Diagnostic Testing and Interpretation of Lab tests for HBV

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Hepatitis B Nomenclature and/or Lab Tests (1)

- **HBV**: Hepatitis B virus.
- **HBsAg**: Hepatitis B surface antigen. Marker of infectivity when found in serum.
- **anti-HBs**: Antibody to HBsAg. Marker of immunity when found in serum.
- **HBcAg**: Hepatitis B core antigen. No commercial test available for this.
- **anti-HBc**: Antibody HBcAg. Marker of past or current infection.

Hepatitis B Nomenclature and/or Lab Tests (2)

- **IgM anti-HBc**: IgM is an antibody subclass of anti-HBc. Indicates recent infection with HBV (<4-6 mos.).
- **IgG anti-HBc**: IgG is a subclass of anti-HBc. Indicates "older" infection with HBV.
- **HBeAg**: Hepatitis B "e" antigen. Can only be present if HBsAg is positive. Marker of high degree of infectivity.
- **Anti-HBe**: Antibody to "e" antigen. May be present in infected or immune person.

Interpretation of Hepatitis B Panel

Hbs Ag	Hbc Ab	Hbs Ab	
-	-	-	Susceptible
-	+	+	Immune due to natural infection
-	-	+	Immune due to vaccination
+	Hbc Ab + HbcAb Igm +	-	Acutely infected
+	Hbc Ab + HbcAb Igm -	-	Chronically infected
-	+	-	4 possible interpretations

Four possible interpretations of isolated antiHBc positive

1. May be recovering from acute HBV infection.
2. May be distantly immune and test not sensitive enough to detect very low level of anti-HBs in serum.
3. May be susceptible with a false positive anti-HBc.
4. May be undetectable level of HBsAg present in the serum and the person is actually a carrier.

Tests for Measuring Clinical Response

Measuring Clinical Response

- Biochemical measurement of response
  - Liver enzymes
    - Normalization of ALT and AST
  - Synthetic tests
    - Normalization of albumin, bilirubin and INR
- Serological measurement of response
  - Loss of HbeAg +/- Gain of anti-HBe (HBeAg seroconversion)
  - Loss of HbsAg +/- Gain of anti-HBs (HBsAg seroconversion)
- Virologic measurement of response
  - HBV DNA suppression
- Histological measurement of response
  - Decrease in inflammation and fibrosis

Lok AS et al. *Gastroenterology*. 2001;120:1828-1853.

Why Focus on HBV Serum DNA?

- Serum HBV DNA is the best available measure of HBV replication
- HBV replication = disease
  - Reduction in HBV DNA precedes biochemical and histological response
  - Rebound in HBV DNA precedes loss of biochemical and histological response
  - Level of HBV replication correlates with incidence of HCC and cirrhosis

