



VHPB Technical meeting

**MULTI TOPIC meeting**

**The impact of viral hepatitis treatment and  
vaccination non-responders and occult  
hepatitis on public health**

Vilnius, Lithuania

25-26 April 2019

# **Occult HBV Infection and HCC**

***Teresa Pollicino***

*Division of Clinical and Molecular Hepatology*

*University Hospital of Messina, Italy*

[tpollicino@unime.it](mailto:tpollicino@unime.it)





ACCEPTED MANUSCRIPT

## UPDATE OF THE STATEMENTS ON BIOLOGY AND CLINICAL IMPACT OF OCCULT HEPATITIS B VIRUS INFECTION

Giovanni Raimondo<sup>1,2</sup>, Stephen Locarnini<sup>3</sup>, Teresa Pollicino<sup>2,4</sup> Massimo Levrero<sup>5</sup>, Fabien Zoulim<sup>5</sup>, Anna S. Lok<sup>6</sup>, and the *Taormina Workshop on Occult HBV Infection Faculty Members\**

***“A still widely debated topic is whether OBI may accelerate the progression toward cirrhosis and the development of HCC in persons with chronic liver disease due to other causes (e.g. HCV, alcohol, nonalcoholic steatohepatitis)”***





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Giovanni Raimondo<sup>1,2</sup>, Stephen Locarnini<sup>3</sup>, Teresa Pollicino<sup>2,4</sup>, Massimo Levrero<sup>5</sup>, Fabien Zoulim<sup>5</sup>, Anna S. Lok<sup>6</sup>, and the *Taormina Workshop on Occult HBV Infection Faculty Members\**

***“In the vast majority of cases, OBI does not appear to lead  
to any clinical sequelae”***



# Selection of studies reporting the association between OBI and HCC

Study	Geographic area	Aetiology
Shafritz <i>et al</i> , <i>NEJM</i> 1981	South Africa	Unknown
Brechot <i>et al</i> , <i>PNAS</i> 1981	France	Unknown
Paterlini <i>et al</i> , <i>NEJM</i> 1990	South Africa, Italy, France and Japan	Unknown
Sheu <i>et al</i> , <i>Gastroenterology</i> 1992	Taiwan	HCV/Cryptogenic
Paterlini <i>et al</i> , <i>Hepatology</i> 1993	France	HCV/Cryptogenic
Yu <i>et al</i> , <i>Hepatology</i> 1997	USA	HCV/Cryptogenic
Koike <i>et al</i> , <i>J Med Virol</i> 1998	Japan	HCV
Kubo <i>et al</i> , <i>Vox Sang</i> 1998	Japan	HCV
Huo <i>et al</i> , <i>Hepatology</i> 1998	Taiwan	HCV/Cryptogenic
Pollicino <i>et al</i> , <i>Gastroenterology</i> 2004	Italy	HCV/Cryptogenic
Yotsuyanagi <i>et al</i> , <i>Alc Cl Exp Res</i> 2004	Japan	Alcoholic
Donato <i>et al</i> , <i>Oncogene</i> 2006	Italy	HCV/Alcoholic
Squadrito <i>et al</i> , <i>Cancer</i> 2006	Italy	HCV
Ikeda <i>et al</i> , <i>Ann Intern Med</i> 2007	Japan	HCV
Miura <i>et al</i> , <i>Hepatol Res</i> 2008	Japan	HCV
Obika <i>et al</i> , <i>Intervirology</i> 2008	Japan	HCV
Shetty <i>et al</i> , <i>Liver Transpl</i> 2008	USA	HCV
Matsuoka <i>et al</i> , <i>Intervirology</i> 2008	Japan	HCV
Kew <i>et al</i> , <i>J Gastr Hepatol</i> 2008	South Africa	Unknown
Ikeda <i>et al</i> , <i>J Viral Hepat</i> 2009	Japan	Alcoholic/Cryptogenic
Chen <i>et al</i> , <i>Int J Cancer</i> 2009	Taiwan	Cryptogenic
Tamori <i>et al</i> , <i>J Med Virol</i> 2009	Japan	HCV
Wong <i>et al</i> , <i>Hepatology</i> 2011	China	Cryptogenic
Kondo <i>et al</i> , <i>Hepatol Res</i> 2013	Japan	Cryptogenic
Pollicino <i>et al</i> , <i>J Hepatol</i> 2013	Italy	Haemochromatosis
Squadrito <i>et al</i> , <i>J Hepatol</i> 2013	Italy	HCV
Shi <i>et al</i> , <i>Liver Int</i> 2012	<i>Meta-analysis</i>	



Liver, Pancreas and Biliary Tract

## Occult hepatitis B virus and the risk for chronic liver disease: A meta-analysis

Loredana Covolo<sup>a,\*</sup>, Teresa Pollicino<sup>b</sup>, Giovanni Raimondo<sup>b</sup>, Francesco Donato<sup>a</sup>

*Digestive and Liver Disease 45 (2013) 238–244*

Pooled risk estimates of chronic liver diseases after stratification.

