**QUESTION 23**

A 42-year-old Asian man has chronic hepatitis B diagnosed four years previously. He presents with the blood test results below. His alanine transaminase (ALT) levels have previously been normal and he has been consistently hepatitis B surface antigen (HBsAg) positive and hepatitis B e antigen (HBeAg) negative. He has not been taking any prescription or over-the-counter medications. He has not travelled internationally for six years.

His current blood test results are as follows:

- Bilirubin 15 μmol/L [3-21]
- Alanine transaminase (ALT) 346 U/L [5-40]
- Aspartate transaminase (AST) 154 U/L [5-40]
- Alkaline phosphatase (ALP) 76 U/L [30-115]
- Gamma glutamyltransferase (GGT) 101 U/L [<65]
- Albumin 40 g/L [38-50]
- HBsAg positive
- HBeAg negative
- Hepatitis B virus (HBV) DNA 3.5 pg/mL [<0.5]
- Hepatitis C virus (HCV) antibody negative
- Ferritin 450 μg/L [25-200]

The most likely explanation for the current blood test results is:

A. hepatitis D superinfection.
B. pre-core mutant disease.
C. hepatitis C co-infection.
D. hepatitis E co-infection.
E. haemochromatosis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbsAg Anti-HBc Anti-HBs</td>
<td>Negative Negative Negative</td>
<td>Susceptible – discuss vaccination</td>
</tr>
<tr>
<td>HbsAg Anti-HBc Anti-HBs</td>
<td>Negative Positive Negative</td>
<td>Immune due to infection</td>
</tr>
<tr>
<td>HbsAg Anti-HBc Anti-HBs</td>
<td>Negative Positive Positive</td>
<td>Immune due to hep b vaccination</td>
</tr>
<tr>
<td>HbsAg Anti-HBc IgM anti-HBc Anti-HBs</td>
<td>Positive Positive Positive negative</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>HbsAg Anti-HBc IgM anti-HBc Anti-HBs</td>
<td>Positive Positive Negative Negative</td>
<td>Chronic carrier</td>
</tr>
<tr>
<td>HbsAg Anti-HBc Anti-HBs</td>
<td>Negative Positive Negative</td>
<td>5 possibilities&lt;br&gt;May be recovering from acute HBV infection&lt;br&gt;May be distantly immune and test not sensitive enough to detect very low level of anti-HBs in serum&lt;br&gt;May be susceptible with a false positive anti-HBc&lt;br&gt;May be undetectable level of HbsAg present in the serum and the person is actually a carrier&lt;br&gt;Maternal antibody</td>
</tr>
</tbody>
</table>

Hepatitis B e antigen and antibody — Hepatitis B e antigen (HBeAg) is a secretory protein that is processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. The presence of HBeAg is usually associated with high levels of HBV DNA in serum and higher rates of transmission of HBV infection from carrier mothers to their babies and from patients to health care workers.
This information therefore confirms a chronic hepatitis B carrier only with not active hep B infection
A. hepatitis D superinfection.
DIAGNOSIS OF HDV INFECTION — Due to the dependence of HDV on HBV, the presence of HBsAg is necessary for the diagnosis of HDV infection. The additional presence of IgM antibody to hepatitis B core antigen (IgM anti-HBc) is necessary for the diagnosis of acute HBV/HDV coinfection
This is incorrect

