Bad Bugs, Broken Red Cells: Recognizing Infection-Triggered Hemolysis in the Clinical Lab

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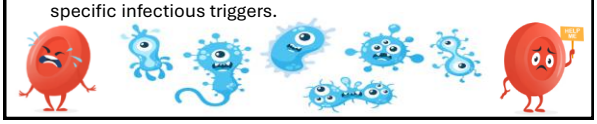
Course Objectives

1. Differentiate immune-mediated hemolysis caused by viral, bacterial, and parasitic infections.
2. Apply transfusion service workflows to investigate suspected immune-mediate hemolytic processes.
3. Recognize unique laboratory patterns associated with specific infectious triggers.



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Why does hemolysis happen?

- Inherited defects within the RBC → **INTRINSIC**
  - Membrane abnormalities (hereditary spherocytosis)
  - Enzyme deficiencies (G6PD deficiency)
  - Hemoglobinopathies (sickle cell disease)
- External factors damage normal RBC → **EXTRINSIC**
  - Autoimmune disorders, transfusion reactions (antibody mediated)
  - Infections (pathogen and/or released toxins)
  - Mechanical damage (artificial heart valves)
  - Toxins, medications (venom, arsenic, lead)

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What happens after a RBC is damaged?

- **INTRAVASCULAR HEMOLYSIS**
  - RBCs are destroyed directly within the bloodstream
  - Releases free hemoglobin (Hgb) into the plasma
  - Antibody-mediated reactions, mechanical damage, infections
- **EXTRAVASCULAR HEMOLYSIS**
  - RBCs are destroyed in the spleen and liver
  - Free Hgb is *not* released into plasma
  - Antibody-mediated reactions, abnormal shapes, inclusion bodies

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Intravascular hemolysis

- Free Hgb dimerizes and is rapidly bound by haptoglobin (Hp)
- Hp/Hgb complex travels to liver, spleen, bone marrow for further processing
  - ➢Hgb → globin (protein), heme (Fe)
  - ➢Bilirubin converted and excreted

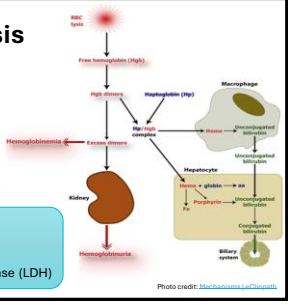

Photo credit: Mechanisms | eClinpath

Key Clinical Signs:
Hemoglobinemia
Hemoglobinuria

Key Lab Findings:
↓ Haptoglobin
↑ Indirect bilirubin
↑ Lactate dehydrogenase (LDH)

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Extravascular hemolysis

- Macrophages "eat" abnormal RBC
  - ➢Spleen
  - ➢Liver
  - ➢Bone marrow
- Breakdown of RBC inside macrophage
  - ➢Hgb → globin (protein), heme (Fe)
  - ➢Bilirubin converted and excreted

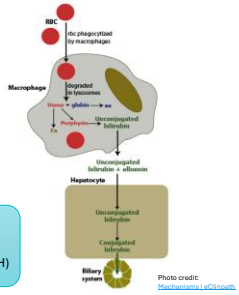

Photo credit: Mechanisms | eClinpath

Key Clinical Signs:
Jaundice
Anemia
Organomegaly

Key Lab Findings:
N Haptoglobin
↑↑ Indirect bilirubin
↑ Lactate dehydrogenase (LDH)
Spherocytes on blood smear

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Types of hemolytic conditions *(from infection)*

Warm autoimmune hemolytic anemia (WAIHA)
- IgG bound to RBC → macrophage phagocytosis → EXTRAVASCULAR

Cold agglutinin disease (CAD)
- IgM on RBC at cold temp → activates complement (C') → EXTRAVASCULAR

Paroxysmal cold hemoglobinuria (PCH)
- IgG to RBC at cold temp → activates C' at 37°C → INTRAVASCULAR

Microangiopathic hemolytic anemia (MAHA)
- Mechanical destruction of RBC from damaged vessels → INTRAVASCULAR

Liver associated hemolysis
- Altered lipid metabolism→ abnormal RBC → EXTRAVASCULAR

