INTRODUCTION TO PATHOLOGY

PRECLINICAL HUMAN BASIC PATHOLOGY

MBS 220 @ (2024)

ALFRED MACHIKO

LEARNING OBJECTIVES

At the end of this lecture, Students should be able to;

- To define Pathology & explain why we study Pathology
- Discuss the history and evolution of pathology
- Discuss the concept of health and disease
- To highlight the branches of pathology
- Familiarise with the basic language pathology
- To understand basic disease nomenclature
- To discuss the factors that affect disease
- To discuss the core aspects of pathology

INTRODUCTION

- Pathos – suffering, logy – study -“Scientific study & diagnosis of disease"
- Pathology may be defined as the "scientific study of the molecular, cellular, tissue, or organ system response to injurious agents or adverse influences.“
- The alterations that occur when abnormal influences (bacteria, viruses, etc.) affect cells, tissues, or body systems
- Provides a logical means of relating the knowledge of normal structure and function **(anatomy and physiology)** to abnormal structure and function as encountered in disease

CONT'D

- Thus, the factual background or knowledge needed for logical reasoning when solving real-life disease conditions is provided.
- Follows the morbid process from its inception to its termination, and investigates the lesions produced
- By the use of molecular, microbiologic, immunologic & morphologic techniques, pathology tries to explain the WHYS & WHEREFORES of signs & symptoms manifested by patients while providing a rational basis for clinical care & therapy
- Therefore, a sound knowledge of pathology is the foundation of a solid understanding of disease as it occurs in the living individual

WHY STUDY PATHOLOGY

- The function of a clinician is to diagnose and treat the disease
- Now several steps are involved in the evolution of a clinician. In the many steps it is necessary to learn the normal development, structure, and function of the body and this occurs through embryology, anatomy, histology, genetics, pharmacology, biochemistry and physiology
- The development of the structural and functional changes in cells, tissues, fluids and organs that result in malfunction and disease is studied in pathology
- A clinician cannot rationally diagnose and treat without understanding the disease process with which he/she is dealing with
- Therefore, pathology is the ‘corner-stone’ of medicine

CONT’D

- “Your practice of medicine will be as good as your understanding of pathology.” – *Sir William Osler*,

HISTORY AND EVOLUTION OF PATHOLOGY

- Pathology as the scientific study of disease processes has its deep roots in medical history
- There has been desire as well as need to know more about the causes, mechanisms and nature of diseases
- Answers to these questions have evolved over the centuries — from supernatural beliefs to the present state of our knowledge of modern pathology

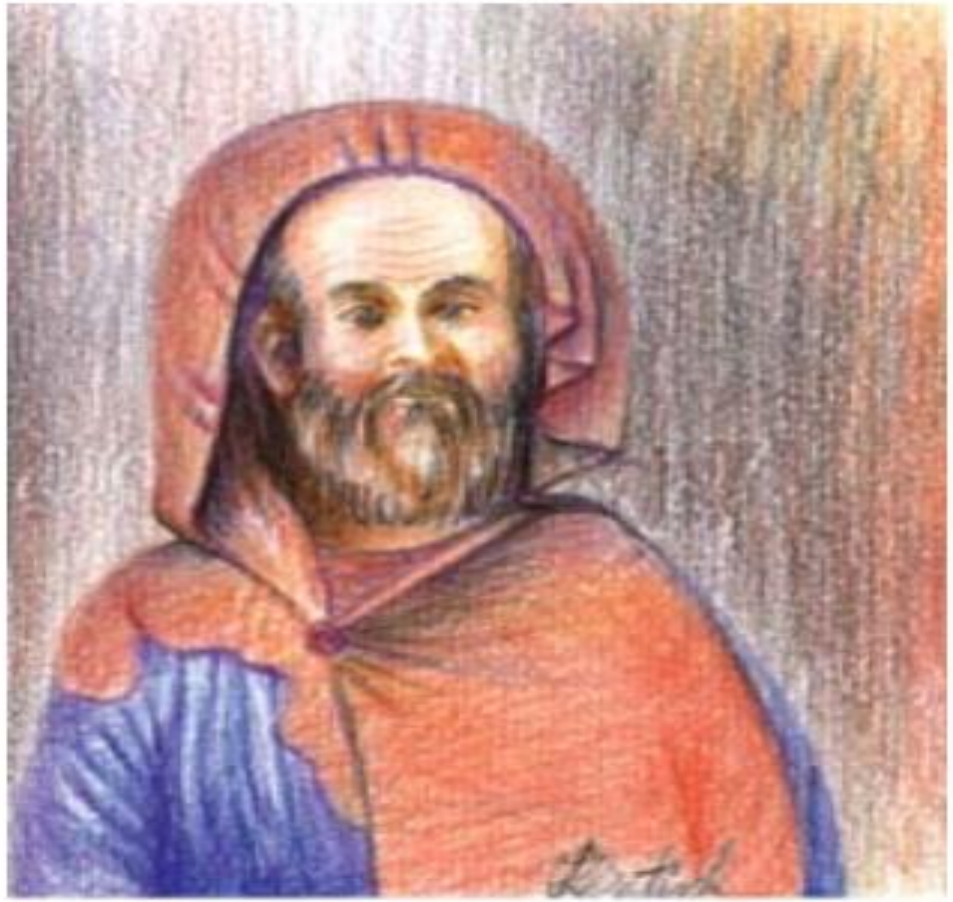
FROM RELIGIOUS BELIEFS AND MAGIC TO RATIONAL APPROACH
(PREHISTORIC TIME TO AD 1500)

