Role of Laboratory Investigations in Etiological Diagnosis of Hemolytic Uremic Syndrome

By

Prof. Amr Sarhan

Mansoura University

Hemolytic Uremic Syndrome (HUS)

- HUS is not a single disease, rather it is a clinical state that occurs during the course of many diseases
- Diagnosis of HUS is simple and easy task
- On the other hand, diagnosis the etiology of HUS is problematic and tough procedure
- However, it should be tried because proper management depends on accurate etiological diagnosis

Objectives

- Introduction
- Laboratory diagnosis of HUS / TTP
- Etiological diagnosis of HUS / TTP

I- Introduction

Hemostasis

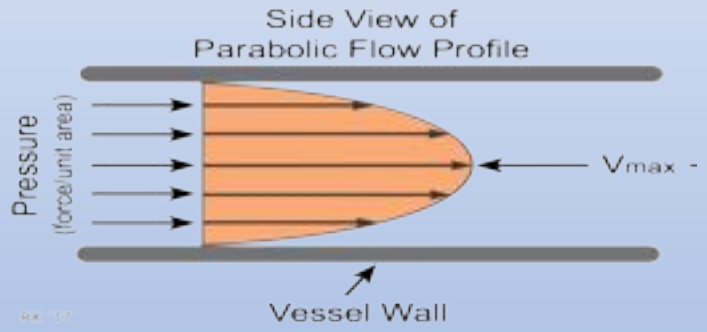
- Hemostasis is the process of maintaining vascular integrity

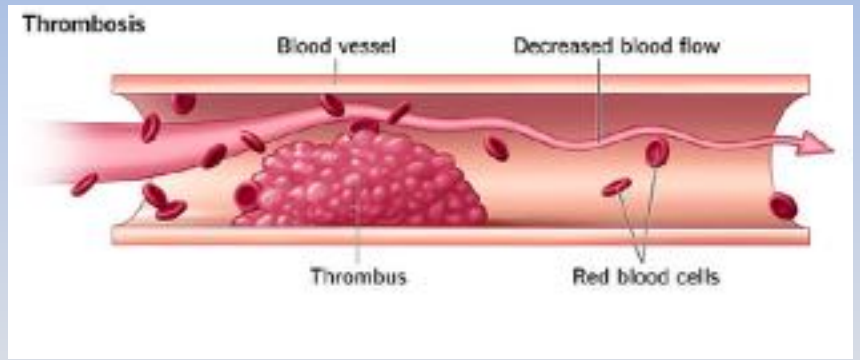
- Vascular integrity means

  1- Blood is a ***fluid*** inside ***intact*** vascular system

  2- When blood vessel is injured ***clot formation*** is formed

  3- Subsequently, ***Recanalization*** occurs





Factors Controlling Hemostasis

- Vessel wall
- Endothelium
- Platelets
- Procoagulant proteins
- Anticoagulant proteins
- Fibrinolytic system

Mechanism of HUS

- Normal endothelium is ***thromboresistant***

- Normally, injury of endothelial cell leads to change of thromboresistant to ***thrombogenic*** with subsequent **rapid**, **local** and **controlled** thrombus formation

- HUS occurs due to **extensive** and **uncontrolled** thrombus formation either due to:
  - Extensive endothelial injury (Shiga toxin, neuraminidase enzyme, ….)
  - Failure of control (down regulation) of thrombus formation
    - Complement mediated HUS
    - ADAMST13 deficiency ….. TTP

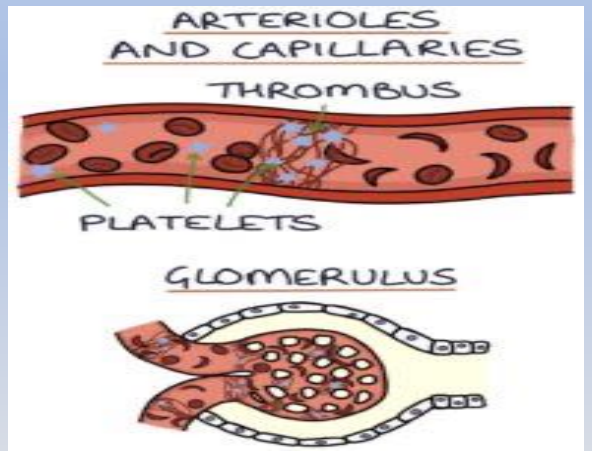
Consequence of Thrombus Formation

- Consequence of thrombi formation irrespective to the etiology or mechanism leads to the classical triad of HUS

Platelets aggregate within thrombus …. ***Consumption thrombocytopenia***

Mechanical disruption of red cells …. ***Microangiopathic hemolytic anemia***

Decrease tissue perfusion …. ***Organ damage (AKI)***



II- Laboratory Diagnosis of HUS/TTP

Is it HUS/TTP?

HUS/TTP are diagnosed by the classical triad of:

1- Microangiopathic hemolytic anemia (MAHA)
2- Thrombocytopenia
3- Acute kidney injury (AKI)

Exclusion of DIC

1- Microangiopathic Hemolytic Anemia

- Acute onset of moderate (Hb 7-9 gm/dl) to sever (Hb < 7gm/dl) normocytic normochromic anemia

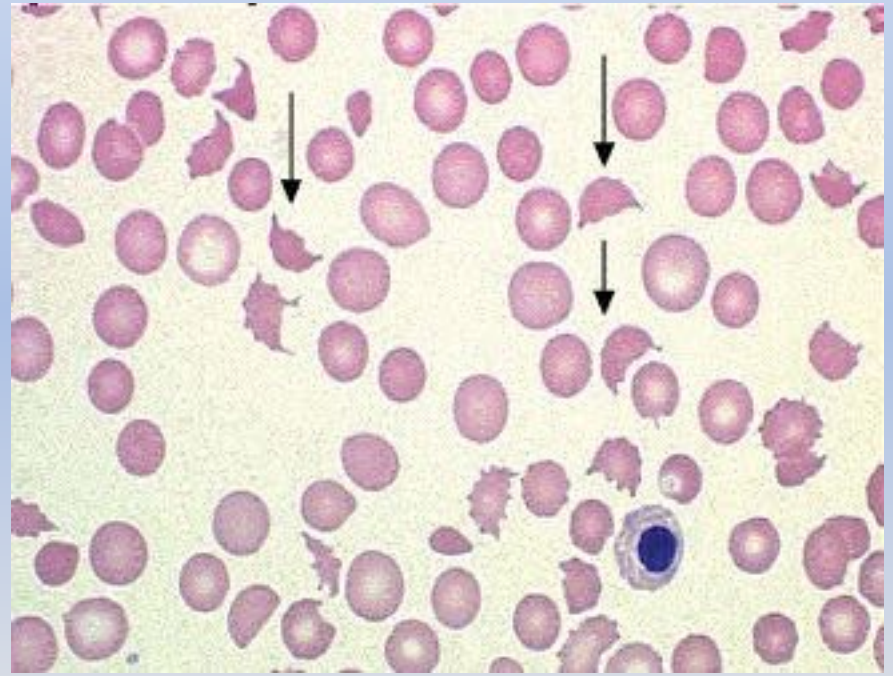
- Negative Coombs test except in pneumococcal HUS

- Blood film shows

  Schistocytes >1-2 %

  Echinocytes, Burr cells, Helmet cells

- Evidence of hemolysis

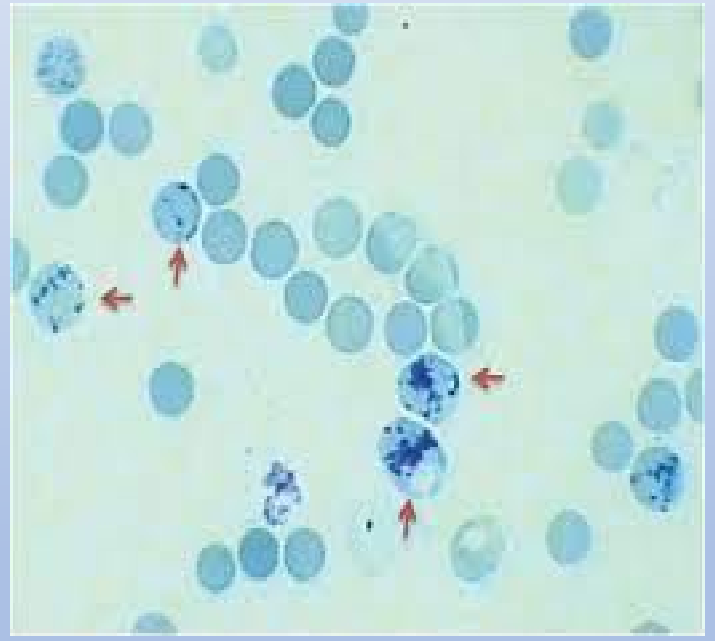


Evidence of Hemolysis

- ❑ High reticulocytes count
- ❑ High serum level of LDH
- ❑ Low serum level haptoglobin
- ❑ Indirect hyperbilirubinemia

Reticulocyte Count

- Reticulocytes are juvenile RBCs, released from bone marrow into blood stream
- They assess erythropoietic activity of bone marrow
- They contains remanent of RNA and ribosomes
- Method of assessment
  - Manual
  - Automated … flow cytometry
- Normal values
  - Adults 0.5 – 2.5 %
  - Infants and children up to3%
  - Newborns up to 6 %
- Corrected values
  - Reticulocyte percentage x Ht of patient/ normal Ht



Lactic Dehydrogenase (LDH)

- LDH is an enzyme found in nearly all living cells
- Increase serum level of LDH indicates cellular destruction and tissue damage (trauma, malignancy, inflammation, infection, hemolysis,…)
- Total LDH assay is not specific test, it can not tell where the damage is located ? or what the etiology of this damage ?
- Normal values of total LDH

  1 -9 years 150 – 500 U/ L

  > 9 years 120 – 300 U / L

2- Thrombocytopenia

- Decreased platelets count …. < 100,000 - 150,000 / mm_3
- Thrombocytopenia is usually detected early, but it can be normal.
- However, If a platelet count obtained within 7 days after onset of illness is not < 150,000/mm, diagnosis of HUS is not considered
- Usually no bleeding tendency
- Coagulation profile is normal

3- Organ Damage

- HUS is systemic disease affecting multiple organs
- HUS is usually affect kidney with
  - Oliguric or anuric AKI
  - Rising serum creatine and BUN
  - Hematuria …. usually microscopic
  - Proteinuria … usually mild to moderate

Extrarenal Organ Damage

- Pancreases …… pancreatitis

  Random blood sugar , serum amylase and lipase

- Heart ……. myocarditis

  Troponin, creatine kinase-myocardial band (CK-MB)

- Skeletal muscles rhabdomyolysis

  Creatine phosphokinase

Differential Diagnosis

- From other causes of thrombotic micro-angiopathy (TMA)

  Disseminated intravascular coagulation ….. DIC

Is it Disseminated Intravascular Coagulation (DIC) ?

- DIC results from failure of both procoagulant and anticoagulant mechanisms of hemostasis

- When do you suspect?

  DIC is a sever life-threating condition

  It is usually associated with sever clinical manifestations (hypoxia, acidosis, shock, tissue necrosis, sepsis, malignancy .. etc.)

  Significant bleeding tendency with history of repeated blood transfusion

- How do you confirm?

