The ABCs of Viral Hepatitis Diagnosis

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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

ALT

Anti-HAV

Anti-HAV IgM

MONTHS AFTER EXPOSURE
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

Hepatitis B virus

- DNA genome
- Envelope glycoprotein
- Lipid membrane
- HbsAg
- HBeAg
- Viral polymerase
- HBc
- Capsid protein
- Anti-HBc
- IgM, IgG
- Anti-HBs
- Anti-HBc

Hepatitis B serology profile

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

HBsAg

- Duration
Laboratory Diagnosis of Viral Hepatitis
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Clinical Pathology/Lab Medicine 2002
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-2α

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

- Injection drug use 60%
- Sexual 15%
- Transfusion 10% (before screening)
- Other* 5%
- Unknown 10%

* Nosocomial, health-care work, perinatal

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

Clinical Pathology/Lab Medicine 2002
### 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

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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 x 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-β
- 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 = 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)
  - Reduces need for liver transplantation
- **No**
  - Slowly progressive disease
  - Not all infected persons will develop serious complications
  - Treatments are expensive, associated with side effects, and not uniformly effective
  - Wait for better drugs
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

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

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?