

capo'07

The Pathologists' Meeting™



CHICAGO

Education by the Experts



New Developments in Hepatitis B and C

D. Robert Dufour, M.D.
Consultant Pathologist
Attending Physician, Liver Clinic
VA Medical Center, Washington DC
Emeritus Professor of Pathology
GWU Medical Center

Disclosure

- I have no significant financial conflicts to disclose for this presentation

Agenda

- Testing for HCV – what's new
- Chronic HBV – what's changed since I learned about HBV
- Treatment for HCV and HBV – new agents, monitoring treatment

Significance

- Chronic HCV affects approximately 2% of US population (2.7 million individuals)
- Chronic HBV affects approximately 1 million US residents (85% born outside US)
- Both may lead to cirrhosis, hepatocellular carcinoma (HCC)

Significance

- Cirrhosis expected to increase 4-fold over next 30 years
- HCC incidence doubled over past 20 years, expected to increase further 3x
- Effective therapies are now available for both HBV, HCV
- Symptoms of chronic infection minimal, most unaware they have disease until complications develop

Brief Update on Hepatitis A

- With use of vaccine, has become rare in US (JAMA 2005;294:246)
- Major risk factors now injection drug use, males having sex with males
- IgM anti-HAV now largely (62%) false positive; CDC recommends use only in clinical setting of acute hepatitis (MMWR 2005;54:453)

Tests for HCV – What's New

Anti-HCV

- Antibody present in $> 99\%$ of HCV RNA positive individuals
- May be persistently negative in immunosuppressed, including those with HIV, renal failure, or on immunosuppressive drugs
- Rarely ($\sim 1/10,000$) has delayed appearance in immunocompetent