  Prolonged PT, APTT

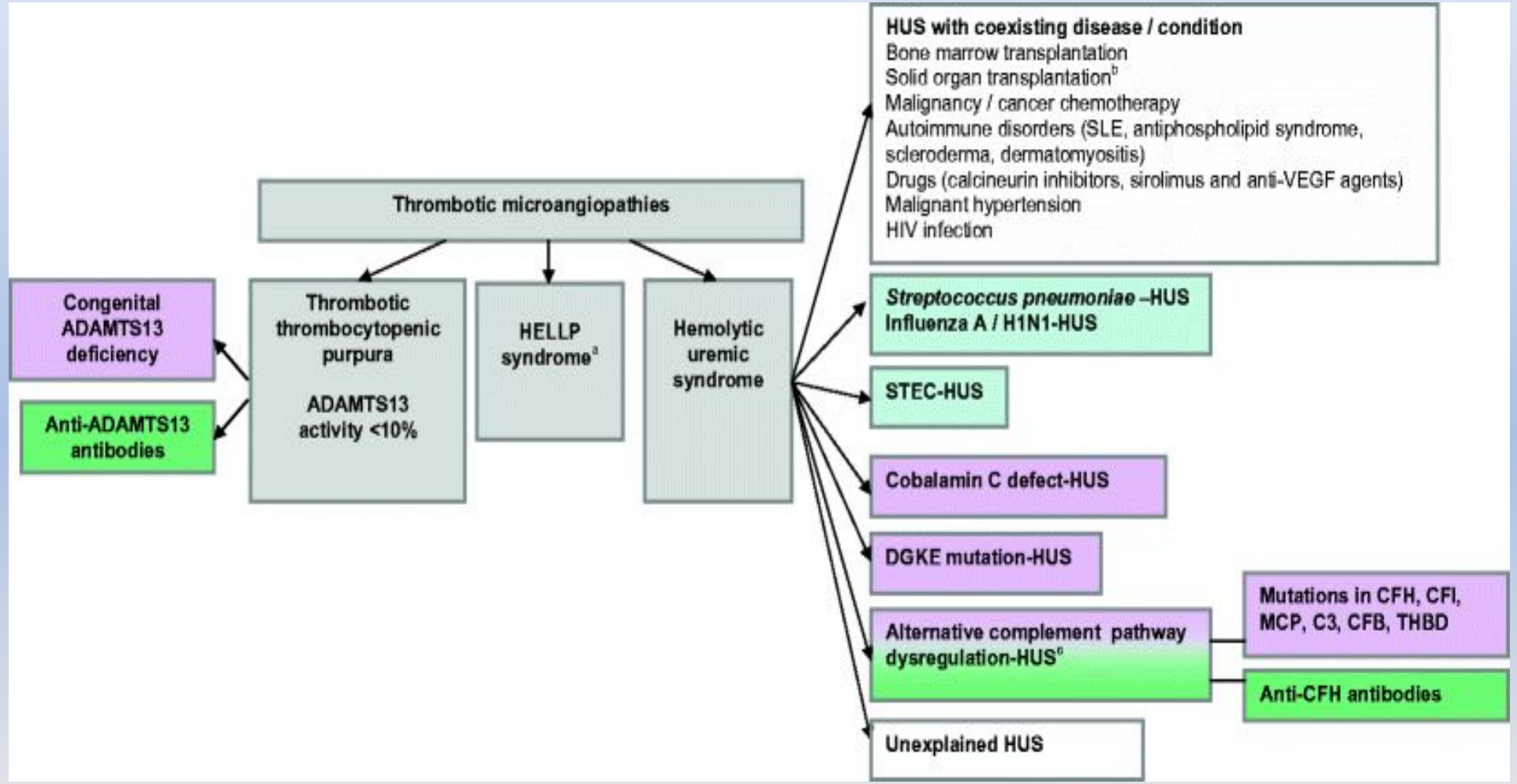
  Elevated INR, FDP and D-dimmers

III- Etiological Diagnosis of HUS/TTP

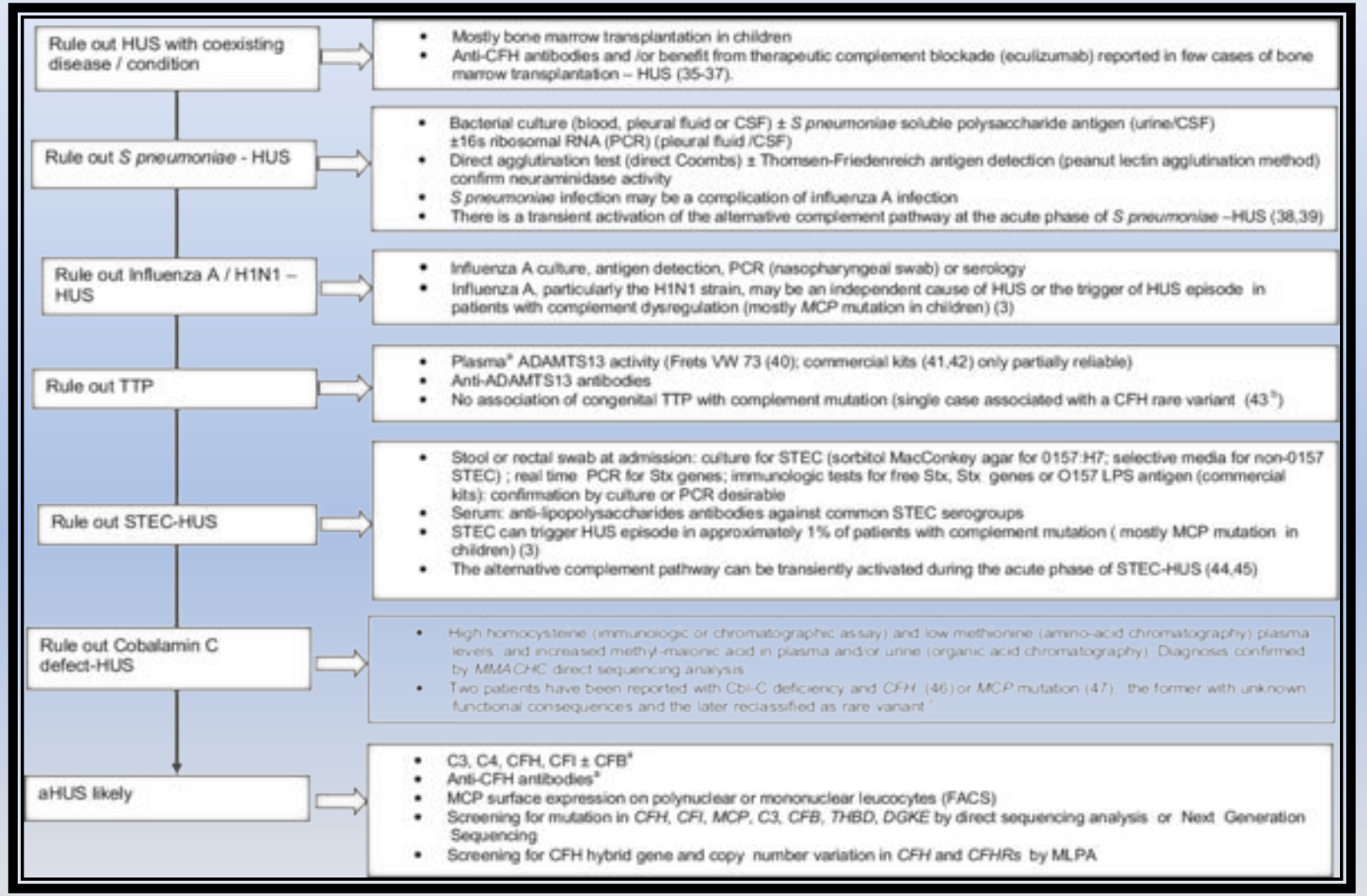
Etiological Diagnosis of HUS/TTP

- Introduction
- TTP
- STEC-HUS
- Pneumococcal HUS
- Complement dysregulation
- Other types

Etiology of HUS/TTP



Laboratory Investigations of HUS/TTP Etiology



Laboratory Diagnosis of HUS Etiology

- Is it important to diagnose the etiology of HUS/TTP?

The answer is yes

- Because proper management depends on accurate etiological diagnosis

Laboratory Diagnosis of HUS Etiology

- There are many laboratory investigations for diagnosis of HUS/TTP etiology

- They include many

  Bacteriologic and viral investigations (cultures, serology, ….)

  Immunological assessment

  Metabolic screen

  Hematologic investigations

  Genetic study PCR, whole exome sequencing

Limitation of Laboratory Investigations

- Meticulous sampling techniques
- Multiple tests
- Time consuming
- Expensive
- Not usually available
- Some results are not standardized
- Sometimes not reliable

Rational of Laboratory Investigations

- The tempo of laboratory investigations should be adjusted to the tempo of clinical situations

- So, 2 questions should be answered

When do you suspect particular etiology ?

How do you confirm this etiology?

1- Thrombotic Thrombocytopenic Purpura TTP

Von Willebrand factor (VWF)

ADAMST-13

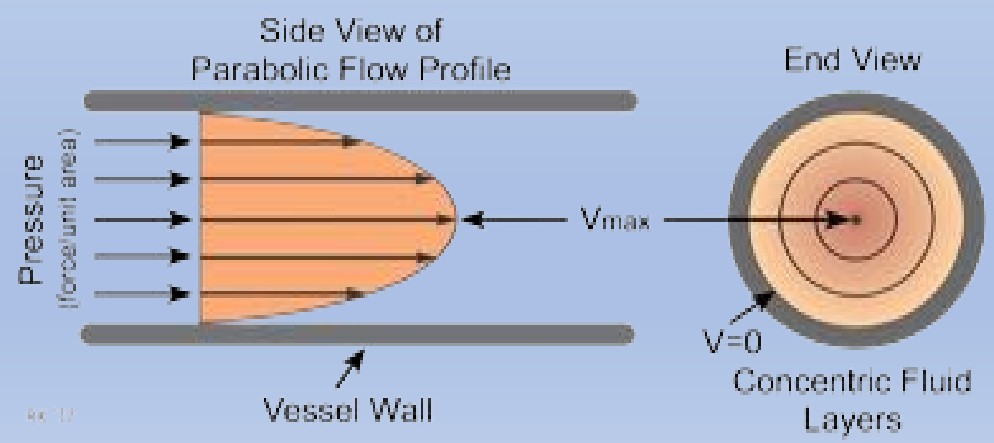
When do you suspect?

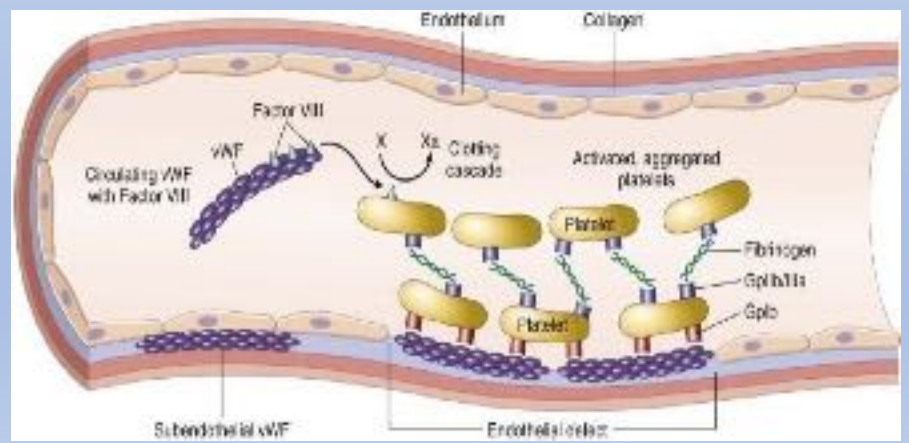
How do you confirm?

Erik Adolf von Willebrand
-1870-1949
Finnish physician

Von Willebrand Factor

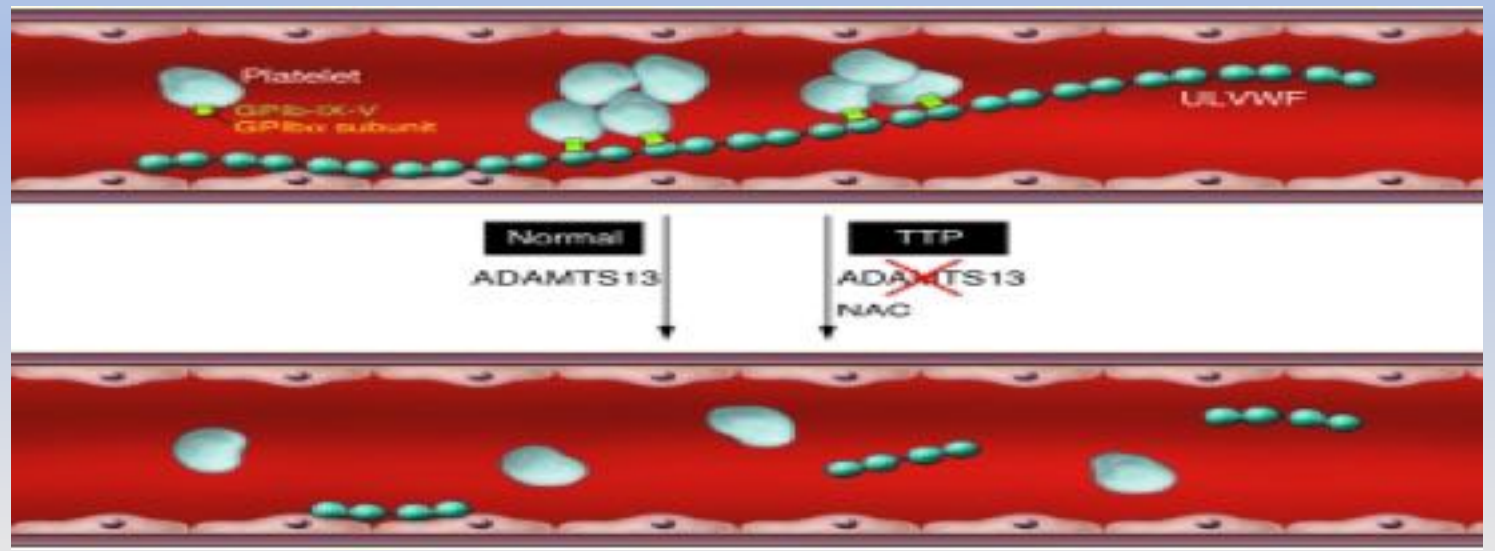
- It is important thrombogenic factor
- It has many domains that combine with factor VIII, platelets, collagen, …
- ***Shear stress*** in blood vessels → endothelial injury → VWF attached to collagen fibers in subendothelial → ***Conformation of VWF*** → ***thrombogenic endothelium***
- Platelets aggregation …. platelets activations …. platelets plug





ADAMTS-13 enzyme

- A Disintegrin And Metalloproteinase with ThromboSpondin type 1 motif no 13
- ADAM 13 cleaves multimeric VWF → monomeric subunits
- Deficient activity of ADAM-13 leads to continues activation of VWF with subsequent exaggerated response to thrombus formation …..TTP



ADAM-13 Reduced Activity

Etiology of ADAM-13 reduced activity

- Congenital
  - Mutation of gene that encode ADAM-13 enzyme … chromosome 9q34
- Acquired
  - Autoimmune diseases
  - Pregnancy, malignancy,
  - Drugs (cyclosporin, mitomycin, irradiations, ..)
  - Idiopathic

When do you suspect TTP?

