Diagnosis and Clinical Complications

David Sullivan, MD
Classical Malaria

- Fever
- Splenomegaly
- Anemia

Hippocrates, 5th Century BC
Comparison of Malaria Fever Curves

Adapted from Thayer and Hewetson
Johns Hopkins Hosp Reports V 1895 p. 3-224

“Tertian” P. vivax

“Quartan” P. malariae

“Aestivo-autumnal “Quotidian” P. falciparum
Diagnosis Based on Clinical Features

Advantages
- Cheap
- Fast

Disadvantages
- Lack of precision
- Over-treatment

CDC/ Dr. Lyle Conrad
Axial temperature is not a good indicator of malaria infection in children under holoendemic conditions, as often less than 10% of infections are associated with fever.
Inaccuracies of Clinical Diagnosis

- Malaria is difficult to diagnose clinically
- In studies > 70% of +ve diagnoses are non-parasitemic
- Beware statistics based on clinical reports
Diagnosis Based on Microscopy

**Advantages**
- Gold standard
- Quantitative
- Useful for other diseases

**Disadvantages**
- Time consuming
- Relies upon good microscopes, reagents, and trained technicians
Useful Web Sites for Training in Blood Film Analysis

This site is presented by the Division of Laboratory Medicine at Royal Perth Hospital.
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• Mr. Graham Icke MSc CBiol FIBiol FIBMS Grad Dip Bus

CDC site
http://www.dpd.cdc.gov/dpdx/HTML/Malaria.asp?body=Frames/M-R/Malaria/body_Malariadiagfind2.htm
Fig. 1: Normal red cell
Figs. 2-18: Trophozoites (among these, Figs. 2-10 correspond to ring-stage trophozoites)
Figs. 19-26: Schizonts (Fig. 26 is a ruptured schizont)
Figs. 27 & 28: Mature macrogametocytes
Figs. 29& 30: Mature microgametocytes (male).


P. falciparum Thick film
Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (rings)
Figs. 6-13: Trophozoites
Figs. 14-22: Schizonts
Fig. 23: Developing gametocyte
Fig. 24: Macrogametocyte (female)
Fig. 25: Microgametocyte (male)

Illustration from: Coatney GR, Collins WE, Warren M, Contacos PG.
P. malariae Thick film

Fig. 1: Normal red cell
Figs. 2-5: Young trophozoites (Rings)
Figs. 6-15: Trophozoites
Figs. 16-23: Schizonts
Fig. 24: Macrogametocytes (female)
Fig. 25: Microgametocyte (male)

P. ovale Thick film

Ken Hobson
Fig. 1: Normal red cell
Figs. 2-6: Young trophozoites (ring stage parasites)
Figs. 7-18: Trophozoites
Figs. 19-27: Schizonts
Figs. 28 and 29: Macrogametocytes (female)
Fig. 30: Microgametocyte (male)


P. vivax Thick Film
# Distinguishing Blood Film Characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>P. falciparum</em></th>
<th><em>P. vivax</em></th>
<th><em>P. ovale</em></th>
<th><em>P. malariae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell size</td>
<td>Normal</td>
<td>Large</td>
<td>Large</td>
<td>Normal</td>
</tr>
<tr>
<td>Merozoites in schizont</td>
<td>Up to 32</td>
<td>Up to 16</td>
<td>Up to 8</td>
<td>Up to 8</td>
</tr>
<tr>
<td>Rings</td>
<td>Fine, delicate</td>
<td>Large, irregular</td>
<td>Large, irregular</td>
<td>Square or band appearance</td>
</tr>
<tr>
<td></td>
<td>double chromatin dots and applique forms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC cytoplasm</td>
<td>Maurer's dots</td>
<td>Schuffner's dots</td>
<td>Schuffner's dots</td>
<td></td>
</tr>
<tr>
<td>Gametocytes</td>
<td>Sickle or banana shape</td>
<td>Round</td>
<td>Round</td>
<td>Round</td>
</tr>
<tr>
<td>Special</td>
<td>Trophozoite and schizonts rare</td>
<td>Amoeboid trophozoites</td>
<td>Comet/oval rbc. Only in Africa</td>
<td>Band form and daisy schizonts</td>
</tr>
</tbody>
</table>
**P. falciparum films**