HBV DNA and Effect on Natural History

Risk for
HCC and Cirrhosis

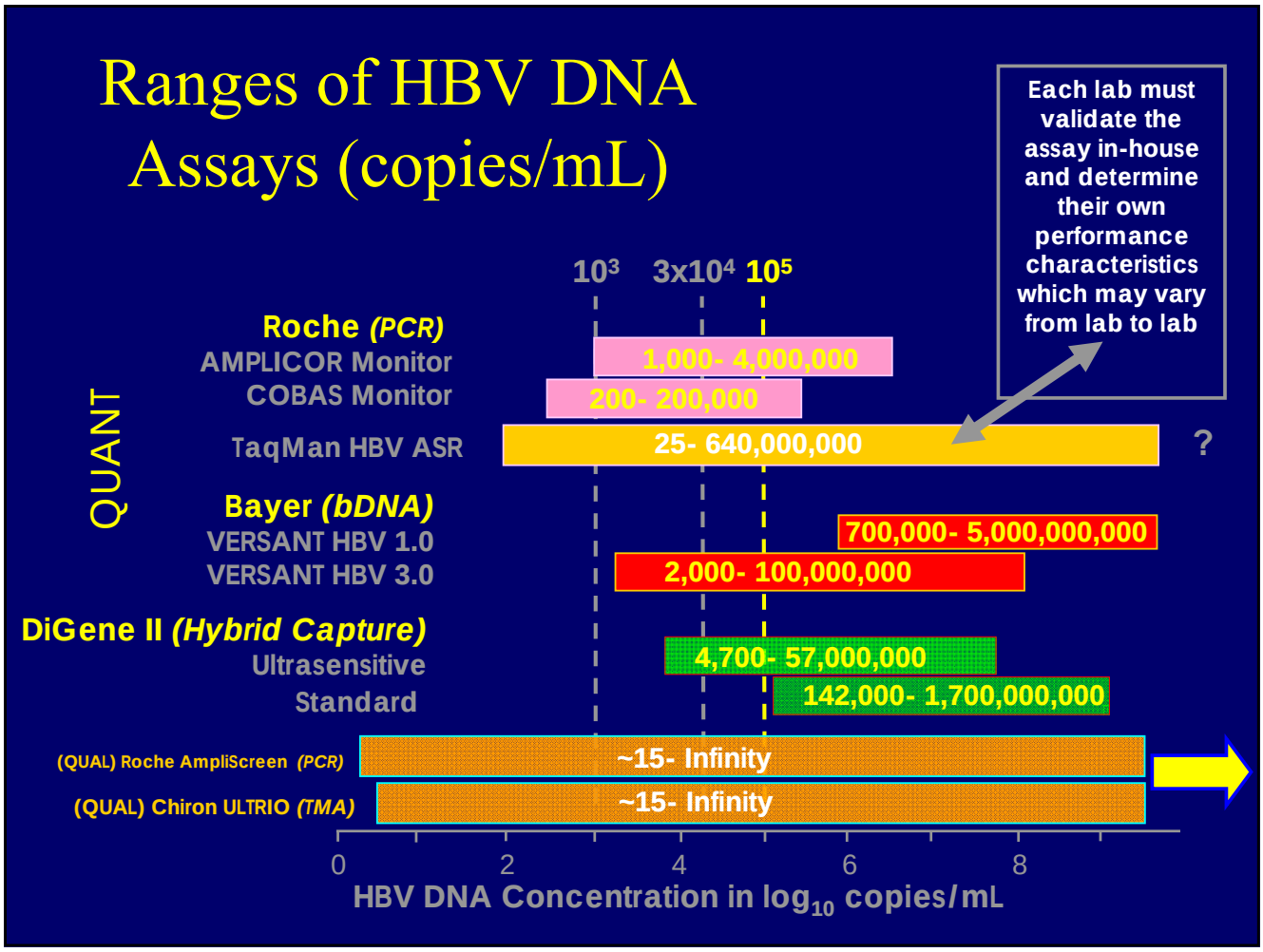
Assays for measuring HBV DNA

Nucleic Acid Tests for HBV DNA

- Signal amplification assays
  - Liquid hybridization assay (Abbott)
  - DNA-RNA
  - Branched DNA, bDNA (Bayer)
  - Hybrid capture (Digene)
- Target amplification assays
  - Polymerase chain reaction assay, PCR
  - Transcription-mediated amplification, TMA (Chiron)
  - Nucleic acid sequence-based amplification, NASBA
  - Ligase chain reaction assay, LCR

Lack of Standardization in HBV DNA Quantification

- Dynamic ranges of detection
  - Lower Limit of Quantification (LLQ)
  - Upper Limit of Quantification (ULQ)
- Presentation of results
  - Mean change in serum HBV DNA as percent reduction in HBV DNA
  - Log change is best format vs linear
  - Comparison vs. baseline
- Units of measurements
  - Number of units to express serum levels of HBV DNA
    - IU is now the standard


Ranges of HBV DNA Assays (copies/mL)
Each lab must validate the assay in-house and determine their own performance characteristics which may vary from lab to lab
QUANT
10³ 3x10⁴ 10⁵
Roche (PCR)
AMPLICOR Monitor
1,000- 4,000,000
COBAS Monitor
200- 200,000
TaqMan HBV ASR
25- 640,000,000
?
Bayer (bDNA)
VERSANT HBV 1.0
700,000- 5,000,000,000
VERSANT HBV 3.0
2,000- 100,000,000
DiGene II (Hybrid Capture)
Ultrasensitive
4,700- 57,000,000
Standard
142,000- 1,700,000,000
(QUAL) Roche AmpliScreen (PCR)
~15- Infinity
(QUAL) Chiron ULTRIO (TMA)
~15- Infinity
0 2 4 6 8
HBV DNA Concentration in log10 copies/mL

HBV DNA Evaluation

- HBV DNA should be evaluated using the assay with the widest dynamic range
- Patient who are negative by a quantitative assay should then be evaluated with the most (qualitative) sensitive assay available
- All patients should be treated until HBV DNA negative by the best assay available

