Lecture 1

Endocrine disease(Thyroid gland)

Graves's disease

Graves' disease is an autoimmune disorder that primarily affects the thyroid gland, leading to hyperthyroidism, which is characterized by the overproduction of thyroid hormones.

Diagnose of Graves' disease

To diagnose Graves' disease, a comprehensive approach is taken, involving a combination of physical examinations, medical history assessments, and various laboratory tests.

1. Physical Examination and Medical History

- Rapid heartbeat
- Weight loss despite normal or increased appetite
- Increased sensitivity to heat
- Tremors
- Changes in menstrual cycles
- Eye problems, such as bulging eyes (Graves' ophthalmopathy)

2. Blood Tests

Blood tests are crucial for diagnosing Graves' disease. The following tests are typically performed:

- **Thyroid-Stimulating Hormone (TSH) Test**: In Graves' disease, TSH levels are usually low due to the overproduction of thyroid hormones.
- **Thyroid Hormone Levels (T3 and T4)**: These hormones are typically elevated in individuals with Graves' disease.
- **Thyroid Antibodies**: Tests for antibodies such as thyroid-stimulating immunoglobulin (TSI) or thyrotropin receptor antibodies (TRAb) can confirm the diagnosis. Elevated levels of these antibodies are indicative of Graves' disease.

3. Imaging Tests

If blood tests suggest hyperthyroidism, imaging tests may be conducted to further evaluate thyroid function:

- **Radioactive Iodine Uptake Test**: This test measures how much iodine the thyroid gland absorbs, which is often increased in Graves' disease.
- **Thyroid Scan**: A scan can visualize the thyroid gland's structure and function, helping to confirm the diagnosis.
- **Ultrasound**: This may be used to assess the thyroid gland's size and blood flow, particularly if there are concerns about nodules or other abnormalities.

Thyroid-Stimulating Hormone (TSH) Test:

- The **Thyroid-Stimulating Hormone (TSH)** test is a crucial diagnostic tool used to assess thyroid function .
- **Hyperthyroidism**: Indicated by low TSH levels, suggesting that the thyroid is overactive and producing excessive hormones.

The measurement of Thyroid-Stimulating Hormone (TSH) is primarily conducted using advanced laboratory instruments. The following instruments are commonly used:

1. Roche Cobas e411 Analyzer

- This instrument utilizes electrochemiluminescent immunoassay (ECLIA) technology to measure TSH levels. It is known for its high sensitivity and specificity, making it a reliable choice for thyroid function tests in clinical settings.

2. Beckman Coulter Access2

- Another instrument used for TSH measurement is the Beckman Coulter Access2. This device operates on a similar immunoassay principle and is employed in various laboratories to determine serum TSH levels accurately.

3. ELISA Kits

- Various ELISA kits, such as the RayBio® Human TSH ELISA kit, are also utilized for measuring TSH levels. These kits are designed for high sensitivity and are suitable for research and clinical diagnostics.

These instruments are part of the standard laboratory equipment in hospitals and diagnostic centers across Iraq, ensuring accurate assessment of thyroid function and aiding in the diagnosis of conditions like hypothyroidism and hyperthyroidism.

Hashimotos Thyroditis

Hashimoto's thyroiditis, also known as Hashimoto's disease, is an autoimmune disorder that primarily affects the thyroid gland, leading to hypothyroidism characterized by high TSH levels, indicating that the thyroid is not producing enough hormones.

The diagnosis of this condition involves several steps:

1. Clinical Assessment

- **Medical History**: includ patient's symptoms, family history of thyroid disease, and any other autoimmune conditions.
- **Physical Examination**: signs of hypothyroidism, such as fatigue, weight gain, cold intolerance, and the presence of a goiter (enlarged thyroid).

2. Blood Tests

- **Thyroid Function Tests**:
  - **TSH (Thyroid-Stimulating Hormone)**: An elevated TSH level indicates that the thyroid is underactive.
  - **Free T4**: A low level of free T4 confirms hypothyroidism.
- **Thyroid Antibody Tests**:

Anti-Thyroid Peroxidase (anti-TPO) Antibodies: TPO is an enzyme found in the thyroid gland that catalyzes the oxidation of iodide ions, which is essential for the production of thyroid hormones, namely thyroxine (T4) and triiodothyronine (T3). These hormones play a vital role in regulating metabolism, growth, and development in the body. Most patients with Hashimoto's have elevated levels of these antibodies.

- **Anti-Thyroglobulin (anti-Tg) Antibodies**: These may also be tested but are less specific.