B. pre-core mutant disease.
Answer by exclusion
Chronic hepatitis due to pre-core hepatitis B virus (HBV) mutants presents as hepatitis B e antigen (HBeAg)-negative chronic hepatitis B (CHB). HBeAg-negative CHB represents a late phase in the natural course of chronic HBV infection that develops after HBeAg loss and seroconversion to anti-HBe. It is usually associated with pre-core stop codon mutation at nucleotide 1896 (mainly selected in non-A HBV genotypes), but also with other pre-core changes or with mutations in the basic core promoter region (mainly in HBV genotype A). In chronic HBV infections, pre-core mutants can be detected both in patients with HBeAg-negative CHB and in inactive hepatitis B surface antigen (HBsAg) carriers. The diagnosis of HBeAg-negative CHB is based on HBsAg positivity, HBeAg negativity, and mainly on increased alanine aminotransferase (ALT) and serum HBV-DNA levels and exclusion of other causes of liver disease. The differential diagnosis between patients with CHB and inactive HBsAg carriers can be made only by close follow-up of aminotransferase activity and viraemia levels, although the cut-off level of serum HBV DNA has not been definitely determined. IgM anti-HBc levels have also been suggested as an index that increases the diagnostic accuracy for transient hepatitis flares, while liver biopsy confirms the diagnosis and evaluates the severity of the liver disease

C. hepatitis C co-infection.
Hep C negative – this is incorrect

D. hepatitis E co-infection.
Not consistent clinical history (endemic area) and no evidence for hep E infection – usually associated with hep A,

E. haemochromatosis.
In 2002 paper two question 46 Jo tells us that the diagnosis of haemochromatosis requires
Test: relatives of index case and symptomatic (eg liver disease, diabetes, heart failure)

Serum ferritin
Fasting Transferrin saturation (TS) > 45% (>90% sensitive)
TS = (serum Fe/2) / (serum TF x100)

HFE gene testing (specific)
Liver biopsy if older, ferritin > 1000 +/- abnormal LFTs or HFE testing non-diagnostic

Hepatic iron index (HII) = hepatic iron concentration (HIC) / age
Usually HII > 1.9 and HIC > 80 microm/g
Incorrect – not enough information for this diagnosis
DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes. HBsAg contain envelope glycoproteins and host-derived lipids outnumber virions by 1000:1. Replication of the DNA genome is by reverse transcription of an RNA intermediate.

The Replication Cycle of HBV.

HBV virions bind to surface receptors and are internalized. Viral core particles migrate to the hepatocyte nucleus, where their genomes are repaired to form a covalently closed circular DNA (cccDNA) that is the template for viral messenger RNA (mRNA) transcription. The viral mRNA that results is translated in the cytoplasm to produce the viral surface, core, polymerase, and X proteins. There, progeny viral capsids assemble, incorporating genomic viral RNA (RNA packaging). This RNA is reverse-transcribed into viral DNA. The resulting cores can either bud into the endoplasmic reticulum to be enveloped and exported from the cell or recycle their genomes into the nucleus for conversion to cccDNA. The small, peach-colored sphere inside the core particle is the viral DNA polymerase.

Pathogenesis of HBV

- The HBV replication cycle is not directly cytotoxic to cells. This fact accords well with the observation that many HBV carriers are asymptomatic and have minimal liver injury, despite extensive and ongoing intrahepatic replication of the virus. It is now thought that host immune responses to viral antigens displayed on infected hepatocytes are the principal determinants of hepatocellular injury.
Cellular Immune Responses to HBV.
HBV replicates in hepatocytes to produce HBsAg particles and virions. Both types of particle can be taken up by antigen-presenting cells, which degrade the viral proteins to peptides that are then presented on the cell surface bound to MHC class I or II molecules. (Antigen-presenting cells can also process and display viral antigens taken up by phagocytosis of killed infected hepatocytes.) These peptide antigens can be recognized by CD8+ or CD4+ T cells, respectively, which are thereby sensitized. Virus-specific CD8+ cytotoxic T cells (with help from CD4+ T cells, green arrow) can recognize viral antigens presented on MHC class I chains on infected hepatocytes. This recognition reaction can lead to either direct lysis of the infected hepatocyte or the release of interferon alpha and TNF – alpha, which can down-regulate viral replication in surrounding hepatocytes without direct cell killing.