- Religion, magic and medical treatment were quite linked to each other in those times
- Earliest concept of disease understood by the patient and the healer was the religious belief that disease was the outcome of ‘curse from God’ or the belief in magic that the affliction had supernatural origin from ‘evil eye of spirits.
- Link between medicine and religion became so firmly established throughout the world that different societies had their gods and goddesses of healing;
- For example: mythological Greeks had *Asclepios* and *Apollo* as the principal gods of healing, *Dhanvantri* as the deity of medicine in India, and orthodox Indians’ belief in *Mata Sheetala Devi* as the pox goddess

CONT'D

- Real practice of medicine began with Hippocrates the great Greek clinical genius of all times and regarded as 'the father of medicine'
- Hippocrates followed rational and ethical attitudes in practice and teaching of medicine as expressed in the collection of writings of that era
- He firmly believed in study of patient's symptoms and described methods of diagnosis

Hippocrates



CONT'D

- Some of the major Hippocratic methods can be summarized as under:
  - Observe all objectively.
  - Study the patient rather than the disease.
  - Evaluate honestly.
  - Assist nature.
- *Celsus* first described four cardinal signs of inflammation— rubor (redness), tumor (swelling), calor (heat), and dolor (pain)
- Galen postulated humoral theory, later called Galenic theory

FROM HUMAN ANATOMY TO ERA OF GROSS PATHOLOGY (AD 1500 to 1800)

- The Renaissance began from Italy in late 15th century and spread to whole of Europe
- Development of human anatomy took place during this period with the art works and drawings of human muscles and embryos by famous Italian painter Leonardo da Vinci
- Dissection of human body was started by *Vesalius* (1514– on executed criminals.
- His pupils, *Gabriel Fallopius* who described human oviducts (Fallopian tubes) and *Fabricius* who discovered lymphoid tissue

CONT’D

- *Antony van Leeuwenhoek* (1632–1723), a cloth merchant by profession in Holland, during his spare time invented the first ever microscope
- He also introduced histological staining in 1714 using saffron to examine muscle fibres
- *Marcello Malpighi* (1624–1694) used microscope extensively and observed the presence of capillaries - Malpighi is known as ‘the father of histology.’