HBV DNA and Effect on Natural History

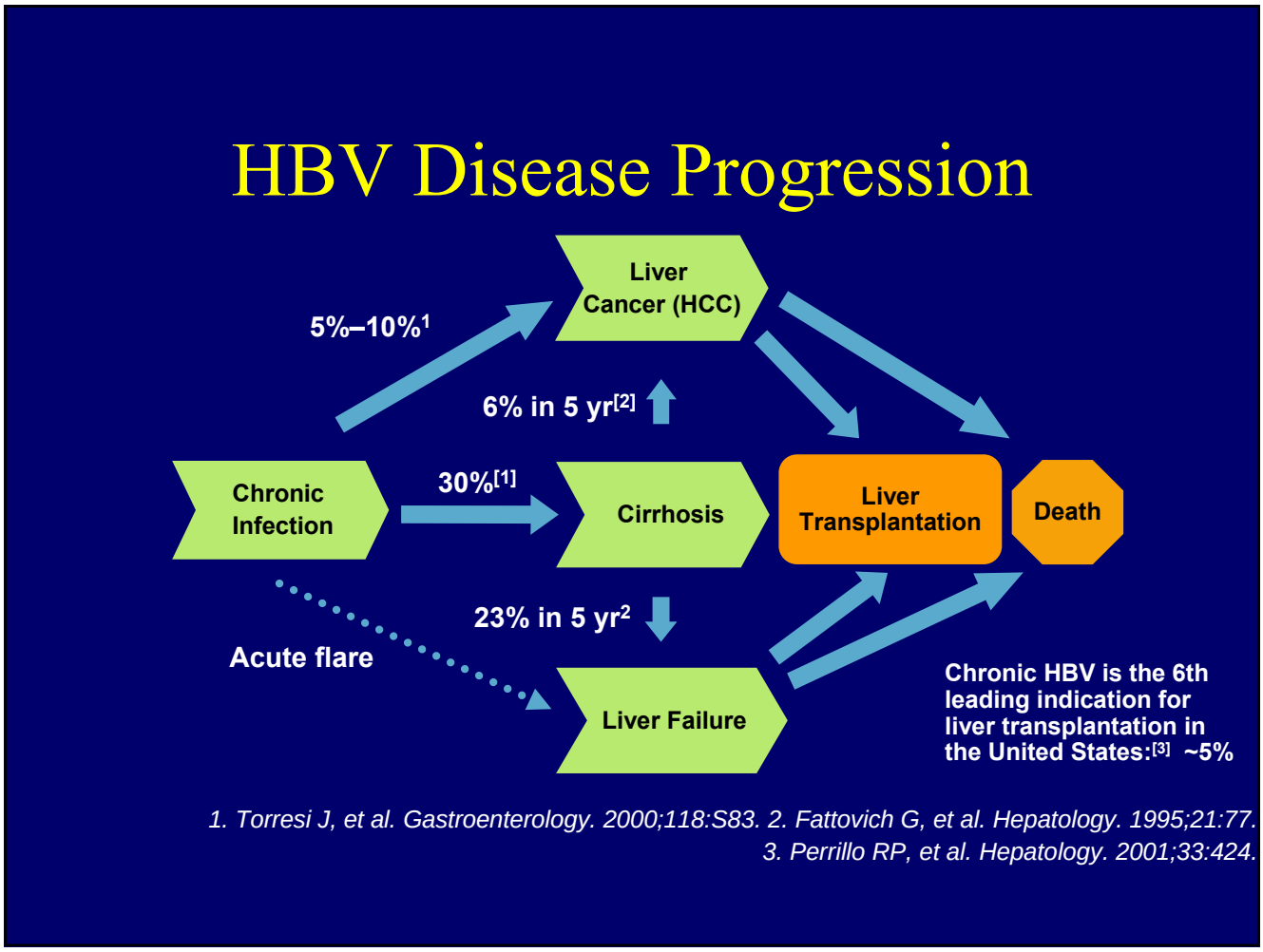
Risk for
HCC and Cirrhosis

Factors Associated With Increased Risks of Progression to Cirrhosis

Host Factors	Virus Factors	Environmental Factors
Older age (longer duration)*	High levels of HBV replication*	Concurrent infection (HCV*, HDV, HIV)
Male*	Genotype (C > B)*	Alcohol consumption*
Immune status	HBV variant (core promoter)	Diabetes mellitus† Obesity †

*Supported by strong evidence.
†Further studies needed.

Yim, Lok. Hepatology 2006;43:s173-s181

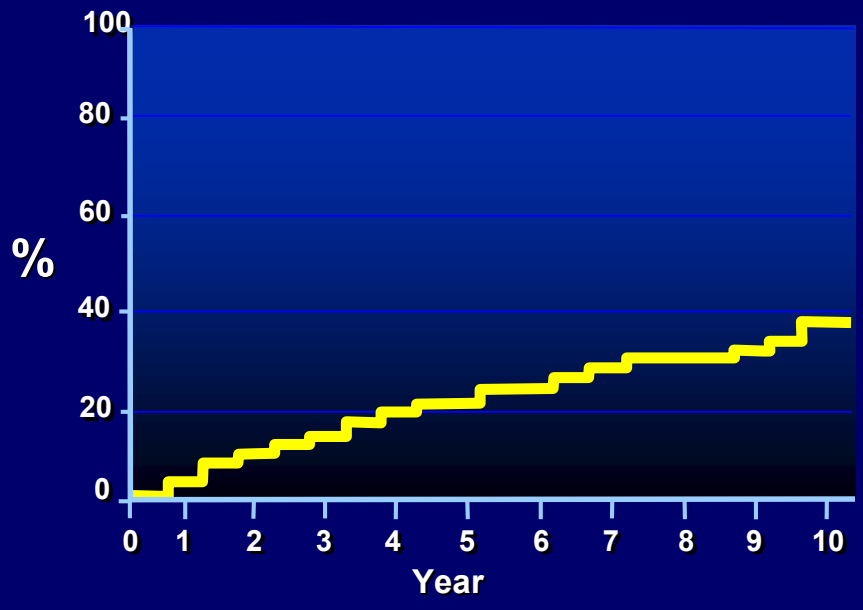

HBV Disease Progression
Liver Cancer (HCC)
5%–10%[1]
6% in 5 yr[2]
Chronic Infection
30%[1]
Cirrhosis
Liver Transplantation
Death
23% in 5 yr[2]
Acute flare
Liver Failure
Chronic HBV is the 6th leading indication for liver transplantation in the United States:[3] ~5%
1. Torresi J, et al. Gastroenterology. 2000;118:S83. 2. Fattovich G, et al. Hepatology. 1995;21:77.
3. Perrillo RP, et al. Hepatology. 2001;33:424.