- Criteria of thrombotic thrombocytopenic purpura

  Microangiopathic hemolytic anemia

  Thrombocytopenia …. Usually sever < 30,000

  Ischemic organ damage … More CNS insult, mild AKI
- TTP is associated with fever
- No bleeding tendency
- Normal coagulation profile …. PT, APTT, INR, FDP and-D dimmers

How do you confirm diagnosis of TTP

- Assessment of VWF Ag or activity has no role in diagnosis of TTP
- Diagnosis depends on assays of plasma ADAMTS-13
- Blood sampling for ADAMTS-13 assessment should be taken before plasma exchange or infusion
- There are several ***biomarkers for ADAMTS-13 assay***

Biomarkers of ADAMTS13

- Plasma ADAMTS-13 activity

  Most commonly used, diagnostic level of ADAM13 activity is <10%

- Anti ADAMTS13 autoantibody …….. ELISA

  Commonly used for diagnosis of secondary TTP

- Plasma level of ADAMTS13 antigen ….. ELISA

  It is not used in routine clinical practice, rarely used to detect relapse

- Genetic assessment …

  Mutation of gene encoding ADAM-13 enzyme .. whole exome sequencing

2- Shiga-toxin producing E coli (STEC HUS)

E-coli

Shiga toxin

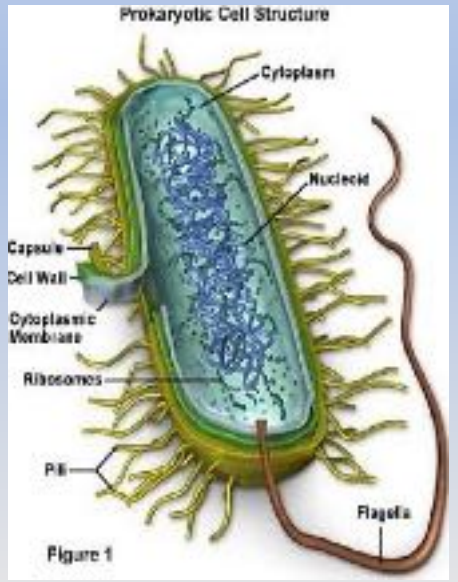
GB_3 receptor

When do you suspect?

How do you confirm?

E-coli

- Theodor Escherich in 1885 discovered this organism in feces of healthy individuals, he named it “Bacterium coli”
- His collogues in 1895 renamed it “Escherichia coli”



Theodor Escherich
German pediatrician
1857-1911

E- Coli

- Gram –ve bacilli known to be a part of normal intestinal flora
- Most intestinal strains of E-coli are not pathogenic
- Some intestinal strains are pathogenic, they include the following strains

  Enteropathogenic E coli (EPEC)

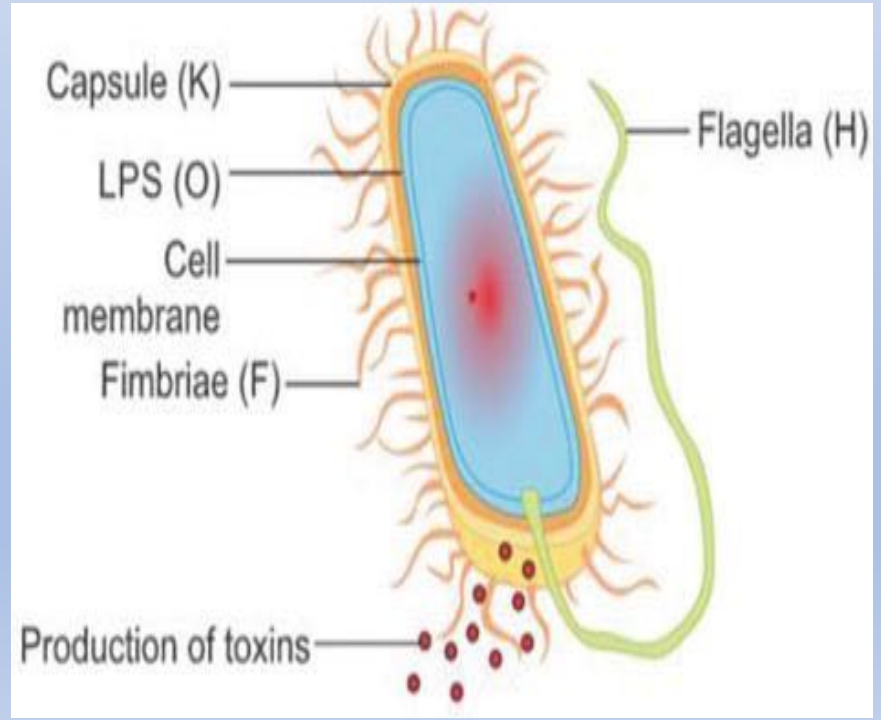
  Enterohemorrhagic E coli (EHEC)

  Enterotoxigenic E coli (ETEC)

  Enteroinvasive E coli (EIEC)

  Enteroaggregative E coli (EAEC)
- Extraintestinal strains are pathogenic

  UTI, neonatal meningitis, sepsis



E- Coli

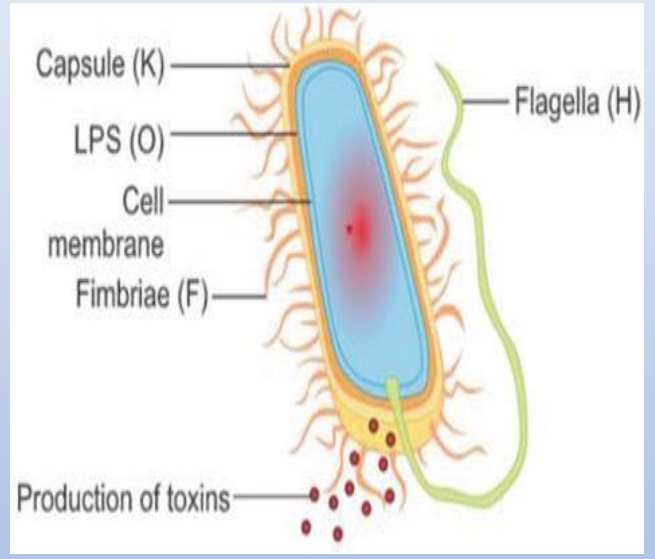
- More than 190 serotypes … recently 470 (2022)

  Cell wall … somatic … O

  Flagellar ……H

  Capsular …… K

  Fimbria …….. F



- The O157: H7 STEC is the most commonly implicated in STEC HUS
- Non O157:H7 serotypes

  Big 6 serotypes O26, O11, O121, O45, O145 …. 75% of all STEC infections in human (USA)

  In 2011 Germany outbreak of STEC-HUS was caused by O104:H4

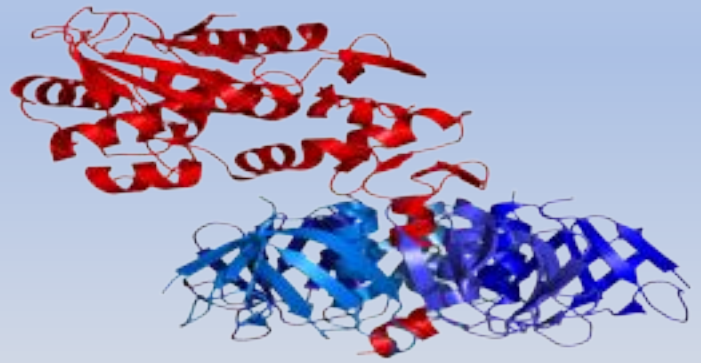
  In 2021 in Europe, 365 cases of HUS were reported, serotype O26 was the most common

- Shiga toxin-producing E-coli (STEC)
- Only 5-10 % of patients with STEC develop HUS

Shiga toxins

- In 1897, Kiyoshi Shiga discovered the microorganism that caused the dysentery outbreak in this year and isolated the toxin produced by these organism
- Organism is named Shigella and the toxin was named

  Shiga toxins



Kiyoshi Shiga
1871-1957

Shiga toxins

- Some strains of E coli (STEC) produce 2 types of Shiga toxins:

  - Shiga toxin 1 (Stx 1)

    It is similar to Shiga toxins that produced by other Gram -ve organism, these toxins cause hemorrhagic colitis but not HUS

  - Shiga toxin 2 (Stx 2)

    It is responsible for HUS

Shiga toxins

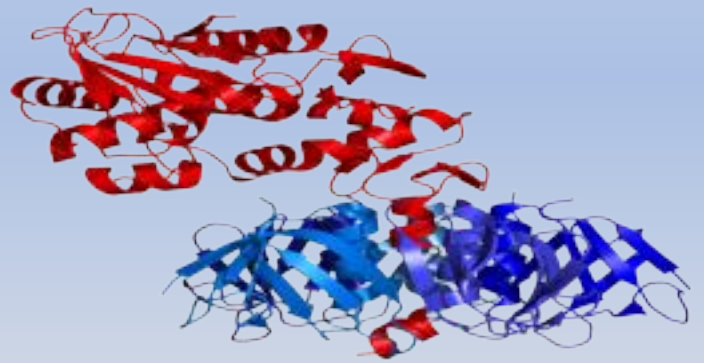
- Shiga toxins have 2 subunits

  Large A subunit

  It enters cell by endocytosis, decrease binding of t RNA to ribosome leading to inhibition of protein synthesis, cytopathic change and finally endothelial cell damage → thrombogenic

  5 small B subunits

  They bind toxin to GB3 receptors



GB3 receptor

- GB3 receptor, due to unknown reason, is highly expressed in renal tissue
- It is expressed mainly at endothelial cells
- Recent studies demonstrate that GB3 is also expressed at mesangial cells, tubular epithelial cells, monocytes, polymorphonuclear leukocytes

When do you suspect STEC-HUS?

- History of bloody diarrhea
- Only 5% of children with bloody diarrhea have +ve Shiga toxins (STEC)
- Only 5- 10% of children with STEC develop HUS

How do you confirm?

- Bacteriological investigations of stool is the gold standard for diagnosis of STEC infection
- Stool sample should be collected early after the onset of diarrhea
- Stool sample is subjected to:

    Microscopic examination and Gram stain … Gram –ve bacilli, motile, ……

    Culture on selective, differential and indicator media

        Mac Conkey agar …. Gram –ve bacteria

        Lactose fermenter ….. E coli, klebsiella

        Indole +ve …. E-coli

        Sorbitol Mac Conkey agar for STEC serotype O157: H7

        Other selective media for STEC non-serotype O157:H7

How do you confirm?

- Immunological assessment

  Detection of free Shiga toxins in stool by enzyme immunoassay (EIA)

  Detection of “O” and “H” antigens of STEC by known antisera (agglutination) XXXX

- Genetic study

  Real time PCR for genes encoding Stx1 and Stx2

  Genetic assessment for gene encoding lipopolysaccharides antigen of O157 XXXX

How do you confirm?

❑ Antibodies of STEC or Stx

- Serological test for detection of serum IgM anti- lipopolysaccharide of serotype O157 xxxx

- Shiga toxins is neither detected in serum nor their antibodies

3- Pneumococcal Associated HUS

Invasive pneumococcal infection

Neuraminidase enzyme

Sialic acid

T- antigen

Neuraminidase enzyme

- Neuraminidase (sialidase) is enzyme secreted by pneumococci
- It catalyze the hydrolysis of glycoside linkage of sialic acid
- Cleavage of sialic acid uncover T antigen

Sialic acid

- Sialic acid is monosaccharide with nine- carbon atoms

- It combines with many organic molecules to form important compounds

  amino acid …. Proteins ……glycoproteins

  fatty acid …… lipid ……. glycolipids

  DNA and RNA

- Usually sialic acid of these compounds decorate the surface of cell membrane

T- antigen

- T antigen is first described as RBCs antigen
- It is one of the oncofetal antigens
- It is one of the membrane transport proteins
- It is normally masked from immune system

Pathogenesis of pneumococcal associated HUS

- Neuraminidase, produced by pneumococci, cleaves sialic acid on membrane of endothelial cells, platelets and red blood cells.

- Sialic acid cleavage, reveals the underlying cryptic T antigen

- Normally circulating antibodies (IgM) against T antigen on RBCs, platelets, and endothelium trigger antibody antigen reaction → inflammatory response → microvascular angiopathy → HUS

When do you suspect?

- Clinically, sever invasive pneumococcal infections

  Pneumonia with plural effusion

  Meningitis

  Septicemia
- Direct +ve Coombs test
- Leukocytosis
- Sever clinical presentation of HUS
- Age of onset < 2 years

How do you confirm?