Direct RBC destruction
- Bacterial toxins, parasitic invasion → lyse RBC → INTRAVASCULAR

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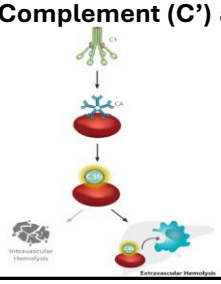
Complement (C') and hemolysis



- Enzymatic proteins attach to IgM or IgG on target cell
- Cascading actions eliminate target cell by lysis (intravascular hemolysis)
- Target cell is phagocytized by macrophage (extravascular hemolysis)

Photo credit: C4 activation of the classical complement pathway on RBCs results in... | Download Scientific Diagram

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Laboratory indicators of hemolysis

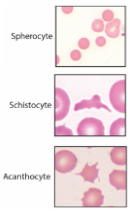

Photo credit: RBC morphology | eClinpath

Hemoglobinemia
Hemoglobinuria
↑ LDH
↑ Indirect bilirubin
↓ Haptoglobin
Abnormal RBCs on smear
+ DAT result (IgG or C3)

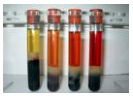

Photo credit: Blood - HEMATOLOGY

Photo credit: BLOOD IN URINE: HAEMATURIA BASICS | All Things Kidney ~ Official

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How do infections cause hemolysis?

Molecular mimicry	Polyclonal B-cell activation	Complement activation	Direct RBC invasion
• Evolutionary strategy • Pathogens express proteins that resemble host proteins • Triggers an autoimmune response	• Super antigens • Loss of tolerance for autoreactive B-cell clones • Flood of non-specific antibodies	• Initiated by Ab attachment • Will cause direct cell lysis and/or phagocytic removal	• Physical rupture upon pathogen exit • Removal by the spleen • Bacterial toxins damage membrane • Eryptosis

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Why is this important to Blood Bank?

- Premature destruction of stored RBC
- Mimics hemolytic transfusion reactions
- Complicates compatibility testing
  - ➢Strict screening processes
  - ➢Careful component handling
  - ➢Accurate differentiation of infectious cause

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Blood Bank investigation workflow

1. Clerical check and visual inspection
2. Direct antiglobulin test (DAT): detects Ab or C' bound to RBCs
3. Repeat ABO/Rh typing on pre- and post-transfusion samples and donor unit
4. Repeat crossmatch with more sensitive method
5. Blood cultures, serological testing, peripheral blood smear
6. Confirmatory hemolysis markers: haptoglobin, LDH, indirect bilirubin, reticulocyte count

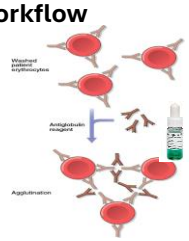

Photo credit: Coombs' Testing and Its Diagnostic Significance in Dogs and Cats - Veterinary Clinics: Small Animal Practice

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Common testing pitfalls

- Blood banker must be able to distinguish between true agglutination and false agglutination
- Interferences can mimic true agglutination or mask underlying, clinically significant alloantibodies

Panagglutination	Rouleaux	Cold agglutinins
• Patient serum reacts with ***all*** RBC • Masks important alloAb	• Non-immunologic phenomenon • RBC resemble stack of coins	• Usually from IgM autoAb • Pathological in some conditions

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Rouleaux versus agglutination

Tube testing | Rouleaux | Agglutination

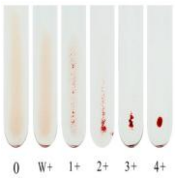
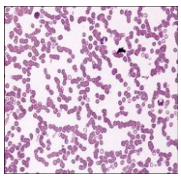


Photo credit: https://www.mdpi.com/2073-4468/14/3/78



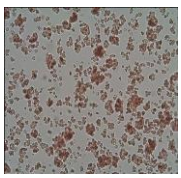


Photo credit: 2: Red Blood Cells | Veterian Key

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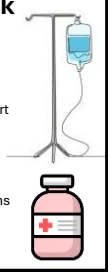
Different pathogens, shared playbook