- **Ring**
- **Schizonts**
- **Gametocyte**

- **Ring**
- **Schizonts**
- **Gametocyte**

- **Ring**
- **Ring**
- **Gametocyte**

- **Ring**
- **Trophozoite**
- **Gametocyte**

- **CDC**
P. vivax Films

- Ring
- Trophozoite
- gametocytes
- Schizonts
- CDC
P. ovale Films

Ring

Ring

Schizonts

Schizonts

gametocytes

gametocytes

Trophozoite

CDC
P. malariae Films

Ring forms

Mature trophozoites (band forms)

Schizonts

Basket trophozoite

gametocytes

CDC
Rapid Diagnostic Tests

Advantages
• Sensitive
• Fast
• Simple to perform
• No need for special equipment or electricity

Disadvantages
• HRPII:
  – Not suitable for non Pf species
  – Remains positive for 2 weeks after treatment
• Not quantitative
• Expensive (US$0.60-2.50 per test)
Why Target HRP II for Detection?

- Multiple His-Ala repeating regions for antibody epitopes
- Present in infected RBC cytoplasm and parasite digestive vacuole
- Secreted in plasma
- Directly related to parasitemia, parasite biomass, and parasite developmental stage
- Aldolase and lactate dehydrogenase enzymes
- Abundant production by parasites
- Aldolase has over 90% identity at amino acid level
- LDH has less and enables species specific monoclonal antibodies
- LDH is basis for Optimal test
- Aldolase is in ICT test as non HRP II band
- Both aldolase and LDH have short half life and go away within 1-2 days of treatment
- HRP II can linger for more than a week
Result

- Other than *P. falciparum*
- *P. falciparum*
- *P. falciparum/mixed*
- Negative

Control aldolase

HRP II
RDTs in Africa?
Current situation

Problems
• Asymptomatic parasitaemia
• Expense

Special situations
• Complex emergencies
• Malaria epidemics
• Low transmission settings
• Military
• Travellers
RDTs in Africa
Future options

• Changing cost-benefit
  – Rising drug costs

• Possible uses
  – Confirmation of treatment failure (pLDH)
  – Severe disease in peripheral settings

• BUT…
  – Will RDT diagnosis change clinical practice?

• Need for operational studies
Malaria Rapid Diagnostic Tests

WHO site: http://www.wpro.who.int/sites/rdt

Contains

• Explanation of RDT
• Use of RDT
• Guidelines on purchasing an RDT including an important table that compares good manufacturing practice on known suppliers
• Collections of published reviews and trials
• Collection of publications and committee documents
• Useful links pertaining to malaria diagnosis
http://www.wpro.who.int/sites/rdt/links.htm
Clinical Complications of Malaria

**P. Falciparum**
- Cerebral coma
- Anemia
- Pulmonary edema
- Shock
- Lactic acidosis
- Hypoglycemia
- Tropical splenomegaly
- Pregnancy
  - Maternal Death
  - Stillbirth
  - Low birth weight
  - Anemia

**P. vivax (P. ovale)**
- Splenic rupture
- Anemia (mild)
- Debilitating fevers
- Higher TNF-alpha per parasite

**P. malariae**
- Immune complex
- Glomurulonephritis leading to nephrotic syndrome
Cerebral Malaria

Adherent parasites release cytokines. In one study 94% of persons with cerebral malaria had adherent parasites compared with 13% of those without change in mental status. Steroids have no effect on mortality, no increase in vascular permeability is observed, anaerobic glycolysis in brain tissue predominates.
Cerebral Malaria: Signs and Symptoms

• About 90% become comatose before dying
• Gradual impairment or coma following seizure
• Extensor posturing
• Immobile or tossing about

**Neurologic Sequelae**

• Uncommon in adults or non-immunes
• Common in African children
  – Psychosis
  – Extrapyramidal tremor
  – Cranial nerve lesions
  – Polyneuropathy
  – Mononeuritis multiplex
  – Guillain-Barre syndrome
  – Focal epilepsy
Modified Glasgow Scale