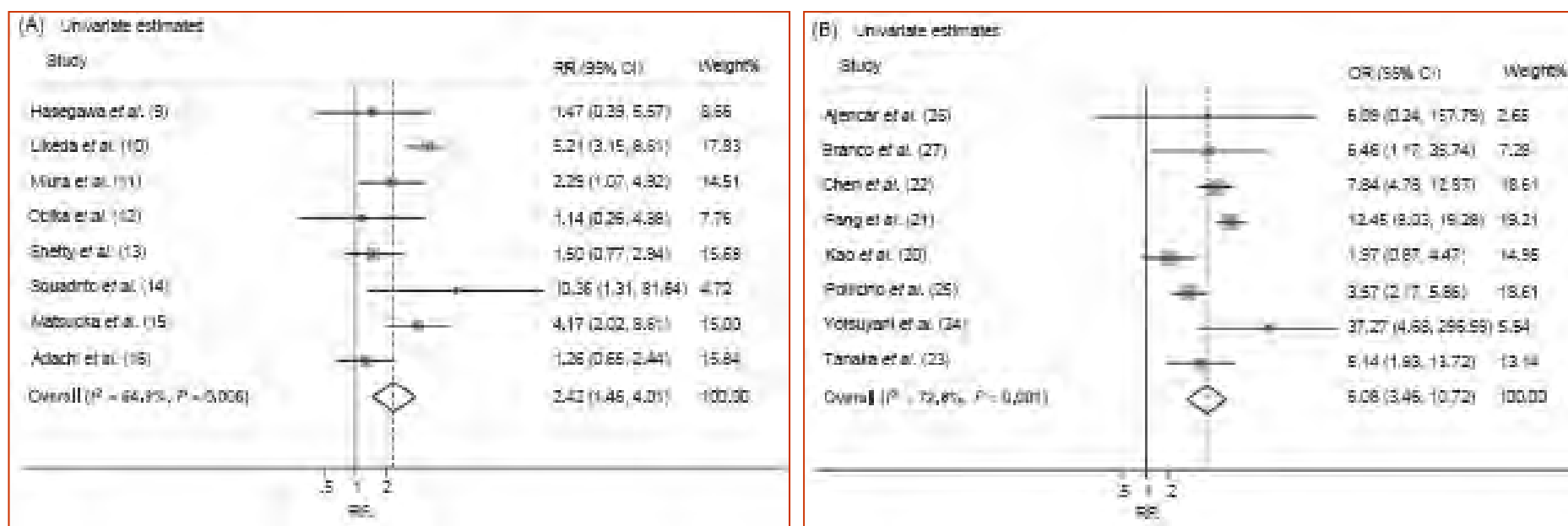
	No. of studies	Pooled OR (95% CI)	Heterogeneity ( $I^2$ , $p$ value)
All studies	14	8.9 (4.1–19.5)	81%, $p < 0.001$
HCV <sup>a</sup>			
Positive	9	7.4 (2.7–20.4)	83%, $p < 0.001$
Negative	8	14.4 (4.2–49.3)	85%, $p < 0.001$
Subjects without CLD			
Blood donors	5	23.3 (6.8–79.9)	69%, $p = 0.007$
Healthy subjects	6	2.9 (1.1–7.5)	76%, $p = 0.003$
Hospital controls	3	10.3 (1.6–68.5)	83%, $p = 0.003$
HBVDNA detection quality score			
High	6	4.5 (1.7–12.0)	86%, $p < 0.001$
Low	8	14.8 (5.6–39.3)	64%, $p = 0.007$
HBV endemicity <sup>b</sup>			
High endemic	5	7.7 (1.7–35.0)	92%, $p < 0.001$
Medium/low endemic	9	10.3 (3.8–27.6)	76%, $p < 0.001$
Matching for age and gender <sup>c</sup>			
Yes	6	22.2 (3.7–132.1)	81%, $p < 0.001$
No	8	6.2 (2.2–17.7)	88%, $p < 0.001$
% Weight of study			
>8.0%	7	3.9 (1.7–9.0)	87%, $p < 0.001$
<8.0%	7	36.8 (14.1–96.0)	0%, $p = 0.5$



## CLINICAL STUDIES

# Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis

Yu Shi<sup>1</sup>, Yi Hua Wu<sup>1,2\*</sup>, Wei Wu<sup>1</sup>, Wan Jun Zhang<sup>3</sup>, Jun Yang<sup>1</sup> and Zhi Chen<sup>1</sup>



**Conclusion:** Our findings suggest that occult HBV infection was associated with an increased risk of HCC. Occult HBV may serve as a cofactor in the development of HCV-related HCC, and it may also play a direct role in promoting Non-B and Non-C HCC growth





# Association between OBI and HCC in patients with chronic hepatitis C

## Studies considering anti-HBc positivity as evidence of OBI

First author, year	No. of patients	Country	Type of study	Liver disease	HCC, positive/tested, n/n (%)		P value
					Anti-HBc <sup>+</sup>	Anti-HBc <sup>-</sup>	
Takano 1995 <sup>[84]</sup>	61	Japan	Cohort	CH	9/36 (25.0)	2/25 (8.0)	NS
Chiba 1996 <sup>[76]</sup>	412	Japan	Cohort	CH/cirrhosis	47/198 (23.7)	16/214 (7.5)	0.020
Chiba 1996 <sup>[77]</sup>	204	Japan	Cross-sectional	cirrhosis	92/128 (71.9)	36/76 (47.4)	0.0005
Shiratori 1997 <sup>[86]</sup>	502	Japan	Case-control	CLD	111/263 (42.2)	81/239 (33.9)	NS
IIHCSG 1998 <sup>[85]</sup>	451	Italy	Cohort	CLD	34/206 (16.5)	32/245 (13.1)	NS
Dutta 1999 <sup>[78]</sup>	51	Australia	Case-control	CH/cirrhosis	10/17 (58.8)	7/34 (20.6)	0.010
Marusawa 1999 <sup>[34]</sup>	2366	Japan	Cross-sectional	CH/cirrhosis	363/1047 (34.7)	248/1319 (18.8)	< 0.01
Hiraoka 2003 <sup>[48]</sup>	202	Japan	Case-control	CLD	109/250 (43.6)	93/342 (27.2)	NS
Imazeki 2003 <sup>[79]</sup>	459	Japan	Cohort	CH/cirrhosis	37/160 (23.1)	26/299 (8.7)	< 0.05
Hasegawa 2005 <sup>[87]</sup>	140	Japan	Cohort	CH/cirrhosis	9/64 (14.0)	9/76 (11.8)	NS
Tanaka 2006 <sup>[80]</sup>	74	Japan	Cohort	CLD	13/53 (24.5)	0/21 (0.0)	0.012
Bruno 2007 <sup>[49]</sup>	160	Italy	Cohort	Cirrhosis	29/86 (33.7)	25/74 (33.8)	0.390
Ikedo 2007 <sup>[81]</sup>	846	Japan	Cohort	CH/Cirrhosis	130/392 (33.1)	107/454 (23.6)	IRR: 1.03 (0.66-1.56) <sup>1</sup> IRR: 1.58 (1.12-2.22) <sup>2</sup>
Adachi 2008 <sup>[82]</sup>	123	Japan	Cohort	Cirrhosis	57/96 (59.3)	10/27 (37.0)	0.0039
Alencar 2008 <sup>[89]</sup>	50	Brazil	Cross-sectional	Cirrhosis	5/12 (41.7)	12/38 (31.6)	NS
Miura 2008 <sup>[94]</sup>	141	Japan	Cohort	CH	22/83 (26.5)	11/58 (19.0)	0.700
Ramia 2008 <sup>[88]</sup>	3364	Lebanon	Cross-sectional	CH/cirrhosis/ healthy controls	7/408 (1.7)	2/2956 (0.07)	0.507
Stroffolini 2008 <sup>[47]</sup>	693	Italy	Cohort	Cirrhosis	44/303 (14.5)	57/390 (12.0)	0.900
Ohki 2010 <sup>[90]</sup>	1262	Japan	Cohort	CLD	160/522 (30.6)	179/740 (24.2)	0.630
Lok 2011 <sup>[43]</sup>	273	United States	Case-control	CH/Cirrhosis	38/121 (31.4)	53/152 (35.0)	0.540
Reddy 2013 <sup>[83]</sup>	459	United States	Case-control	CLD	95/229 (41.5)	27/230 (11.7)	0.010
Tsubouchi 2013 <sup>[91]</sup>	400	Japan	Cohort	CLD	24/213 (11.3)	14/187 (7.5)	0.280