- Cultures (blood, CSF, pleural fluid) are +ve for pneumococci
- Microscopic examination reveals Gram +ve capsulated diplococci
- Neuraminidase activity is confirmed by detection of serum T antigen by agglutination test
- PCR (pleural fluid or CSF) is +ve for pneumococci

4- Complement Dysregulation

Complement system

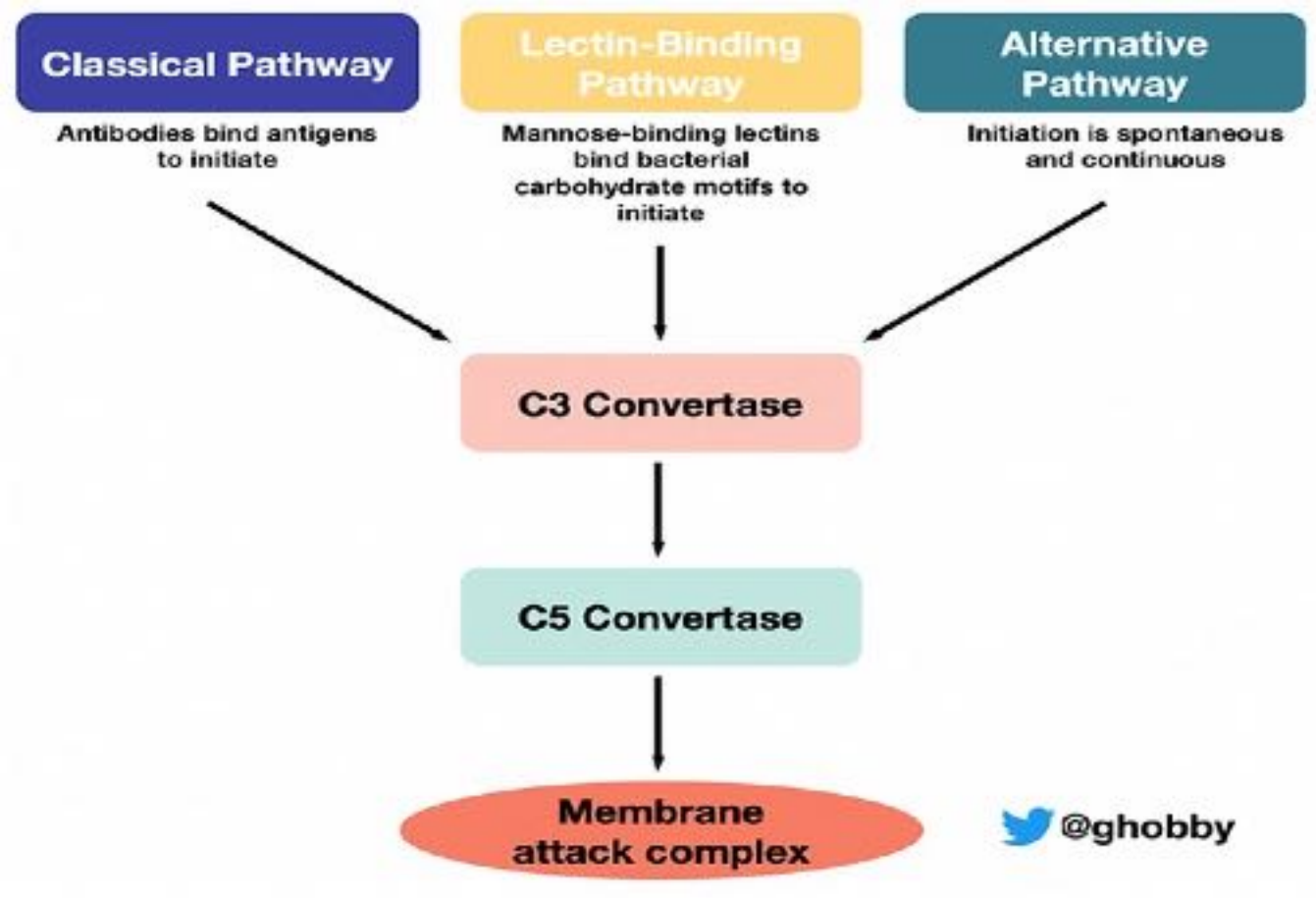
- Complement system consists of more than 30 different proteins
- They are synthetized in liver
- They are distributed across plasma and cell surface
- Their main function is

  Cell lysis (microorganism)

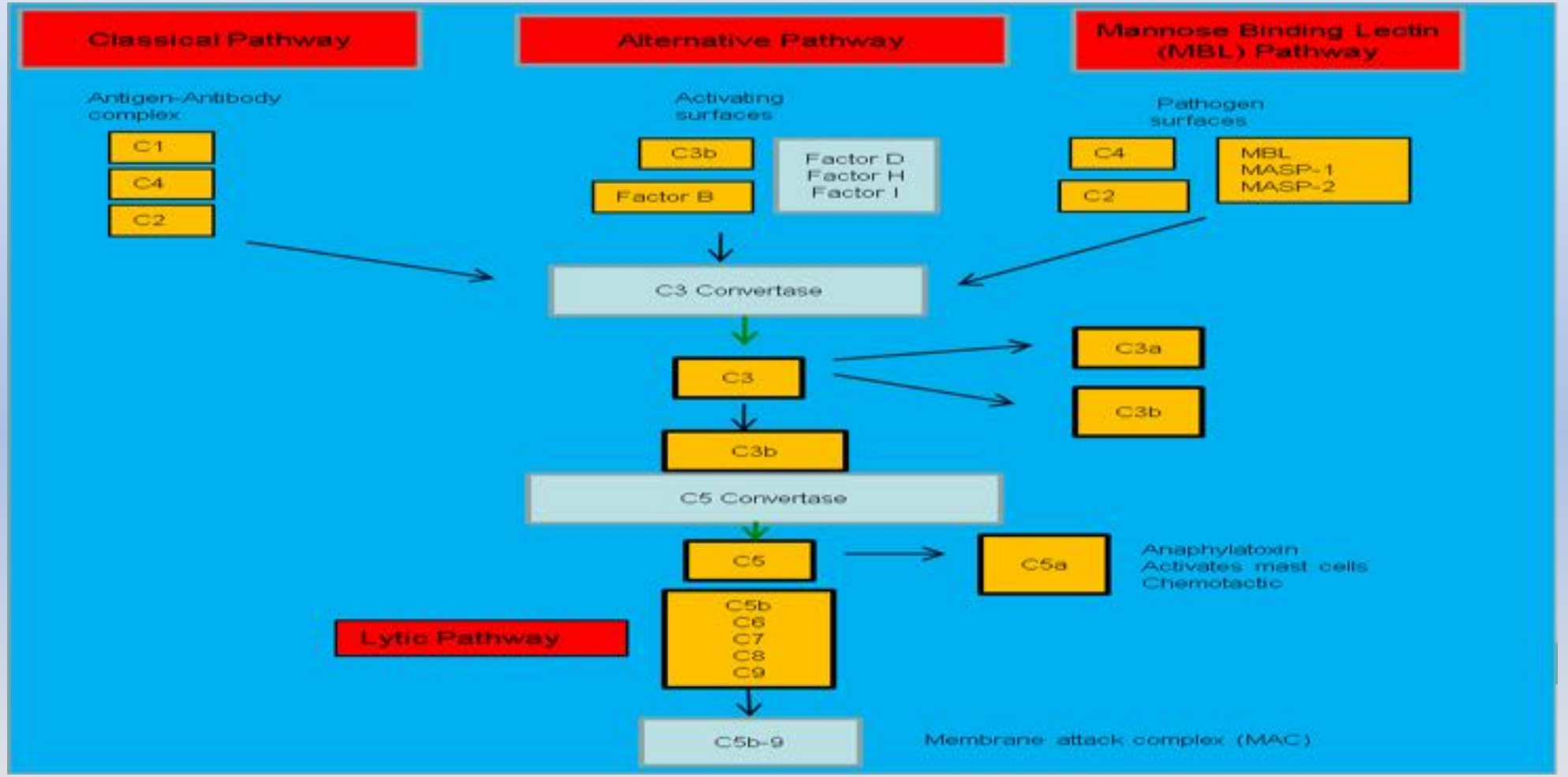
  Generation of inflammatory mediators

  Modulation of adaptive immune response

Complement system



Complement system

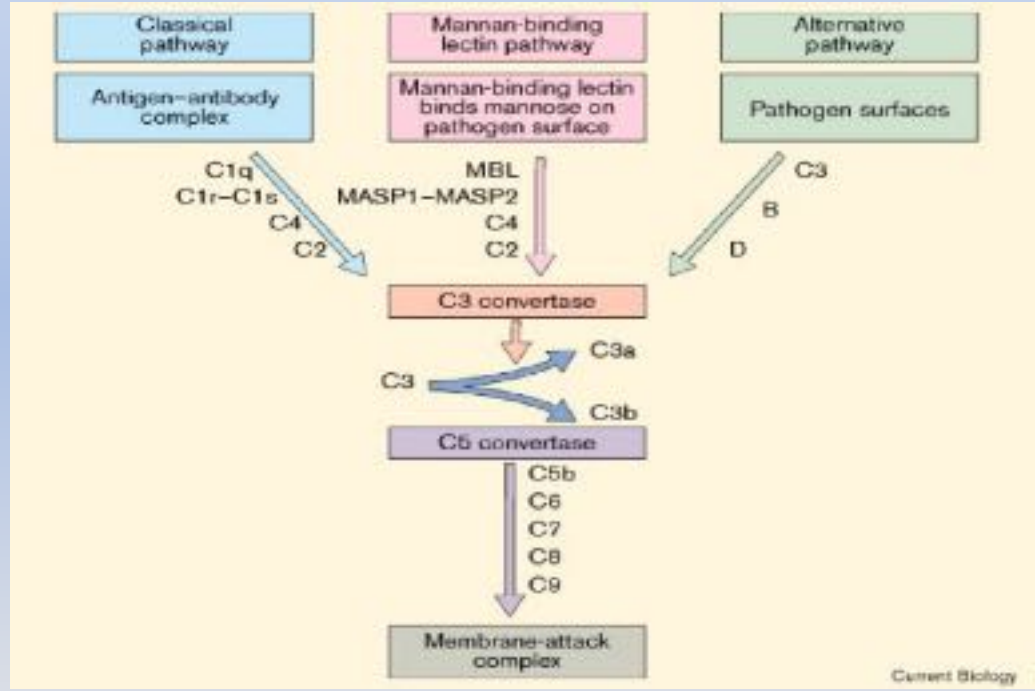


Pathogenesis

- Alternative complement pathway is continuously active at a low level
- This activation is under control of regulatory proteins of complement system (CFH, CFI, membrane cofactor protein, …) that down regulate continuous activity
- Alteration function of this regulatory proteins → continuous generation of C3 and C5 convertase → uninhibited complement activation → resulting in formation of membrane attack complex (MAC)

Pathogenesis

- This uninhibited activation of complement cascade at the sit of vascular endothelium results in thrombogenic state → thrombus formation



Etiology of complement dysregulation

- Inherited mutation of :
  - CFH…….. 30%
  - MCP CD46 …. 10%
  - CFI …….. 5%
  - Others
    - C3, CFB, DGKE, thrombomodulin, CFH Related protein1-5
- Acquired
  - Anti factor H antibodies

Molecular genetic assessment

- Diagnosis by molecular genetics techniques are expensive, time consuming and still have limited clinical validity, although it have 100% lab. validity
- Sequencing techniques become available, they includes:

  Single gene sequencing

  Panel of genes sequencing

  Whole exome sequencing

  Whole genome sequencing

5- HUS with coexisting disease / condition

- Viral infections

  HIV, H_1:N_1, covid-19, …
- Systemic diseases

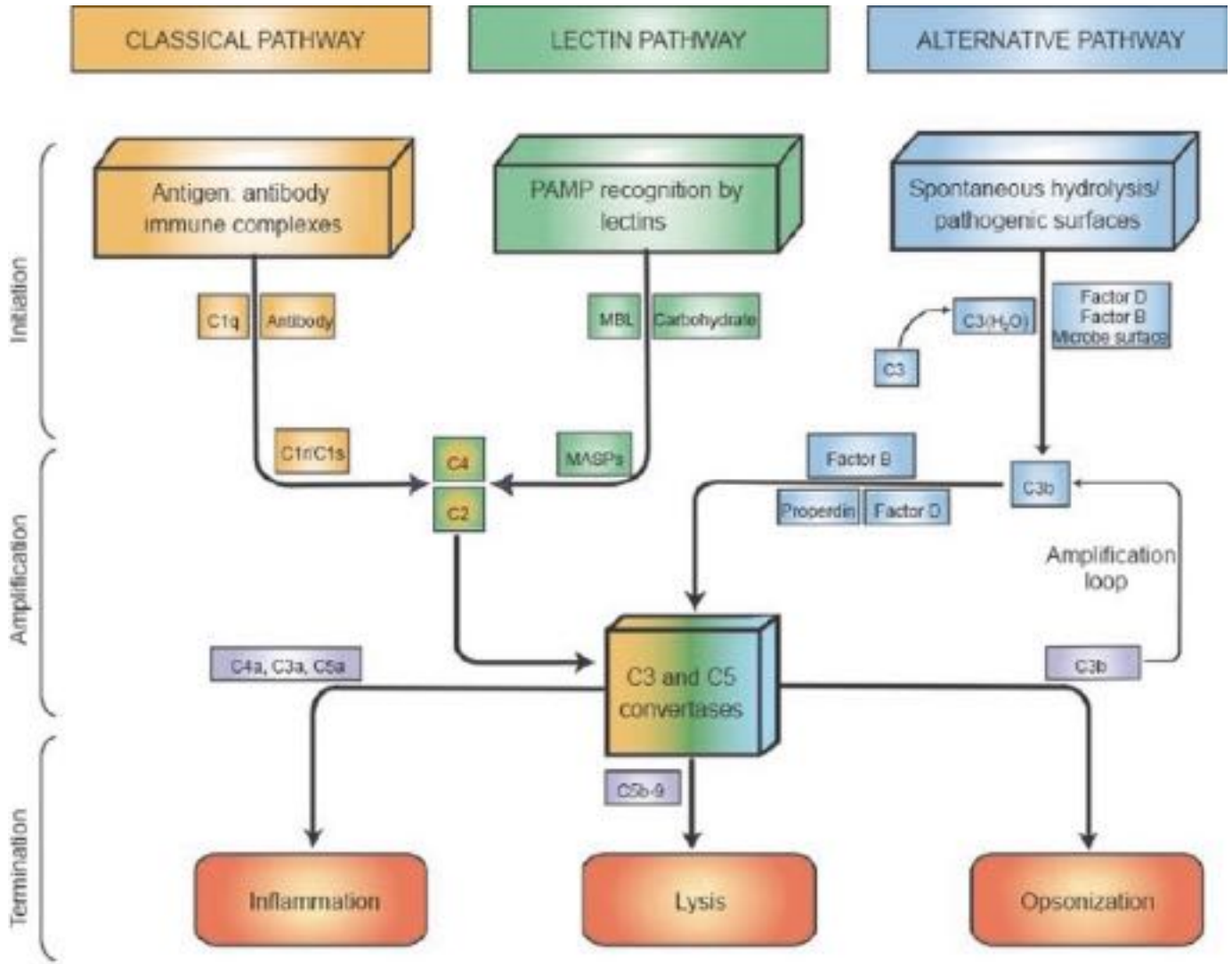
  SLE, antiphospholipid syndrome, advanced malignancy, ….
- Drugs and ionizing radiations

  calcineurin inhibitors, mitomycin C, …..
- Pregnancy

Conclusions

- HUS is not a single disease
- It is serious, life-threatening condition
- Diagnosis of TTP/HUS is easy
- Etiological diagnosis is tough and difficult, but should be tried because proper management depends on etiological diagnosis

Thank you


CLASSICAL PATHWAY
LECTIN PATHWAY
ALTERNATIVE PATHWAY
Initiation
Amplification
Termination
Antigen: antibody immune complexes
PAMP recognition by lectins
Spontaneous hydrolysis/ pathogenic surfaces
C1q
Antibody
MBL
Carbohydrate
C3
C3(H2O)
Factor D
Factor B
Microbe surface
C1r/C1s
C4
C2
MASPs
Factor B
Properdin
Factor D
C3b
Amplification loop
C4a, C3a, C5a
C3 and C5 convertases
C3b
C5b-9
Inflammation
Lysis
Opsonization

Thrombotic Microangiopathies (TMA)

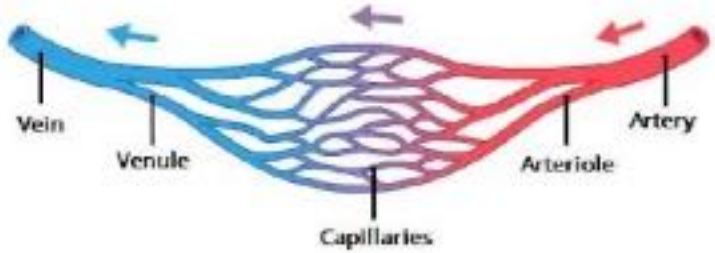
- Definition

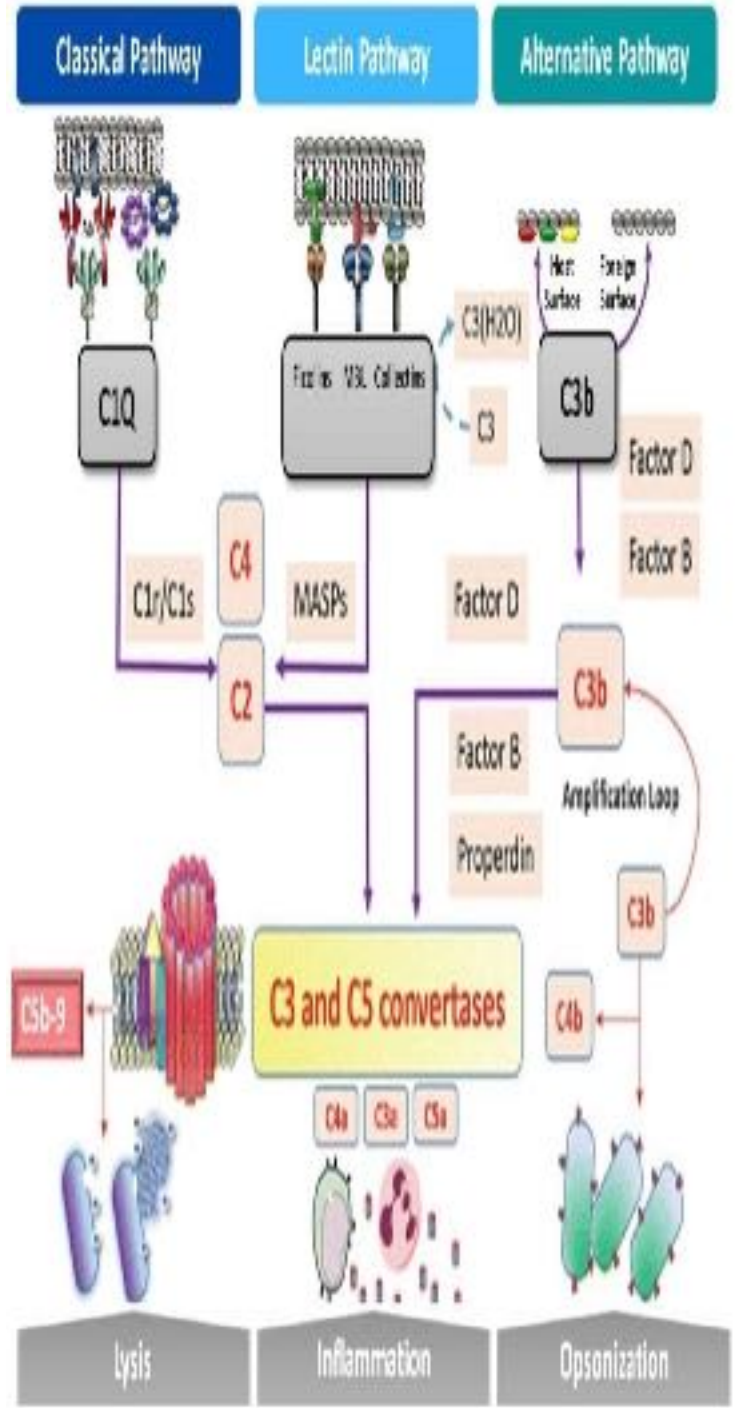
  TMA is a spectrum of clinical syndromes result from widespread formation of platelets reach-thrombi in the ***microcirculation***, leading to:

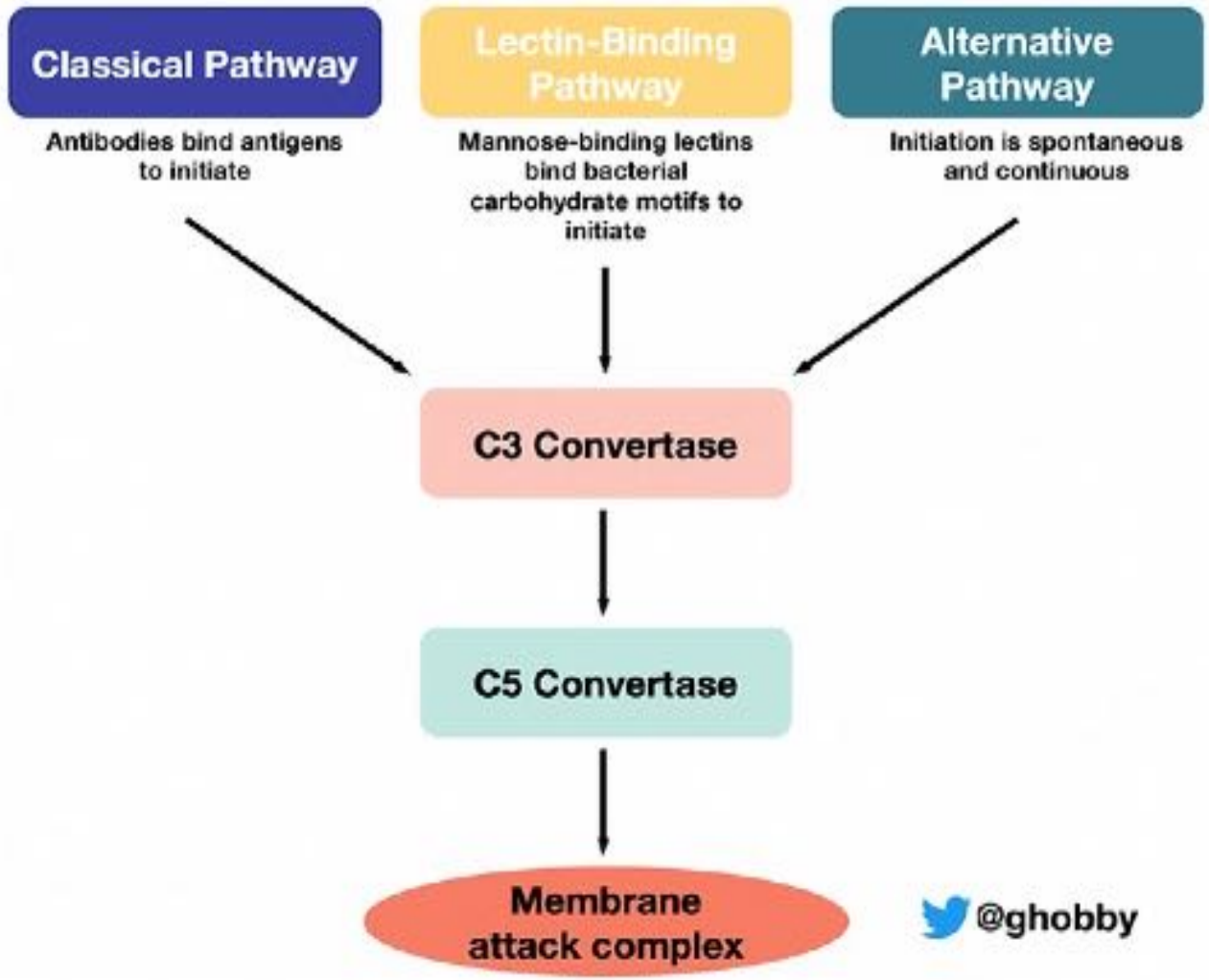
  Microangiopathic hemolytic anemia ….. MAHA
  Thrombocytopenia
  Ischemic organ damage

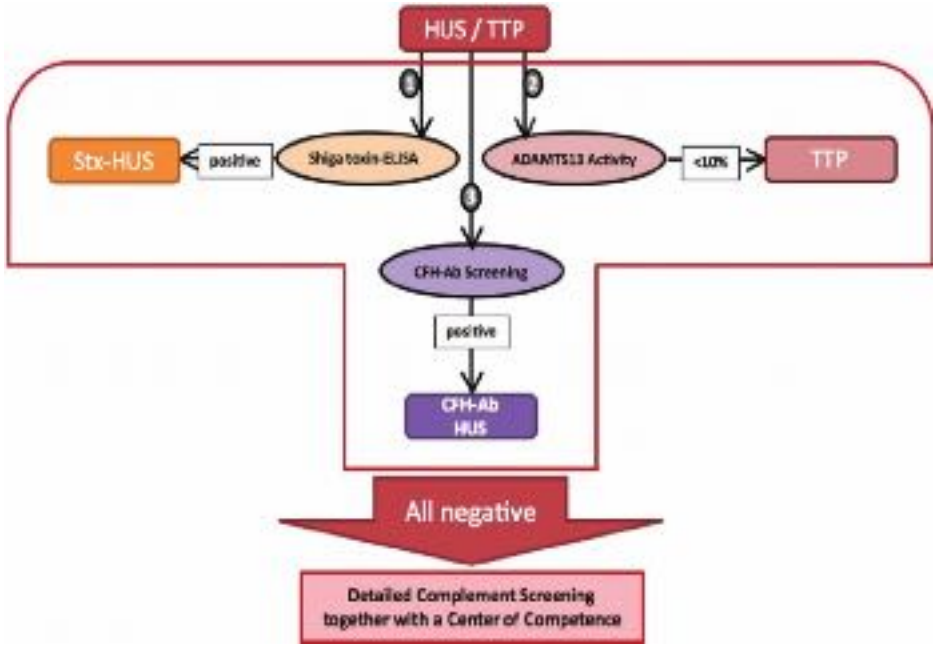
  TMA includes the following syndromes

  Thrombotic thrombocytopenic purpura ….. TTP
  Hemolytic uremic syndrome ……HUS
  Disseminated intravascular coagulation ….. DIC


