

## The ABCs of Viral Hepatitis Diagnosis

Ila Singh, M.D., Ph.D.  
P & S 14-453  
is132@columbia.edu

## Viral Hepatitis

- Hepatotropic viruses
  - ◆ Hepatitis A, B, C, D, E and G viruses
- Generalized infection plus infection of liver
  - ◆ EBV, CMV and HSV

## Some basic serology...

- ◆ Presence of Viral Proteins/Nucleic acid (mostly called 'antigens')
  - ☞ Virus is present
  - ☞ Virus might be replicating
  
- ◆ Presence of antibodies to Viral proteins
  - ☞ Virus may be currently present (or not)
  - ☞ Could indicate either immunity or ongoing infection

## Hepatitis A infection

- Non-enveloped RNA virus
- Fecal-oral transmission
- Usually self-limited illness
- No carrier state
- In rare cases, fulminant hepatic necrosis

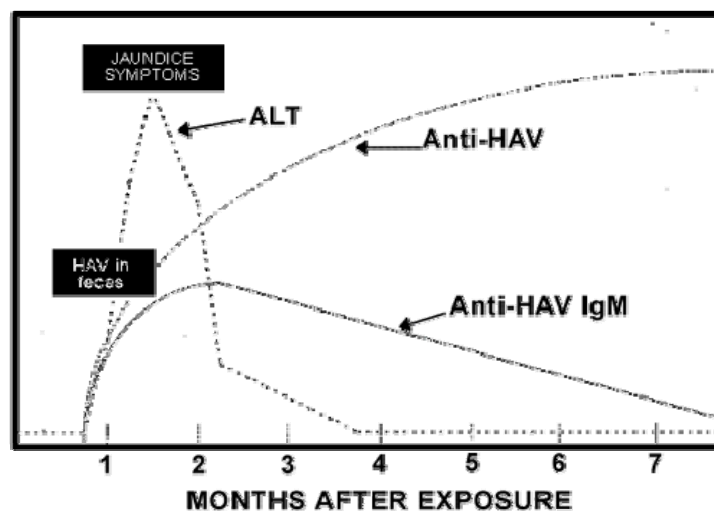
## Hepatitis A infection

200,000 cases/year in the US

~800,000 cases of HIV

cumulative through 2002 in the US

## Hepatitis A serology

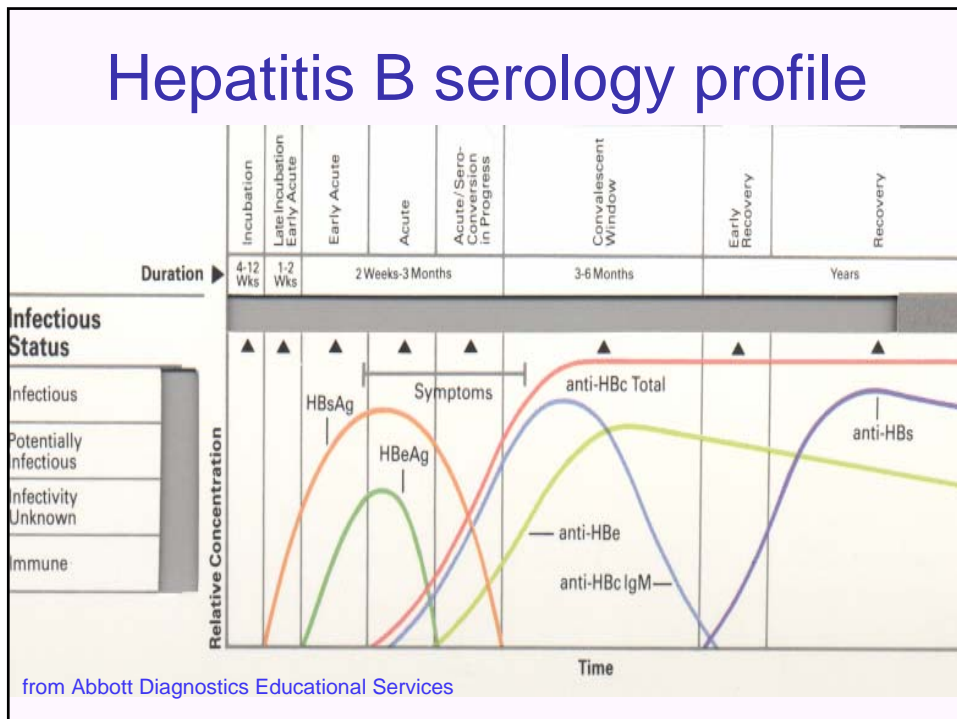
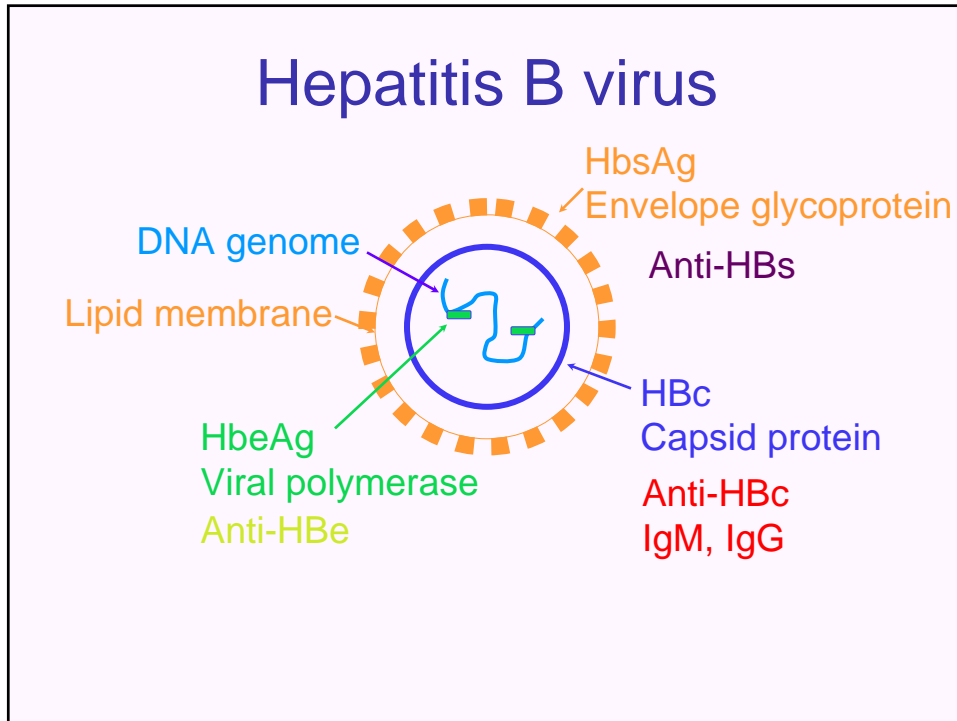


## Diagnosis of hepatitis A

- **IgM anti-HAV**: appears 4 wks after exposure and disappears by 3 -6 months. Indicates **acute** infection
- **IgG anti-HAV**: peaks during convalescence and persists for life. Indicates **exposure and immunity**