# Association between OBI and HCC in patients with chronic hepatitis C

## Studies requesting HBV DNA in serum or liver as evidence of OBI

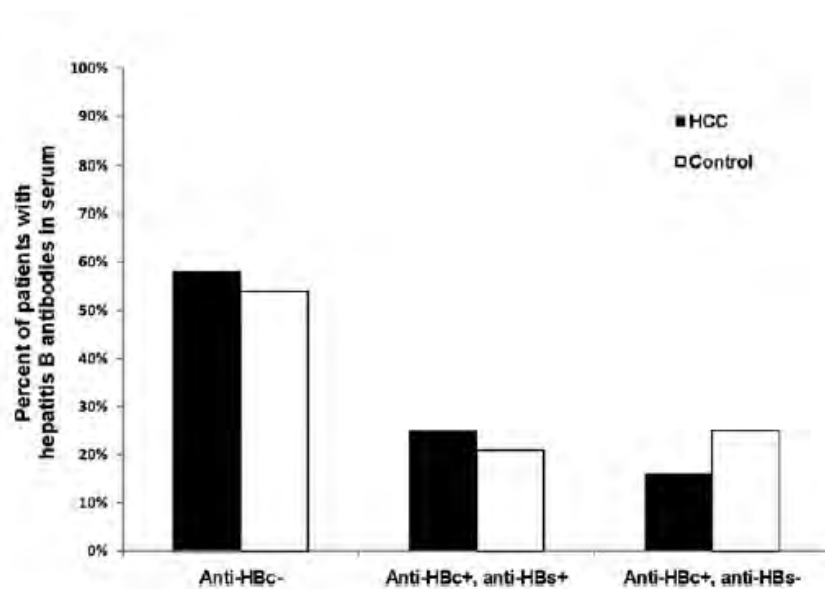
First author, year	No. of patients	Country	Type of study	Liver disease	Sample for HBV-DNA detection	HCC, positive/tested, n/n (%)		P value
						OBI +	OBI -	
Pollicino 2004 <sup>[39]</sup>	226	Italy	Case-control	CH/cirrhosis/ HCC	Liver	45/101 (44.5)	28/125 (22.4)	< 0.001
Tanaka 2004 <sup>[80]</sup>	93	Japan	Cross-sectional	CH/cirrhosis/ HCC	Serum	25/32 (78.1)	25/61 (41.0)	< 0.001
Hasegawa 2005 <sup>[87]</sup>	140	Japan	Cohort	CH/cirrhosis	Serum	2/11 (18.2)	16/129 (12.4)	NS
Squadrito 2006 <sup>[40]</sup>	134	Italy	Cohort	CH/cirrhosis	Liver	8/53 (15.1)	1/81 (1.2)	0.002
Branco 2007 <sup>[93]</sup>	66	Brazil	Cross-sectional	CH/HCC/health controls	Serum/liver <sup>1</sup>	7/10 (70.0)	13/56 (23.2)	0.029
Adachi 2008 <sup>[82]</sup>	123	Japan	Cohort	Cirrhosis	Serum	6/14 (42.9)	60/109 (55.0)	NS
Matsuoka 2008 <sup>[68]</sup>	468	Japan	Cohort	CH/cirrhosis	Serum/liver <sup>1</sup>	29/204 (14.2)	9/264 (3.4)	0.0001
Miura 2008 <sup>[94]</sup>	141	Japan	Cohort	CH	Serum	4/8 (50.0)	29/133 (21.8)	0.0036
Obika 2008 <sup>[41]</sup>	167	Japan	Cohort	CLD	Liver	2/25 (8.0)	10/142 (7.0)	NS
Shetty 2008 <sup>[42]</sup>	44	United States	Cross-sectional	cirrhosis	Liver	12/22 (54.5)	8/22 (36.3)	NS
Lok 2011 <sup>[43]</sup>	83	United States	Case-control	CH/Cirrhosis	Liver	3/16 (18.7)	25/67 (37.3)	NS
Squadrito 2013 <sup>[69]</sup>	94	Italy	Cohort	CH/cirrhosis	Liver	13/37 (35.1)	5/57 (8.1)	< 0.01



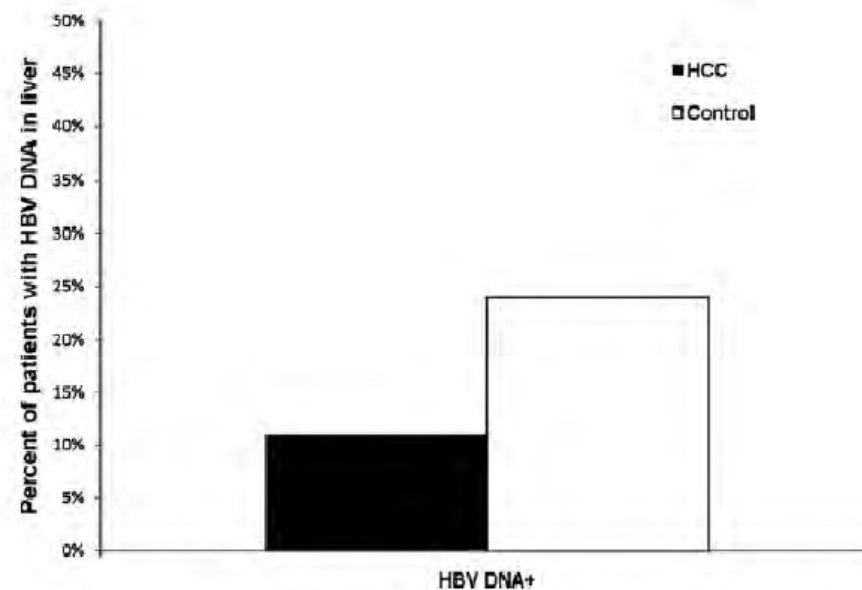


## Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C

	HCC Cases	Controls	Odds Ratio	95% Confidence Interval	P Value
HBV antibodies in serum	n = 91	n = 182			
Anti-HBc—	53 (58%)	99 (54%)	Ref.		
Anti-HBc+, anti-HBs+/-	38 (42%)	83 (46%)	0.85	0.51-1.43	0.54
Anti-HBc+, anti-HBs—	15 (16%)	45 (25%)	0.63	0.33-1.12	0.12
Anti-HBc+, anti-HBs+	23 (25%)	38 (21%)	1.13	0.60-2.138	0.27
HBV DNA in liver	n = 28	n = 55			
HBV DNA—	25 (89%)	42 (76%)	Ref.		
HBV DNA+	3 (11%)	13 (24%)	0.42	0.12-1.52	0.18



Prevalence of anti-HBc and anti-HBs in the sera of HCC patients (n = 91) and matched controls (n = 182).



