QUESTION 93

A 16yo adolescent male presents with lethargy and lower respiratory tract infection. Physical examination shows him to be febrile, icteric and pale in addition to respiratory findings.

Full blood examination shows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>67g/L</td>
<td>[128-175]</td>
</tr>
<tr>
<td>Mean cell volume</td>
<td>86fL</td>
<td>[80-97]</td>
</tr>
<tr>
<td>White cell count</td>
<td>13.0 x 10^9/L</td>
<td>[3.9-12.7]</td>
</tr>
</tbody>
</table>

**Differential:**

- Neutrophils: 10.2 x 10^9/L [1.9-8.0]
- Lymphocytes: 0.8 x 10^9/L [0.9-3.3]
- Monocytes: 1.6 x 10^9/L [0.3-1.1]
- Eosinophils: 0.3 x 10^9/L [0-0.5]
- Basophils: 0.1 x 10^9/L [0-0.1]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>390 x 10^9/L</td>
<td>[150-396]</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>213 x 10^9/L</td>
<td>[9-116]</td>
</tr>
</tbody>
</table>

The blood film is shown below.

The most likely cause of the anaemia is:

A. Paroxysmal cold haemoglobinuria  
B. Glucose-6-phosphate dehydrogenase (G6PD) deficiency  
C. Red cell aplasia  
D. Cold agglutinin haemolysis  
E. Sickle cell anaemia

The raised reticulocyte count and jaundice suggest haemolytic anaemia – can immediately remove red cell aplasia from the list. Sickle cell anaemia is diagnosed in early childhood (and there are no sickle cells on film anyway).
Table 93–2. Red Blood Cell Morphology in the Diagnosis of Hemolytic Anemia (Harrison’s)

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Cause</th>
<th>Syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherocytes</td>
<td>Loss of membrane</td>
<td>Hereditary spherocytosis, immunohemolytic anemia</td>
</tr>
<tr>
<td>Target cells</td>
<td>Increased ratio of RBC surface area to volume</td>
<td>Hemoglobin disorders: thalassemias, hemoglobin S, C, etc.; liver disease</td>
</tr>
<tr>
<td>Schistocytes</td>
<td>Traumatic disruption of membrane</td>
<td>Microangiopathy, intravascular prostheses</td>
</tr>
<tr>
<td>Sickled cells</td>
<td>Polymerization of hemoglobin S</td>
<td>Sickle cell syndromes</td>
</tr>
<tr>
<td>Acanthocytes</td>
<td>?Abnormal membrane lipids</td>
<td>Severe liver disease (spur cell anemia)</td>
</tr>
<tr>
<td>Agglutinated cells</td>
<td>Presence of IgM antibody</td>
<td>Cold agglutinin disease</td>
</tr>
<tr>
<td>Heinz bodies</td>
<td>Precipitated hemoglobin</td>
<td>Unstable hemoglobin, oxidant stress</td>
</tr>
</tbody>
</table>

HAEMOLYSIS

- 3 categories:
  - Molecular defect inside RBC (enzymopathies or haemoglobinopathies)
  - Abnormal membrane structure and function
  - Environmental factor (trauma, autoantibody)
- Hereditary or acquired
- Hereditary haemolytic anaemias due to membrane abnormalities:
  - Hereditary spherocytosis
  - Hereditary elliptocytosis
  - Hereditary stomatocytosis
- Hereditary haemolytic anaemias due to RBC enzyme defects:
  - Defects in Embden-Meyerhof pathway
  - Defects in hexose-monophosphate shunt including G6PD deficiency
- Hereditary Haemoglobinopathies:
  - Structural (HbS, altered O2 affinity, unstable Hbs)
  - Thalassaemia
  - Thalassaemia haemoglobin variants
  - Hereditary persistence of fetal haemoglobin
  - Acquired haemoglobinopathies (eg: toxic exposure, carboxyhaemoglobin)
- Acquired Haemolytic Anaemias:
  - Entrapment (hypersplenism)
  - Immune
    - Warm-reactive (IgG) antibody
    - Cold-reactive IgM antibody (cold agglutinin disease)
    - Cold-reactive IgG antibody (paroxysmal cold haemoglobinuria)
    - Drug-dependent antibody
  - Traumatic
    - Impact haemolysis
    - Macrovascular – prostheses
    - Microvascular – TTP/HUS, others
  - Toxic effects on membrane
    - Spur cell anaemia
    - External toxins (eg: spider bites, metals)
    - Paroxysmal nocturnal haemoglobinuria
G6PD DEFICIENCY