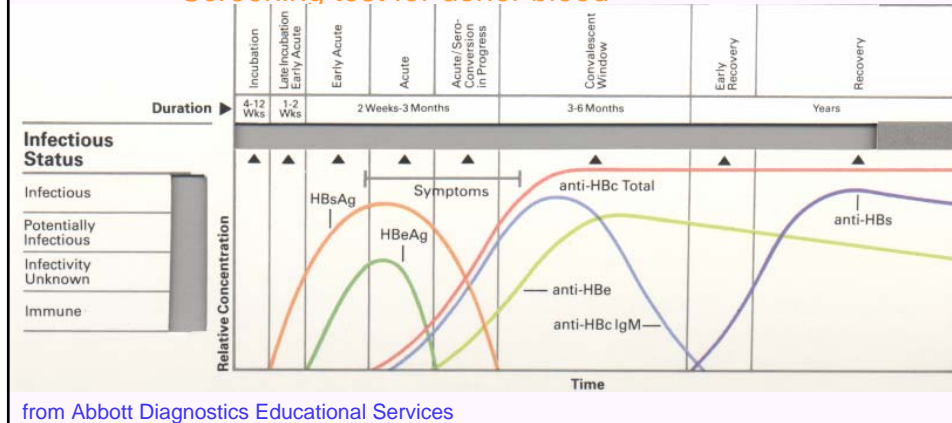
## Hepatitis B virus infection

- Transmission
  - ◆ Parenteral
  - ◆ Sexual
  - ◆ Vertical
- Clinical: incubation period of 1-6 mo.
  - ◆ 25% acute hepatitis, 1% fulminant hepatic necrosis
  - ◆ 10% chronic carriers: CAH, cirrhosis and hepatocellular carcinoma



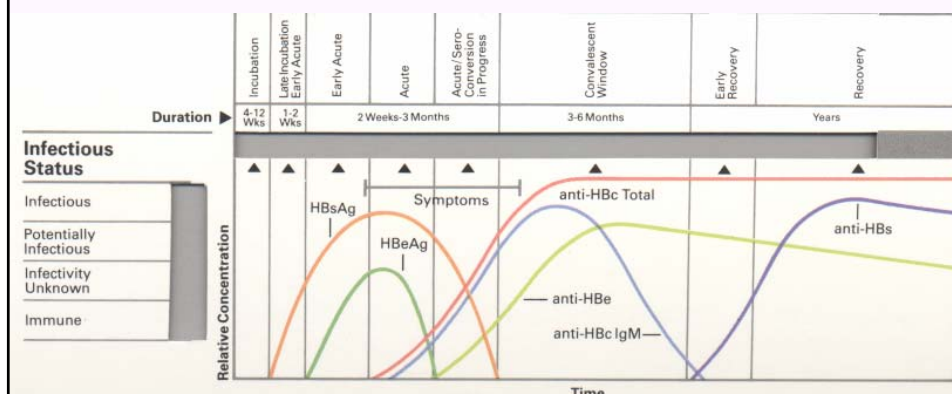
## HBsAg

- First specific marker
- Detectable during incubation, peaks in acute stage
- Declines upon recovery
- Elevated in carriers
- Screening test for donor blood



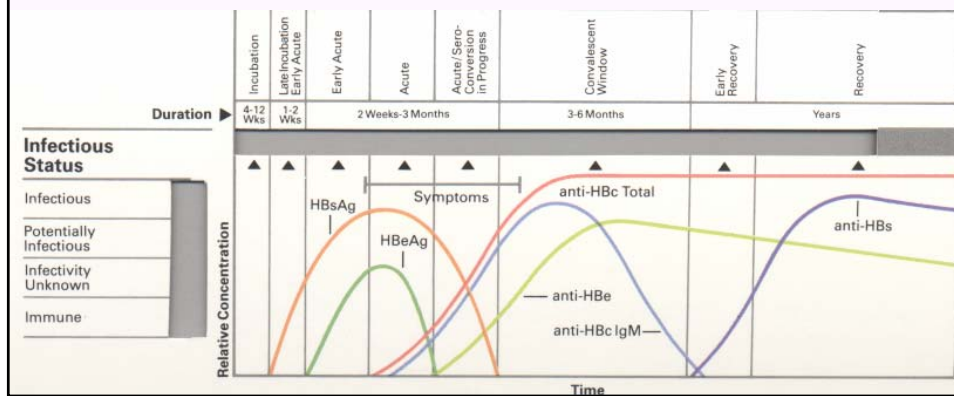
## HBeAg

- Appears shortly after HBsAg and parallels HBsAg
- Present during active replication of virus (most infectious phase)