Prevalence of HBV DNA in the livers of HCC patients (n = 28) and matched controls (n = 55).

*Lok ASF et al, Hepatology 2011; 54:434-42.*



## Occult and previous hepatitis B virus infection are not associated with hepatocellular carcinoma in United States patients with chronic hepatitis C

*Lok ASF et al, Hepatology 2011; 54:434-42.*

### *Limitations*

- The limited number of HCC cases evaluated
- A relatively long storage duration and limited size of biopsy specimens examined
- A smaller number of HBV genomic regions tested (polymerase and surface genes), as compared to previous studies
- The X genomic region, which expresses the oncogenic HBV transcription factor, was not assessed

# Histology in chronic hepatitis C patients according to presence/absence of intrahepatic HBV DNA

<i>Histology</i>	<b>HBV +</b>	<b>HBV –</b>
Minimal Changes	<b>0</b>	<b>5</b>
Chronic Hepatitis	<b>44</b>	<b>103</b>
Cirrhosis	<b>22</b>	<b>26</b>

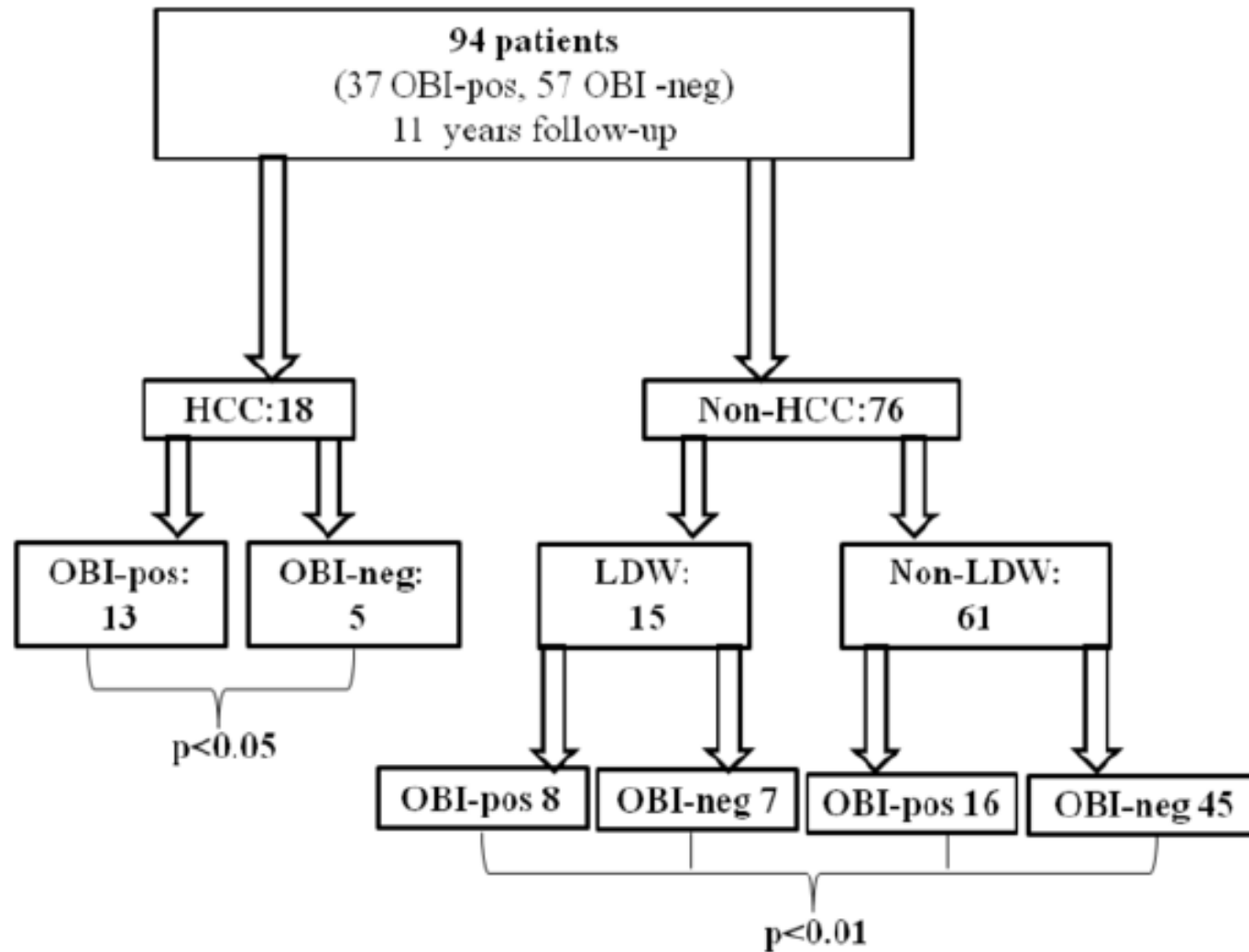
P = 0.04

*Cacciola et al, NEJM 1999*





## HCC development and liver disease worsening (LDW) events in patients with chronic hepatitis C



OBI positivity was associated with HCC development as well as with decompensated cirrhosis



***The use of different technical approaches for the diagnosis of OBI is reflected in the discordant datasets on several aspects of OBI.***





## UPDATE OF THE STATEMENTS ON BIOLOGY AND CLINICAL IMPACT OF OCCULT HEPATITIS B VIRUS INFECTION

Giovanni Raimondo<sup>1,2</sup>, Stephen Locarnini<sup>3</sup>, Teresa Pollicino<sup>2,4</sup>, Massimo Levrero<sup>5</sup>, Fabien Zoulim<sup>5</sup>, Anna S. Lok<sup>6</sup>, and the *Taormina Workshop on Occult HBV Infection Faculty Members\**

- *The ideal method of diagnosis of OBI is the detection of replication competent HBV DNA in the liver.*
- *The recommended methods include nested-PCR techniques to amplify at least 3 different viral genomic regions, real-time PCR assays, or droplet digital PCR assays. In each case the assay must include primer sets that allow to detect replication competent HBV DNA.*