Probability of Decompensation in Patients with Compensated Hepatitis B Cirrhosis

N = 349

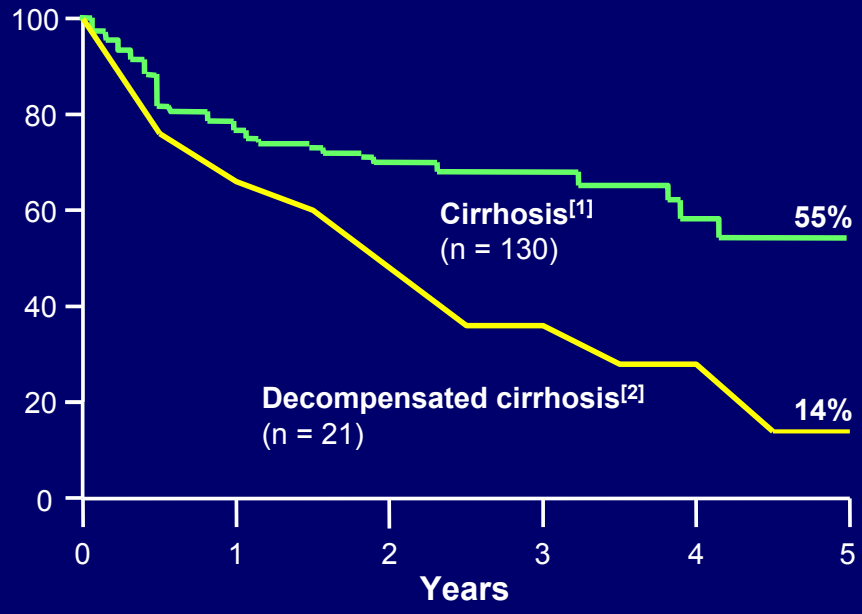
Mean f/u period of 73 months

At 5 years, probability of decompensation was 23%



Fattovich G, et al., Hepatology 1995; 21:77

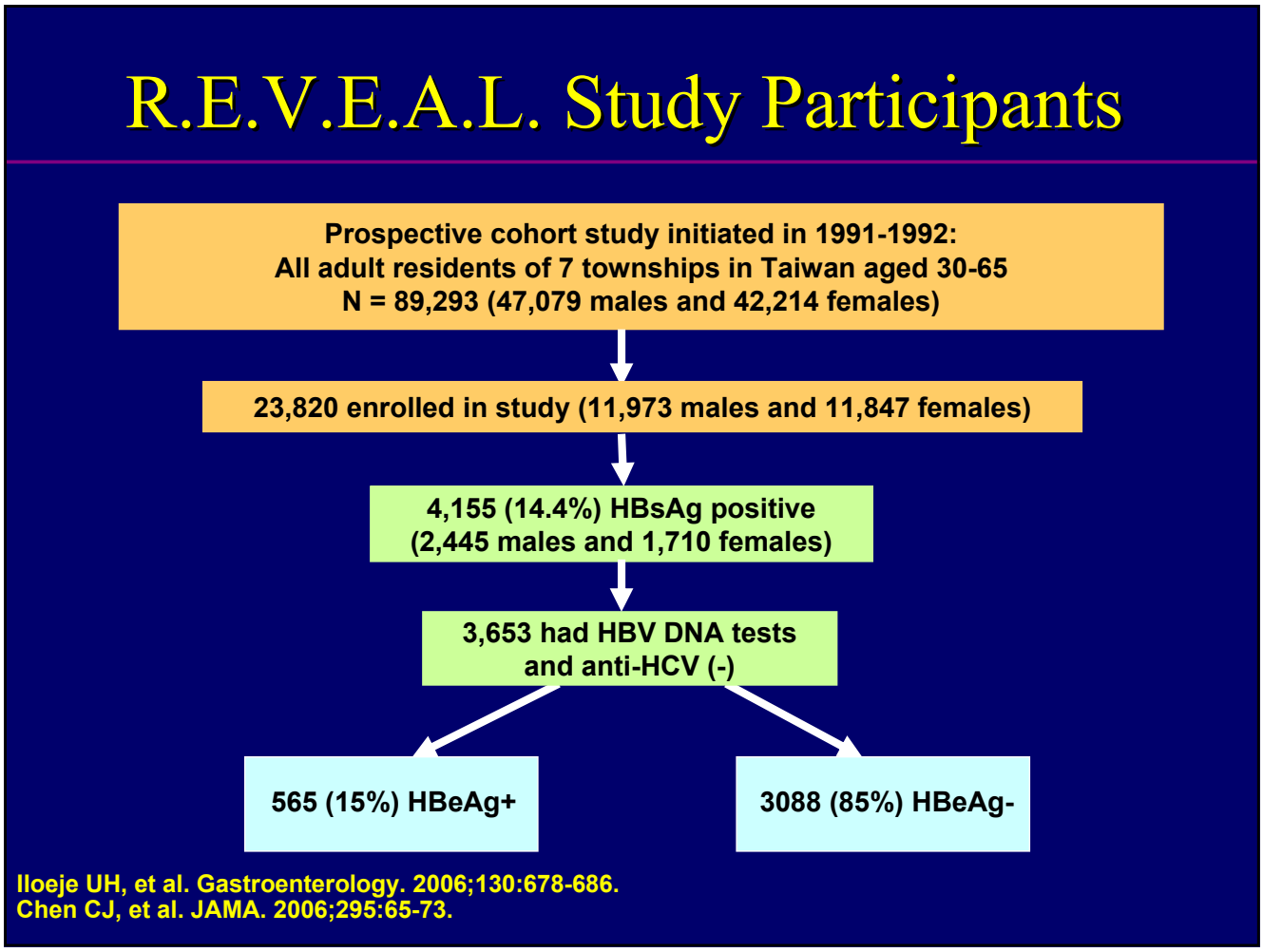
Actuarial Survival in ESLD
Historical Studies



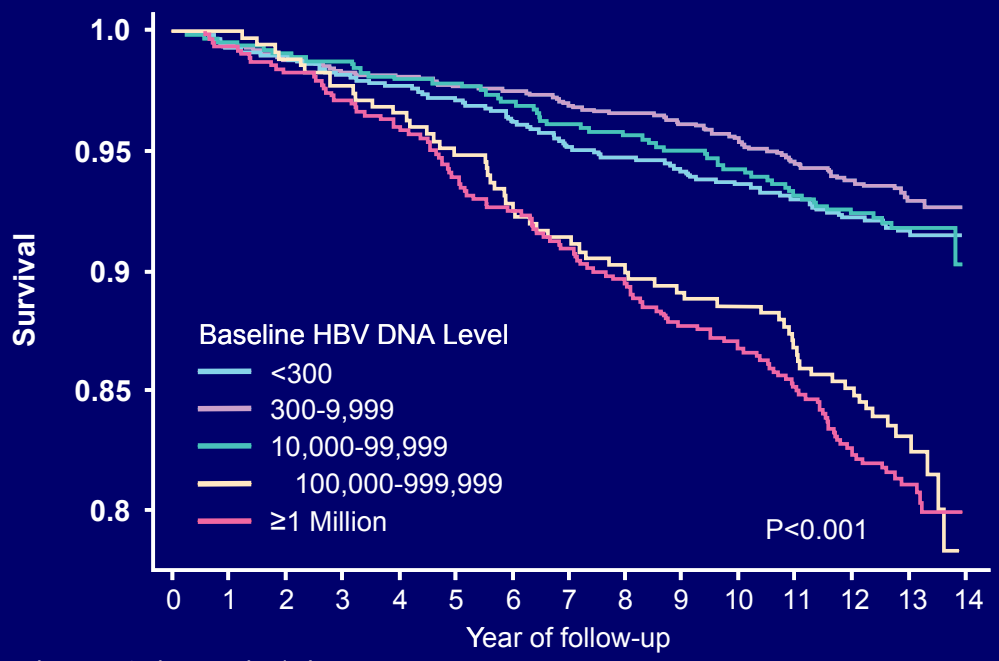
1. Weissberg JI, et al. Ann Intern Med. 1984;101:613. 2. De Jongh FE, et al. Gastroenterology. 1992;103:1630.