1. **Treat the infection**
   - Address the trigger: antivirals, antibiotics, antiparasitics
2. **Support the hemolysis**
   - Monitor hemolysis markers, provide hydration and renal support
3. **Transfuse when necessary**
   - pRBC transfusion or exchange if severe anemia
4. **Add immunosuppression when necessary**
   - Corticosteroids, monoclonal Ab infusion/injection, IgG infusions
5. **Escalate for mechanism-specific emergencies**
   - Targeted therapies for medical emergencies



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Viral triggers of hemolysis

HIV	CMV	Sars-CoV-2	EBV
Hepatitis B, C, D	Rubeola	Mumps	Varicella Zoster

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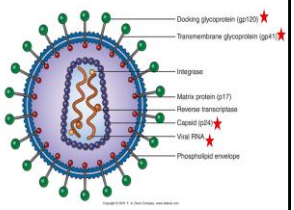
HIV: Human Immunodeficiency Virus

- Retrovirus
- Two strains: HIV-1 and HIV-2
- HIV-1 responsible for worldwide AIDS pandemic
- HIV-2 primarily in West Africa
- Both strains can cause *acquired immune deficiency syndrome* (AIDS)
- Primarily infects CD4+ cells (T-helper cells, macrophages)



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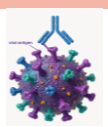
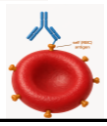
HIV: Human Immunodeficiency Virus

- Increases incidence of **WAIHA**
  - ➢Molecular mimicry
  - ➢Immune dysregulation
  - ➢Complement activation
- Medications can cause **drug-induced hemolysis**
  - ➢Antiretrovirals, various antibiotics, antifungals
- Opportunistic infections with other pathogens
- HIV may alter RBC structure to be prone to rupture
- Mostly extravascular hemolysis
  - ➢Anemia, jaundice, spherocytes, DAT IgG+





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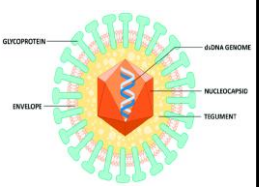
CMV: Cytomegalovirus

- Large virus of Herpesviridae family
- Replicates only in human cells
- Spread via close contact
- Shed in saliva, tears, urine, stool, breastmilk, semen, vaginal secretions, blood, and blood products
- Establishes latent infection in myeloid cells
- Most infections are mild, "mono-like"
- Special concern for immunocompromised



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CMV: Cytomegalovirus

- Most commonly: triggers an autoimmune response
  - ➢May be **cold agglutinins (CAD) or warm-reacting IgG antibodies (WAIHA)**
  - ➢Mostly extravascular hemolysis mechanism/pathway
- May have a **direct cytotoxic effect** on mature RBC
  - ➢Can lead to intravascular hemolysis, even in absence of autoAb
- Rarely: triggers a hyper-inflammatory state
  - ➢Overactive macrophages in liver and spleen phagocytize healthy RBC
  - ➢Extravascular hemolysis
  - ➢Self-limiting in immunocompetent host

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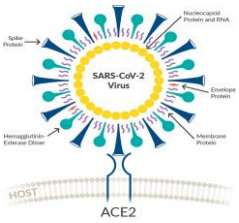
SARS-CoV-2: Coronavirus/COVID-19



- Spread by direct contact, droplet, and airborne routes
- Asymptomatic to severe, fatal infections
- High risk groups: immunocompromised, multiple co-morbidities
- Cytokine storm (PRO-inflammatory)
- Pulmonary infiltrates
- Leukopenia
- Hypercoagulable state (microthrombi)

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SARS-CoV-2: Coronavirus/COVID-19

- Can trigger both **WAIHA (IgG-mediated) and CAD (IgM-mediated)**
  - Molecular mimicry: structural similarities with RBC membrane
  - Cryptic antigens: "hidden" parts of RBC are revealed and attacked
- Direct interaction with RBC and precursors
  - Binding to receptors on RBC disrupts the cytoskeleton
  - Can infect erythroid precursor cells in bone marrow
- Potent activator of the complement system
  - Can activate all 3 C' pathways
  - Leads to intravascular hemolysis
  - Widespread inflammation of blood vessels