Epidemiology
- X-linked inherited disorder
- Primarily men affected but homozygous women found in some populations
- Carrier women can also have haemolytic attacks

Pathophysiology
- G6PD enzyme catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate while concomitantly reducing NADP+ to NADPH
- NADPH is a required cofactor in many reactions and maintains glutathione in its reduced form
- Red cells rely on G6PD as it is the only source of NADPH
- Reduced glutathione acts as a scavenger for oxidative metabolites within the cells and converts hydrogen peroxide to water
- In G6PD deficiency the red cells are at risk of oxidative stress and rapid haemolysis can occur (eg: when exposed to oxidative drugs)

Clinical Manifestations
- Variable
- Most often asymptomatic
- Can present with neonatal jaundice or acute haemolytic anaemia
- Haemolytic anaemia can be triggered by oxidative drugs, infection or ingestion of fava beans
- Gallstones common
- Splenomegaly may be present

Diagnosis
- G6PD enzyme activity
- Usual signs of haemolysis during an acute attack
- Associated with Heinz bodies which are denatured haemoglobin

Treatment
- Avoid precipitants including antimalarials, nitrofurantoin, ciprofloxacin, norfloxacin, vitamin K, sulfamethoxazole, mothballs

SICKLE CELL ANAEMIA
- Mutant haemoglobin
- Most common form in Western world is Hb S
- Arises from mutation substituting thymine for adenine → valine instead of glutamine in Hb beta chain
- Resulting Hb forms polymers under deoxy conditions and has changes in solubility and molecular stability
- Under deoxy conditions, Hb S has decreased solubility, increased viscosity and forms polymers
- After recurrent episodes of sickling the membranes are damaged and cannot reoxygenate (irreversibly sicked cells)
- Cardinal signs:
  - Haemolytic anaemia
  - Painful vasoocclusive crises
Multiple organ damage with microinfarcts

- Sickle cells cause vasoconstriction and microthrombi for a number of reasons:
  - Express very late antigen (VLA)-4 which adheres to vascular cell adhesion molecules (VCAM)-1
  - VCAM-1 upregulated by hypoxia and inhibited by NO (which is also a vasodilator)
  - Hypoxia inhibits NO and free Hb scavenges NO
  - Deformed sickle cells express CD18 which adheres to the endothelium
  - Sickle cells also adhere to macrophages
  - Sickle cells have increased IgG which plays a role in vasoocclusive crises by activating clotting factors (triggered by infection)

Clinical Features

- Common in black population
- Presents in childhood with chronic haemolytic anaemia and painful vasoocclusive crises
- Infants largely protected in first 6 months of life by HbF
- Crises can be triggered by infection, change in temperature or there may be no precipitant
- Severe deep pain in extremities
- Severe abdominal pain (resembling acute abdomen)
- May be accompanied by fever, malaise and leukocytosis
- Can last hours to days
- Anaemia is universal
- Aplastic crisis can occur with parvovirus B19 infection – infects RBC progenitor cells in BM → cessation of erythropoiesis
- Splenomegaly → infarcts → fibrosis → autosplenectomy → risk of infection with encapsulated organisms
- Hand-foot syndrome = dactylitis = swelling of dorsum of hands and feet, cortical thinning and destruction of metacarpal and metatarsal bones
- Acute chest syndrome = chest pain, tachypnoea, leukocytosis, pulmonary infiltrates (medical emergency)
- CNS involvement – stroke
- Haemosiderin deposits in myocardium, dilated ventricles, microinfarcts
- Gallstones
- Bone and joint infarctions → stunted growth, aseptic necrosis, chronic pain
- Microinfarcts of lungs, pulmonary HT (?2’ low NO)
- Loss of concentrating ability of kidneys
- Retinal vascular changes, proliferative retinopathy
- Leg ulcers
- Priapism
- High rates of fetal loss