REVEAL

Taiwan Cohort Study

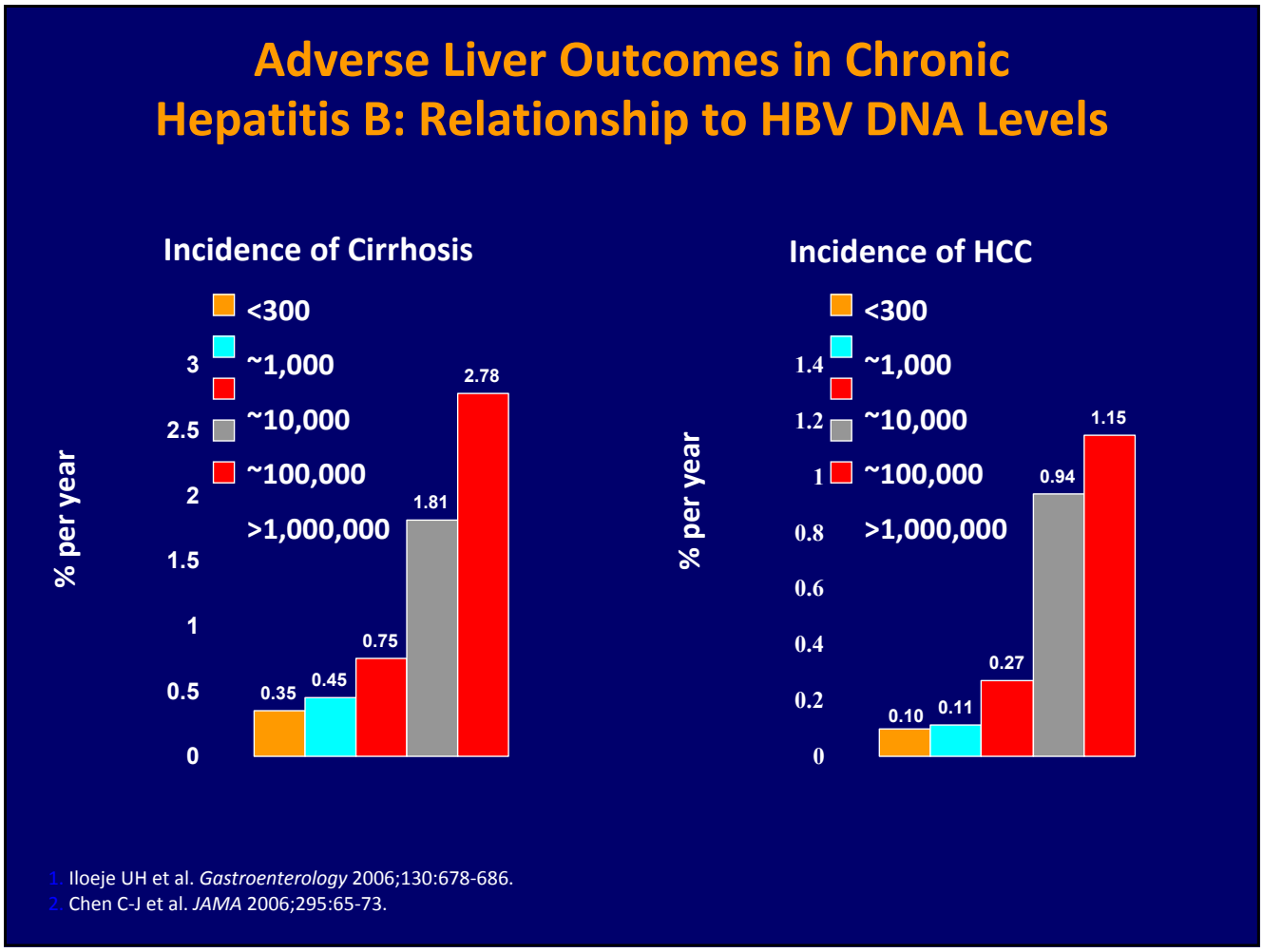

R.E.V.E.A.L. Study Participants
Prospective cohort study initiated in 1991-1992:
All adult residents of 7 townships in Taiwan aged 30-65
N = 89,293 (47,079 males and 42,214 females)
23,820 enrolled in study (11,973 males and 11,847 females)
4,155 (14.4%) HBsAg positive
(2,445 males and 1,710 females)
3,653 had HBV DNA tests
and anti-HCV (-)
565 (15%) HBeAg+
3088 (85%) HBeAg-
Iloeje UH, et al. Gastroenterology. 2006;130:678-686.
Chen CJ, et al. JAMA. 2006;295:65-73.