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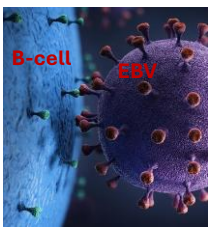
EBV: Epstein Barr Virus



- Highly prevalent worldwide, by adulthood >90% of us have been infected with EBV
- Tropism for oropharyngeal epithelial cells and B-lymphocytes
- Member of Herpesviridae family, so establishes latency
- Most common outcome: infectious mononucleosis (mono)
- Other outcomes: cancer, MS, post-transplant disorders

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EBV: Epstein Barr Virus

- Triggers hemolysis in 0.5% - 3% of cases
- **Cold Agglutinin Disease (CAD)** most common
  - Auto-anti-i (IgM) binds to RBC in lower temperature body sites
  - C' proteins recruited and may elicit intravascular hemolysis
  - Extravascular hemolysis occurs in the spleen (splenomegaly common)
  - Macrophages may become hyper-aggressive and devour healthy cells
  - Spherocytes common, increased reticulocytes in some cases
- Usually becomes apparent in 2nd – 3rd week of infection
- Typically self-limiting and resolves as the viral infection clears

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Hepatitis B, C, D (HBV, HCV, HDV)

- Bloodborne hepatitis viruses: have affinity for hepatocytes of liver
- Acute infection (≤ 6 mths) may develop into chronic infection (lifelong)
- Chronic infections yield cirrhosis, liver cancer, and liver failure
- HBV and HCV prevalent worldwide
- HDV required HBV for infection; most prevalent in Asia and Africa

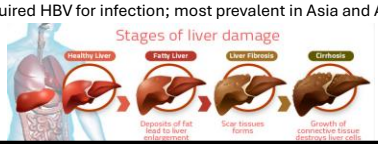

Photo credit: https://txliver.com/

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Hepatitis B, C, D (HBV, HCV, HDV)

- **HBV: non-immune hemolysis during acute phase**
  - ➢Toxin build-up due to decreased liver clearance, RBC become fragile
- **HCV: immune-mediated and drug-induced**
  - ➢Triggers production of cryoglobulins and autoAb (IgM or IgG)
  - ➢Ribavirin (older drug) major cause of drug-induced hemolysis
- **HDV: severe liver dysfunction and accelerates liver failure**
  - ➢Rapid cirrhosis and fulminant liver failure
  - ➢Mechanical damage to RBC from fibrin strands

- Liver-associated hemolysis, mostly **extravascular**
- Acanthocytes often indicate worsening liver disease
- Schistocytes may be observed in severe cirrhosis

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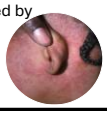
Rubeola virus: Measles

- First infects upper respiratory tract, then lungs and lymph nodes
- Highly contagious by respiratory droplets and fomites
- Prodromal period followed by maculopapular rash
- May cause immunosuppression



Mumps virus

- Infection of parotid glands
- Will also spread to brain, pancreas, testes, ovaries
- Maculopapular rash is rare

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Rubeola virus: Measles & Mumps virus

- Both viruses can directly invade RBC and cause damage and lysis
- **Primarily cause Paroxysmal Cold Hemoglobinuria (PCH)**
  - ➢Formation of Donath-Landsteiner (D-L) antibody = biphasic IgG
  - ➢Viruses express proteins that mimic the P-antigen on RBC
  - ➢D-L antibodies cross react and attack RBC
    - ➢Attaches to RBC in areas of body at cooler temps (< 37°C)
    - ➢Activates complement when RBC return to warmer core temp (37°C)
    - ➢Immediate intravascular hemolysis
    - ➢Sudden hemoglobinuria and constitutional symptoms

- Transient after viral infection (shortly after prodromal period)
- Can confirm with Donath-Landsteiner test

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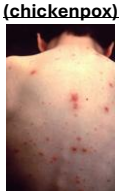
VZV: Varicella Zoster Virus

- Causes chickenpox and shingles
- Herpes virus; establishes latency in nerve cells
- Highly contagious
- Prodromal period followed by asynchronous, painful, itchy rash
- Chickenpox rash is widespread
- Shingles rash is unilateral
- Both are infectious until the last scab falls off