3. Additional Diagnostic Tools

- **Ultrasound**: While not always necessary, a thyroid ultrasound can help assess the size and structure of the thyroid gland, particularly if nodules are present.
- **Fine-Needle Aspiration (FNA)**: If nodules are detected, FNA may be performed to rule out malignancy.

4. Monitoring and Follow-Up

- Patients with positive thyroid antibodies but normal TSH levels may not require immediate treatment but should be monitored regularly for any changes in thyroid function. Follow-up tests are typically done every 6-12 months.

principle of the Anti-TPO (Thyroid Peroxidase) antibody test

The principle of the Anti-TPO (Thyroid Peroxidase) antibody test is based on the detection of antibodies produced by the immune system against the enzyme thyroid peroxidase, which is crucial for the synthesis of thyroid hormones.

Procedure for Detection of Anti-TPO Antibodies (ELISA)

1. Sample collection.

2. Antigen coating (Microtiter wells are pre-coated with thyroid peroxidase (TPO) antige) .

3. Addition of patient serum (Patient serum is added to the wells) If Anti-TPO antibodies are present, they bind to the TPO antigen

4. Washing step (Wells are washed to remove unbound serum components)

5. Addition of enzyme-linked secondary antibody (An enzyme-conjugated anti-human IgG antibody is added)This binds to the patient's Anti-TPO antibodies (if present)

6. Second washing (Excess unbound conjugate is removed by washing)

7. Substrate addition (A chromogenic substrate (e.g., TMB) is added) The enzyme reacts with the substrate to produce a color change

8. Reaction termination (Stop solution is added to halt the enzymatic reaction)

9. Measurement (Color intensity is measured using an ELISA reader) Optical density is proportional to Anti-TPO antibody concentration .

10. Interpretation (Elevated levels indicate autoimmune thyroid disease)

Lecture 2

Insulin-dependent diabetes mellitus (IDDM) or type1

Diagnosis of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1D) is a chronic condition characterized by the body's inability to produce insulin, leading to elevated blood glucose levels. Diagnosing

T1D involves several tests that measure blood glucose levels and assess the presence of specific antibodies.

Diagnostic Tests

1. **Fasting Plasma Glucose (FPG) Test**:
    - This test measures blood glucose levels after fasting for at least 8 hours.
    - A fasting blood glucose level of **126 mg/dL (7.0 mmol/L)** or higher indicates diabetes.
2. **Oral Glucose Tolerance Test (OGTT)**:
    - Involves fasting overnight, followed by drinking a sugary solution.
    - Blood glucose levels are measured before and 2 hours after consumption.
    - A 2-hour blood glucose level of **200 mg/dL (11.1 mmol/L)** or higher confirms diabetes.
3. **Hemoglobin A1C Test**:
    - This test reflects average blood glucose levels over the past 2 to 3 months.
    - An A1C level of **6.5% or higher** indicates diabetes.
4. **Random Plasma Glucose Test**:
    - A blood sample is taken at any time, regardless of when the patient last ate.
    - A blood glucose level of **200 mg/dL (11.1 mmol/L)** or higher, along with symptoms of hyperglycemia (such as increased thirst, frequent urination, and fatigue), suggests diabetes.
5. **Insulin and C-Peptide Levels**:
    - In T1D, insulin and C-peptide levels are typically low or undetectable, distinguishing it from type 2 diabetes, where these levels are often normal or high.
6. **Autoantibody Testing**:
    - Testing for specific autoantibodies (such as GAD65, IA-2, and insulin autoantibodies) can help confirm a diagnosis of type 1 diabetes.
    - The presence of these antibodies indicates an autoimmune response against pancreatic beta cells, which produce insulin.

- Islet Cell Cytoplasmic Autoantibody (ICA)

- Glutamic Acid Decarboxylase Autoantibody (GADA)
- Insulinoma-Associated Autoantibody (IA-2A)
- Insulin Autoantibody (IAA)

Lecture 3

Addison's disease

Addison's disease is a chronic disorder caused by **destruction or dysfunction of the adrenal cortex**, leading to **deficiency of glucocorticoids, mineralocorticoids, and androgens**.

Measurement of Anti–21-Hydroxylase Antibodies

Anti–21-hydroxylase antibodies are measured using **immunoassay techniques** on a **blood (serum) sample**.

Common Methods

1. **ELISA (Enzyme-Linked Immunosorbent Assay)** – most commonly described in exams
2. **Radioimmunoassay (RIA)** – older but highly sensitive
3. **Chemiluminescent immunoassay (CLIA)** – modern automated labs

Principle (ELISA-based)

The test detects **autoantibodies in patient serum directed against the enzyme 21-hydroxylase**, which is essential for cortisol and aldosterone synthesis.

