Infectious Disease Definitions

- **Infection** – when a microorganism invades a host and multiplies enough to disrupt normal function by causing signs and symptoms
- **Pathogenicity** – ability of an organism to cause disease
- **Incubation period** – time immediately before the onset of acute disease (1 to 2 days)
- **Acute phase** – most severe signs and symptoms of disease occur
- **Convalescence phase** – signs and symptoms are receding and person is returning to normal health
- **Convalescent titers** - antibody level drawn weeks after symptoms appear

Infectious Diseases

- Infectious Mononucleosis (IM)
- Hepatitis
- Rubella
- Cytomegalovirus (CMV)
- Human Immunodeficiency Virus (HIV)

EBV and Infectious Mononucleosis
Etiology of Infectious Mononucleosis (IM)

- Caused by the DNA Epstein Barr virus (EBV)
- Part of the herpes virus group
- Disorder is an acute, benign, and self-limiting lymphoproliferative condition
- Once infected, a lifelong carrier state develops whereby a low grade infection is kept in check by the immune defenses.
- EBV infections complications can involve:
  - Cardiac, ocular, respiratory, hematologic, digestive, renal and neurologic system

Epidemiology of IM

- Reactive (atypical, variant) lymphs seen in peripheral blood smear are T Lymphs
- Viral genome persists in B lymph and epithelial cells of oropharynx
- Transmitted primarily by contact with oral-pharyngeal secretions - salvia (kissing disease)
- After primary exposure a person is considered to be immune

Signs and Symptoms of IM

- Seroconvert without any significant clinical signs and symptoms of disease
- In children under 5, infection is either asymptomatic or frequently characterized by mild, poorly defined signs and symptoms
- Incubation period 10 – 50 days
- Extreme fatigue, sore throat, malaise, fever, cervical lymphadenopathy
**Signs and Symptoms of IM**

- Splenomegaly 50% of cases
- Jaundice is infrequent, although the most common complication is hepatitis
  - Abnormal liver function test: elevated liver enzymes (AST, ALT, possibly GGT, elevated bilirubin)
- Significant number of cases do not manifest classic signs and symptoms: most common sign – fatigue.

**Diseases associated with Epstein-Barr Virus (EBV)**

- **Burkitt’s lymphoma**: a malignant neoplasm of B lymphs
  - Found in children or immunocompromised
- **Nasopharyngeal carcinoma**: squamous cell carcinoma is caused by EBV and found mainly in southern China
- Other assoc.: Neoplasms of thymus, parotid gland, and supraglottic larynx
- Neurologic syndromes include: Bell’s palsy, Guillain-Barre syndrome, meningocerebralitis, Reye’s syndrome, myelitis, cranial nerve neuritis, and psychotic disorders
- **Infectious mononucleosis**
  - 95% of the world’s population is exposed to the virus, which makes it the most ubiquitous virus known to humans.

**Serological Tests for IM**

- Heterophile Antibody Assay:
  - Nonspecific agglutination assay
  - Screening tests:
    - Paul-Bunnell screening test
    - Davidson Differential Assay
- EBV-Specific Antibody Assay:
  - Identification of Ab produced against specific EBV antigens.
**Immunologic Manifestations**

- EBV induces the production of heterophile antibodies

- **Heterophile antibodies**: antibodies that are stimulated by one antigen and react with an entirely unrelated surface antigen present on cells from different mammalian species
  - Present in normal individuals in low titer (<56)
  - Such causes are due to febrile agglutinins

**Immunologic Manifestations**

- **Three** types of heterophile antibodies distinguished in IM testing:
  - Antibodies against Forssman antigens.
  - Serum sickness Antibodies
  - IgM Heterophile Antibodies to Infectious mononucleosis (EBV) antigens

**Immunologic Manifestations**

- **1. Forssman antigen**: In 1911, Forssman revealed that emulsions of guinea pig organs injected into rabbits provoked the formation of antibodies that lysed sheep RBCs in the presence of complement – **Forssman antigen**
  - found on RBCs of: horse, sheep, dog, cat, mouse, fowl, guinea pig, some bacteria
  - Absent from: humans, monkeys, rabbits, rats, ducks, cows

- Forssman heterophile antibodies can cross react in experiments testing for IM causing False Positive IM results.
Immunologic Manifestations

- 2. Serum sickness
  - Hypersensitivity reaction following a single, large injection of serum from an animal of another species
    - Historically observed after administration of antitoxin containing foreign serum such as antitetanus or antipertussis serum which were made from horse.
    - The immune system recognizes the horse serum as foreign and is activated to produce antibodies against it.
  - Heterophile antibodies result from sensitization to animal serum (usually horse)
  - Serum Sickness heterophile antibodies can cross react in experiments testing for IM causing False Positive IM results.