CONT'D

- Italian anatomist-pathologist, *Giovanni B. Morgagni* - published his life-time experiences based on 700 postmortems and their corresponding clinical findings
- Laid the foundations of clinicopathologic methodology in the study of disease and introduced the concept of clinicopathologic correlation (CPC), establishing a coherent sequence of cause, lesions, symptoms, and outcome of disease
- Sir Percival Pott (1714–1788), famous surgeon in England
- John Hunter (1728 – 1793), a student of Sir Percival Pott, rose to become greatest surgeon-anatomist of all times and he, together with his elder brother William Hunter (1718–1788) who was a reputed anatomist-obstetrician

CONT'D

- *Richard Bright* (1789–1858), *Thomas Addison* (1793–1860), *Thomas Hodgkin* (1798–1866)
- *Xavier Bichat* (1771–1802) in France described that organs were composed of tissue and divided the study of morbid anatomy into General Pathology and Systemic Pathology
- *R.T.H. Laennec* (1781–1826), another French physician - described several lung diseases (tubercles, caseous lesions, miliary lesions, pleural effusion, bronchiectasis), chronic sclerotic liver disease (later called Laennec's cirrhosis) and invented stethoscope

ERA OF TECHNOLOGY DEVELOPMENT AND CELLULAR PATHOLOGY (AD 1800 TO 1950s)

- Correlation of clinical manifestations of disease with gross pathological findings at autopsy became the major method of study of disease
- Pathology started developing as a diagnostic discipline in later half of the 19th century with the evolution of cellular pathology
- The discovery of existence of disease-causing micro-organisms was made by French chemist *Louis Pasteur*
- *G.H.A. Hansen* (1841–1912) in Germany identified Hansen's bacillus as causative agent for leprosy (Hansen's disease) in 1873
- *Edward Jenner*, immune tolerance and allergy

CONT'D

- *Ilya Metchnikoff* (1845-1916), a Russian zoologist, introduced the existence of phenomenon of phagocytosis
- *Paul Ehrlich* (1854–1915), German physician
- *Christian Gram* (1853–1938), Danish physician, who developed bacteriologic staining by crystal violet
- *D.L. Romanowsky* (1861–1921), Russian physician, who developed stain for peripheral blood film using eosin and methylene blue derivatives
- *Robert Koch* (1843–1910), German bacteriologist who, besides Koch's postulate and Koch's phenomena, developed techniques of fixation and staining for identification of bacteria

CONT’D

- *May-Grunwald* in 1902 and Giemsa in 1914 developed blood stains
- *Sir William Leishman* (1865–1926) who described Leishman’s stain for blood films in 1914
- *Robert Feulgen* (1884–1955) who described Feulgen reaction for DNA staining and laid the foundations of cytochemistry and histochemistry
- *Rudolf Virchow* (1821–1905) in Germany is credited with the beginning of microscopic examination of diseased tissue at cellular level and thus began histopathology as a method of investigation.
- Virchow gave two major hypotheses:
  - All cells come from other cells.
  - Disease is an alteration of normal structure and function of these cells

CONT’D

- *Karl Landsteiner* (1863–1943) described the existence of major human blood groups in 1900
- *Ruska* and *Lorries* in 1933 developed electron microscope
- *George N. Papanicolaou* (1883–1962), a Greek-born American pathologist, in 1930s who is known as ‘father of exfoliative cytology’
- Another pioneering contribution in pathology in the 20th century was by an eminent Canadian pathologist teacher-author, *William Boyd* (1885–1979)

MODERN PATHOLOGY (1950s TO PRESENT TIMES)

- Possible to study diseases at molecular level, and provide an evidence-based and objective diagnosis and enable the physician to institute appropriate therapy
- Major impact of advances in molecular biology are in the field of diagnosis and treatment of genetic disorders, immunology and in cancer
- Description of the structure of DNA of the cell by Watson and Crick in 1953.
- Identification of chromosomes and their correct number in humans (46) by Tijo and Levan in 1956

CONT'D

- Identification of Philadelphia chromosome t(9;22) in chronic myeloid leukaemia by *Nowell* and *Hagerford* in 1960 as the first chromosomal abnormality in any cancer
- In Situ Hybridization introduced in 1969 in which a labelled probe is employed to detect and localize specific RNA or DNA sequences 'in situ' (i.e. in the original place).
- Recombinant DNA technique developed in 1972 using restriction enzymes to cut and paste bits of DNA.
- In 1983, *Kary Mullis* introduced polymerase chain reaction (PCR) i.e. "xeroxing" DNA fragments which revolutionized the diagnostic molecular genetics.