Procedure (ELISA – simplified steps)

1. Microtiter wells are **coated with recombinant human 21-hydroxylase antigen**
2. Patient serum is added
    - If antibodies are present, they bind to the antigen
3. Wells are washed to remove unbound substances
4. **Enzyme-labeled anti-human IgG antibody** is added
5. After washing, a **chromogenic substrate** is added

6. Color development occurs
7. Optical density is measured using an ELISA reader
8. Antibody concentration is calculated using standards

Interpretation

- **Positive** → Autoimmune Addison's disease
- **Negative** → Suggests non-autoimmune cause (e.g., TB, metastasis)

Laboratory findings

- Low morning serum cortisol
- Plasma ACTH elevated
- Hyponatremia
- Hyperkalemia
- Hypoglycemia
- Elevated plasma renin
- Low aldosterone
- Anti–21-hydroxylase antibodies positive

Lecture 4 & 5

Hypersensitivity

Asthma

Asthma is a **chronic inflammatory disease of the airways** characterized by **variable, reversible airflow obstruction** and **bronchial hyperresponsiveness**. lead to **recurrent episodes of wheezing, breathlessness, chest tightness, and cough**, especially at night or early morning .

Diagnosed of Asthma

1. Clinical history (suggestive features)

- Wheezing
- Shortness of breath
- Chest tightness
- Cough (often worse at night or early morning)
- Symptoms triggered by **dust, pollen, exercise, cold air, smoke, infections**

Clinical immunology/ practical

2. Physical examination

- Expiratory **wheeze**
- Prolonged expiration
- May be normal between attack

3. Pulmonary function tests

A-Spirometry (gold standard)

B-Reversibility test

- Give inhaled **short-acting bronchodilator**
- Repeat spirometry after 15 minutes

4. Bronchial provocation test (if spirometry normal)

5. Allergy testing (supportive)

- **Total serum IgE** ↑
- **Specific IgE** or skin prick test positive
- Eosinophilia

Total Serum IgE Estimation

Total Serum IgE estimation is a **laboratory test to measure the overall concentration of IgE antibodies in blood**, mainly used in **allergic disorders and parasitic infections**.

Common Methods

- **ELISA**
- **CLIA (Chemiluminescent immunoassay)** – most common in automated labs
- **RIA** (older method)

Principle (Sandwich immunoassay)

Total IgE is measured using a **sandwich immunoassay**, where:

- IgE in patient serum binds to **anti-IgE antibodies** fixed on a solid phase
- A second **enzyme- or chemiluminescent-labeled anti-IgE antibody** binds to IgE
- The generated signal is **directly proportional** to total IgE concentration

Procedure (ELISA – simplified)

1. Wells are coated with **anti-human IgE antibodies**
2. Patient serum is added → IgE binds to the antibody
3. Wash to remove unbound proteins
4. Add **enzyme-labeled anti-IgE antibody**
5. Wash again
6. Add chromogenic substrate
7. Color develops
8. Measure absorbance using an ELISA reader
9. Compare with standards to calculate IgE level

Clinical significance

Increased IgE seen in:

- Allergic diseases (asthma, allergic rhinitis, eczema)
- Parasitic infestations
- Atopic dermatitis
- Hyper-IgE syndrome
- Some immunodeficiency states

Decreased IgE:

- Rarely clinically significant

Lecture 6

Allergic Rhinitis

A **chronic inflammatory condition of the nasal mucosa** caused by an **IgE-mediated immune response** to inhaled allergens, characterized by symptoms such as **sneezing, nasal congestion, rhinorrhea (runny nose), and nasal/ocular itching**.

Key points in simpler terms:

- "Allergic" → triggered by allergens (pollen, dust, pets, mold)
- "Rhinitis" → inflammation of the nasal lining
- Immune system overreacts → histamine release → typical symptoms

Diagnosing of allergic rhinitis (AR)

Diagnosing **allergic rhinitis (AR)** involves a combination of **history, physical exam, and sometimes allergy testing**

Clinical immunology/ practical

1. Clinical History

- Timing: seasonal vs year-round
- Triggers: pollen, pets, dust, mold, perfumes
- Symptoms: sneezing, nasal congestion, runny nose, itchy eyes/nose/throat, post-nasal drip
- Family or personal history of allergies, asthma, or eczema
- Response to antihistamines or avoidance measures

2. Physical Examination

typical signs:

- Pale, bluish or boggy nasal mucosa
- Swollen nasal turbinates
- Clear nasal discharge
- "Allergic shiners" (dark circles under eyes)
- Dennie-Morgan lines (folds under eyes)
- Eye redness or watery eyes
- Sometimes associated signs: nasal polyps, eczema, or signs of asthma

3. Allergy Testing (to confirm the specific allergen)

Used if diagnosis is unclear, or for targeted treatment like immunotherapy:

a) Skin Prick Test

- Small amount of allergen placed on skin → pricked lightly
- Positive reaction: redness and swelling in 15–20 minutes
- Quick and reliable

b) Serum-specific IgE Blood Test (RAST or ImmunoCAP)

- Measures antibodies to specific allergens
- Useful if skin testing isn't possible (eczema, antihistamines, etc.)