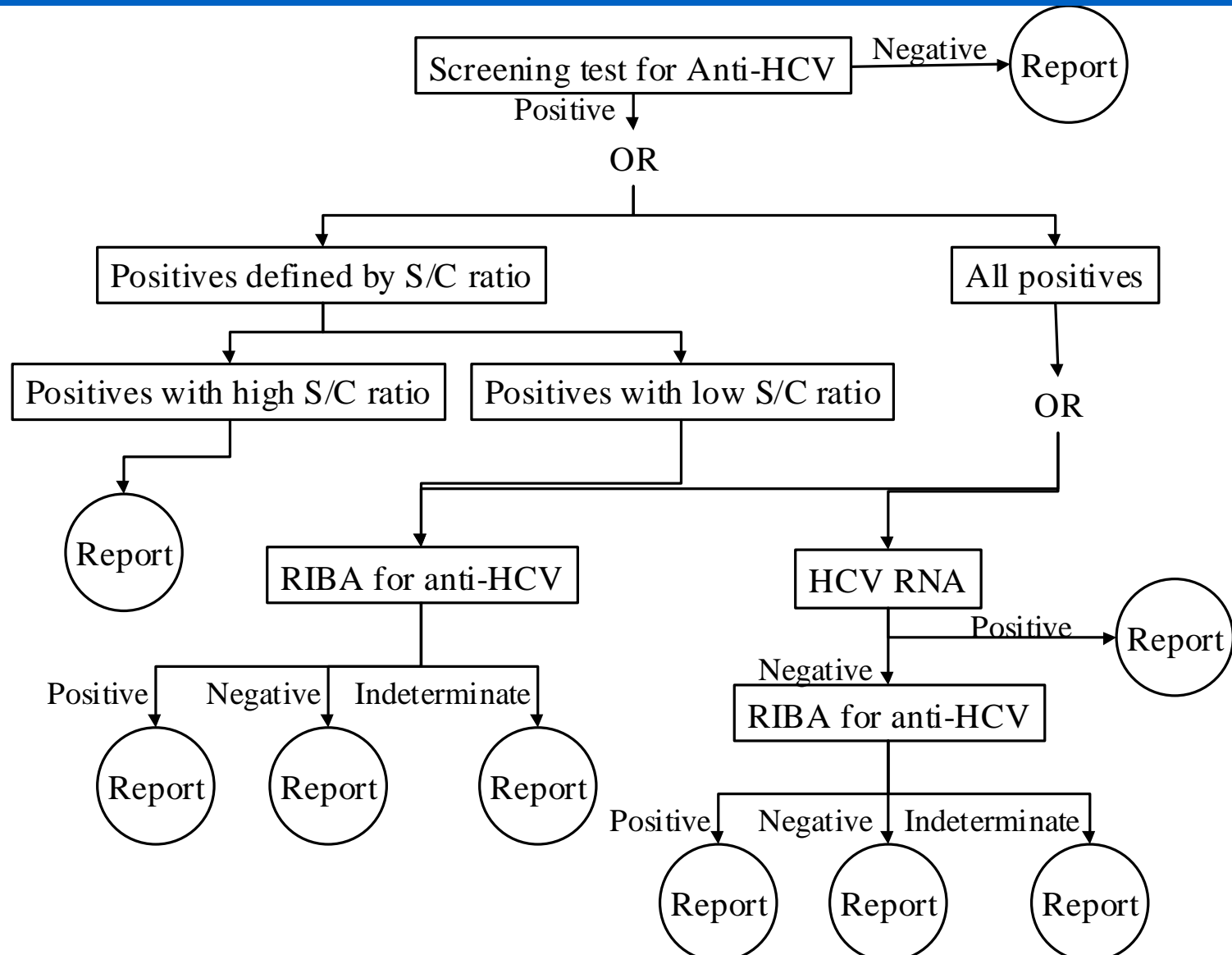
Anti-HCV

- Major screening test for HCV, detects antibody to ≥ 1 of 4 HCV antigens
- Two basic versions (2nd, 3rd generation) in use; 2nd sl less sensitive, not positive till avg 12 wk after exposure, 3rd sl less specific, pos. avg 9 wk.
- EIA tests less specific than CA, MEIA versions for same generation

Anti-HCV

- Most false positive are weakly positive
- Weak positive defined as ≤ 3.8 by EIA, ≤ 8.0 by Vitros, ≤ 10.0 by Architect/ImX
- Majority of weakly positive are negative on other anti-HCV assays or on confirmatory tests
- CDC recommends performance of RIBA on all weakly positive before reporting

CDC HCV + Algorithm



RIBA

- Uses same antigens as anti-HCV but separated on blot
- Positive - $\geq 1+$ antibody to 2 or more
- Negative - $\leq 1+$ antibody to all 4
- Indeterminate - $\geq 1+$ antibody to 1 or to more than one plus non-specific SOD
- In our experience, $< 0.5\%$ positive for SOD

HCV RNA

- Historically, qualitative (pos/neg), quantitative (viral load) used
- Until recently, detection limit of qual assays 1-2 log lower than quant
- Newer real-time PCR quant assays have detection limit \approx qual assays; in VA survey, 44% no longer offer qual RNA
- Used to diagnose active infection, monitor treatment

HCV RNA

Assay	Method	LDL IU/mL	UDL IU/mL	Kit?
		Quantitative		
Amplicor Monitor 2.0	PCR	600	6×10^5	Yes
Versant 3.0*	BDNA	615	8×10^6	Yes
Super Quant	PCR	33	2×10^6	No
Heptimax	TMA/PCR	5	5×10^7	No
QuantaSure	PCR	10	1×10^8	No
Celera	PCR	50	5×10^6	ASR
TaqMan	PCR	50	1×10^7	ASR
		Qualitative		
Amplicor*	PCR	50	N/A	Yes
Versant*	TMA	5	N/A	Yes

HCV RNA

- HCV RNA degraded by RNase in WBC
- Rapid (< 1 h) separation of serum from cells critical
- EDTA whole blood stable 24 h
- If separated, serum stable 3 d at RT, 7 d in refrigerator, indefinitely at - 70° C
- Most important factor in reliable HCV RNA results

HCV Genotype

- Six major genotypes (1-6); genotype 1 most common (70-75%) in North America, Europe, Japan
- While sub-types present (eg, 1a, 1b), not important distinctions
- Genotypes 2,3,4 respond much better to therapy
- Genotype 1 over 90% of infections in African-Americans

HCV Genotype

- Differences found throughout genome, most reliably in NS5b, 5'-UTR regions
- Line probe assay most widely used, direct sequencing also available using 5'-UTR region; sometimes fail to identify subtype
- Real time PCR available, direct sequencing being developed for NS5b, more reliable for subtype

Chronic HBV – What's Changed Since I First Learned About It?