Vein
Venule
Capillaries
Arteriole
Artery

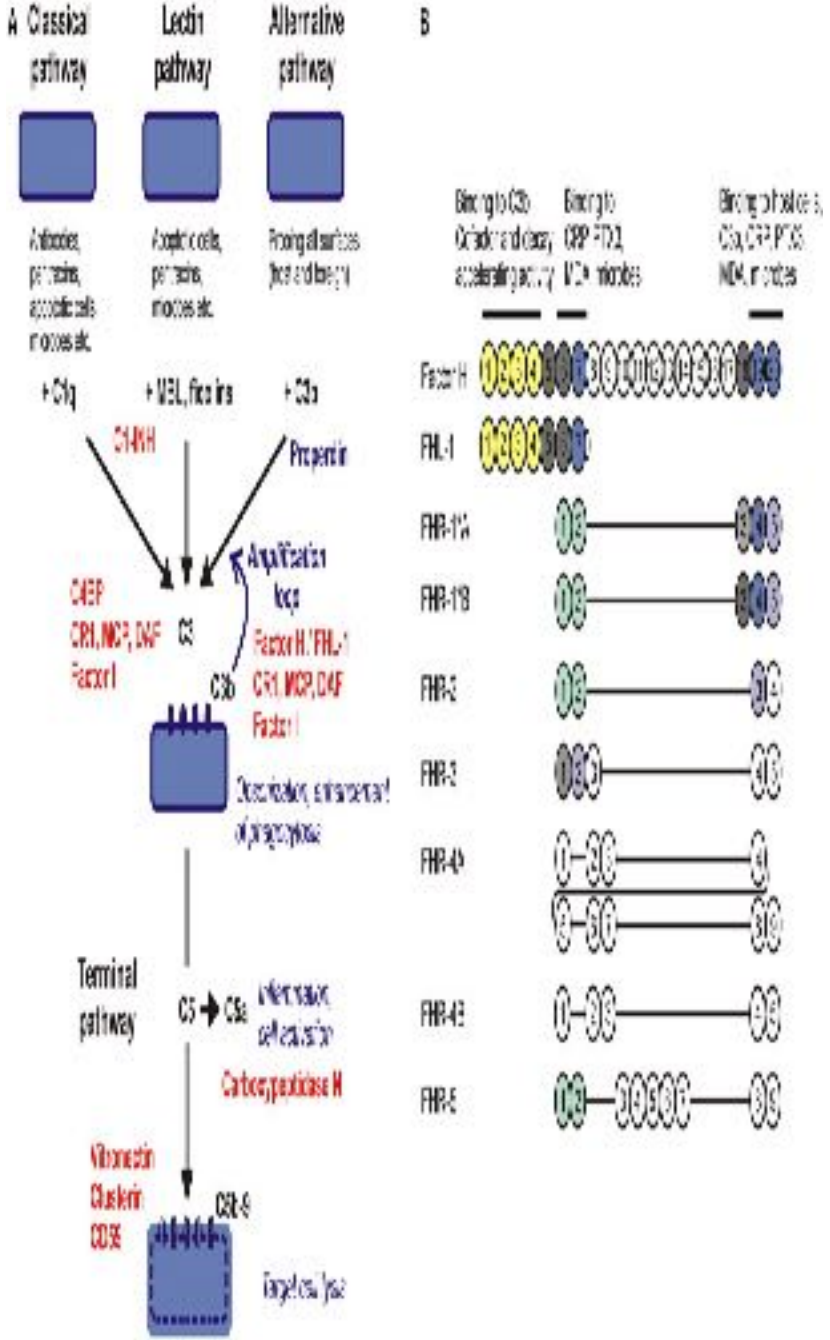

Classical Pathway
Lectin Pathway
Alternative Pathway
C1Q
C3(H2O)
C3
C3b
Factor D
Factor B
C4
C1r/C1s
MASPs
Factor D
C2
C3b
Factor B
Amplification Loop
Properdin
C3b
C5b-9
C3 and C5 convertases
C4b
C4a
C3a
C5a
Lysis
Inflammation
Opsonization

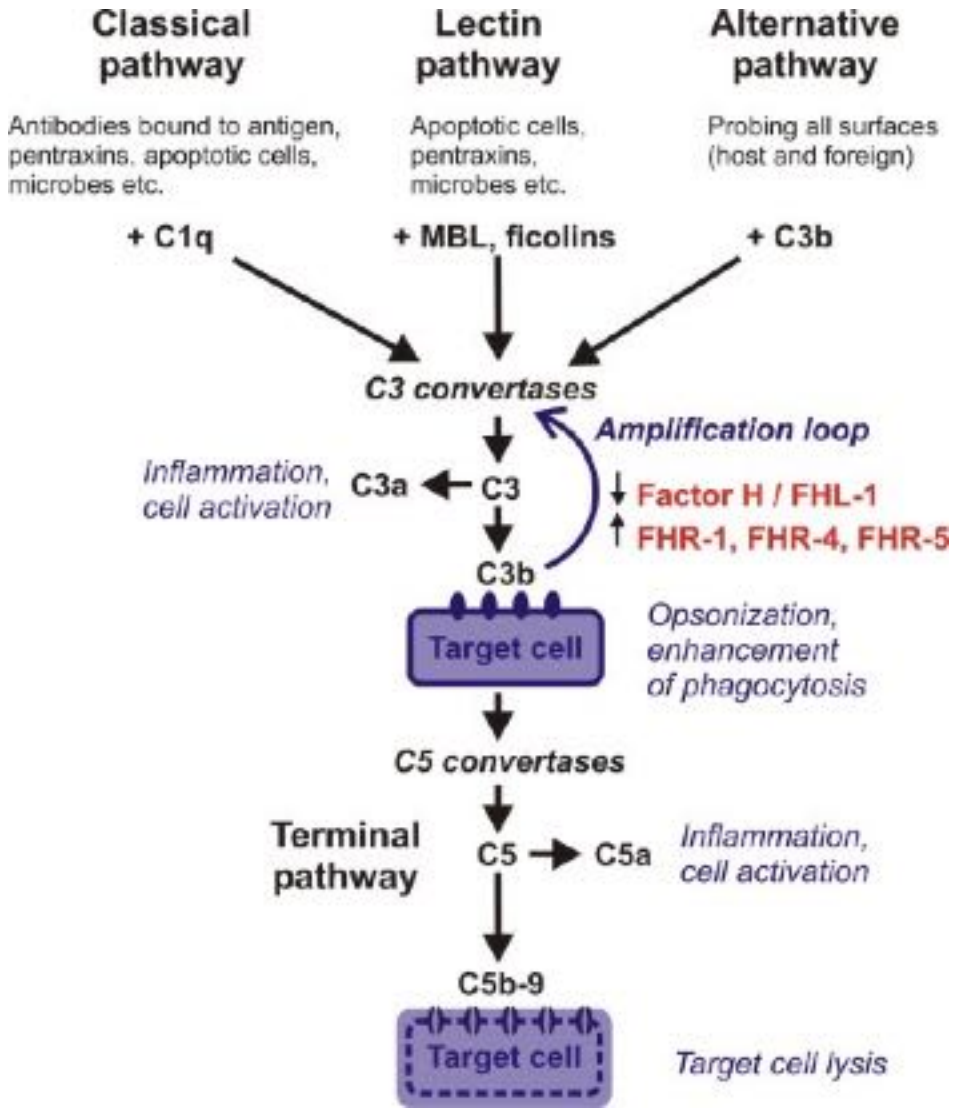

Classical Pathway
Antibodies bind antigens to initiate
Lectin-Binding Pathway
Mannose-binding lectins bind bacterial carbohydrate motifs to initiate
Alternative Pathway
Initiation is spontaneous and continuous
C3 Convertase
C5 Convertase
Membrane attack complex
@ghobby

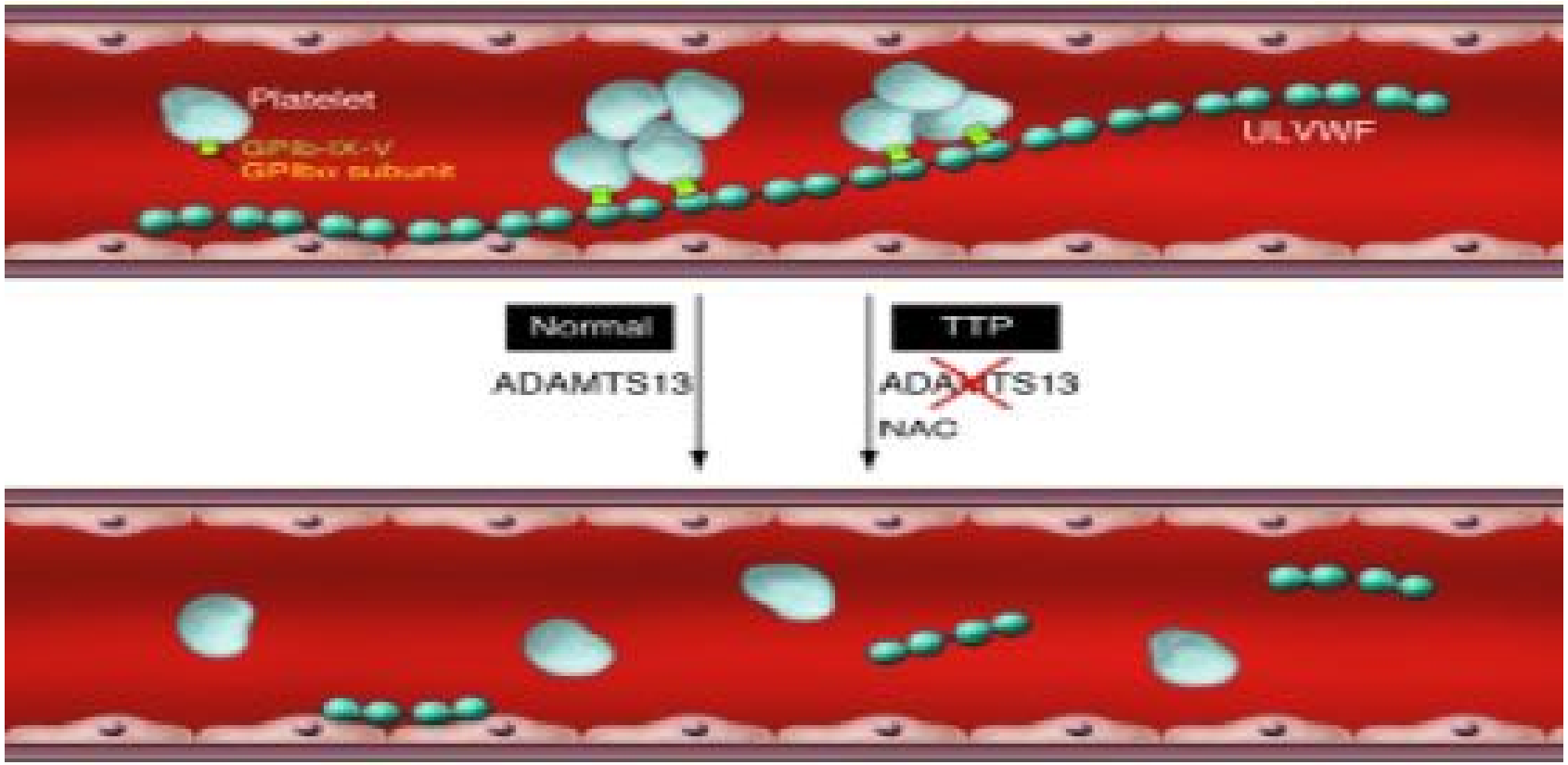


Category	Defining Characteristic
Disseminated intravascular coagulation (DIC)	Coagulation abnormality with elevated INR and aPTT
Thrombotic thrombocytopenic purpura (TTP)	ADAMTS13 <5%-10%; autoantibody inhibitor of ADAMTS13 (unless one of the rare congenital forms, with no inhibitor)
Atypical hemolytic uremic syndrome (aHUS)	ADAMTS13 >5%-10% (exact cut-off as specified by the laboratory and assay technique employed); associated with a recognized complement-activating condition in two-thirds of cases; congenital mutation in complement system recognized in 70% of cases
Shiga toxin–producing *E. coli* HUS (STEC-HUS)	Stool sample or rectal swab positive for *E. coli*–producing Shiga toxin by culture and/or PCR (both should be performed)

ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aPTT, activated partial thromboplastin time; INR, international normalized ratio;


A
Classical pathway
Lectin pathway
Alternative pathway
B
Factor H
FHL-1
FHR-1A
FHR-1B
FHR-2
FHR-3
FHR-4A
FHR-4B
FHR-5
Terminal pathway
C5 → C5a
C5b-9


Classical pathway
Antibodies bound to antigen, pentraxins, apoptotic cells, microbes etc.
Lectin pathway
Apoptotic cells, pentraxins, microbes etc.
Alternative pathway
Probing all surfaces (host and foreign)
+ C1q
+ MBL, ficolins
+ C3b
C3 convertases
Amplification loop
Inflammation, cell activation
C3a
C3
Factor H / FHL-1
FHR-1, FHR-4, FHR-5
C3b
Target cell
Opsonization, enhancement of phagocytosis
C5 convertases
Terminal pathway
C5
C5a
Inflammation, cell activation
C5b-9
Target cell
Target cell lysis