4. Other Investigations (if needed)

- **Nasal endoscopy**: to look for polyps or other causes of congestion
- **Imaging**: usually only if sinus disease suspected

Clinical immunology/ practical

Lecture 7

Atopic dermatitis

A **chronic, relapsing inflammatory skin disorder** characterized by **pruritus (itching), eczematous lesions, and a personal or family history of atopy** (such as asthma, allergic rhinitis, or food allergies).

main points:

- Often appears in **infancy or childhood**, but can persist into adulthood.
- Skin barrier dysfunction + immune dysregulation → increased susceptibility to irritants and allergens.
- Lesions are **red, dry, scaly, and itchy**, often affecting **flexural areas** (elbows, knees) in older children and adults, and **cheeks, scalp, trunk** in infants.

Diagnosis of Atopic Dermatitis

Diagnosis is **mainly clinical**—there is **no single definitive test**. It is based on **history, physical examination, and established criteria**.

1. Clinical History

- Chronic or relapsing course
- Intense itching (pruritus)
- Personal or family history of **atopy** (eczema, asthma, allergic rhinitis)
- Triggers: soaps, detergents, heat, stress, allergens

2. Physical Examination

- Acute lesions: red, oozing, crusted, vesicular
- Chronic lesions: thickened (lichenified), dry, scaly
- Distribution pattern:
    - Infants: face, scalp, trunk, extensor surfaces
    - Children/adults: flexural areas (elbows, knees, neck, wrists, ankles)
- Signs of scratching: excoriations, pigmentation changes

3. Diagnostic Criteria

Major criteria:

- Pruritus
- Typical morphology and distribution of lesions

- Chronic or relapsing course
- Personal or family history of atopy

Minor criteria (examples):

- Xerosis (dry skin)
- Ichthyosis
- Elevated serum IgE
- Early age of onset
- Susceptibility to skin infections

4. Additional Tests (if needed)

- **Serum IgE** or **allergy testing** (to identify triggers, not for diagnosis)
- **Skin biopsy**: rarely needed, only to rule out other conditions .

Lecture 8

Contact dermatitis

A **localized inflammatory skin reaction** caused by **direct contact with an irritant or allergen**, resulting in **redness, itching, and sometimes vesicles or blisters** at the site of exposure.

Key points:

- Two main types:
    1. **Irritant Contact Dermatitis (ICD):** non-immune reaction caused by direct chemical or physical damage to the skin (e.g., soaps, acids, detergents).
    2. **Allergic Contact Dermatitis (ACD): immune-mediated (type IV hypersensitivity)** reaction to allergens (e.g., nickel, poison ivy, cosmetics).
- Usually **localized** to the area of contact, but can spread if severe.

Diagnosis of Contact Dermatitis

Diagnosis is primarily **clinical**, based on **history and physical examination**, and sometimes **allergy testing**.

1. History

- Recent **exposure** to chemicals, plants, metals, cosmetics, or topical medications
- Timing: **minutes to hours** for irritant dermatitis; **24–72 hours** for allergic contact dermatitis
- History of **recurrent or worsening rash** at the same site after exposure
- Occupational or hobby exposures

2. Physical Examination

- Acute stage: erythema, swelling, vesicles, oozing
- Chronic stage: thickened, dry, scaly, lichenified skin
- Distribution: corresponds to **contact site** (hands, wrists, face, neck common)
- Borders may be sharp (allergic) or more diffuse (irritant)

3. Diagnostic Tests

- **Patch testing:**
  - Gold standard for **allergic contact dermatitis**
  - Small amounts of allergens applied on the skin for 48 hours
  - Readings at 48–72 hours for delayed hypersensitivity reaction
- **Skin biopsy:** rarely needed, only to rule out other skin conditions

Patch Testing Procedure Steps

1. Patient Preparation

- **Avoid antihistamines**: They don't usually interfere much, but corticosteroid creams on the back should be avoided for 1–2 weeks.
- **Clean skin**: The test is usually applied on **upper back or outer arm**, free from lesions, scarring, or sunburn.