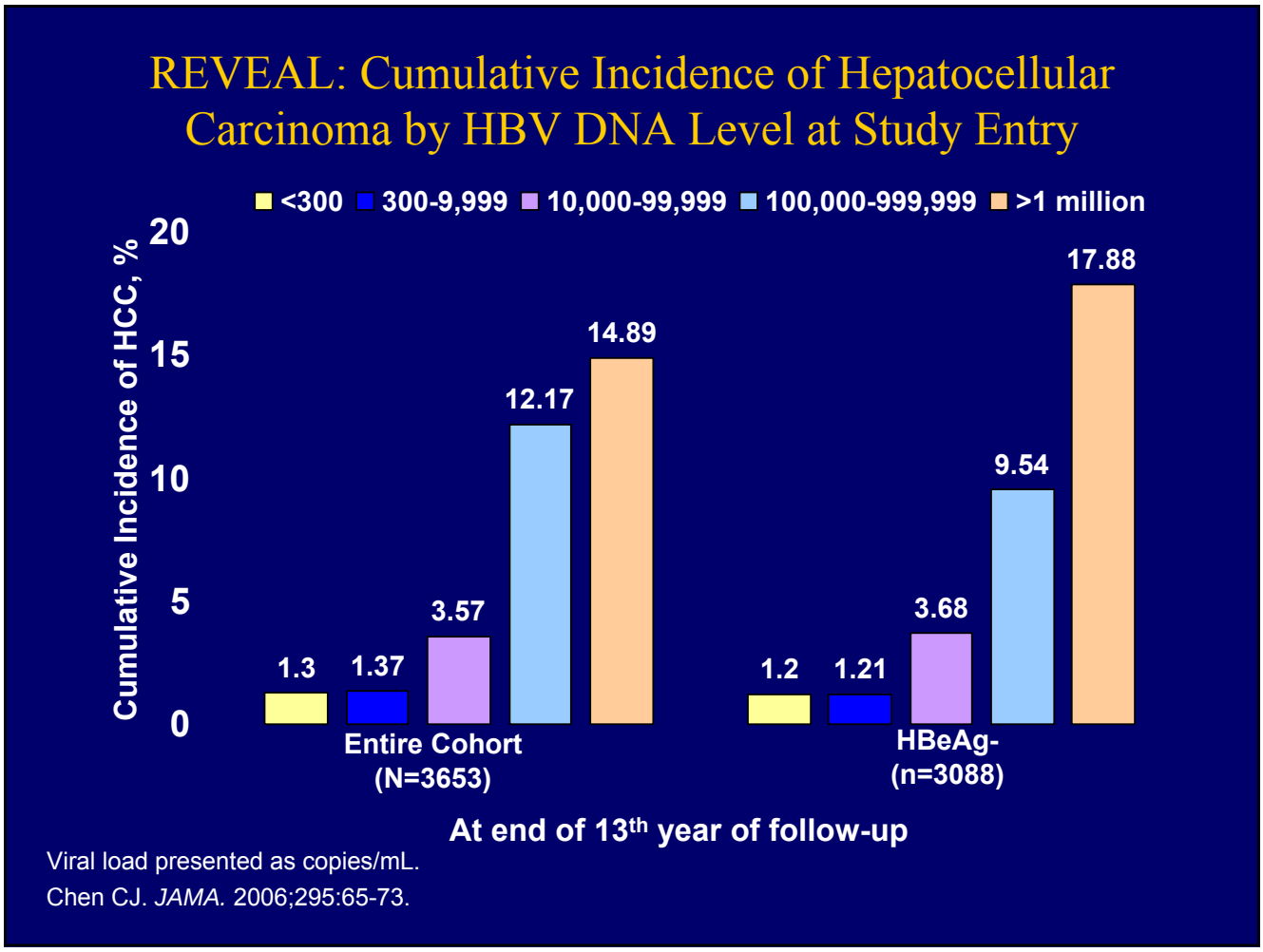
REVEAL: Relationship Between HBV DNA Level and All-Cause Mortality



Viral load presented as copies/mL.

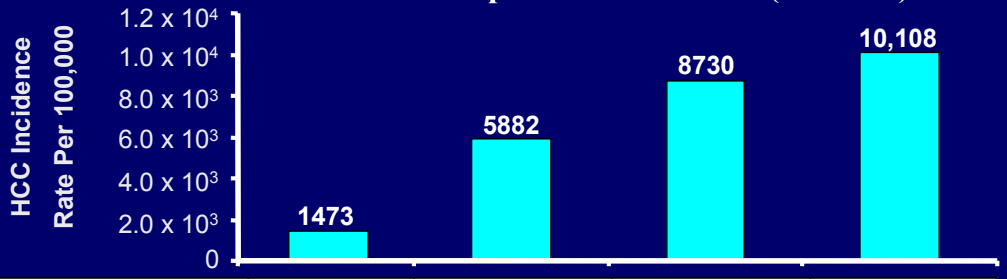
Iloeje UH. *Gastroenterology.* 2006;130:678-686.


Adverse Liver Outcomes in Chronic Hepatitis B: Relationship to HBV DNA Levels
Incidence of Cirrhosis
% per year
<300
~1,000
~10,000
~100,000
>1,000,000
0
0.5
1
1.5
2
2.5
3
0.35
0.45
0.75
1.81
2.78
Incidence of HCC
% per year
<300
~1,000
~10,000
~100,000
>1,000,000
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.10
0.11
0.27
0.94
1.15
1. Iloeje UH et al. Gastroenterology 2006;130:678-686.
2. Chen C-J et al. JAMA 2006;295:65-73.


REVEAL: Cumulative Incidence of Hepatocellular Carcinoma by HBV DNA Level at Study Entry
<300
300-9,999
10,000-99,999
100,000-999,999
>1 million
Cumulative Incidence of HCC, %
20
15
10
5
0
1.3
1.37
3.57
12.17
14.89
1.2
1.21
3.68
9.54
17.88
Entire Cohort (N=3653)
HBeAg- (n=3088)
At end of 13th year of follow-up
Viral load presented as copies/mL.
Chen CJ. JAMA. 2006;295:65-73.

REVEAL: Relationship Between Persistent Viremia and HCC Incidence

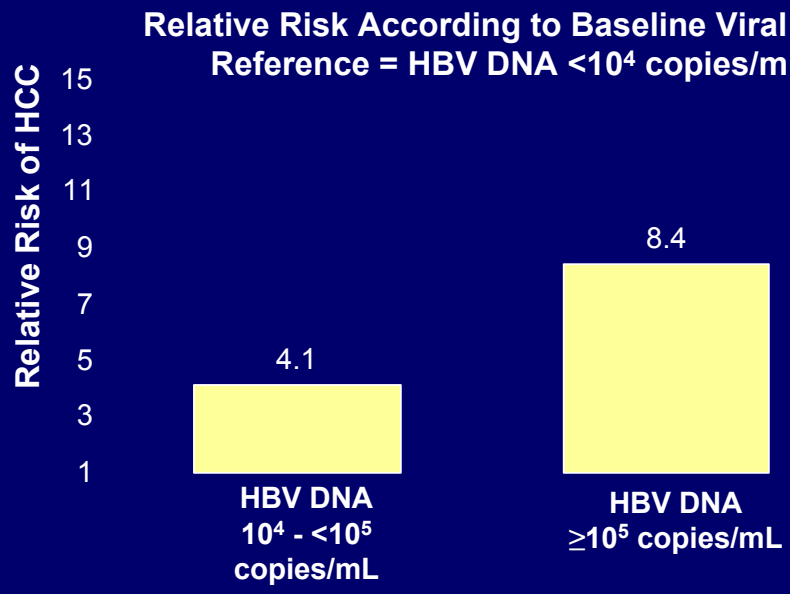
- **Persistent HBV DNA associated with greater risk of HCC**
  - **Examined in those with levels ≥ 10^4 copies/mL at baseline (n = 1376)**



Baseline HBV DNA, copies/mL	< 10^4	≥ 10^5	≥ 10^5	≥ 10^5
Follow-up HBV DNA, copies/mL	--	< 10^4	10^4 to < 10^5	≥ 10^5
Adjusted RR (95% CI)	1.0 (reference)	3.8 (1.7-8.4)	7.3 (3.5-15.3)	10.1 (6.3-16.2)
P Value	--	< .001	< .001	< .001

Chen CJ, et al. *JAMA* 2006;295:65-73.