- 3. IM (IgM) heterophile antibody: After being infected with EBV, the immune system is activated to produce IgM IM heterophile antibodies against the virus.
  - IM antibodies are characterized by the following features:
    - Reacts with horse, ox and sheep RBCs = agglutination
    - Is absorbed by beef RBCs = No agglutination
    - NOT absorbed by guinea pig kidney cells (agglutinates with)
    - Does NOT react with EBV-specific antigens (no agglutination)

Paul and Bunnell Screening Test

- (1932) - Recognized that heterophile antibodies developed in patients suffering from IM
- The antibodies were found to agglutinate sheep RBCs and ox RBCs but NOT guinea pig kidney therefore, NOT of the Forssman type
- IgM agglutinins observed within 2 weeks after development of symptoms
- Last 4 – 8 weeks
- Maximum titer at 2 – 3 weeks
- Titer DOES NOT correlate with severity of disease
Paul and Bunnell Screening Test

- IM heterophile Ab only appear in 50 – 80% of cases of IM, therefore negative test DOES NOT rule out possible infection
- Hemagglutination test to detect heterophile antibodies
- Dilutions of inactivated patient serum are mixed with sheep RBCs. Inactivated serum is required for this experiment in order to prevent complement from lyse the cells.
  - Improperly inactivated serum will produce hemolysis
  - Inactivate complement by heating diluted samples to 56 degrees
- Positive agglutination with a titer ≥ 56 is clinically significant and considered PRESUMPTIVE evidence of infection with EBV
  - False Positive Results: due to Antigens on sheep RBCs can also be agglutinated by Forssman and serum sickness antibodies
  - Patient is only SUSPECTED of having Infectious Mononucleosis.

False-positives are also observed with hepatitis infections and Hodgkin’s disease

Advantages: Simple, inexpensive

Disadvantages: lacks sensitivity

Only a screening test

Not specific, only indicates the presence or absence of heterophile antibodies

Davidsohn Differential Test

- (1937) - Modified Paul-Bunnell test with an absorption step to remove cross-reacting antibodies (Forssman and serum sickness heterophile Ab) since sheep RBCs react with all three types
  - This procedure removes the Forssman and Serum Sickness Ab to determine if the IM heterophile Abs are present.
- Perform when the Paul-Bunnell titer is ≥ 1:56.
- Distinguishes between the three types of heterophile antibodies
- Cells used in Test: Sheep and beef (ox) RBCs bear some common antigens that are not present on the kidney cells of guinea pigs
- Davidson Differential: Two assays are performed and compared to determine Infectious Mononucleosis.
Davidsohn Differential Test

Assay #1:
1. Guinea pig kidney cells are rich in Forssman antigen
   - When mixed with patient serum, GP cells will ABSORB the Forssman heterophile antibodies out of the patient serum.
2. Then absorbed serum is incubated with sheep RBCs:
   - At this point, ALL Forsman Ab have been absorbed out of the serum; the only heterophile Ab left should be IM Ab:
     - If the sheep RBCs DO NOT agglutinate, No IM antibodies are present
       (No agglutination = No IM antibodies)
     - If the absorbed serum agglutinates the sheep RBCs, then the heterophile antibodies ARE of the IM type
       (Agglutination = IM antibodies)

Assay #2:
1. Bovine RBCs are contain antigens that will absorb out IM heterophile Abs from the patient serum.
   1. Then absorbed serum is incubated with sheep RBCs:
      - At this point, ALL IM heterophile Abs have been absorbed out of the serum; the only heterophile Ab left should be the Forssman Abs:
        - If the sheep RBCs DO NOT agglutinate, IM antibodies ARE possibly present. Need to compare with results from Assay #1 to confirm.
          (No agglutination = IM antibodies)
        - If the absorbed serum agglutinates the sheep RBCs, heterophile Ab ARE NOT IM antibodies.
          (Agglutination = NOT IM antibodies)

Test:

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay #1</td>
<td>Add Sheep RBCs</td>
</tr>
<tr>
<td>IM Ag + Serum</td>
<td>1+</td>
</tr>
<tr>
<td>Beef RBCs</td>
<td>1+</td>
</tr>
<tr>
<td>Assay #2</td>
<td>Add Sheep RBCs</td>
</tr>
<tr>
<td>IM Ag + Serum</td>
<td>1+</td>
</tr>
<tr>
<td>Beef RBCs</td>
<td>1+</td>
</tr>
</tbody>
</table>