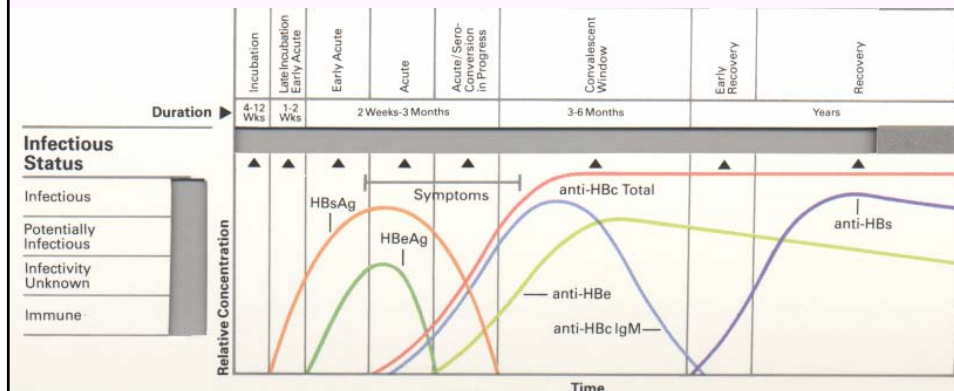
## Anti-HBc

- First detectable antibody
- IgM present in the interval between disappearance of HBsAg and appearance of Anti-HBs (core window)
- IgG produced during convalescence and persists for life



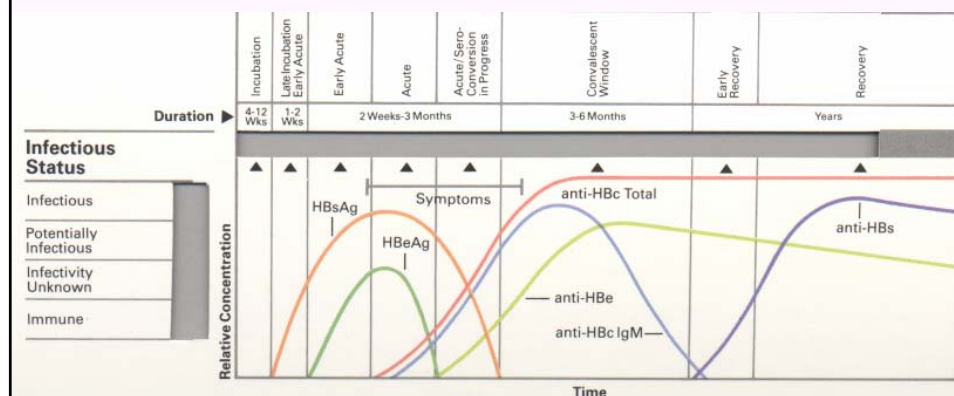
## Anti-HBe

- Appears after disappearance of HBeAg
- Indicates resolution



## Anti-HBs

- Appears during recovery and lasts for years
- Indicates immunity (also produced as a result of vaccination)



## Serum HBV DNA assay

- Assess candidacy for viral therapy. High pretreatment levels ( $> 200\text{pg/ml}$ , by liquid hybridization assay, Abbott) - less likely to respond to IFN-2 $\alpha$
- Clearance used as an endpoint in therapy -30-40% respond
- Rarely, to identify HBV as the etiology of liver disease in HBsAg negative patients, especially in patients with fulminant hepatitis B, who may have cleared HBsAg by the time they present or in patients with AIDS



## Markers for different phases of infection

HBsAg	HBeAg	IgM Anti-HBc	IgG Anti-HBc	Anti-HBs	Anti-HBe	HBV DNA	
+	+	+				+	Early
+	+		+			+	Chr, repl
		+				+	Window
+			+		+		Low, non-rep
+	+		+			+	Flare-up of chronic
+					+	+	Core mutants
			+	+	+		Recovery

## Genotyping Hep B

- Resistance to Lamivudine develops 1 year after therapy in 20% patients
- Resistance is associated with mutations in the catalytic domain of the HBV polymerase

## Hepatitis D infection

- Hepatitis D virus is an incomplete small RNA virus that needs HBV to survive
- Only occurs in the presence of HBV
- Test for D if suspicion that it might be a cause of disease exacerbation in chronic hepatitis B
- Can occur initially as a co-infection, where it runs the same course as hepatitis B
- Also treated with IFN-2 $\alpha$ .