High HBV DNA Associated With Increased Risk of Hepatocellular Carcinoma



Nested case-control series. 37 cases matched with 61 controls. Median viral load was 9.3×10^5 copies/mL in the HCC cases and undetectable ($<3 \times 10^4$ copies/ml) in the controls ($P<0.001$).

Evans AA. 55th AASLD, October 29 - November 2, 2004, Boston, MA.

Impact of Viral Replication on Disease Progression: Taiwan Cohort Study Results

- The incidence of hepatocellular carcinoma (HCC) and liver cirrhosis is correlated with level of viral replication
- Persistent elevation of viral load over time has the greatest impact on HCC risk
- Viral load predicts risk of future HCC independent of HBeAg status and serum ALT level
- This risk increases with increasing viral load

EASL April 2005

HBV Genotype?

HBV Genotype

Currently eight genotypes (A-H) defined

Clinical Outcomes
- Genotype B: better than genotype C
- Genotype A: better than genotype D
- Genotypes E to H: the clinical significance remains to be examined

Sustainted response to standard interferon
- Genotype B better than genotype C
- Genotype A better than genotype D.

Response to PegIFN
- A and B, better rates of HBeAg seroconversion than C and D

Responses to nucleos(t)ide analogues
- No significant difference between HBV genotypes have been identified

HBV Genotype Conclusion

Currently

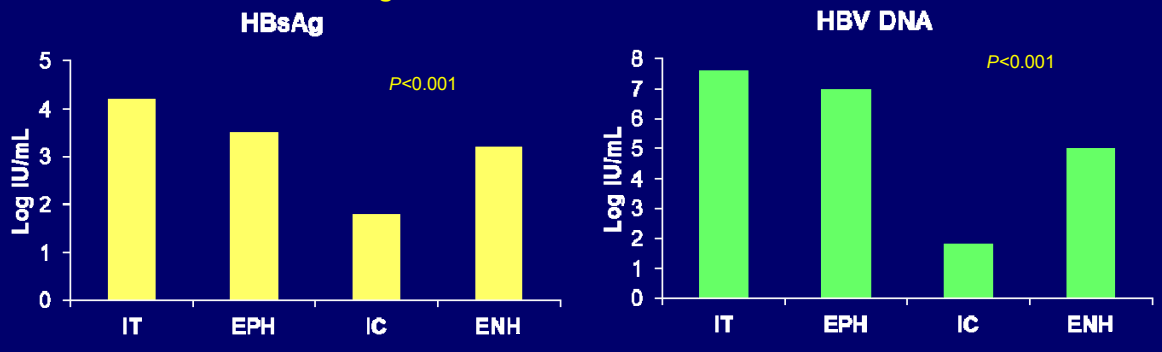
- HBV genotyping is not routine
- but this is likely to change if additional studies confirm that genotyping has prognostic value or strongly predicts response to therapy

HBsAg levels?

HBsAg Levels in the Natural Course of HBV

- HBsAg levels have been recently studied as markers of response to treatment
- Study evaluating HBsAg level variance with stage of disease
- Cross sectional retrospective study, N = 601
- HBsAg correlates with HBV DNA except in HBeAg(-) patients
- In untreated patients, higher HBsAg levels may predict recurrence as well as HBV DNA

Mean HBsAg and HBV DNA Level in Each Phase of CHB

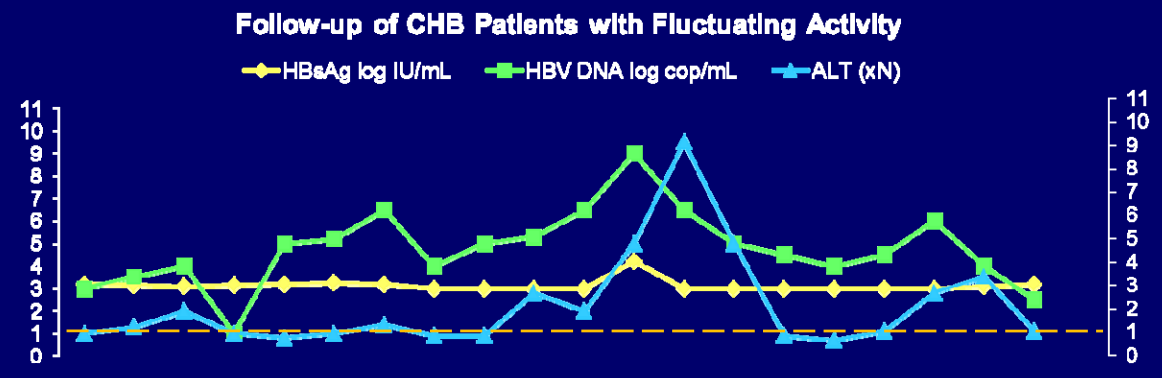


Kim Y, et al. 61st AASLD; Boston, MA; October 29-November 2, 2010; Abst. 1396.

Adapted from Simply Speaking HBV 2011

Quantitative HBsAg: New Marker for Diagnosis of HBsAg Inactive Carriage

- Quantitative HBsAg is new marker for diagnosis of HBV inactive carriage
- HBsAg ≤2,000 IU/mL and HBV DNA ≤2,000 copies/mL identifies true inactive carriers with 100% specificity
- Yearly measurement of HBsAg to identify patients that will demonstrate HBsAg seroconversion
- Conclusion: Quantitative HBsAg should be considered for future HBV guidelines



Martinot-Peignoux, M., et al. 61st AASLD; Boston, MA; October 29-November 2, 2010; Abst. 1397.