**Best Verbal Response**
- Oriented: 5
- Confused: 4
- Inappropriate Words: 3
- Incomprehensible sounds: 2
- None: 1

**Best Motor Response**
- Obeys commands: 6
- Localizes pain: 5
- Flexion to pain:
  - Withdrawal: 4
  - Abnormal: 3
- Extension to pain: 2
- None: 1
Blantyre Scale

Eye Movements
- Directed: 1
- Not Directed: 0

Verbal Response
- Appropriate cry: 2
- Moan or inappropriate cry: 1
- None

Best Motor Response
- Localizes painful stimulus: 2
- Withdraws limb from pain: 1
- Non-specific or absent response: 0

Total = 0-5
Unrousable coma <2
Severe Anemia

- Not only red blood cell destruction, but also decreased production
- Due to iron deficiency and ineffective erythropoiesis, Rouleaux formation of unininfected erythrocytes increases spleen destruction
- Peak incidence in African children from holoendemic areas between ages of 6 months and 2 years
- Associations with secondary bacterial infections
- Transfusion is life saving
Pathogenesis of Severe Anemia

- Degree of anemia corresponds to duration and severity of parasitemia.
- Parasitemia does not predict risk of death in severe anemia and in Kenya over half of children with severe anemia had less than 10,000 parasites per ul.
- Treated uncomplicated *P. falciparum* malaria will decrease the hematocrit by one seventh.
- Severe anemia kills as hemoglobin falls below 5g/dl
- Mortality rises with tissue hypoxia and metabolic acidosis.
- Another infection can tip to catabolic metabolism.
Placental Malaria

Unstable epidemiology
  Maternal death, abortion, stillbirth, premature delivery, low birthweight
Stable (Holoendemic) epidemiology
  Clinical symptoms and parasitemia is higher in primigravida
  Low birthweight
Non-immunes
  Higher mortality
  Progressive anemia
  Quinine induced hypoglycemia

Primigravida women expose chondroitin sulfate A on placenta endothelial cells to which a new population of *P. falciparum* parasites predominates and causes microvascular sequestration in the placenta, disrupting its function.
**Vertical transmission**

Congenital
- Parasitemic neonate within 7 days of birth

Blood transfusion

*P. malariae*

**Pulmonary edema**

May develop at any stage of disease

Iatrogenic (presents as patient recovering)

Increased RR, dyspnea, crepitations are first clinical signs

ARDS with normal right heart pressures

CXR
- Bronchopneumonia
- Metabolic acidosis
- ARDS
Tropical Splenomegaly Syndrome

• Also known as Hyperreactive malarial splenomegaly
• Progressive, massive, splenic enlargement
• 80% of some areas of PNG
• Past medical history of repeated attacks of fever or malaria
Tropical Splenomegalgy Syndrome

- Abdominal distention, vague dragging sensation, sharp abdominal pains
- Peritonism suggesting perisplenitis
- Cachexia
- Lower leg ulcerations
- NC/NC Anemia with hemolytic episodes
- Very low or undetectable parasitemia
Tropical Splenomegaly Syndrome

• Untreated morality rate is high
• Death due to overwhelming pulmonary or skin infections
• Definition:
  – Gross splenomegaly
  – Elevated IgM (polyclonal)
  – Clinical and immunological response to anti-malarial prophylaxis
Why Is *P. falciparum* So Dangerous?

- Ability to infect all age of RBCs
- Higher multiplication capacity
- Sequestration (cytoadherance and rosetting)
- Capillary leak syndromes
- End organ failure
The Numbers

- 70 kg person has @ 5 liters of blood = $5 \times 10^3$ ml = $5 \times 10^6$ µL times $5 \times 10^6$ RBCs per µL of blood = $2.5 \times 10^{13}$ RBCs
- 1% parasitemia = 1 in 100 iRBCs = $2.5 \times 10^{11}$ parasites = 250 billion parasites
- *P. vivax* invades predominately reticulocytes and so has a built-in ceiling, but *P. falciparum* can invade all ages of RBCs.
- Pyrogenic density *P. falciparum* 10,000/uL nonimmune; 100,000/uL immune; *P. vivax* 100/uL