CONT'D

- Flexibility and dynamism of DNA invented by *Barbara McClintock* for which she was awarded Nobel prize in 1983
- In 1997, *Ian Wilmut* and his colleagues at Roslin Institute in Edinburgh, successfully used a technique of somatic cell nuclear transfer to create the clone of a sheep; the cloned sheep was named Dolly
- In 1998, researchers in US found a way of harvesting stem cells, a type of primitive cells, from embryos and maintaining their growth in the laboratory
- In April 2003, Human Genome Project (HGP) consisting of a consortium of countries

CONCEPT OF HEALTH & DISEASE

HEALTH

- As generally used, the term **"health"** refers to the **"state in which an individual is living in complete harmony with his/her environment,"** it is a relative state.
- All body functions are performed normally even though lesions may be present in organs and/or tissues.
- It should be remembered that the transitional zone between health and disease is difficult to define.

CONCEPT OF HEALTH & DISEASE – CONT'D

DISEASE

- A disease may be defined as a **"state in which an individual exhibits an anatomical, physiological, or biochemical deviation from the normal.**"
- As generally used, the term "disease" is employed to describe a state in which there is **sufficient departure** from the normal for clinical signs or symptoms to be produced.
- It may affect the whole body or any of its parts, and the disease's aetiology, pathology and prognosis may be known or unknown

BRANCHES OF PATHOLOGY

General pathology

- Refers to the study of the basic alterations in tissues. These are changes that apply to most of the organs or tissues of the body and include such things as atrophy, necrosis and inflammation.

Systemic pathology

- Refers to the study of the diseases of the organ systems of the body such as the respiratory system, digestive system and nervous system. It is the application of knowledge of general pathology in order to study specific diseases, organ by organ.

BRANCHES OF PATHOLOGY – CONT'D

Gross pathology (macroscopic pathology, pathological anatomy, morbid anatomy)

- Refers to the study of disease in which tissues and organs are examined with the unaided eye.

Cellular pathology (microscopic pathology, histopathology)

- Refers to the study of diseased tissues and organs with the aid of a microscope.

Surgical pathology

- Refers to the study of tissues removed at the time of surgery.

Clinical pathology (Laboratory Medicine)

- Refers to the study of disease by examination of blood, urine, faeces, skin scrapings, etc.

BRANCHES OF PATHOLOGY – CONT'D

Immunopathology

- Refers to the study of diseases associated with abnormalities of the immune mechanisms of the body.

Chemical pathology

- The study of chemical alterations in body fluids and tissues that result from disease e.g. the analysis of liver enzymes (ALT, AST) in hepatitis.

TWO MANY CATEGORIES OF DISEASE

- **Acute disease -** characterised by sudden onset and short duration. The outcome of acute disease may be:
  - Resolution due to host defence response or clinical therapy
  - Progression to chronic disease
  - Death
- **Chronic disease -** characterised by insidious onset and protracted course. The outcome of chronic disease may be:
  - Progressive destruction of tissue, which compromises function and endangers life
  - The halting of the course of disease, with tissue repair by scarring

TYPES OF AGENTS CAUSING DISEASE

1. Infectious organisms

- Viruses
- Bacteria
- Fungi
- Parasites

2. Physical

- Trauma
- Pressure
- Heat
- Cold
- Radiation

TYPES OF AGENTS CAUSING DISEASE – CONT'D

3. Chemical

- Toxic organic and inorganic substances
- Toxins produced by infectious organisms

4. Nutritional

- Deficiencies of vitamins and trace elements
- Excess of particular diets e.g. lipids, sugar, etc.

5. Genetic defects

- There is a very wide range of potential defects; some are incompatible with life whilst others affect specific systems within the body

BASIC LANGUAGE OF PATHOLOGY

- Lesion - **"structural or morphological alterations associated with a diseased state in an individual“**
- Lesions may be recognized with the naked-eye (**gross lesions**), with the aid of a light microscope (**microscopic lesions**), or with the aid of an electron microscope (**ultra-structural lesions**).
- Biochemical or functional “lesions” are recognized as changes that result from disturbed function
- Pathognomonic Lesion**:** is a change that is specifically characteristic of a disease.