Diagnosis

- Sickle cells on peripheral blood smear
- Hb electrophoresis

Treatment

- Manage anaemia (balanced diet, Fe tablets if deficient, transfusions only in certain situations eg: acute chest syndrome, stroke)
- Manage pain (opioids)
- Avoid infections (vaccinations especially pneumococcal)
- Prevent and treat complications (eg: stroke)
- Acute vasooclusive crisis managed with IVT and analgesia, supportive care
- ?hydroxyurea
- ?BM transplant

**PAROXYSMAL COLD HAEMAGLOBINURIA**

- Due to cold-reacting IgG antibody
- Does not cause much agglutination
- Readily fixes complement
- Antigen is P antigen on red cells which is present on RBCs of almost all people
- Occurs primarily in 2 situations:
  - In children 7-10 days following a viral infection or following other infections including Mycoplasma pneumonia and Klebsiella pneumonia
  - In adults PCH can occasionally occur in association with other autoimmune abnormalities and rarely in lymphomas or CLL – usually chronic, lasting several years
- As blood circulates to the peripheries and cools the antibody and complement are fixed to the RBC surface
- Complement cascade is completed when the RBCs are subsequently warmed to 37 degrees
- Results in lysis by complement, intravascular haemolysis, haemoglobinuria and haemosiderinuria

**Clinical Manifestations**

- Cardinal features are haemolysis with dark urine (haemoglobinuria) beginning a few minutes to several hours after exposure to cold
- Constitutional symptoms, back pain, leg pain, abdominal cramps, chills and fever can occur
- Raynaud phenomenon and urticaria can also occur
- Haemolysis does not persist once cold exposure ended
- Spherocytes, erythrophagocytosis by neutrophils can be seen on film
- Degree of anaemia variable, can be severe in childhood, usually moderate in adults
- Coombs’ test positive during haemolytic episode but may be negative between crises

**Diagnosis**

- IgG antibody that reacts with red cells at reduced temperature but not at 37 degrees
- Radiolabeled monoclonal anti-IgG is more sensitive

**Treatment**

- Treatment of acute attack in children is supportive
- Warm environment
- For chronic condition – prednisolone, or if this fails, cyclophosphamide or azathioprine
- Avoid cold environments

**COLD AGGLUTININ HAEMOLYSIS**

**Pathogenesis**

- IgM antibodies generally react with polysaccharide antigens on the RBC surface at lower temperatures
- IgG antibodies generally react at body temperature (and are therefore associated with warm agglutination)
- A single IgM can bind 2 RBCs together which can result in agglutination
- When blood circulates to colder parts of the body interaction occurs between the cell and antibody leading to complement activation and haemolysis
- Amount of haemolysis varies depending on the amount of antibody in the plasma, the thermal amplitude, the degree of inhibition of antibody binding by C3d (present on red cell surfaces) and the degree of complement fixation
- Antigens are polysaccharides present on all red cells
- Antibodies are produced in response to either infection or paraneoplastic or neoplastic growth of a single immunocyte clone
- Occur commonly with mycoplasma pneumonia and infectious mononucleosis, less commonly with viral infections
- Peak titre usually occurs 2-3 weeks after onset of infection with haemolysis more likely to occur at this time
- Antibodies due to paraneoplastic causes, however, remain in circulation over long periods → chronic cold agglutinin disease

**Clinical Features**

- Anaemia
- Blue peripheries
- Haemolysis
- Splenomegaly
- Look for underlying lymphoma

**Laboratory Findings**

- Anaemia
- Haemolysis
- Coomb’s positive
- Cold agglutinins

**Treatment**

- Avoid cold
- Cyclophosphamide, steroids
- Rituximab (?)
- Plasmapheresis

The answer is B – G6PD deficiency but I don’t know why it couldn’t be cold agglutinin or paroxysmal cold haemoglobinuria associated with mycoplasma infection. Perhaps because the haemolysis tends to occur a week or two after the infection with cold haemolysis disorders?