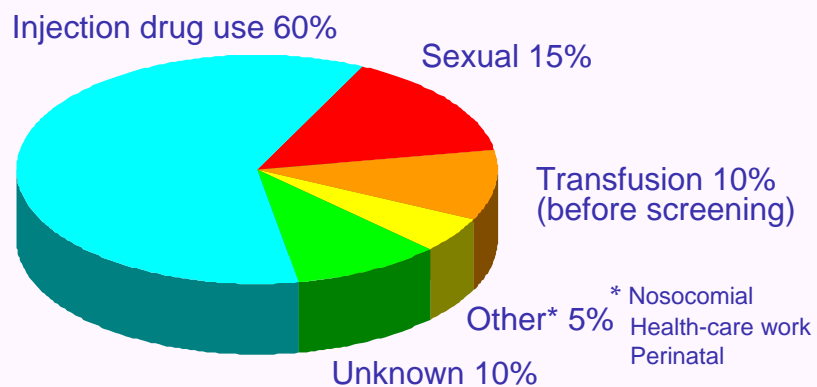
## Hepatitis D tests

- HDV Ag
  - ◆ Present only during prodrome, not tested for
- Anti-HDV IgM
  - ◆ Acute and chronic
- Anti-HDV IgG
  - ◆ Appear during convalescence
  - ◆ But remain elevated in carriers

## Hepatitis C infection

- Enveloped RNA virus
- Not possible to grow virus in culture
- 4 million people infected in the US (~2%)
- Parenteral infection, sexual transmission may play a small role
- 60-85% get chronic infection
- Treatment with interferon+ribavirin cures virus in only 25-40%

## Sources of infection for persons with newly-diagnosed Hepatitis C



CDC

## Who Should be Screened for Hepatitis C?

- History of IDU, even if remote and if only once
- History of receiving clotting factors prior to 1987
- History of blood transfusion or organ transplantation prior to July 1992
- History of percutaneous or mucosal exposure to HCV-infected blood
- Infants born to HCV-positive mothers
- Person with chronically elevated liver enzymes
- All HIV-infected persons

MMWR 1998;47:20-26, 1999 USPHS/IDSA Guidelines

## Other Potential Exposures to Blood

- No or insufficient data showing increased risk
  - ◆ Intranasal cocaine use, tattooing, body piercing, acupuncture, barbering, military service, foreign travel
- No association in acute case-control or population-based studies
  - ◆ Limited number of studies in highly selected groups (e.g., blood donors)
- Risk factor or high prevalence identified in selected subgroup cannot be extrapolated to the population
  - ◆ May be limited to certain settings and account for small fraction of cases, e.g., prisons, unregulated practitioners

## Risk of HCV

- Transmission to fetus ~4% if mother viremic
- C-section? Not recommended
- breast feeding No increased risk
- To sexual partner 0-0.6%/yr if monogamous, 1-2%/yr if multiple partners
- Blood Transfusion 1:103,000 per unit
- Accidental stick, HCV RNA+ patient? ~1.8%, greater for hollow-bore needle than other sharps

## HCV testing

- HCV Antibody Tests
  - ◆ EIA to detect
  - ◆ Antibodies to various recombinant HCV proteins
  - ◆ Present in acute and chronic stages and following recovery

## EIA

- Third generation EIA:
  - ◆ sensitivity > 99%, specificity = 99%, in immunocompetent patients
  - ◆ No need for confirmatory test in pts with clinical liver disease
    - ☞ False positives: autoimmune disorders
  - ◆ No need for further testing in case of negative EIA in immune-competent patients
    - ☞ False negatives: hemodialysis, immune-deficiencies

## HCV ?confirmatory? tests

- ALT
- RIBA (recombinant immunoblot assay)
- HCV RNA test

## ALT

- very variable in HCV infection
- Weak association between ALT levels and severity of histopathology
- Resolution of high levels is good indicator of response to therapy
- Pegylated IFN can cause ALT increase

## HCV RNA test-qualitative

- Used to confirm positive EIA
- Not necessary if evidence of liver disease and obvious risk factors for HCV
- Test should have a lower limit of detection of 50 IU/ml =100 viral genes/ml
- Specificity >98%

Single +ve: confirms infection,  
-ve: may just be below the level of detection.