CONT'D

- "Clinical signs" refer to any "functional evidence of disease that can be determined objectively by the observer" e.g. lameness, salivation, increased respiratory efforts, etc. (*clinical signs are seen only in the living individual*).
- The term clinical symptoms should be reserved for any "functional evidence of disease that can be determined subjectively by the patient" (feeling of abdominal discomfort, etc.).
- "Syndrome" refers to a group of symptoms and other changes in the body's functions which, when taken together, show that a particular disease is present.

CONT’D

- “Prognosis" refers to the “**probable outcome of a disease in a living individual**”.
- A pathologist’s/clinician's estimate of the severity and possible result of a disease.
- Outcomes include:
  - Complete recovery
  - Partial healing
  - Death

CONT’D

- “Diagnosis" refers to the **"determination of the nature of a disease expressed in a concise manner.“**
- **A morphological or anatomical** diagnosis is based on the location and nature of the lesion e.g. **haemorrhagic enteritis, etc**.
- **Aetiologic diagnosis** is made on the basis of the cause e.g. **fungal pneumonia, etc**.
- **Definitive diagnosis** is made on the basis of the specific disease entity involved e.g. **aspergillosis etc**
- A **clinical diagnosis** is made on the basis of clinical signs observed in the living individual e.g. **bloody diarrhoea**

CONT'D

- **Autopsy** - Refers to gross examination of the cadaver by systematic dissection in order to evaluate any abnormal changes **(lesions)** that may be present.
- A complete autopsy refers to all post-mortem examinations including gross, microscopic, toxicological, and microbiological examinations.
- **Biopsy** refers to the removal and examination of tissue obtained from the living body.

CONT’D

- “**Somatic death**" refers to death of the entire body; there is cessation of all body functions.
- The absence of **heart beat, pulse, respiration or brain waves** has been used to define somatic death
- "**Postmortem changes**" refer to cell death which accompanies or occurs after death of the entire body (**somatic death).**
- Ante mortem changes refer to those alterations that occur in cells, tissues, organs, etc. prior to somatic death or in the living individual

CONT'D

- **Post mortem autolysis** refers to self-digestion by **enzymes** that are present within or released into the cytoplasm of cells after death. It is due to total diffuse anoxia.

- **Post mortem putrefaction (rotting)** refers to the decomposition of tissues by bacterial **enzymes** after death of the entire body
- **Rigor mortis** refers to stiffening of all muscles after death. It is related to a progressive decrease in oxygen, ATP, creatinine phosphate, and pH of muscles

CONT'D

- **Livor mortis** refers to the discolouration of dependent parts of the body after death as a result of red blood cell destruction. It is seen as the "gravitational settling of blood" in the lower (dependent) parts of the body after death.
- **Algor mortis** refers to the cooling of the body after death.

MEDICAL TERMINOLOGY

Breaking it down: Word parts

- Beginning = Prefix
  - Description: Number/amount, size, location, colour etc
- Middle = Root
  - Subject: relating to a body part
- Ending = Suffix
  - Condition: Pertaining to a process or procedure; amount, location et

CONT'D

Prefixes: Size and Amount

- Macro - Large, Visible to the naked eye
- Micro - Small, not visible to the naked eye
- Hyper - High, above, normal, elevated
- Hypo - Low, below, decreased, deficient
- Eu/Norm- Normal
- Pan/omini - All
- A/An - Absent
- Megalo - Large, larger than average

Prefixes: Numerical and Speed

- Mono = Single
- Di = two, double
- Tri = three, triple
- Quad = four
- Poly = many, a lot
- Oligo = few
- Tachy = Rapid, fast
- Brady/brachy = slow

CONT’D

Prefixes: Location

- Epi/Peri/Circum = around
- Endo/intra = Inside, inner,interior
- Exo/extra = Outside, outer, exterior
- Inter = between
- Trans = across
- Dia/Per = through, complete
- Para = near by
- Juxta = Next to, beside, adjacent

Prefixes: Movements

- Ab = away
- Ad = toward
- Dis = separate

CONT’D

Prefixes: Colour

- Chromo/chromate = colour
- Leuko = white
- Erythro = Red
- Cyano = blue
- Chloro = green
- Melano = black