Adapted from Simply Speaking HBV 2011

HBsAg Levels in the Natural Course of HBV

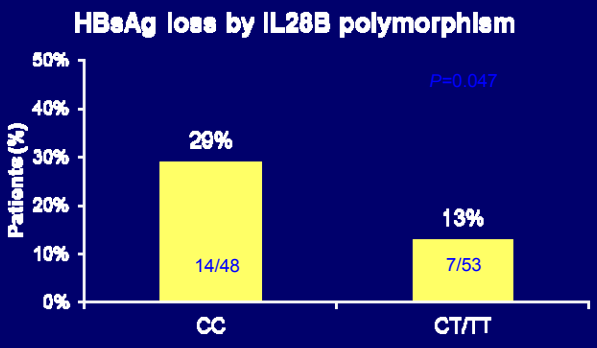
Key Message:

- HBsAg levels are known to correlate with treatment success with interferon.
- This study demonstrates that they parallel HBV DNA and can be used to predict the course of HBV infection, even when not on treatment.
- measurement of HBsAg to identify patients that will demonstrate HBsAg seroconversion
- Conclusion: There may be clinical utility in following HBsAg levels

IL28B Testing?

IL28B Polymorphism May Predict HBsAg Clearance in Genotype D, HBeAg(-) Patients Treated with Interferon Alfa

- Retrospective study of role of IL28B polymorphism in interferon treatment in HBeAg(-) CHB pts
  - Treatment: standard IFN for 23 months, 11 year follow-up
  - Endpoint HBsAg loss
- Low baseline HBV DNA, high ALT levels and genotype CC of IL28B independently predicted HBsAg clearance
- IL 28B polymorphism may represent an additional pretreatment predictor of interferon response in HBeAg(-), genotype D patients with CHB



Predictors of HBsAg Loss by Multivariate Analysis

Factors	OR	(95% CI)	*P* value
HBV DNA levels <6 log cp/mL	9.9	(2.5 - 38.7)	0.001
ALT levels >136 IU/mL	5.3	(1.5 -17.9)	0.007
IL28B genotype CC	3.9	(1.2 -12.4)	0.023

Lampertico P, et al. 61st AASLD; Boston, MA; October 29-November 2, 2010; Abst. 237.

Adapted from Simply Speaking HBV 2011

Treatment Options

Natural Clearance of HBeAg

- Occurs in 4% to 12% of carriers per year
- 40% to 50% of HBeAg+ carriers will clear HBeAg in 5 years
- 70% to 80% will clear HBeAg in 10 years
- Occurs more frequently in older carriers and those with elevated aminotransferase levels
- 20% of carriers who clear HBeAg will have one or more reversions to HBeAg+

Alaska HBV Carrier Study

McMahon Ann Int Med 2001;135:759-68

- **Population based cohort study: all ages, genders**
- **1536 HBV carriers tested semiannually**
- **F/u 19,430 person years (average 12.3)**
- **641 initially HBeAg-positive**
- **Probability of HBeAg clearance 73% by 10 years**

Clearance of HBsAg

- **Occurs in ~ 0.5% of carriers per year**
- **Occurs in 10% to 15% of carriers who clear HBeAg with interferon therapy**
- **No evidence HBsAg clearance occurs more frequently in carriers who clear HBeAg during Lamivudine therapy**

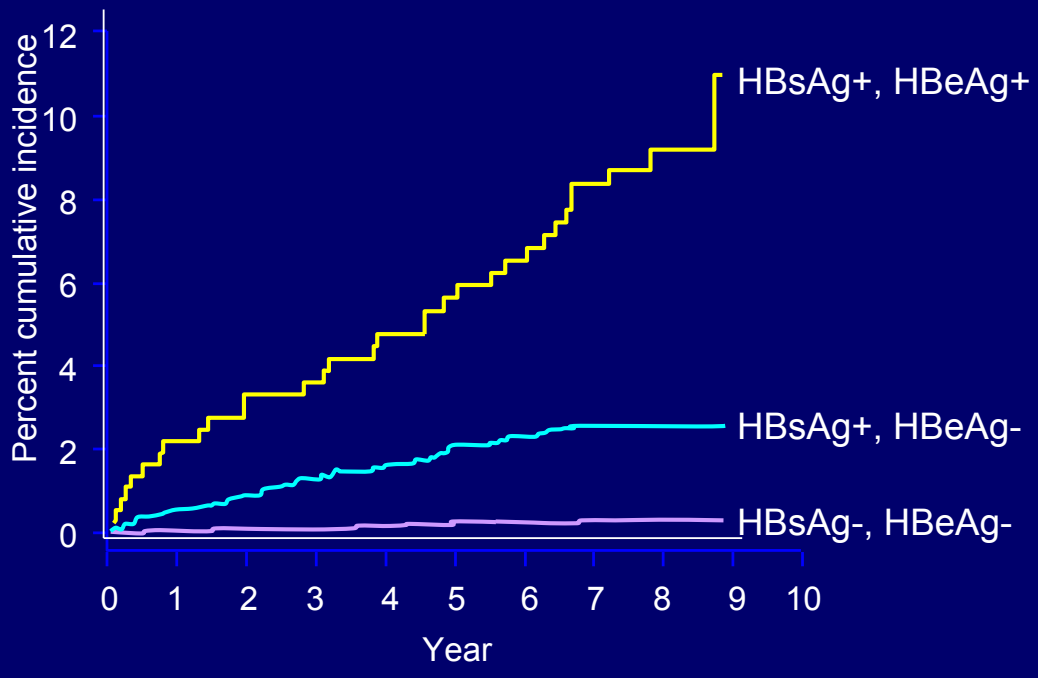
HBV Natural History

Progression to cirrhosis
and decompensation

REVEAL

HBeAg Status and HCC

Hepatitis B and Risk of HCC



Yang HI et al. *N Engl J Med*. 2002;347:168-174.