2. Selection of Allergens

- Use standard allergen panels (e.g., **European or North American baseline series**)
- Additional **custom allergens** may be added based on patient history (cosmetics, metals, occupational chemicals).

3. Application

- **Allergens are placed in small chambers** on adhesive strips (called **patches**)
- Usually **20–30 allergens** per strip
- **Patches applied** to the **upper back**
- Label each allergen carefully

4. Occlusion Period

- **Leave patches on skin for 48 hours** (sometimes 24–48 depending on protocol)
- Patient must **avoid sweating, bathing, or vigorous activity** that might dislodge patches
- Avoid scratching the area .

5. First Reading (48 hours)

- **Remove patches carefully**
- Inspect skin for **erythema, papules, vesicles, or swelling**
- Record reaction using a **grading system** (e.g., +, ++, +++)

6. Second Reading (72–96 hours)

- Some reactions appear **delayed**, so a second reading is essential
- Record any new or persistent reactions

7. Interpretation

- **Positive reaction:** localized erythema, infiltration, papules, or vesicles at the site of allergen
- **Negative reaction:** no change from baseline skin
- **Irritant reaction:** may be diffuse, not sharply demarcated

8. Post-Test Care

- Patient may apply emollients or mild corticosteroid cream if skin is irritated
- Avoid scratching the area
- Discuss results and possible allergen avoidance

Lecture 9

Respiratory disease

Drug induced Respiratory disease

A **spectrum of respiratory disorders caused or worsened by medications**, through **allergic (immune-mediated) or non-allergic mechanisms**, affecting the **airways, lung parenchyma, pleura, or pulmonary vasculature**.

Common causative drugs:

- NSAIDs, aspirin
- Beta-blockers
- ACE inhibitors (cough)
- Chemotherapy drugs (e.g., bleomycin)
- Antibiotics (e.g., nitrofurantoin)

Diagnosis of Drug-Induced Respiratory Disease

Diagnosis is mainly **clinical**, based on **history, exclusion of other causes, and response to drug withdrawal**.

1. Clinical History (Most Important Step)

- Recent **initiation or dose change** of a drug
- Onset of respiratory symptoms after drug exposure
- Symptoms improve after stopping the drug
- Previous similar reactions to the same or related drug
- History of asthma, allergy, or aspirin sensitivity

2. Physical Examination

- Wheezing (bronchospasm)
- Crackles (interstitial involvement)
- Signs of hypoxia or respiratory distress
- Nasal inflammation (if rhinitis present)

3. Investigations

Used to support diagnosis and rule out other causes:

- **Pulmonary function tests (PFTs):**
  - Obstructive pattern in asthma/bronchospasm
- **Chest X-ray / HRCT:**
  - Interstitial infiltrates, pulmonary edema
- **Blood tests:**
  - Eosinophilia (drug-induced eosinophilic lung disease)
- **Arterial blood gases:** if severe respiratory compromise

Lecture 10

Eosinophilic Pneumonia

A group of lung disorders characterized by accumulation of eosinophils in the lung parenchyma and airspaces, leading to pulmonary inflammation and respiratory symptoms, with or without peripheral blood eosinophilia.

main points:

- Often associated with **allergic reactions, drugs, infections, or autoimmune diseases**
- Eosinophils cause lung inflammation and impaired gas exchange

Types:

- **Acute Eosinophilic Pneumonia (AEP)** – rapid onset, severe
- **Chronic Eosinophilic Pneumonia (CEP)** – gradual onset, relapsing

Diagnosis of Eosinophilic Pneumonia

Diagnosis is based on **clinical features, imaging, laboratory findings, and exclusion of other causes**

1. Clinical History

- Symptoms:
  - Dyspnea
  - Cough
  - Fever
  - Chest pain
  - Wheezing (sometimes)
- Acute (days–weeks) or chronic (weeks–months) onset
- History of:
  - Drug exposure
  - Smoking (especially new-onset smoking in AEP)
  - Atopy or asthma (common in CEP)
  - Parasitic infection or travel

2. Physical Examination

- Tachypnea
- Crackles on lung auscultation
- Signs of hypoxia
- Fever may be present

3. Laboratory Findings

- **Peripheral blood eosinophilia** (common in CEP; may be absent early in AEP)
- Elevated **IgE** (often present)
- Exclude parasitic infections (stool tests if indicated

4. Imaging :- **Chest X-ray**

5. Bronchoalveolar Lavage (BAL)

- **Diagnostic test**
- **Eosinophils >25%** of BAL cells strongly supports diagnosis

6. Lung Biopsy

- Rarely required
- Shows eosinophilic infiltration of alveoli and interstitium
- Done only if diagnosis remains uncertain

Lecture 11

Occupational lung diseases

Is a respiratory disorders caused by inhalation of harmful dusts, fumes, gases, vapors, or biological agents in the workplace, leading to **airway, interstitial, pleural, or alveolar lung damage**.