DISEASE PREFIXES - General

- Ana –back, again, up (anaplasia
- Dys – bad , difficult, defective, abnormal (dysplasia)
- Meta – after, beyond, change (metaplasia)
- Neo – new (neoplasia)
- Homeo – body
- Cephalo –head
- Cerv- neck
- Throaco – chest
- Abdomino – abdimonal
- Perito – peritoneum
- Derm- skin
- Neuro - neuron

DISEASE SUFFIXES

- -itis – inflammation (arthritis)
- -oma – tumour or mass (teratoma)
- -oid – resemblance (rheumatoid arthritis)
- -osis – condition or abnormal condition (ketoacidosis)
- -penia – lack of /deficiency (thrombocytopenia)
- -cytosis – increased number of cells (leucocytosis)
- -ectasis – dilation (bronchiectasis)
- -plasia – disorder growth (hyperplasia)
- -opthy – abnormal state (neuropathy)

FACTORS INVOLVED IN THE DEVELOPMENT OF DISEASE

- The host - the individual's nutritional and immune status, which may be modified by recent or concurrent disease, and/or previous exposure to the agent(s) responsible. The host defences are important in determining the **presentation** of the disease
- The disease-causing agent(s**) -** An aetiological agent's capacity to produce disease depends upon the dose and the virulence of the agent.
- Environmental factors – poor sanitation and interaction with carriers provide necessary conditions for disease causation

FOUR ASPECTS OF DISEASE PROCESS THAT FORM THE CORE OF PATHOLOGY

1. **Etiology/Aetiology/Cause** – “**study of the cause of a disease."**

- **Predisposing causes of diseases -** factors that make an individual more susceptible to a disease (**damp weather, poor ventilation, etc.**)

- **Exciting causes of disease:** refer to those factors that are directly responsible for a disease **(bacteria, viruses, hypoxia, chemical agents, etc**.)

CONT'D

- Classes of Aetiologic factors ;
- **Intrinsic** (genetic e.g. inherited mutations & disease-associated gene variants or polymorphisms) e.g. Down syndrome
- **Extrinsic** (acquired e.g. infectious, nutritional, chemical, physical) e.g. broken bones, allergy
- **Multifactorial** (Combination of genetic & acquired) e.g. Cancer, diabetes

CONT'D

2. Pathogenesis – Sequence of events in the response of cells or tissues to etiologic agent; mechanism of its deviation from **initial stimulus** to the ultimate expression of disease

- Examples
  - Inflammation
  - Degeneration
  - Neoplasm
  - Immune response
- Remains one of the main domains of pathology
- Develops in latent or incubation periods
  - Carcinogenesis (Years)
  - Infection (Days or Weeks)

CONT'D

3. Molecular & Morphological Changes - Structural alterations induced in cells and tissues of body that either characteristic of disease or diagnostic of an etiologic process

- Examples;
  - Spaces occupying lesions
  - Deposition
  - Abnormally sited tissue
  - Loss of healthy tissue
  - Obstruction
  - Distension or rupture