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- ***Given that HBV DNA is present in low concentrations in persons with OBI, adequate size samples, and fresh frozen – but not formalin fixed - liver tissue should be used.***
- ***Detection of anti-HBc in the blood may be used as a surrogate marker for identifying OBI.***

## Prevalence of occult HBV infection in HCC and chronic hepatitis (CH) patients

	No. of Cases	HBV + (%)
HCC	107	68 (63.5)
CH	192	63 (32.8)

$P < 0.0001$

O.R. = 3.6 (95% CI: 2.2 – 5.9)





## Hepatitis B Virus Maintains Its Pro-oncogenic Properties in the Case of Occult HBV Infection

TERESA POLLICINO,\* GIOVANNI SQUADRITO,\* GIOVANNI CERENZIA,\* IRENE CACCIOLA, GIUSEPPINA RAFFA,\* ANTONIO CRAXÌ,\* FABIO FARINATI,§ GABRIELE MISSALE,¶ ANTONINA SMEDILE,|| CLAUDIO TIRIBELLI,# ERICA VILLA,\*\* and GIOVANNI RAIMONDO\*

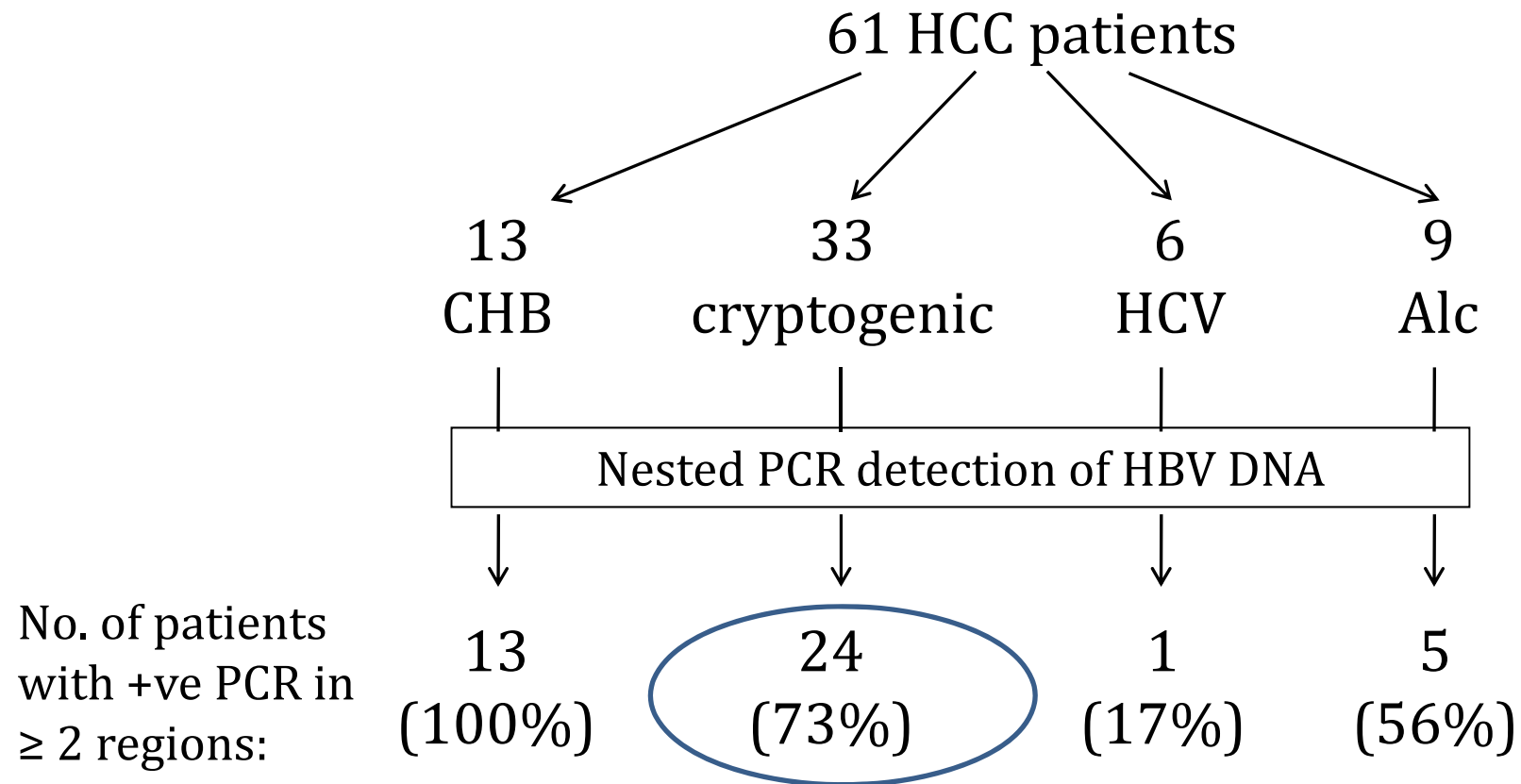
### **Molecular status of occult HBV in tumor and non-tumor tissues of HCC patients**

	<b>Samples examined</b>	<b>Positive cases</b>
- Integrated HBV	<b>10</b>	<b>2</b>
- HBV cccDNA	<b>30</b>	<b>20</b>
- HBV RNA	<b>10</b>	<b>10</b>



# Occult Hepatitis B Infection and HBV Replicative Activity in Patients with Cryptogenic Cause of Hepatocellular Carcinoma

Danny Ka Ho Wong,<sup>1,2</sup> Fung Yu Huang,<sup>1</sup> Ching Lung Lai,<sup>1,2</sup> Ronnie Tung Ping Poon,<sup>2,3</sup> Wai Kay Seto,<sup>1</sup> James Fung,<sup>1</sup> Ivan Fan Ngai Hung,<sup>1</sup> and Man Fung Yuen<sup>1,2</sup>





# Patients with unknown history of hepatitis B: OBI

## Intrahepatic HBV DNA and pregenomic RNA quantification

Intrahepatic HBV DNA – 30/39 (77%)

Median: 0.22 copies/cell (<0.001 – 18.0)

cccDNA – 1/39 (3%)

0.005 copies/cell

Pregenomic RNA – 5/39 (13%)

Range: <0.0004 – 0.06 copies/cell

Serum HBV DNA – 18 (45%)