## HCV RNA test-qualitative

- RT-PCR or Branched DNA
- Indications
  - ◆ Acute HCV, before antibodies made (+in 1 - 3 wks)
  - ◆ Chronic hepatitis with indeterminate serology
  - ◆ Chronic hepatitis and autoantibodies, with false positive serology
  - ◆ Persistent HCV replication after liver transplantation, when antibodies persist

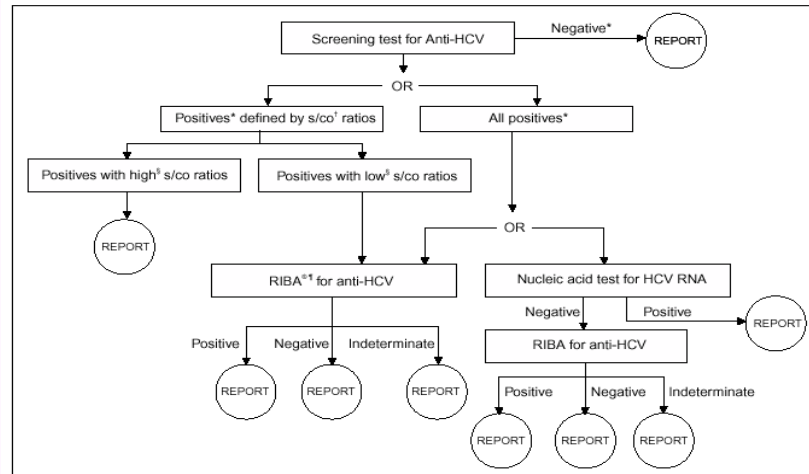
## RIBA

- No liver symptoms, +ve EIA
  - ◆ negative test implies false +ve EIA
- +ve EIA, but -ve HCV RNA
- Problem: Presence of antibody does not indicate if the virus is replicating
- Advantage?
  - Can be ordered on the same sample as the original EIA



## Diagnostic Algorithm for HCV

FIGURE 4. Laboratory algorithm for antibody to hepatitis C virus (anti-HCV) testing and result reporting



\* Interpretation of screening immunoassay test results based on criteria provided by the manufacturer.

† Signal-to-cut-off.

‡ Screening-test-positive results are classified as having high s/co ratios if their ratios are at or above a predetermined value that predicts a supplemental-test-positive result  $\geq 95\%$  of the time among all populations tested; screening-test-positive results are classified as having low s/co ratios if their ratios are below this value.

\* Recombinant immunoblot assay.

## Needlestick exposure

- Risk estimated as 2%
- Source and exposed individual be tested for HCV by EIA
- If source EIA positive, then exposed individual tested for
  - ◆ RNA
  - ◆ Ab
  - ◆ ALT at time zero, 2 weeks and 8 weeks after injury
  - ◆ No post-exposure prophylaxis recommended
  - ◆ Recommend seroconverted people to experts

## HCV RNA test-quantitative

- Treatment of patients with chronic HCV disease
  - ◆ HCV RNA levels do NOT correlate with disease activity
  - ◆ Pretreatment levels less than  $2 \times 10^6$  RNA copies/ml serum- more likely to have sustained response
  - ◆ Change in viral load in the first four weeks following therapy- good predictor
  - ◆ Loss or reduction - primary indicator of response to therapy
  - ◆ Significant variability among tests - Use the SAME test for serial monitoring