Natural Course - HBV

- Course of HBV infection is quite variable; major factors include age at infection, immune status
- In healthy teens/adults, over 95% recover after exposure (acute hepatitis)
- In neonates, about 90% develop chronic infection
- In immunosuppressed, about 10-20% develop chronic infection

Natural Course - HBV

- Acute infection produces icteric hepatitis in 30-50% of adults, almost never in infants
- With recovery, significant viral replication ceases, but low level viral replication persists
- With immunosuppression, infection may reactivate; with immune reconstitution, stable disease may exacerbate

Natural Course - HBV

- Chronic HBV infection:
 - 1) Immune control - virus in liver, no immune damage, no circulating virus
 - 2) Immunotolerant phase - virus in liver and circulation, no immune damage
 - 3) Chronic hepatitis - virus in liver and circulation plus immune damage
 - 4) “Occult” hepatitis – negative HBsAg, but low level virus in liver, ? blood

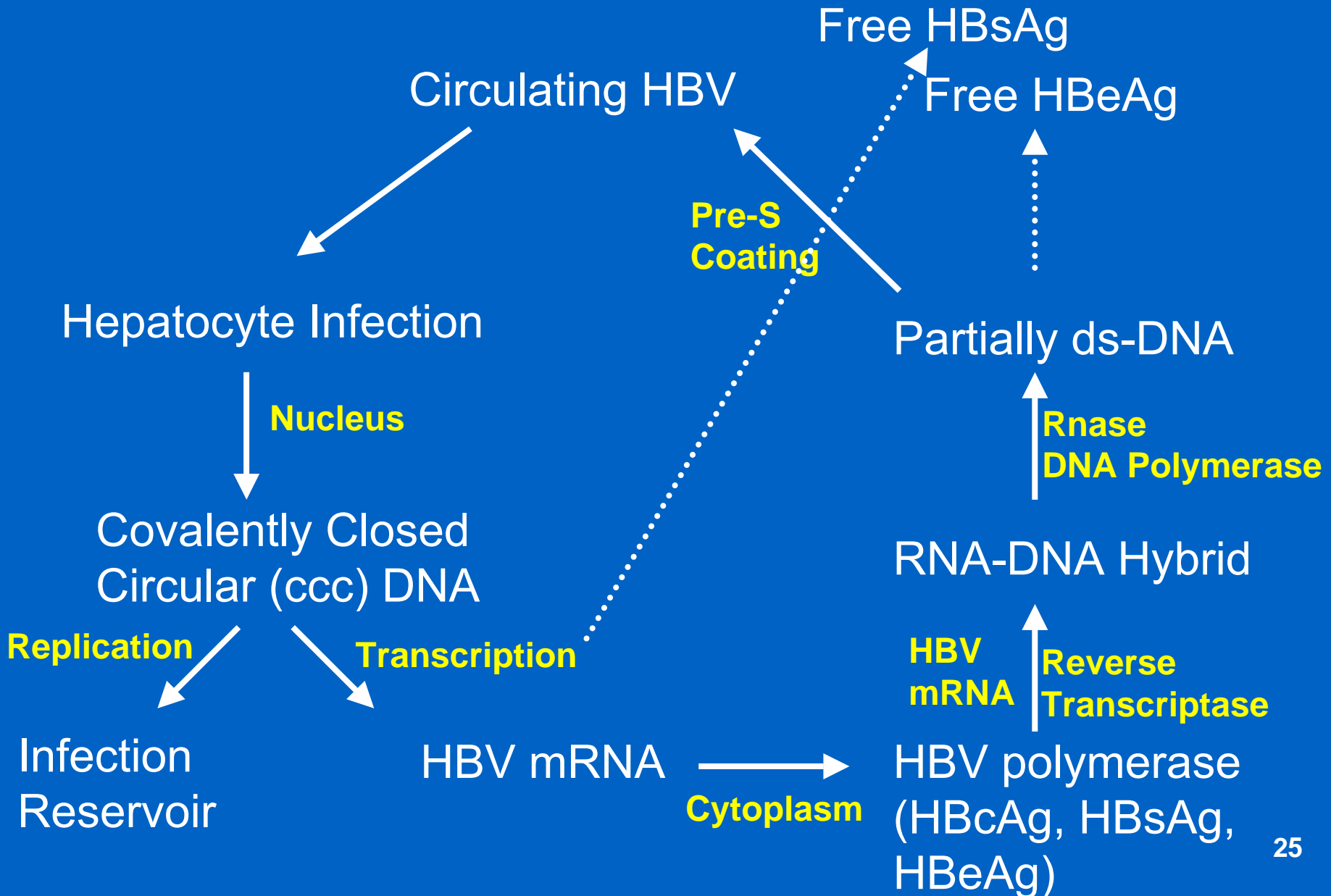
Natural Course - HBV

- Spontaneous conversion between states may be accompanied by acute flares of activity, mimicking acute hepatitis B
- Chronic hepatitis B progresses to cirrhosis in 20-30% of cases, higher with immunosuppression
- Chronic carrier state has lower risk but may lead to hepatocellular carcinoma
- Viral load predictive (risk \uparrow as $VL > 10^4$)

HBV Biology

- Partially double-stranded DNA virus, belongs to family hepadnaviridae
- Peculiar pattern of coding, replication unique among viruses
- Virus is not hepatotoxic; damage occurs from T-cell response to the virus
- Virus may be carcinogenic (? Related to HBV X antigen)

HBV Replication

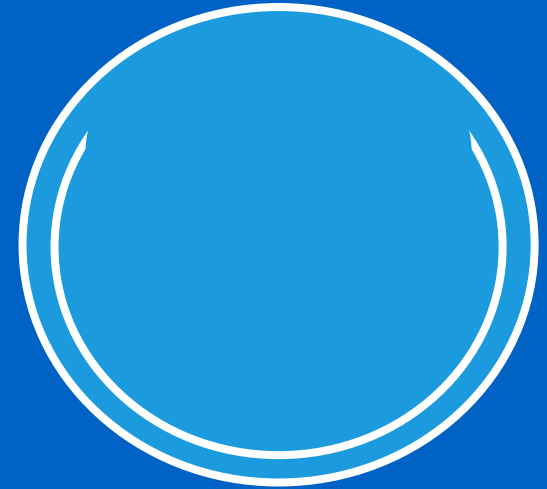


GENES OF HBV

X Ag

HBcAg

eAg



Polymerase

Pre-S

HBsAg

HBV Biology

- RNA replication leads to high rate of mutations (not as high as HIV, though)