Varicella (chickenpox)



Herpes zoster (shingles)

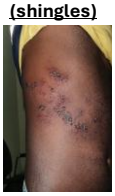


Photo credits: CDC.gov (chickenpox), Dr. Misheck Ruwende FaceBook (shingles)

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VZV: Varicella Zoster Virus

- Triggers **autoimmune hemolytic anemia**
  - ➢Warm AIHA via IgG autoAb → extravascular hemolysis in spleen
  - ➢Cold Agglutinin Disease via IgM antibodies → intravascular hemolysis
- Exhibits **molecular mimicry** and over-activates immune response
  - ➢Inappropriate activation of complement cascade → bystander hemolysis
  - ➢Macrophages become hyper-aggressive and consume healthy cells
- Damage to lining of blood vessels in severe, disseminated cases
- Special concern for those with G6PD deficiency and B12 deficiency

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Bacterial triggers of hemolysis

Mycoplasma pneumoniae

Bartonella bacilliformis

Treponema pallidum

Shiga-toxin *Escherichia coli*

Clostridium perfringens

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Mycoplasma pneumoniae

- Highly contagious bacterium that causes "walking pneumonia"
- Lacks a cell wall, making it resistant to common antibiotics like PCN
- Common symptoms: fever, sore throat, persistent dry cough
- Children < 5 yrs: may have wheezing, vomiting, diarrhea
- Spread through respiratory droplets
- Will not be seen on gram stain; notoriously difficult to culture
- Usually diagnosed based on clinical presentation

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Mycoplasma pneumoniae

- Bacterial surface molecules mimic the I antigen on RBC
- IgM antibodies elicit **Cold Agglutinin Disease (CAD)**
  - ➢Extravascular and intravascular hemolysis
  - ➢Hemoglobinuria, jaundice, acrocyanosis

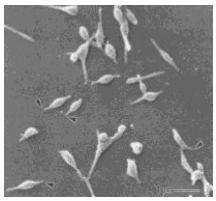

Photo credit: Mycoplasma pneumoniae and Its Role as a Human Pathogen | Clinical Microbiology Reviews

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Bartonella bacilliformis

- Extremely aggressive bacterium that causes Carrion's disease
- Restricted almost entirely to high-altitude valleys of Andes mountains
- Unique among other *Bartonella* spp. as a trigger for intense hemolysis
- Spread by bite from sandfly; humans are the only natural reservoir
- 2 phases of disease: 1) Oroya fever, 2) Verruga Peruana

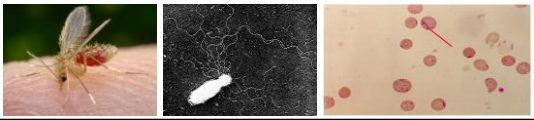

Photo credits: CDC.gov, www.sciencedirect.com (US Armed Forces Institute of Pathology), www.researchgate.net (Cesar Ugarte-Gil)

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Bartonella bacilliformis

Direct RBC invasion, destruction, and immunosuppression

- Use a protein called **deformin** to create pits in RBC
- Invade RBC through pits and multiply
- Up to 90% of circulating RBC can become infected simultaneously
- Spleen and liver must then filter out and destroy infected cells
- In addition to antibiotics, pRBC transfusion is life-saving measure

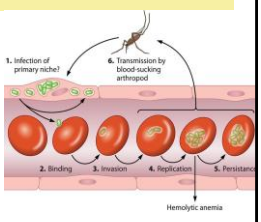

Photo credit: www.researchgate.net (Joaquim Ruiz)

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Treponema pallidum: Syphilis

- Spirochete bacterium

4 stages of disease:

1) Primary (chancre)
2) Secondary (rash)
3) Latent (no symptoms)
4) Tertiary (CNS involvement)

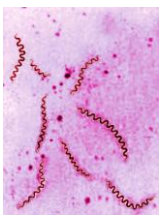

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Photo credit: www.microbiologyinpictures.com

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Treponema pallidum: Syphilis