Interpretation:

- **POSITIVE IM Result:**
  - (+) = agglutination
  - (-) = no agglutination

- **NEGATIVE IM Result:**
  - (+) = agglutination
  - (-) = no agglutination

- Agg. with both Assays = you need to dilute the sample & repeat
- No agglutination with both Assays = Neither IM heterophiles or Forssman Ab are present; Interpretation: Possible Serum Sickness
Davidsohn Differential Test

Interpretation of Differential Patterns

<table>
<thead>
<tr>
<th>Paul-Bunnell Test (Titer)</th>
<th>Davidsohn Differential</th>
<th>Beef erythrocytes</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 16</td>
<td>(+) Agglutination</td>
<td>(-) No agglutination</td>
<td>Infectious Mononucleosis (IM) antibody</td>
</tr>
<tr>
<td>≥ 16</td>
<td>(-) No agglutination</td>
<td>(+) Agglutination</td>
<td>Forssman antibody</td>
</tr>
<tr>
<td>≥ 16</td>
<td>(-) No agglutination</td>
<td>(-) No agglutination</td>
<td>Serum Sickness</td>
</tr>
<tr>
<td>≤ 16</td>
<td>(-) No agglutination</td>
<td>(-) No agglutination</td>
<td>Negative for Heterophile antibodies</td>
</tr>
<tr>
<td>≤ 16</td>
<td>(+) Agglutination</td>
<td>(+) Agglutination</td>
<td>Dilute the sample &amp; repeat</td>
</tr>
</tbody>
</table>

EBV-Specific Ab Assays

- In diagnostically inconclusive cases of IM, a more definitive assessment of immune status may be obtained through an EBV serologic Ab panel

- Candidates for EBV serology include those who:
  - Do not exhibit classic symptoms for IM
  - Are heterophile negative
  - Are immunosuppressed

- EBV infected B lymphs express a variety of “new” antigens encoded by the virus
  - VCA – viral capsid antigen
  - EA – early antigen
  - EBNA – nuclear antigen

  - All of the above can elicit an antibody response.
  - Assays are available for IgM and IgG antibodies to these EBV antigens.
**EBV-Specific Ab Assays**

- **Onset of symptoms:** Anti-VCA IgM, disappears in 3 mo.; Anti-VCA IgG, persists for life.
- **Acute infection:** anti-VCA IgM, anti-VCA IgG, anti-EA.
- **Present during convalescence:** Anti-VCA IgG, Anti-EBNA
- **Past infection:** anti-EBNA, anti-VCA IgG, neg anti-VCA IgM.

**EBV-Specific Ab Assays**

![Graph showing antibody response over time]

**Epstein-Barr Virus Serology**

- **VCA:**
  - Found in the cytoplasm of B cells
  - Anti-VCA IgM is usually detectable early in the course of infection – disappears within 2-4 months
  - Anti-VCA IgG detectable within 4-7 days after onset of signs and symptoms – persists for an extended amount of time, perhaps lifelong
Epstein-Barr Virus Serology

- **EA (Early Antigen)**
  - Made of two components:
    - **EA-D** (early antigen-diffuse) found in nucleus and cytoplasm of B cells
      - Anti-EA-D IgG is highly indicative of acute infection
    - **EA-R** (early antigen-restricted) found in cytoplasm of B cells
      - Anti-EA-R IgG is not usually found in young adults during acute phase, but it is sometimes demonstrated in the serum of very young children during the acute phase

Epstein-Barr Virus Serology

- **EBNA (Epstein-Barr Nuclear Antigen)**
  - Found in nucleus of all EBV-infected cells
  - EBVNA does not become available for Ab stimulation until after the incubation period of IM.
  - Activated T-lymphs destroy the EBV-infected B cells, as a result:
    - antibodies to NA are **absent** or barely detectable during acute IM infections

Hematologic Studies

- 66% pt. WBC = 10 – 20,000/mm³
- 10% pt. Leukopenia
- Relative lymphocytosis: with 5 – 55% variant or atypical/reactive lymphs that persists for 1 – 6 months
<table>
<thead>
<tr>
<th>Hematologic Studies</th>
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<td>Normal Lymph</td>
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![Images of Normal and Atypical Lymphs]