- Certain sites of mutation more common - #1 is stop codon in HBeAg coding region (“pre-core”)
- Mutations commonly induced by reverse transcriptase inhibitors (lamivudine, famcyclovir; less common with adefovir, rare with entecavir)

HBV Mutations

- Resistance - currently, line probe assay to detect lamivudine, adefovir resistance available
- Pre-core mutants - Can be detected, but clinically diagnosed (HBeAg negative with high HBV DNA)
- HBsAg mutants - no commercial assays available to detect these rare mutant strains, but HBV DNA positive

HBsAg

- Responsible for genotypes of HBV; common “a” determinant in all genotypes
- Vaccine response mainly to “a” determinant
- Mutants in “a” determinant may not be recognized by vaccine, or by HBsAg serologic tests

Isolated Anti-HBc

- Early in viral clearance (“Core window” in acute hepatitis, during recovery)
- Many years following infection; especially common in HCV infected
- Failure to develop anti-HBs (?especially in immune deficient)
- False positive result (post-influenza vaccine, other states)
- False positive more common with EIA

HBeAg

- Produced along with viral particles, but not part of virus; not needed for replication, function uncertain
- If positive, correlates with presence of replicating virus (VL > 10^6); loss may indicate lack of (or lower level) viremia
- Not produced by pre-core mutants, may not be reliable in infection by genotypes other than A

Anti-HBe

- Appears with loss of HBeAg, indicating loss of circulating virus
- Formerly used to indicate transition to carrier state
- Also present if HBeAg lost due to development of pre-core mutant strains
- Probably persists for life

HBV Serologic Markers

Marker	Acute Infection	Occult Infection	Chronic Infection	Pre-core Mutant	Immune Control
HBsAg	+	-	+	+	+
HBeAg	+	-	+	-	-
HBV DNA	+	- ^b	+	+	-
Anti-HBc					
IgM	+	-	-/+ ^c	-	-
Total	+	+	+	+	+
Anti-HBe	-	+	-	+	+
Anti-HBs	-	+	-	-	-

^a+, detectable; -, not detectable; +/- may be detectable.