Diagnosis of Occupational Lung Diseases

Diagnosis requires a **high index of suspicion** and is based on **exposure history, clinical findings, investigations, and exclusion of other causes**.

1. Occupational & Exposure History (Most Important)

- Type of job and industry
- Specific agents exposed to (silica, asbestos, coal dust, chemicals, animal proteins)
- Duration and intensity of exposure
- Use of protective equipment
- Symptoms relation to work (worse during workdays, improve on holidays)
- Co-workers with similar symptoms

2. Clinical History & Examination

Symptoms:

Physical findings:

- Crackles (interstitial disease)
- Wheeze (occupational asthma)
- Clubbing (advanced disease)
- Signs of cor pulmonale (late stages)

3. Pulmonary Function Tests (PFTs)

- **Restrictive pattern** → interstitial lung disease
- **Obstructive pattern** → occupational asthma or Chronic Obstructive Pulmonary Disease (COPD)
- Reduced **DLCO (** Diffusing Capacity of the Lung for Carbon Monoxide **)** in interstitial diseases
- Serial PFTs (Pulmonary Function Tests) help assess progression

4. Imaging

- **Chest X-ray:** Nodules, reticular shadows, pleural plaques

5. Laboratory & Specialized Tests

- **Blood tests:** limited role
- **Immunologic tests:** for hypersensitivity pneumonitis
- **Bronchoalveolar lavage (BAL):** may show specific inflammatory cells
- **Lung biopsy:** rarely needed, only when diagnosis is uncertain .

Lecture 12

Autoimmune hemolytic anemia (AIHA)

A **hematologic disorder in which autoantibodies are produced against the patient's own red blood cells**, leading to **premature destruction (hemolysis)** and resulting in **anemia**.

Key points:

- Hemolysis can be **intravascular or extravascular**
- Caused by **IgG or IgM antibodies**
- May be **primary (idiopathic)** or **secondary** to diseases like SLE, lymphomas, infections, or drugs

Types:

- **Warm AIHA** – IgG antibodies, active at 37 °C (most common)
- **Cold AIHA** – IgM antibodies, active at cold temperatures
- **Mixed type**

Clinical immunology/ practical

Diagnosis of Autoimmune Hemolytic Anemia

Diagnosis is based on **evidence of hemolysis** and **proof of immune-mediated RBC destruction**.

1. Clinical Features

- Fatigue, weakness
- Pallor
- Jaundice
- Dark urine (more common in intravascular hemolysis)
- Splenomegaly (especially in warm AIHA)

2. Laboratory Evidence of Hemolysis

- **Low hemoglobin (anemia)**
- **Increased reticulocyte count**
- **Elevated indirect (unconjugated) bilirubin**
- **Increased LDH**
- **Decreased haptoglobin**

3. Peripheral Blood Smear

- **Spherocytes** (especially in warm AIHA)
- Polychromasia
- RBC agglutination (cold AIHA)

4. Direct Antiglobulin Test (DAT / Direct Coombs Test)

⭐ **Key diagnostic test**

- **Positive DAT** indicates antibodies and/or complement on RBC surface
- IgG positive → warm AIHA
- C3 positive → cold AIHA

5. Additional Tests (to find cause)

- ANA, anti-dsDNA → autoimmune disease
- Viral serology
- Lymphoma work-up
- Drug history

Lecture 13 & 14

Measurement of Warm Gammaglobulins

Warm Antibodies (Warm AIHA)

- Usually **IgG**
- React optimally at **37 °C**
- **Measured indirectly**, not by titration

Cold Antibodies (Cold AIHA)

- Usually **IgM**
- React best at **4 °C**
- **Measured by Cold Agglutinin Titration**

Cold Agglutinin Titration – Procedure Steps

1. Sample Collection

- Collect **patient blood**
- Keep sample **warm (37 °C)** until serum separation to prevents false agglutination before testing

2. Serum Preparation

- Separate **patient serum** by centrifugation
- Use serum (contains antibodies)

3. Serial Dilution :- make **serial two-fold dilutions** of serum (1:2, 1:4, 1:8, 1:16, 1:32, etc.)

4. Addition of Red Blood Cells

- Add **group O red blood cells** (2–5% suspension)

- Mix gently

5. Incubation at Cold Temperature

- Incubate tubes at **4 °C for 30–60 minutes**

6. Observation of Agglutination

- Examine tubes for **visible RBC agglutination**
- Identify the **highest dilution showing agglutination**