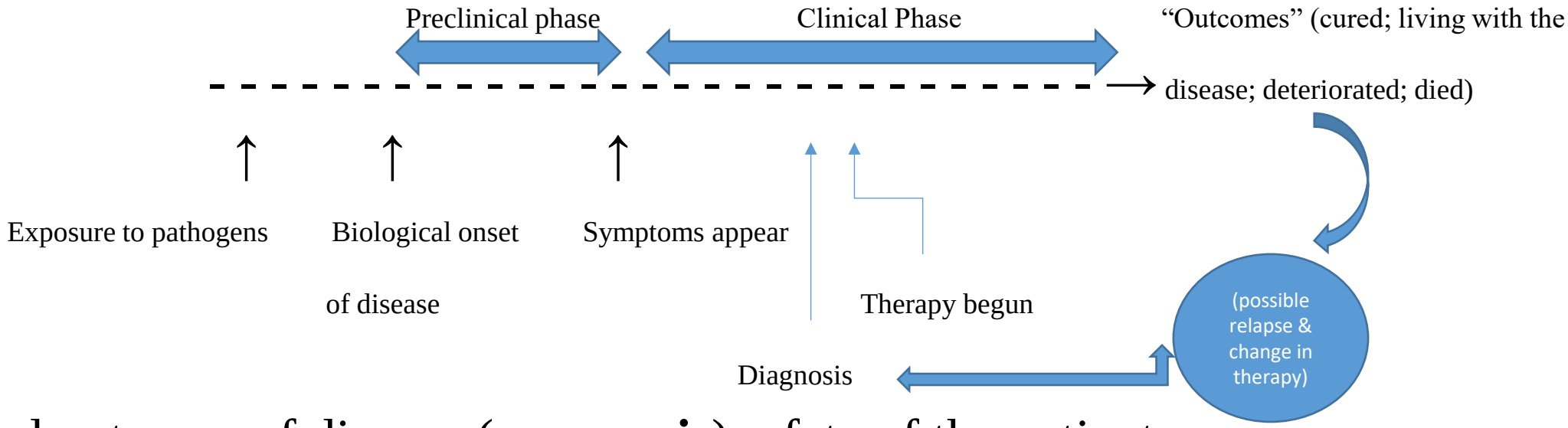
CONT'D

4. Functional derangements and Clinical Manifestations - functional consequences of the morphologic change which determine clinical features(**Symptoms & Signs**),

- Examples;
  - Pain
  - Fever
  - Nausea
  - Malaise
  - Swellings
  - Bowel movements
  - Breathing alteration
  - Skin rash

CONT'D

- Progress (**Clinical course**)



- And outcome of disease (**prognosis**) – fate of the patient
  - A good prognosis – little or no lasting effects
  - A bad prognosis – detrimental effects as a consequences

CONT'D

- Clinical significance of the morphologic and functional changes together with results of other investigations help to arrive at an answer to what is wrong *(diagnosis)*
- What is going to happen *(prognosis)*
- What can be done about it *(treatment)*
- Finally what should be done to avoid complications and spread *(prevention)* (i.e. 'what' of disease)

CONT’D

Complications and sequelae

- Infections spreading to different organs or other areas of the body
- Tumours metastasising
- Long lasting damage e.g. nervous damage leads to paralysis, behavioural changes

METHODS USED IN THE STUDY OF PATHOLOGY

1. Gross examination of organs
    a. Gross examination of organs on the exam has two major components
        i. Determining what organ are you looking at!
        ii. Determining what's wrong (the pathology)
    b. Useful gross features
        i. Size
        ii. Shape
        iii. Consistency
        iv. Colour
2. Microscope examination of tissue
    a. Light microscopy
        i. Hematoxylin and Eosin (H&E) – Gold standard

CONT'D

b. Other histochemical stains (Chemical reaction)
   i. Prussian blue
   ii. Congo red
   iii. Periodic acid-Schiff (PAS)
   iv. Acid fast (ziel-nelson, Fite)
   v. Gram stain
   vi. Trichrome
   vii. Reticulin

c. Immunohistochemical (Antibody) stain
   i. Cytokeratin
   ii. Vimentin
   iii. Desmin
   iv. Prostate specific antigen
   v. Many others

CONT’D

- 3. Ancillary techniques
  a. Immunofluorescence microscopy IFM
     i. Renal diseases
     ii. Autoimmune diseases
  b. Transmission electron microscopy EM
     i. Renal diseases
     ii. Neoplasms
     iii. Infections genetic disorders
- 4. Molecular techniques
  a. Protein electrophoresis
  b. Southern and Western blotting
  c. Polymerase chain reaction

TABLE 2.1: Common Special (Histochemical) Stains in Surgical Pathology (in Alphabetic Order of Constituents).