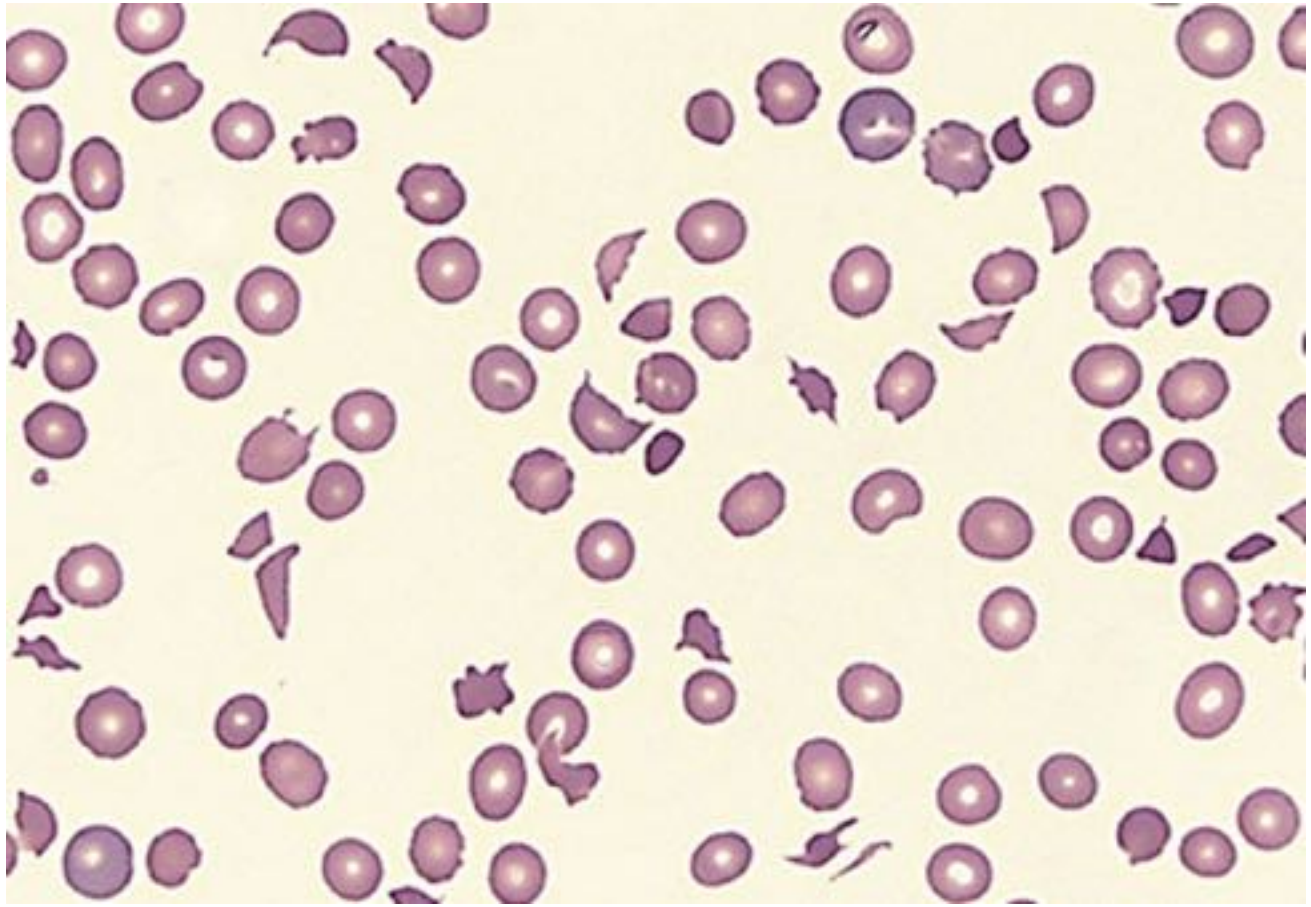

Platelet
GPIb-IX-V
GPIbα subunit
ULVWF
Normal
ADAMTS13
TTP
ADAMTS13
NAC

When do you suspect diarrhea +ve HUS?

- Global annual incidence of diarrheal disease episodes in children <5 years old is 2.5 billion episodes /year

- Annual incidence of diarrheal diseases per child < 5 years old is 2-3 episodes / year

- The global annual incidence of HUS is difficult to estimate, however it is estimated to be around 2-3 cases/ 100,000 in children < 5 years

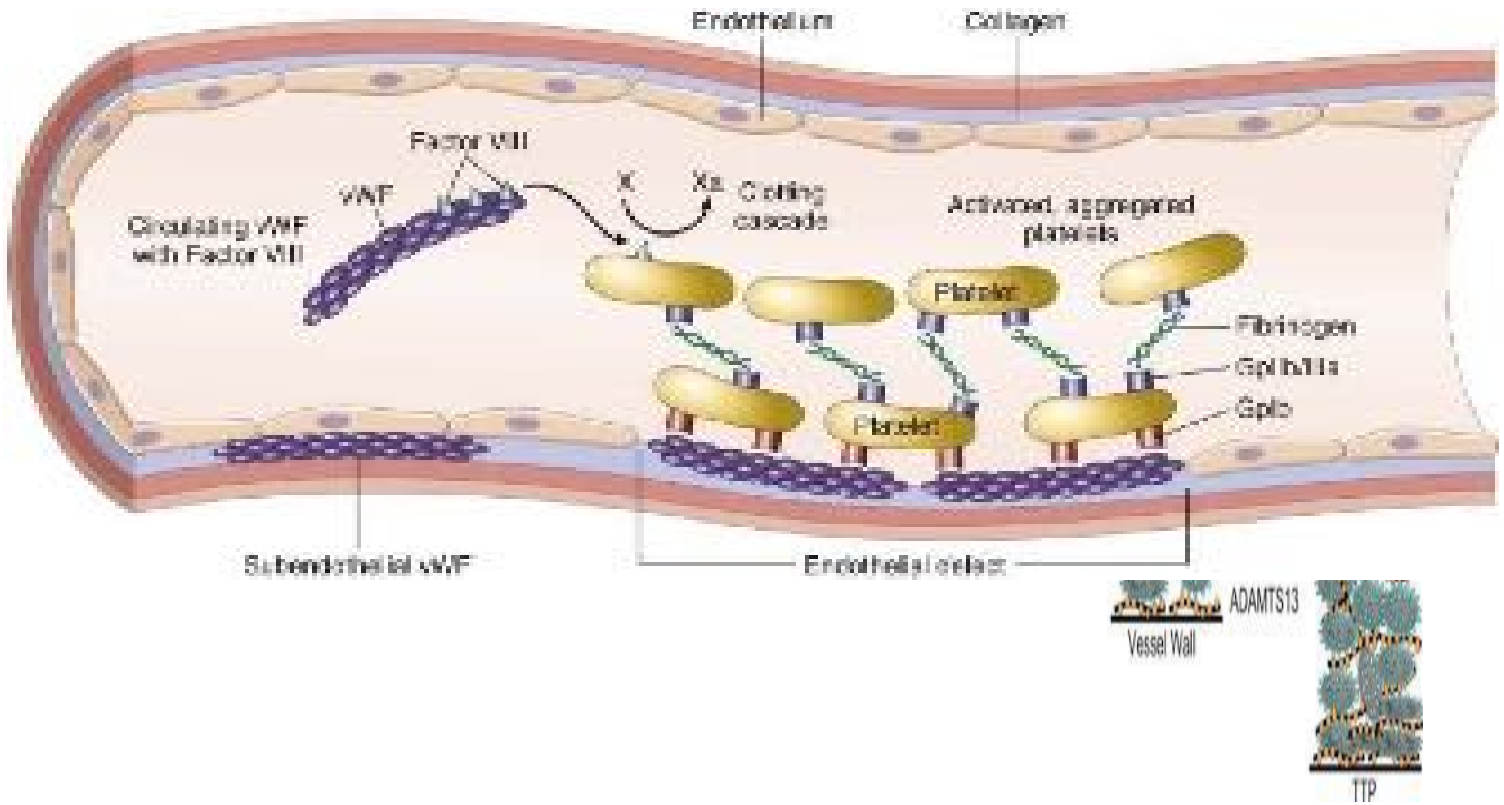


Is it important subject?

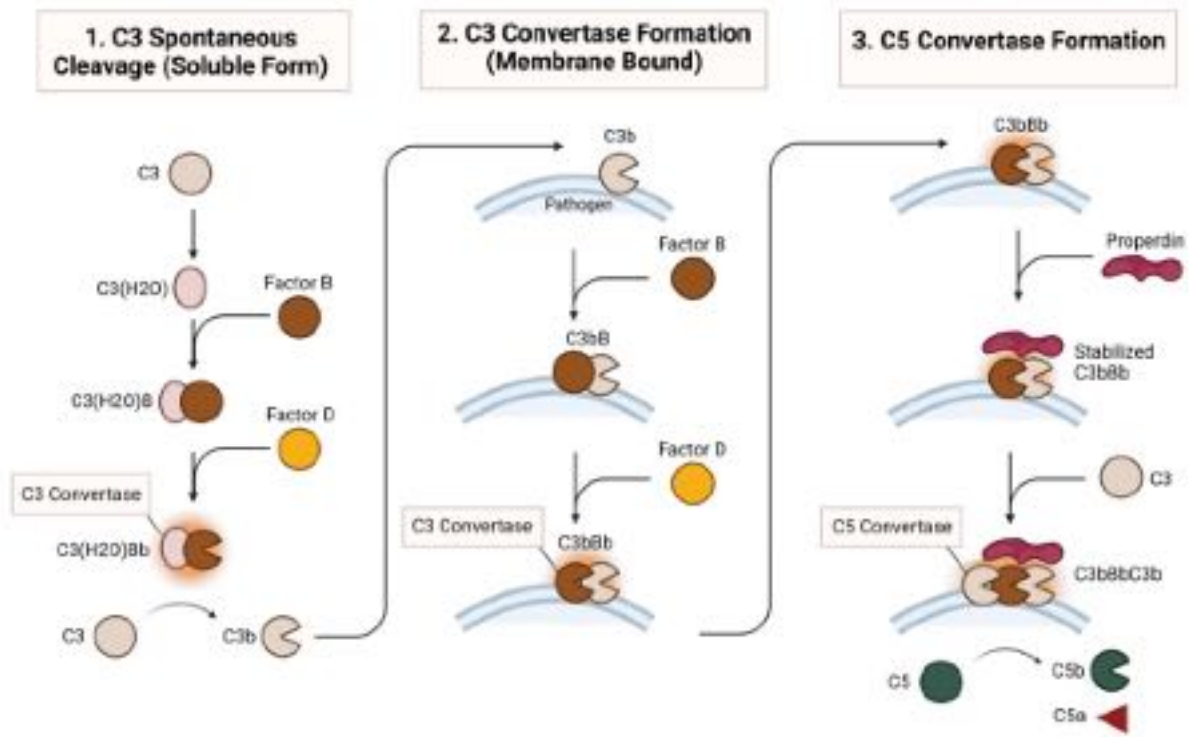
- Not uncommon
- It is a life-threatening condition
- Although HUS is easily diagnosed, the etiological diagnosis of HUS is very problematic
- It is caused by a varieties of etiologic factors
- Prognosis depends on

  Proper etiological diagnosis

  Proper management according to etiology


Endothelium
Collagen
Factor VIII
VWF
Circulating VWF
with Factor VIII
X
Xa
Clotting
cascade
Activated aggregated
platelets
Platelet
Platelet
Platelet
Fibrinogen
GpIIb/IIIa
GpIb
Subendothelial vWF
Endothelial cellular
ADAMTS13
Vessel Wall
TTP

Tickover Initiation - Alternative Complement Pathway



Is it HUS ?