7. Confirmation

- Warm tubes to **37 °C**
- **Disappearance of agglutination confirms cold antibody**

8. Reporting the Titer

- The **titer** = **highest serum dilution showing agglutination at 4 °C**
- Example: - Agglutination up to 1:64 → **Cold agglutinin titer = 64**

Interpretation of Cold Agglutinin Titer

- **Low titer (<1:64)** → often clinically insignificant
- **High titer (≥1:64)** → suggests **Cold Agglutinin Disease**
- Higher titers = more severe hemolysis risk

Warm Antibody Detection (for comparison)

Warm antibodies are detected by:

- **Direct Coombs (DAT)** → IgG on RBC surface
- **Indirect Coombs (IAT)** → free IgG antibodies in serum
  Warm antibodies are NOT measured by titration

Direct Coombs Test (DAT) – Procedure Steps

Purpose

Clinical immunology/ practical

To detect **IgG antibodies and/or complement (C3)** already **bound to the patient's red blood cells in vivo**.

1. Sample Collection

- Collect **patient's venous blood** in an **EDTA tube**
- EDTA prevents complement activation after collection

2. Washing of Red Blood Cells

- Separate **red blood cells (RBCs)** from plasma
- Wash RBCs **3–4 times with normal saline**
  □ removes unbound antibodies and plasma proteins

3. Preparation of RBC Suspension

- Prepare a **2–5% suspension** of washed RBCs in saline

4. Addition of Coombs Reagent

- Add **anti-human globulin (AHG) reagent**
  - Contains antibodies against **human IgG and/or complement (C3)**

5. Centrifugation

- Centrifuge the mixture to bring RBCs together

6. Reading the Result

- Gently resuspend the cell button
- Look for **visible agglutination**

7. Interpretation

- **Positive DAT:** agglutination present
  → IgG and/or C3 is bound to RBCs
- **Negative DAT:** no agglutination

8. Control Check (Validity Test)

- If result is negative, add **Coombs control cells** (IgG-coated RBCs)
- Agglutination must occur to confirm the test is valid
  - If no agglutination → test is invalid

Result Reporting

- Report as:
  - **DAT positive (IgG)**
  - **DAT positive (C3)**
  - **DAT positive (IgG + C3)**

Indirect Coombs Test (IAT) – Procedure Steps

Purpose

To detect **free (unbound) IgG antibodies in the patient's serum** that can react with red blood cells **in vitro**.

1. Sample Collection

- Collect **patient's blood**
- Separate **serum** (contains antibodies)

2. Addition of Test Red Blood Cells

- Add **known antigen-positive RBCs** (screening or donor cells) to patient serum

3. Incubation

- Incubate mixture at **37 °C for 30–60 minutes** , allows antibodies in serum to bind to RBC antigens

4. Washing of Red Blood Cells

- Wash RBCs **3–4 times with normal saline** to removes unbound antibodies

5. Addition of Coombs Reagent

- Add **anti-human globulin (AHG) reagent** (contains anti-IgG ± anti-C3)

6. Centrifugation :- Centrifuge to bring RBCs together

7. Reading the Result

- Gently resuspend cell button
- Observe for **agglutination**

8. Interpretation

- **Positive IAT:** agglutination present→ serum contains antibodies against RBC antigens
- **Negative IAT:** no agglutination

9. Control Check

- If negative, add **Coombs control cells**
- Agglutination confirms test validity

Uses of Indirect Coombs Test

- Antibody screening before blood transfusion
- Cross-matching
- Antenatal screening (Rh incompatibility)
- Detection of warm antibodies in AIHA

Lecture 15

Acute & Chronic Leukemia
&
Lymphoid and Myloid Leukemia

Acute Leukemia

A rapidly progressing malignancy of hematopoietic stem cells, characterized by accumulation of immature blast cells in the bone marrow and blood, leading to bone marrow failure. Rapid onset, life-threatening if untreated

- **Types**:
  - **Acute Lymphoblastic Leukemia (ALL)** – lymphoid lineage

    - **Acute Myeloid Leukemia (AML)** – myeloid lineage
- Symptoms often develop over **days to weeks**

2. Chronic Leukemia

Chronic Leukemia:
A **slowly progressing malignancy of mature but functionally abnormal white blood cells**, leading to **gradual accumulation in blood and bone marrow**.

    - **Chronic Lymphocytic Leukemia (CLL)** – lymphoid lineage
    - **Chronic Myeloid Leukemia (CML)** – myeloid lineage
- Symptoms develop over **months to years**

Diagnosis of Leukemia

Diagnosis is based on **clinical features, laboratory tests, and bone marrow examination**.