^bNon-PCR methods

^cMay be present with acute flares of activity

HBV DNA

- Assays have marked difference in detection limit; reported in pg/mL, copies/mL (1 pg = 285,000 copies)
- WHO standard now used for most assays (IU/mL), but correlation not linear (unresolved issue)
- Most with chronic hepatitis have $> 10^6$ IU/mL (often $> 10^9$); levels $< 10^2$ thought to have low transmission risk

HBV DNA

Lower Detection Limits for HBV DNA

Method	Detection Limit	
	copies/mL	pg/mL
Hybrid Capture	$3.0 * 10^6$	10.5
Branched DNA	$0.7 * 10^6$	2.5
Liquid Hybridization	$4.0 * 10^3$	0.02
Branched DNA (3.0)	$2.0 * 10^3$	0.01
Amplicor PCR	$2.0 * 10^2$	0.001

“Significant” Viremia

- Much of data is old, based on assays with high detection limits
- AASLD, EASL guidelines suggest 2×10^4 IU/mL “significant” (91% HBeAg +, but only 16% of HBeAg – have viral load above this level)
- With treatment, viral load $> 10^2$ usually leads to recurrence
- Many “immune” individuals have low viral loads ($< 10^2$); most HBsAg mutants have viral load $< 10^3$

TREATMENT OF CHRONIC HBV AND HCV

Chronic Hepatitis B

- Six agents available: interferon (std., pegylated), lamivudine, adefovir, entecavir, telbivudine
- Combination treatment not currently used
- Response rate low in those with normal ALT, or viral load $< 10^5$ IU/mL
- Successful treatment clears HBeAg, HBV DNA, and develops anti-HBe
- Rarely, HBsAg is also cleared

Chronic Hepatitis B

- Histologic improvement usually seen with clearance
- Relapses after treatment can occur
- Success rate with 1 yr Rx about 30-40%
- With lamivudine, increasing treatment to 3-4 yrs increases response to $\geq 70\%$, but resistant mutants also increase
- With adefovir, mutants less common (about 30% in long-term follow-up)

Chronic Hepatitis B

- HBV DNA should to decline to $< 10^5$ IU/mL, or fall by at least 2 logs
- Generally evaluated after 3, 6 months of treatment; failure to respond suggests low likelihood of response to further Rx
- Higher likelihood of long term effect if viral load $< 10^2$ copies/mL post-Rx; with oral agents, long term response much more likely if HBeAg -, anti-HBe + for 6 mo

Chronic Hepatitis C

- Current treatment of choice is pegylated interferon plus ribavirin
- Treatment for 24 wks with genotype 2 or 3, 48 wks for other genotypes
- Goal of treatment is clearance of virus that persists after therapy stopped
- Response rate is about 40% with genotype 1, 70% with genotypes 2, 3, 4

