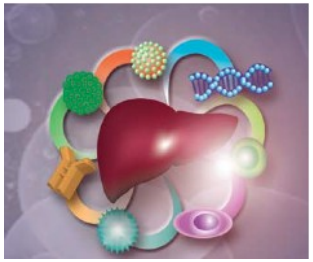
University Hospital of Pisa, Italy

Antonella Cristofani
Simonetta Ferretti *(part-time)*
Simona Giannetti
Teresa Crisponi
Isabella Rossi *(Chief)*

Nurses

Pietro Ciccorossi
Barbara Coco
Piero Colombatto
Filippo Oliveri
Veronica Romagnoli
Carlotta Rastelli
Beatrice Cherubini

Medical Doctors



Administrative Personel

Barbara Giorgi

Biologists

Daniela Cavallone
Francesco Moriconi

Bio-Physics

Luigi Civitano
Ranieri Bizzarri

INNOVATIVE MODEL OF
IMPC
PERSONALIZED CARE

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Gatroenterology Chair
Pisa University