1. Clinical Features

Acute Leukemia:

- Fatigue, pallor (anemia)
- Fever, infections (neutropenia)
- Bleeding/bruising (thrombocytopenia)
- Bone pain (marrow expansion)
- Lymphadenopathy, hepatosplenomegaly

Chronic Leukemia:

- Often asymptomatic early
- Fatigue, weight loss, night sweats
- Lymphadenopathy, splenomegaly
- Easy bruising or bleeding (late)

2. Laboratory Findings

- **Complete Blood Count (CBC):**
    - **Acute:** anemia, thrombocytopenia, leukocytosis with **blasts**
    - **Chronic:** leukocytosis with **mature but abnormal cells**
- **Peripheral blood smear:**

  - **Acute:** many immature blasts
  - **Chronic:** predominance of mature cells, e.g., lymphocytes in CLL
- **Other labs:**
  - Elevated LDH
  - Coagulation abnormalities in some acute leukemias (esp. AML M3/APL)

3. Bone Marrow Examination

- **Acute leukemia:** ≥20% blasts in bone marrow
- **Chronic leukemia:** hypercellular marrow with mature abnormal cells
- Bone marrow cytochemistry, flow cytometry, cytogenetics help subtype leukemia

4. Immunophenotyping & Cytogenetics

- **Flow cytometry:** distinguishes **myeloid and lymphoid lineage**
- **Cytogenetics / molecular testing:**
  - **Philadelphia chromosome (BCR-ABL)** in CML
  - **t(15;17)** in AML M3 (APL)
  - Other translocations help prognosis and treatment

5. Ancillary Tests

- Lumbar puncture in ALL to check CNS involvement
- Imaging if organomegaly is suspected

Diagnosing Chronic Lymphocytic Leukemia (CLL)

Diagnosing Chronic Lymphocytic Leukemia (CLL) involves a combination of medical history, physical examinations, and various diagnostic tests.

1. Medical History and Physical Examination

Medical History: The doctor will review your personal and family medical history, symptoms, and risk factors.

Physical Examination: The doctor will check for signs such as swollen lymph nodes, an enlarged spleen, or an enlarged liver.

2. Blood Tests

Blood tests are often the first step in diagnosing CLL and may include:

Complete Blood Count (CBC): Measures the number of red blood cells, white blood cells, and platelets. A high lymphocyte count (lymphocytosis) is a key indicator of CLL.

Peripheral Blood Smear: Examines the size, shape, and appearance of blood cells under a microscope.

Flow Cytometry and Immunophenotyping: Identifies specific markers on the surface of lymphocytes to confirm CLL.

Cytogenetic Tests (e.g., FISH): Detects chromosomal abnormalities in leukemia cells, which can help predict disease progression.

Beta-2 Microglobulin (B2M): Measures protein levels that may indicate disease severity.

Lactate Dehydrogenase (LDH): Assesses cell turnover and disease activity.

3. Bone Marrow Aspiration and Biopsy

Purpose: If blood tests are inconclusive, a bone marrow biopsy may be performed to confirm the diagnosis.

Procedure: A hollow needle is used to extract a small sample of bone marrow and bone, typically from the pelvic bone. The sample is analyzed for abnormal cells and other markers of leukemia.

4. Imaging Tests

Imaging tests are used to assess the extent of the disease and detect any organ involvement:

CT Scan: Provides detailed images of lymph nodes and organs.

MRI: Offers high-resolution images of soft tissues.

PET-CT Scan: Highlights areas of active cancer or inflammation.

Ultrasound: May be used to evaluate organ enlargement

5. Lymph Node Biopsy

When Needed: If lymph nodes are significantly enlarged, a biopsy may be performed to confirm the presence of leukemia cells.

Procedure: A portion or the entirety of a lymph node is removed for microscopic examination.

6. Staging

Once CLL is diagnosed, staging is performed to determine the extent of the disease. The Rai staging system is commonly used:

Stage 0: Lymphocytosis without organ enlargement or anemia.

Stage I: Lymphocytosis with enlarged lymph nodes.

Stage II: Lymphocytosis with an enlarged spleen or liver.

Stage III: Lymphocytosis with anemia.

Stage IV: Lymphocytosis with thrombocytopenia (low platelet count).

Key Notes

CLL is often detected incidentally during routine blood tests before symptoms appear.

Some patients may not require immediate treatment and are monitored through "active surveillance" or "watchful waiting."

Advanced diagnostic techniques, such as molecular genetics, can provide insights into the disease's aggressiveness and guide treatment planning.

Accurate diagnosis is critical for determining the appropriate course of action and ensuring effective management of CLL.