Range: <1.1 – 14 IU/mL

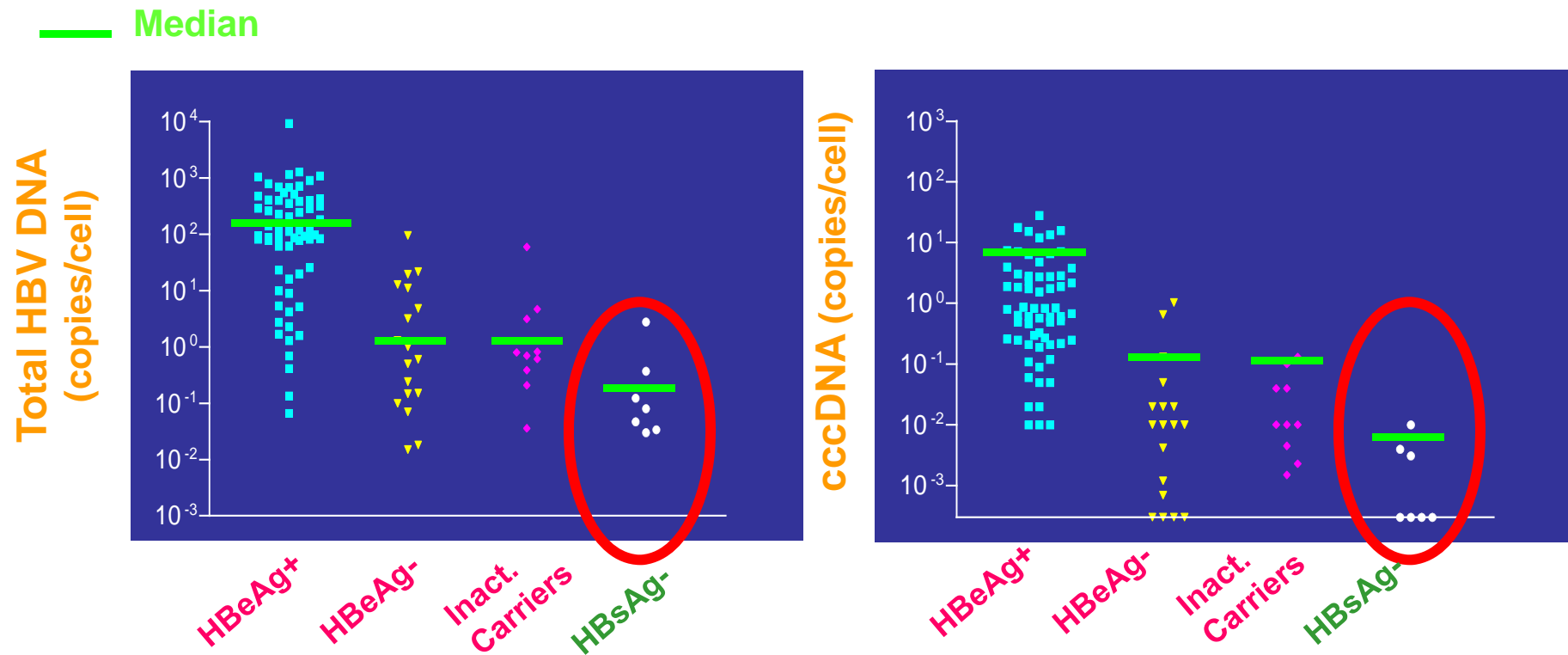
### **Lower limit of detection**

Intrahepatic HBV DNA 0.001 copies/mL

cccDNA 0.005 copies/mL

Pregenomic RNA <0.0004 copies/mL

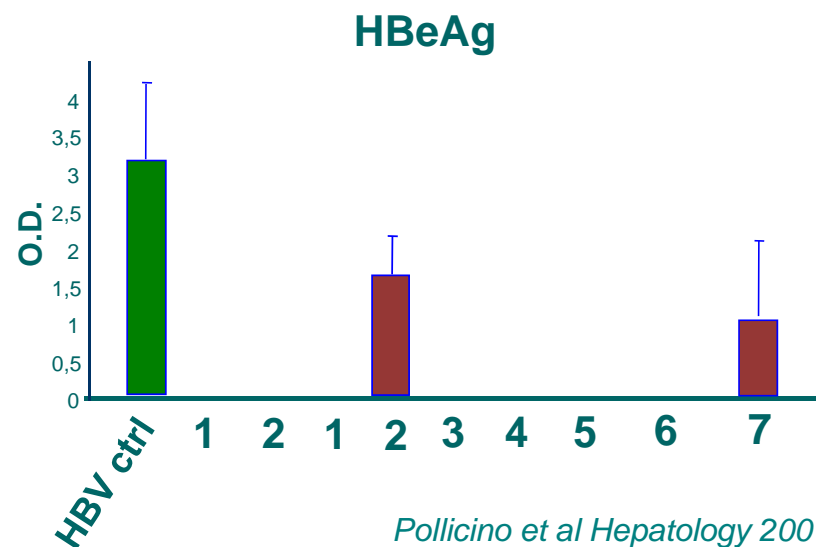
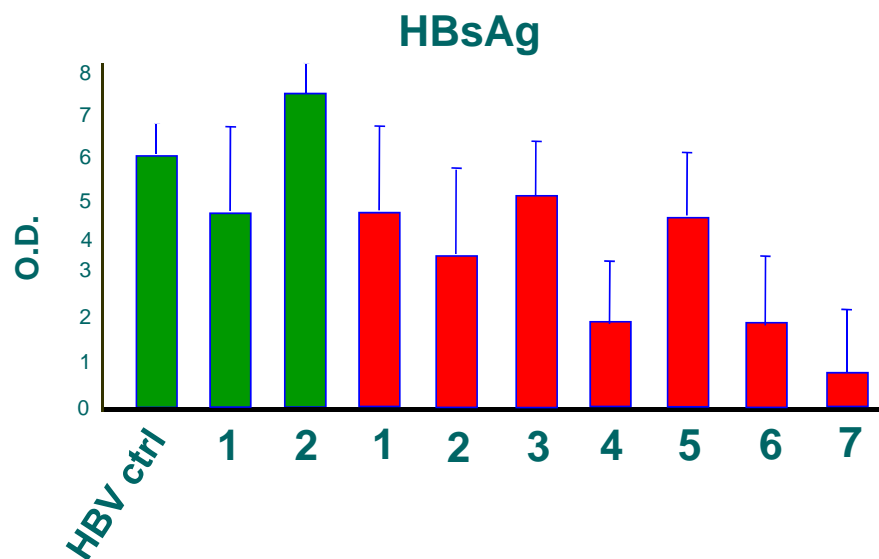
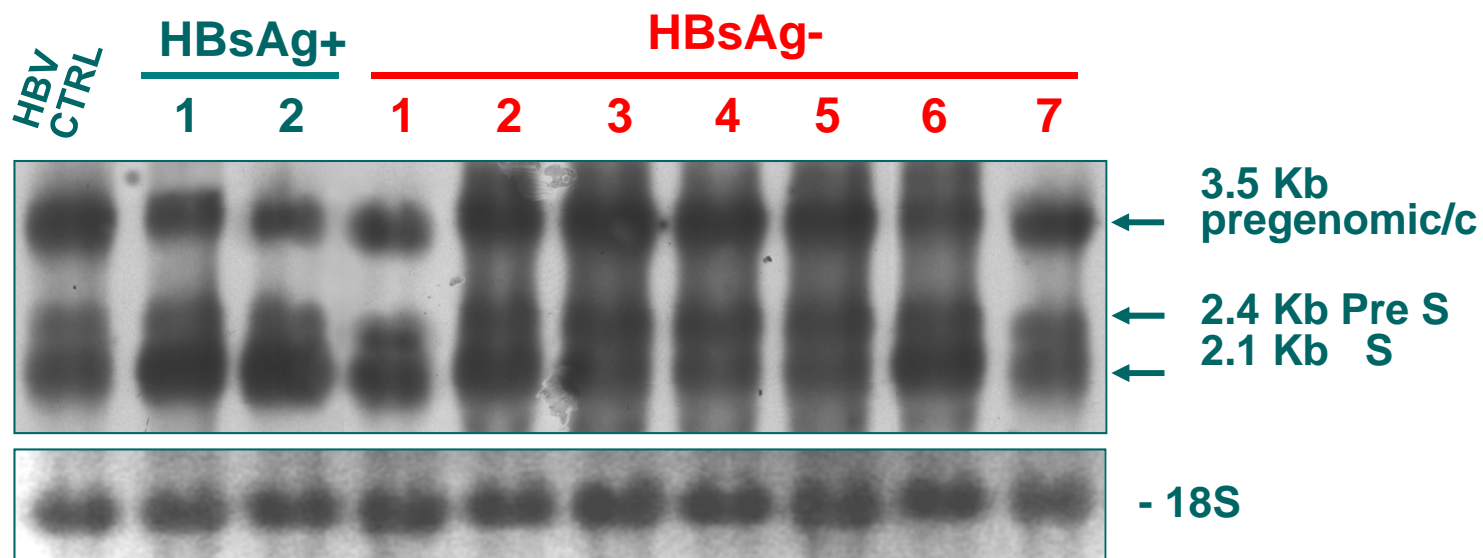
# Real Time PCR Quantification of Total HBV DNA and cccDNA in Different Groups of Patients