Natural History
- Primary HBV infection in susceptible (nonimmune) hosts can be either symptomatic or asymptomatic
- Most primary infections in adults, whether symptomatic or not, are self-limited, with clearance of virus from blood and liver and development of lasting immunity to reinfection
- Less than 5% - persistant infection
- Asymptomatic chronic HBV carriers
  - Subclinical persistent infection
  - Normal serum aminotransferase level
  - Normal or nearly normal findings on liver biopsy
- Chronic hepatitis B
  - Abnormal LFTs
  - Histologic features
- Cirrhosis with severe liver injury develops in about 20% of people with chronic hep B

Primary Infection
- HBsAg detectable in blood after an incubation period of 4-10 weeks
- Followed shortly by antibodies against the HBV core antigen (anti-HBc antibodies), which early in the infection are mainly of the IgM isotope
- Viremia well established by the time HBsAg is detected very high titers of virus $10^9$ virions per millilitre
- HBeAg detectable in most cases
- High rates of both vertical and horizontal transmissibility during acute HBV infection

Persistant Infection
- Mostly low levels persistent viremia
- HBsAg detectable for life in most cases
Year 2003 Paper two: Questions supplied by Tricia

- Tendency over time for HBeAg to disappear from blood
- Tendency over time for seroconversion to positivity for anti-HBe antibodies 5-10% per year in persistently infected people
- Evidence of ongoing immune mediated destruction of infected hepatocytes

**Hepatocellular Carcinoma**
- Risk
  - 100 times as high as noncarriers
  - Twice yearly screening of chronically infected patients with measurements of serum alpha fetoprotein +/- ultrasound
  - Problems AFP excellent negative predictive value, but PPV 9-30%

**Therapy**
- **Goals**
  - Reduction level of viremia
  - Amelioration of hepatic dysfunction
- **Treatment**
  - Clear indication for therapy in HBeAg - positive patients – increased risk of early progression to chronic active hepatitis and cirrhosis, higher risk of hepatocellular carcinoma
  - Asymptomatic HBeAg-negative chronic carriers with viral loads below 10 to 5 per millilitre and normal LFTs usually relatively stable course with low rates of progression – therapy currently not offered
  - Usually markers of successful therapy
    - Loss of HBeAg
    - Seroconversion to anti-HBe antibodies
    - Reduction of the circulating viral load
    - True cure – loss of HBsAg and complete disappearance of viremia measured by PCR) is achieved infrequently 1-5%
- **Interferon**
  - Previously mainstay of therapy
  - 30% response
  - Problems: SE – fever, myalgias, thrombocytopenia and depression make it difficult to continue in many patients
  - Many patients undergo a flare of liver injury during administration of interferon alfa, often just before or during clearance of HBeAg
  - Contraindicated in very advanced liver disease – may precipitate overt liver failure and patients with advanced cirrhosis and splenomegaly usually have base-line leukopenia and thrombocytopenia which may be exacerbated
- **Antiviral drugs**
  - Lamivudine
    - Directly blocks replication of HBV genome
    - Nucleoside or nucleotide analogues selectively targets the viral reverse transcriptase
    - Usually 3 to 4 log reduction in circulating levels of HBV DNA in 1st 3 months of therapy
    - Well tolerated
    - Not immunomodulatory and can be used in patients with decompensated cirrhosis
    - Limitation = development of drug resistance, which is mediated largely by point mutations at the YMDD motif at the catalytic center of the viral reverse transcriptase
  - Other nucleotide analogues
    - Adefovir – nucleotide (adenosine monophosphate) analogue prodrug undergoes two intracellular phosphorylations to yield the active drug / nephrotoxic in high doses (required for HIV) lower doses effective in HBV
    - Tenofovir
- **Liver transplantation**
  - 80% recurrent viral infection in association with immunosuppressive drugs
  - Post transplantation prophylaxis of both hepatitis B immune globulin and lamivudine reduces rate of reinfection to about 10% and boosted 5 year rate of HBV-free survival to 80%
  - Prob - expense
Year 2003 Paper two: Questions supplied by Tricia