- Historically, syphilis was the most common cause of PCH (>90%)
- Today, many patients treated with PCN before reaching tertiary stage
- Critical infectious trigger of **extravascular hemolysis** for newborns
  - Classic hematological manifestation in infants infected in utero
  - Bacteria are highly invasive and disseminate through bloodstream
  - Widespread spirochetemia = extravascular hemolysis and organ damage
  - Anemia characterized by jaundice, hepatosplenomegaly, and BM suppression

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Shiga-toxin producing *E. coli* (STEC)

- Most common strain: *E. coli* O157:H7 (gram negative rods)
- Highly contagious, spread via fecal-oral route
- Contaminated food and water, animal contact, person-to-person
- About 5-10% of cases develop **Hemolytic Uremic Syndrome (HUS)**
  - Toxin enters bloodstream and damages RBC and kidneys
  - Life-threatening hemolytic condition
  - Children < 5 yrs and older adults
  - Decreased urination, fatigue, unusual bruising
  - Can lead to kidney failure, seizures, stroke, and death
  - Treatment includes fluid/electrolyte management, blood transfusion, dialysis, blood pressure control; AVOID antibiotics and antidiarrheals

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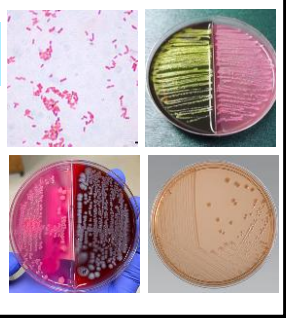
Shiga-toxin producing *E. coli* (STEC)

1. **Thrombocytopenia:** toxin injures capillary cells; microthrombi form; low platelet count due to excessive clotting/consumption in capillaries
2. **Microangiopathic Hemolytic Anemia (MAHA):** RBC are sliced and shredded as they push through clot-filled capillaries; lysis and schistocytes formed
3. **Acute kidney injury:** toxin binds to receptors on kidney cells; inflammation & microthrombi block blood flow; filtration rate plummets; renal tubular cells die from lack of oxygen



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Clostridium perfringens

- Gram positive, spore-forming, anaerobic bacterium (rods, "box-car")
- Leading cause of food poisoning and gas gangrene
- Produces toxins that lyse RBC and cause major tissue damage (gas gangrene)
- Important risk for immunocompromised and underlying conditions

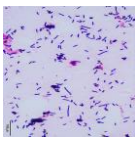

Photo credit: Clostridium perfringens sepsis in three patients with acute leukemia and review of the literature | International Journal of Hematology | Springer Nature Link; www.researchgate.net (Aasim Habib Wani)

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Clostridium perfringens

- Produces 2 very potent exotoxins (causes double zone on BAP)
  - ➢Alpha-toxin (Phospholipase C): hydrolyzes lipids of RBC membrane
  - ➢Theta-toxin (Perfringolysin O): forms large pores in RBC membrane
- Leads to massive **intravascular hemolysis** and tissue damage
  - ➢Black serum/urine (free hemoglobin)
  - ➢Spherocytes and ghost cells
  - ➢Medical emergency = septic shock
  - ➢High mortality rate ~ 70-80%

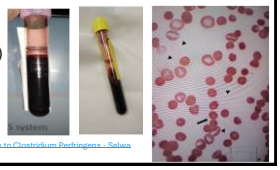

Photo credit: Black Urine and Methemoglobinemia in the Setting of Sepsis Due to Clostridium Perfringens – Salwa A Koubaissi, Reem G Al Assaad, Ziad Itani, Imad Bouaki, 2020

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Parasitic triggers of hemolysis

Plasmodium spp.	*Babesia microti*	*Trypanosoma brucei* spp.