## Molecular and Functional Analysis of Occult Hepatitis B Virus Isolates



# OBI and severity of liver disease

*Which is the pathobiological significance of  
HBV-DNA persistence ?*



*Journal of Hepatology* 2000; 33: 992–997

## **Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation**

Jonas Bläckberg and Karin Kidd-Ljunggren

*Department of Infectious Diseases, Lund University, Lund, Sweden*

HEPATOLOGY, 2003;37:1172-1179.

## **Long-Term Histologic and Virologic Outcomes of Acute Self-Limited Hepatitis B**

Nobukazu Yuki,<sup>1</sup> Takayuki Nagaoka,<sup>2</sup> Masatoshi Yamashiro,<sup>2</sup> Kiyoshi Mochizuki,<sup>3</sup> Akira Kaneko,<sup>1</sup> Keiji Yamamoto,<sup>1</sup>  
Masao Omura,<sup>2</sup> Kazumasa Hikiji,<sup>2</sup> and Michio Kato<sup>1</sup>

***Mild necro-inflammation at liver histology analysis have been observed in individuals up to 30 years after the resolution of acute HBV infection.***



# WHV lifelong persistence after SLAH

TABLE 1. Serological Features of WHV Infection in Woodchucks  
Convalescent From Experimental Acute Viral Hepatitis

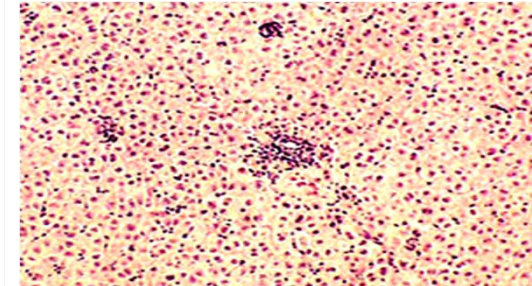
Animal No./Sex (M/F)	Observation Period* (mo)	Duration of WHsAg (mo)	Duration of Anti-WHs (mo)	Duration of Anti-WHc (mo)	Duration of Serum WHV DNA Positivity (mo)	Reason of Autopsy
1/M	45	1	neg	44	44.5	senility
2/F	47	4	6	46.5	46.5	senility
3/F	60	3	7	59	59	HCC
4/F	63	1.5	1	62	62.5	senility
5/M	48	1	36	47	47.5	senility
6/M	60	2	neg	59	59.5	senility
7/F	37	0.5	4	35.5	36	HCC
8/F†	71	1.5	11.5	70	70.5	senility
9/F†	57	2	1	56.5	56.5	ascites

\*From inoculation with WHV.

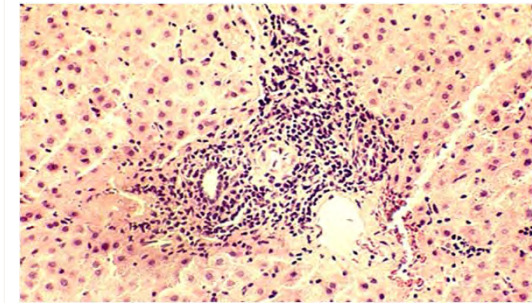
†Animals challenged with WHV after 25 months of follow-up.