Stain	Component/Tissue	Dyes	Interpretation
A. AMYLOID			
1. *Congo red with polarising light*	Amyloid	Congo red	Green-birefringence: amyloid
2. *Toluidine blue*	Amyloid	Toluidine blue	Orthochromatic blue: amyloid
B. CARBOHYDRATES			
3. *Periodic acid-Schiff (PAS)*	Carbohydrates (particularly glycogen), all mucins	Periodic acid, Schiff reagent (basic fuchsin)	Glycogen and other carbohydrates: magenta Nuclei: blue
4. *Mucicarmine/Best's carmine*	Acidic mucin	Carmine	Mucin: red Nuclei: blue
5. *Alcian blue (AB)*	Acidic mucin	Alcian blue (at pH 2.5)	Acid mucin: blue Nuclei: red
6. *Combined AB-PAS*	Neutral mucin	Alcian blue	Acid mucin: blue Neutral mucin: magenta Nuclei: pale blue
C. CONNECTIVE TISSUES			
7. *Van Gieson's*	Extracellular collagen	Picric acid, acid fuchsin, celestin blue-haemalum	Nuclei: blue/black Collagen: red Other tissues: yellow
8. *Masson's trichrome*	Extracellular collagen	Acid fuchsin, phospho-molybdic acid, methyl blue, celestin blue-haemalum	Nuclei: blue/black Cytoplasm, muscle, red cells: red Collagen: blue
9. *Phosphotungstic acid-haematoxylin (PTAH)*	Muscle and glial filaments	Haematoxylin, phosphotungstic acid, permanganate, oxalic acid	Muscle striations, neuroglial fibres, fibrin: dark blue Nuclei: blue Cytoplasm: pale pink
10. *Verhoeff's elastic*	Elastic fibres	Haematoxylin, Ferric chloride, iodine, potassium iodide	Elastic fibres: black Other tissues: counter-stained
11. *Gordon and Sweet's*	Reticular fibres	Silver nitrate	Reticular fibres: black Nuclei: black or counterstained

E. MICRO-ORGANISMS

15. *Gram's*	Bacteria (cocci, bacilli)	Crystal violet, Lugol's iodine, neutral red	Gram-positive, keratin, fibrin: blue Gram-negative: red
16. *Ziehl-Neelsen's (Acid-fast)*	Tubercle bacilli	Carbol fuchsin, methylene blue (differentiate in acid-alcohol)	Tubercle bacilli, hair shaft, actinomyces: red Background: pale blue
17. *Fite-Wade*	Leprosy bacilli	Carbol fuchsin, methylene blue (decolorise in 10% sulfuric acid)	Lepra bacilli: red Background: blue
18. *Grocott's silver methanamine*	Fungi	Sodium tetraborate, silver nitrate, methanamine	Fungi, *Pneumocystis*: black Red cells: yellow Background: pale green
19. *Giemsa*	Parasites	Giemsa powder	Protozoa: dark blue Nuclei: blue
20. *Shikata's orcein*	Hepatitis B surface antigen (HBsAg)	Acid permanganate, orcein, tetrazine	HBsAg positive: brown to black Background: yellow

Stain	Component/Tissue	Dyes	Interpretation
F. NEURAL TISSUES			
21. *Luxol fast blue*	Myelin	Luxol fast blue, cresyl violet	Myelin: blue/green Cells: violet/pink
22. *Bielschowsky's silver*	Axons	Silver nitrate	Axon and neurofibrils: black
G. PIGMENTS AND MINERALS			
23. *Perl's Prussian blue*	Haemosiderin, iron	Potassium ferrocyanide	Ferric iron: blue Nuclei: red
24. *Masson-Fontana*	Melanin, argentaffin cells	Silver nitrate	Melanin, argentaffin, chromaffin, lipofuscin: black Nuclei: red
25. *Alizarin red S*	Calcium	Alizarin red S	Calcium deposits: orange red
26. *von Kossa*	Mineralised bone	Silver nitrate, safranin O	Mineralised bone: black Osteoid: red
27. *Rubeanic acid*	Copper	Rubeanic acid	Copper: greenish-black Nuclei: pale red
28. *Pigment extraction*	Removal of formalin pigment and malarial pigment	Alcoholic picric acid	Formalin pigment/malarial pigment: removed
29. *Grimelius'*	Argyrophil cells	Silver nitrate	Argyrophil granules: brown-black
H. PROTEINS AND NUCLEIC ACIDS			
30. *Feulgen reaction*	DNA	Potassium metabisulphite	DNA: red purple Cytoplasm: green
31. *Methyl green-pyronin*	DNA, RNA	Methyl green, pyronin-Y	DNA: green-blue RNA: red

PRESCRIBED TEXTBOOKS

- 1. Macsween, R., (2012) Muir’s textbook of Pathology. Edward Arnold. London

- 2. Robbins, S.L. and Kumar, V. (2013). Basic Pathology. W.B. Saunders Co., London.