## SVR - sustained viral response

- Absence of detectable HCV RNA in the serum as shown by a QUALITATIVE HCV RNA test 24 weeks after end of treatment
- Test should have a lower limit of detection of 50 IU/ml

## EVR - early viral response

- Minimum 2 log decrease in viral load during first 12 weeks of treatment
- Predictive of SVR
- Should be a routine part of monitoring therapy in genotype 1 patients

## HCV Genotypes

- Genetic heterogeneity among different HCV isolates within a population. Genotypes vary by 31-35% of nucleotides over the entire length of the genome.
- Six genotypes identified
- Subtypes (a or b) vary by ~ 20%
- Association between mode of transmission and genotype: type 3 more prevalent in iv drug users

## HCV Genotypes in the US

- >70% are genotype 1a or 1b,
- Genotype 1 has a higher rate of chronic disease, more severe disease, lower response to treatment and ? higher rates of carcinoma

## HCV Quasispecies

- Refers to genetic heterogeneity of the HCV population within an individual.
  - ↳ Vary by 1-9% of nucleotides.

## Role of Liver biopsy

- Gold standard for assessing the severity of liver disease --> *prognosis*
  - Determines amount of inflammation and fibrosis
  - Serves as guide to determine *urgency* of initiating therapy
- Histology helps predict the likelihood of response to therapy.
  - Lower rates of response in patients with fibrosis/cirrhosis
- R/O alternative or co-existing conditions
  - e.g. alcohol, NASH, iron overload

## Non-invasive markers of fibrosis

TGF - $\beta$

Matrix metalloproteinases, etc

Using microarray technology to determine which genes are up-regulated - look for their products in the serum - correlate with biopsy

## Hepatocellular carcinoma screening

AFP and ultrasound every six months

DID NOT increase HCC identification!

No better option.

Certainly should not be done in absence of cirrhosis because HCC extremely rare

## HIV screening

■ HIV  $\rightleftharpoons$  HCV

## Should Everyone be *Considered* for Antiviral Treatment?

### Yes

- Treatment reduces the pool of infected individuals
- Treatment stabilizes disease and reduces risk of HCC (perhaps improves survival)
- Reduce need for liver transplantation

### No

- Slowly progressive disease
- Not all infected persons will develop serious complications of disease
- Available treatments are expensive, associated with side effects, and not uniformly effective

## HCV - Treatment

- Treatment should be selective ?
- Not all patients need to be treated (at least in short-term)
  - Patients with mild disease and minimal fibrosis may choose to await more efficacious, less toxic therapies
- Current therapies are highly effective in some patients - notably those with HCV genotype 2 or 3 infection
- For patients with genotype 1, response rates are lower (<50%) and new therapies are needed

## Additional References

NIH Consensus Final Statement on Management of  
Hepatitis C *Sept. 12, 2002*

[www.consensus.nih.gov/cons/116/116cdc\\_intro.htm](http://www.consensus.nih.gov/cons/116/116cdc_intro.htm)

### CDC MMWR

Guidelines for the Management of Occupational Exposures  
to HBV, HCV and HIV and Recommendations for Post-  
Exposure Prophylaxis.

[www.cdc.gov/mmwr/preview/mmwrhtml/rr5011a1.htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5011a1.htm)

## Hepatitis E infection

- RNA virus
- Present in animals without causing disease (60% of urban US rats have HEV)
- Human HEV infection rare in the US. Endemic in many countries.
- Fulminant hepatic necrosis in pregnant women (case fatality rate is 10-50%)
- IgM antibodies to HEV, HEV RNA assay



## Hepatitis G virus

- Hepatitis G virus or GBV-C is closely related to HCV
- Common in HCV infected patients
- Mode of transmission: ?parenteral ?sexual
- Role in human disease is controversial. Usually mild acute or chronic hepatitis.
- May *delay* progression of HIV disease (Sep 6, 2001, NEJM)

## Approach to diagnosis of viral hepatitis

- **Answer 3 key questions**
- Does the patient have hepatitis infection NOW?
- What kind of infection?
- Does the patient need treatment?