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Plasmodium species: Malaria

- Protozoan parasites that cause malaria
- Transmitted from bite of *Anopheles* mosquito (female)
- > 100 species; only 5 infect humans
  - ➢*Plasmodium falciparum*
  - ➢*Plasmodium vivax*
  - ➢*Plasmodium ovale*
  - ➢*Plasmodium malariae*
  - ➢*Plasmodium knowlesi*

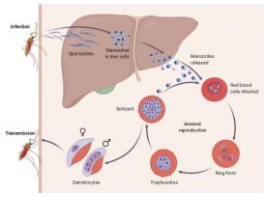

Photo credit: Malaria. Plasmablasts are the real parasites. — Breaking Down Biology

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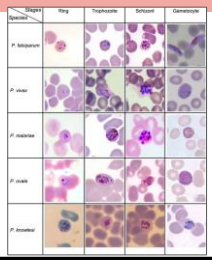
Plasmodium species: Malaria



Intravascular hemolysis (direct damage)
- ➢Mature schizont ruptures RBC to release merozoites

Extravascular hemolysis (clearance)
- ➢Macrophages in spleen and liver "eat" infected AND healthy cells

- Jaundice, hemoglobinuria ("Blackwater Fever")
- Acute kidney injury from free hemoglobin
- ↓ blood flow to vital organs

- Giemsa (thick & thin), rapid antigen tests, PCR

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Babesia microti

- Parasite transmitted by blacklegged tick (*Ixodes scapularis*)
- Causes babesiosis, a malaria-like illness
- Northeast and Upper Midwest (NY, MA, CT, NJ, RI, MN, WI)
- Symptoms appear 1-4 weeks after exposure: high fever, chills, sweats, fatigue, headaches, myalgias, jaundice, dark urine, anemia
- Severe disease risk for those >50 yrs, immunocompromised, and those without a spleen

Photo credits: www.mypmp.net; www.lymemow.org

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Babesia microti

- Sporozoites directly invade, replicate, burst out
  - ➢**Intravascular hemolysis**
- Infection triggers complex immune response, destroys healthy RBC too
  - ➢Warm autoimmune hemolytic anemia (WAIHA)
  - ➢IgG autoAb target antigens on RBC
  - ➢Macrophages in liver and spleen eliminate marked RBC
  - ➢**Extravascular hemolysis**
- Blood smear and PCR best for diagnosis
- Will NOT show brown pigment like *Plasmodium*

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Trypanosoma brucei spp.

- EXTRAcellular protozoan parasite
- Transmitted by tsetse fly in Sub-Saharan Africa
- Causes "sleeping sickness"
  - ➢*Trypanosoma brucei gambiense* (West)
  - ➢*Trypanosoma brucei rhodesiense* (East)
- Progresses through 2 distinct phases:
  - ➢Hemolymphatic phase
  - ➢Meningoencephalitic phase

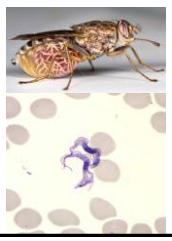

Photo credit: https://theconversation.com, www.cdc.gov

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Trypanosoma brucei spp.

- **Destruction via extravascular hemolysis**
  - ➢Erythrophagocytosis in spleen and liver
  - ➢Parasites release vesicles that fuse with RBC, alerts immune system
- **Direct parasite activity**
  - ➢Highly motile, flagella damage RBC
  - ➢Secrete hemolysins that directly rupture RBC
  - ➢Oxidative stress weakens RBC membrane
- **Iron theft**
  - ➢Parasite requires iron for survival
  - ➢Steals iron from host RBC

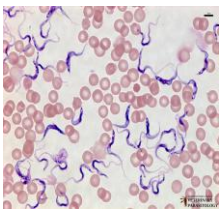

Photo credit: Lance Wheeler, Texas A&M School of Veterinary Medicine

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Handling hemolysis triggered by infection

- Distinguish in-vivo hemolysis from in-vitro hemolysis
- Monitor for sudden drops in Hgb/Hct (absence of blood loss)
- Evaluate the typical hemolysis markers and interferences
- Analyze RBC morphology on blood smears
- Understand notification protocols
- Recognize individual patient needs
- Manage high-volume transfusion needs

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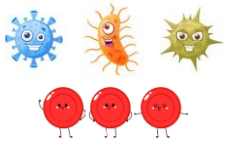
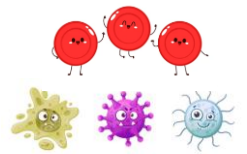
THANK YOU!!!





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