Chronic Hepatitis C

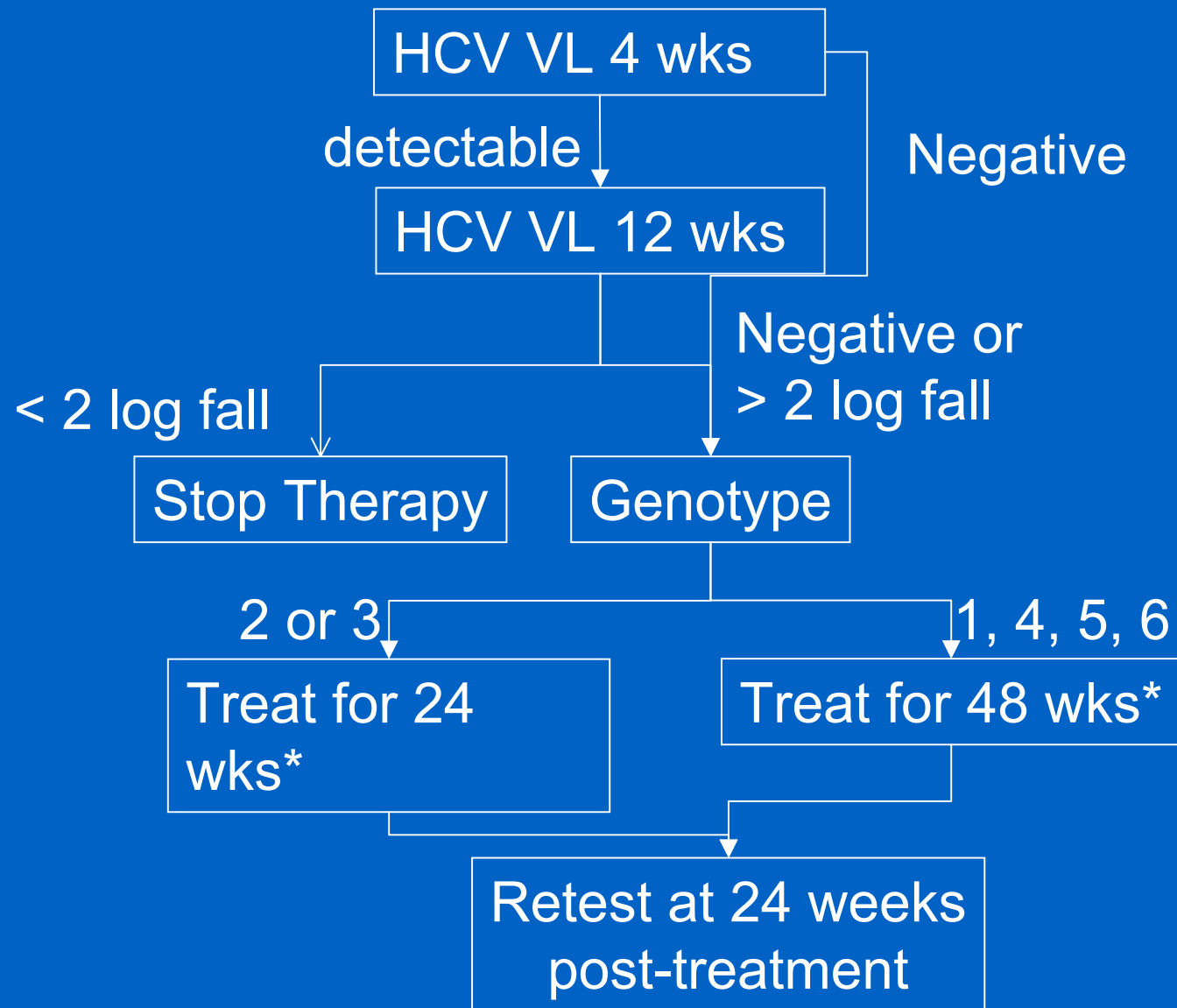
- Effectiveness monitored at 12 wk; failure to clear virus or fall by > 2 logs (early virologic response) indicates $< 5\%$ likelihood of success
- Success evaluated 6 mo post-Rx as absent HCV RNA by sensitive method (sustained virologic response, SVR)
- In those with SVR, likelihood of recurrent viremia $< 1\%$; however, virus persists in mononuclear cells in most

Chronic Hepatitis C

Mangia: N Engl J Med 2005;352:2609

- Defined rapid virologic response (RVR) as absent HCV RNA after 4 weeks of treatment with peg interferon/ribavirin
- Randomized genotype 2/3 patients with RVR to 12 versus 24 wk treatment
- No significant difference in SVR (76 vs 77%) with 12 versus 24 wk therapy
- Fewer complications with 12 wk, slightly more relapsers with 12 wk

Chronic Hepatitis C



Contraindications

- Interferon can precipitate liver failure in decompensated HBV or HCV cirrhosis
- Interferon may worsen autoimmune disease, uncontrolled HIV infection
- Ribavirin in renal failure, pregnancy
- Adefovir in renal failure
- HBV inhibitors - abrupt discontinuation may rarely precipitate acute hepatitis

Recent Guidelines

- HBV:
 - AASLD - Hepatology 2001;34:1225, updated Hepatology 2007;45:507
 - EASL - J Hepatol 2003;38:533
- HCV:
 - AASLD - Hepatology 2004;39:1147
 - NIH - Hepatology 2002;36 (Supplement 2)
 - CDC (on lab testing) MMWR 2003;52 RR-3
- General:
 - NACB - Clin Chem 2000;46:2027, 2050

Thank you for
participating!

Please complete the course
evaluation before you leave.

Education by the Experts