Liver histology at the end of lifetime follow-up

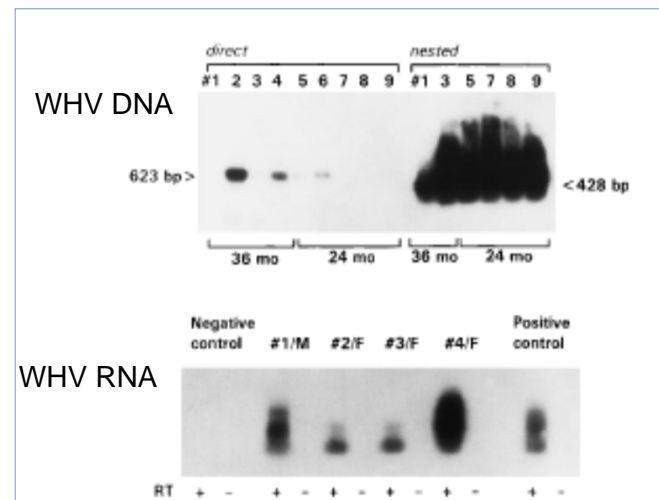
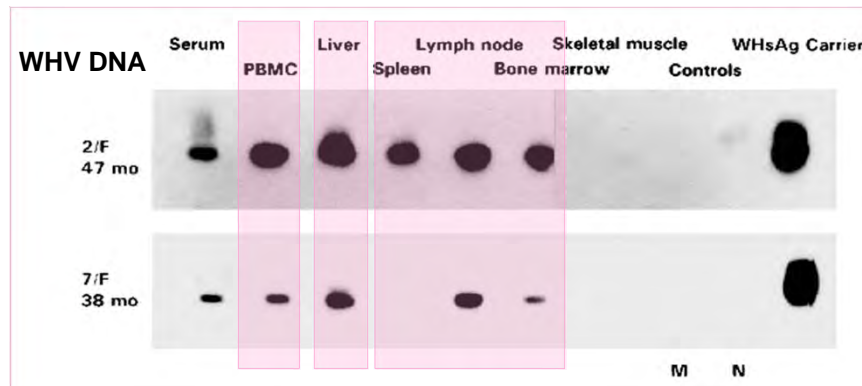
5/M 48 mo



6/M 60 mo



## WHV DNA and RNA expression in liver after lifetime follow-up







# SEQUELA ON WHV INFECTION

Woodchuck Viral Hepatitis Research Facility, Memorial University, Canada

n = >300 animals

## WHV INOCULATION

WHV doses i.v.:  $\geq 10^6$  virions

88%

### ACUTE HEPATITIS

12%

### SILENT INFECTION (WHsAg neg)

85%

#### SELF-LIMITED ACUTE HEPATITIS

15%

#### CHRONIC HEPATITIS

100%

#### OCCULT LIFE-LONG WHV CARRIAGE

80%

#### HEPATOCELLULAR CARCINOMA

18%

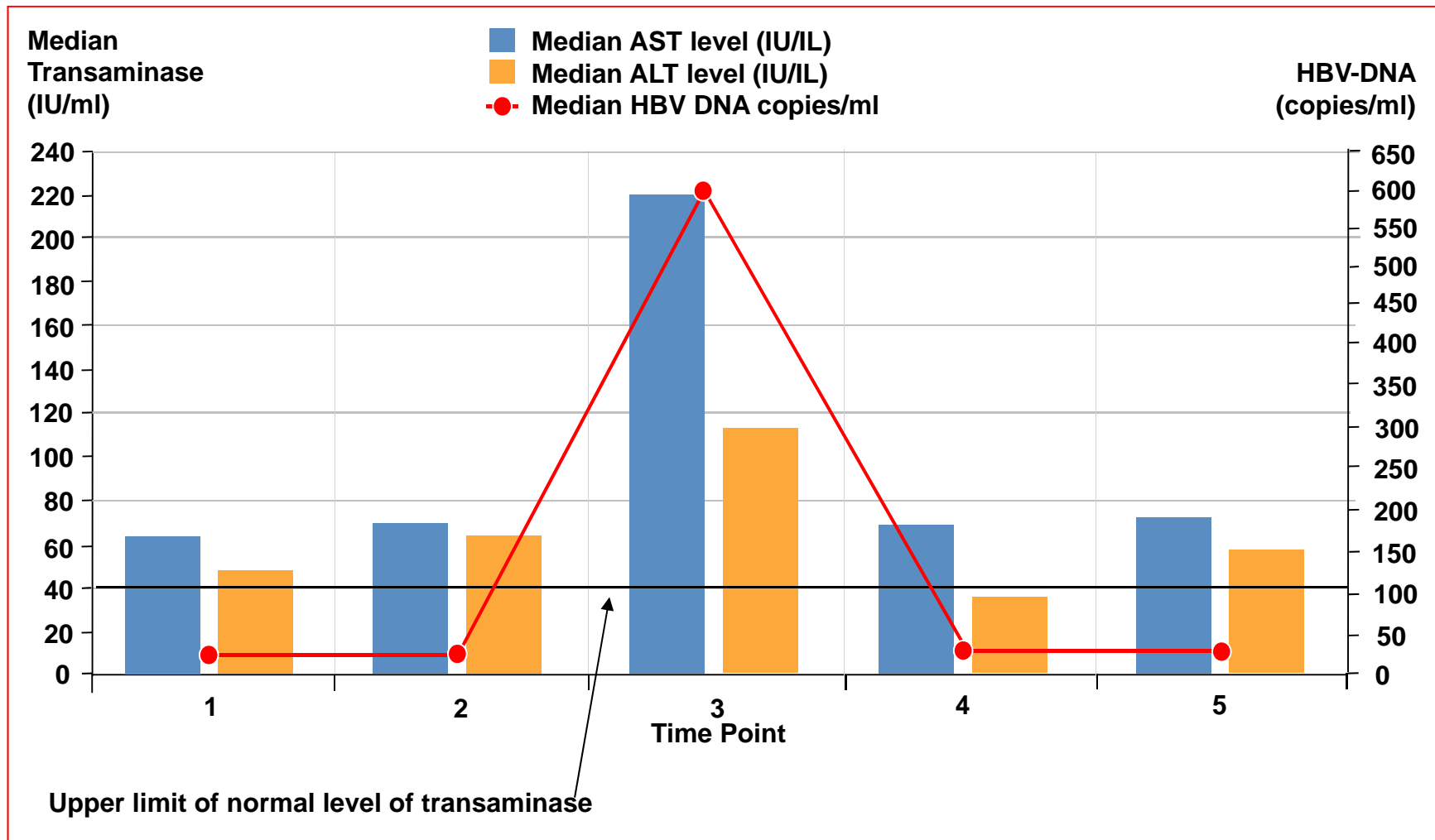
#### HEPATOCELLULAR CARCINOMA

~ 100%

#### OCCULT ILIFE-LONG WHV CARRIAGE

Courtesy from Michalak TI

# Liver enzyme flares and occult hepatitis B in persons with chronic hepatitis C infection



**A**



**Chemin et al, J Hepatol 2009**

## HBV Integration in HCC Patients

### OBI vs Overt HBV infection

	Overt	OBI
Prevalence of integration > 75%	✓	✓
Random distribution into the host genome	✓	✓
Functional genes often involved	✓	✓
X and preS/S viral genomic regions most frequently involved	✓	✓





# Overt HBV Infection

# Occult HBV Infection