1- Exclude TTP

Sever thrombocytopenia (platelets count < 30,000)

Mild AKI

CNS insult

ADAMTS 13 activity < 10 %

Anti - ADAMST 13 antibodies is positive

Etiology of HUS

- Diarrhae +ve HUS ….. Shiga toxin-producing *Escherichia coli (*STEC-HUS)
- Infections associated HUS

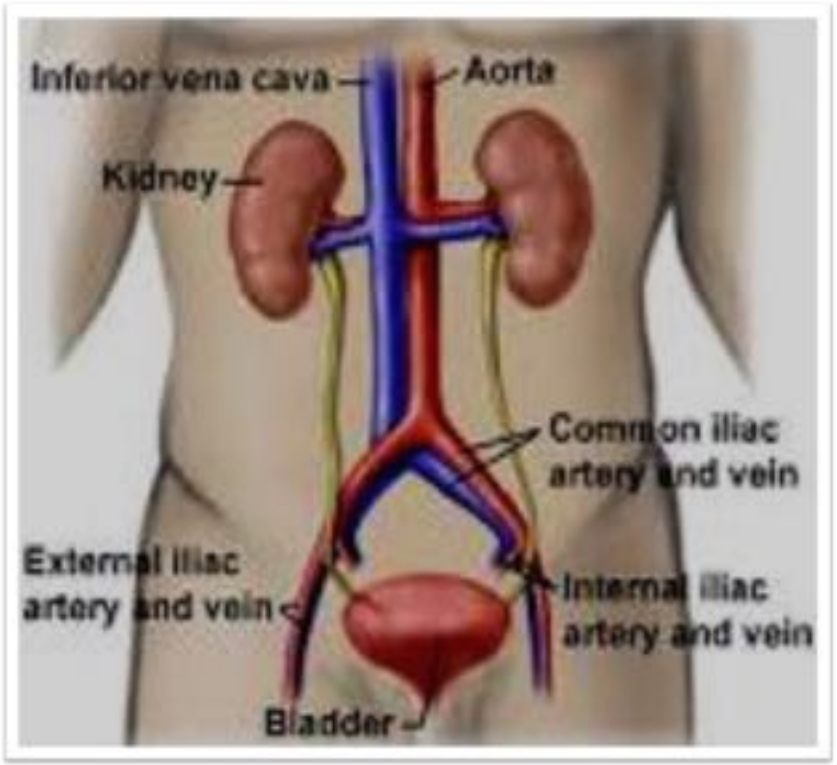
  strept. Pneumonia, N1H1, HIV, covid 19, ….
- Secondary to systemic diseases

  SLE, malignancy, antiphospholipid syndrome, organ transplantation ….
- Drugs

  CNI,
- Cobalamin C deficiency
- Atypical HUS

Urinary system

- **It is clinically useful to classify urinary system into:**
  - **Kidneys.**
  - **Urinary tract**



objectives

- Review of renal anatomy, histology, and physiology
- Classification of renal diseases
- Ultrastructure of glomerular components
- Normal glomerular structure by light microscope
- Glomerular diseases

Urinary system

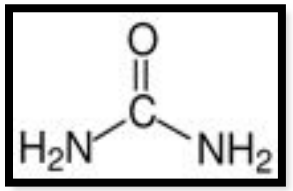
What is the main function of kidney and UT ?

Urinary system

- **The main function of the kidney is *urine formation* while *urine transmission, storage, and evacuation* are the function of the urinary tract.**

Function of Kidney

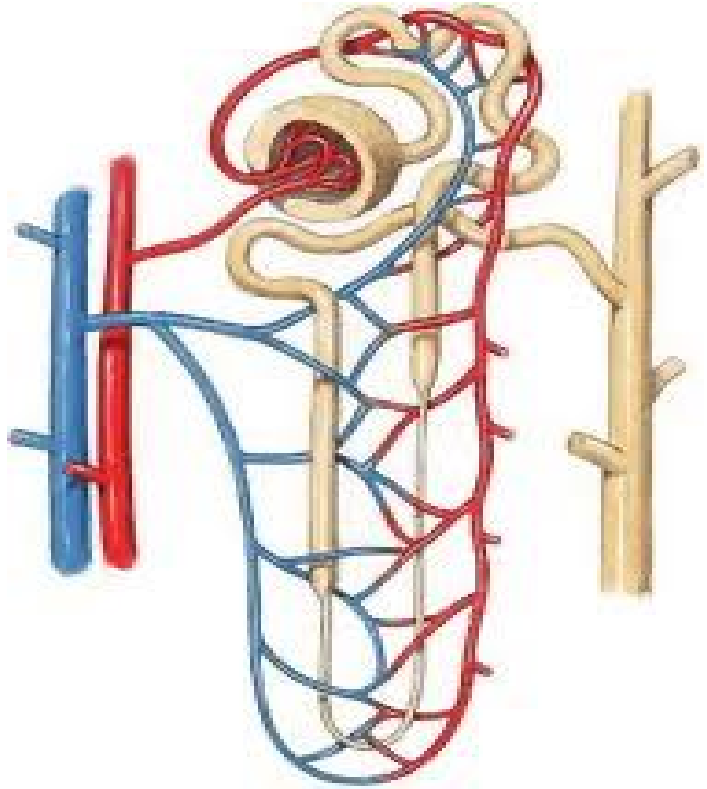
- **By urine formation the kidney**
  - ***Regulate water, electrolytes and acid base balance.***
  - ***Excrete non-volatile waste products, mainly nitrogenous compounds.***



- **Other functions include:**
  - ***Erythropoietin secretion.***
  - ***Regulation of blood pressure through renin.***
  - ***Activation of Vit. D***

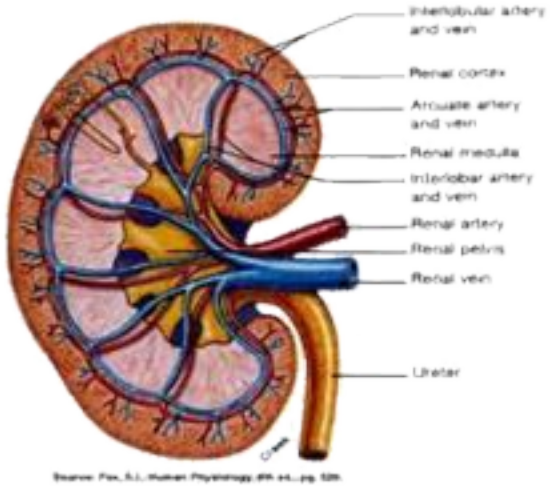
How does the kidney form urine?

- Adequate renal blood flow
- Proper glomerular filtration
- Efficient tubular reabsorption



Renal Blood Flow

- Renal blood flow = 20-25% total cardiac output
- In adult, it is more than one liter /min (1250 ml / min)
- Renal plasma flow = 700 ml / min
- About 90% of RBF supplies cortex of kidney
- RBF is subjected to restricted autoregulation



Glomerular Filtration

- **Glomerular filtration is the initial step of urine formation**
- **Glomerular filtrate (GF) is formed by filtration across the glomerular membrane an ultrafiltrate identical to plasma minus its proteins.**
- **Driving force is pressure gradient between intracapillary blood pressure and Bowman's space pressure**

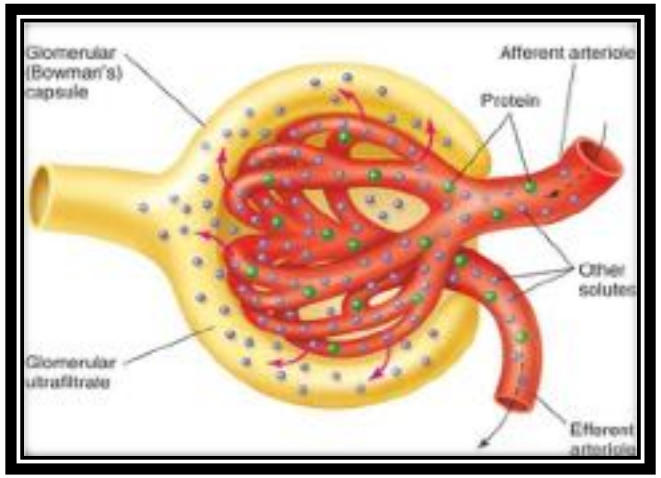
➢ **Glomerular blood flow and pressure is under restrict autoregulation**

- **In adults**

  GFR /min = 125ml/min …. 10% of RBF

  GFR /hour = 7.5 L/h

  GFR /day = 180 L/day



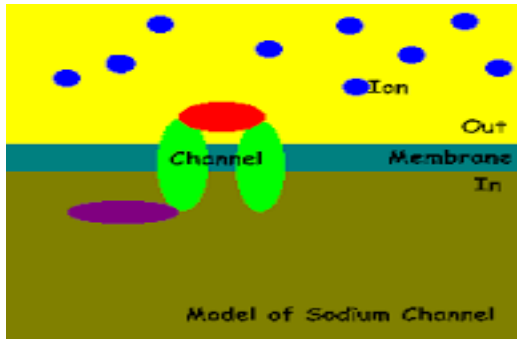
Tubular Function

- **Reabsorption** is the main function of renal tubules, although there is a limited *excretory* tubular function

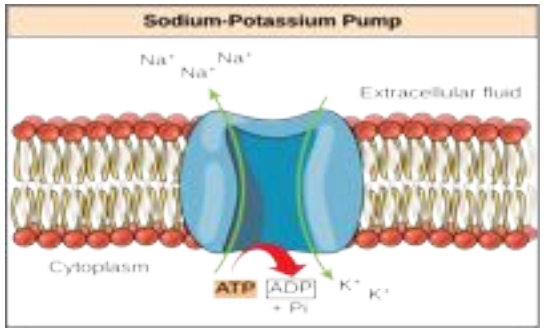
- Solutes reabsorption is an active and controlled process. In certain sites, it is under hormonal control *(ADH, aldosterone, ANP ….)*

- Solutes are transported across cell membrane through:

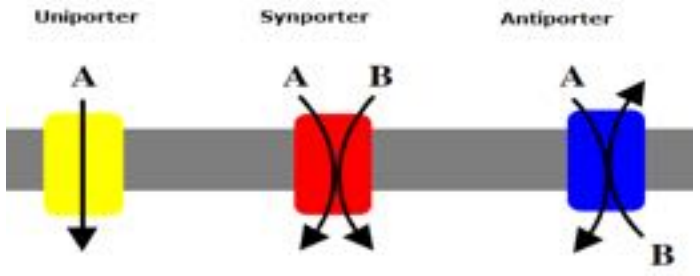
ionic channels



pumps



transport (carrier) proteins



Renal Diseases

- It is clinically useful and, in most instances, theoretically sound to consider renal diseases from the stand point of primary site of injury or of disturbed physiology Thus, renal diseases can be classified to:
    - Glomerular diseases.
    - Tubular renal diseases.
    - Interstitial renal diseases.
    - Renal vasculature diseases.

Glomerular structure

- Capsule

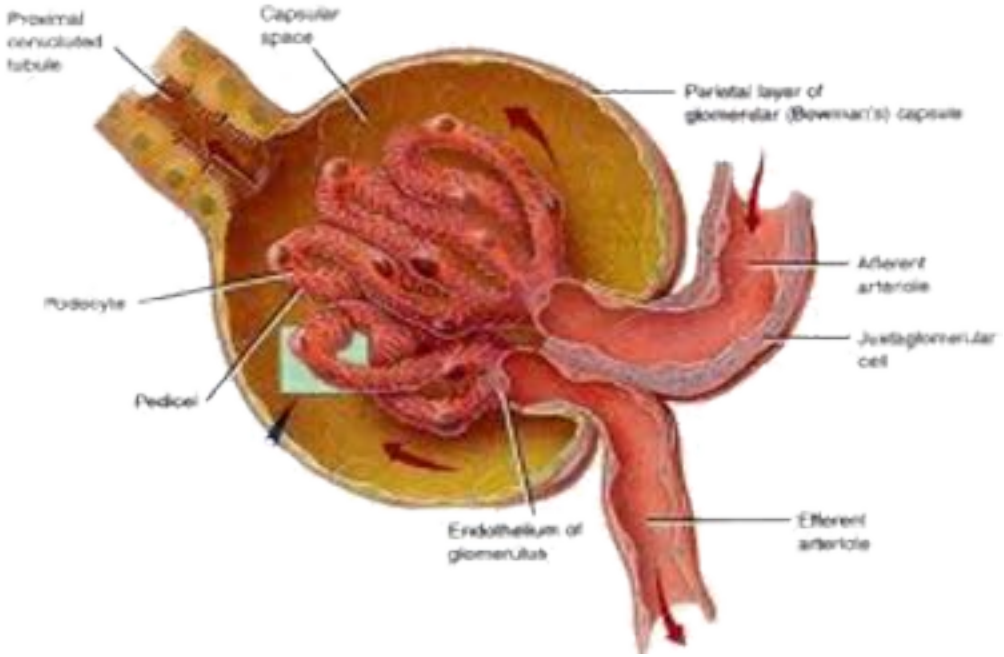
  Lined by parietal epithelium

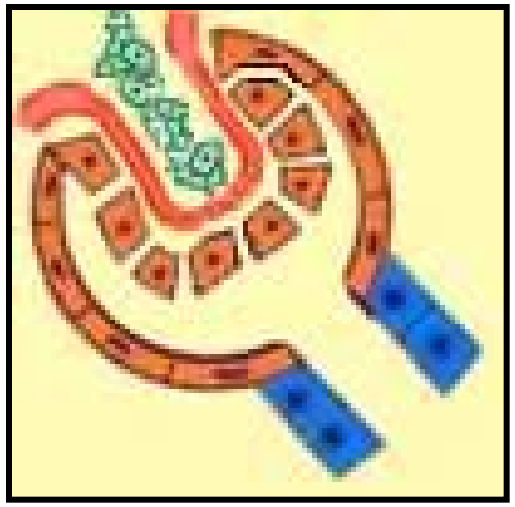
- Bowman`s space

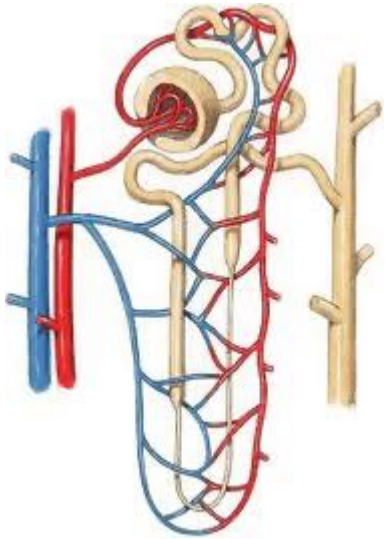
- Tuft

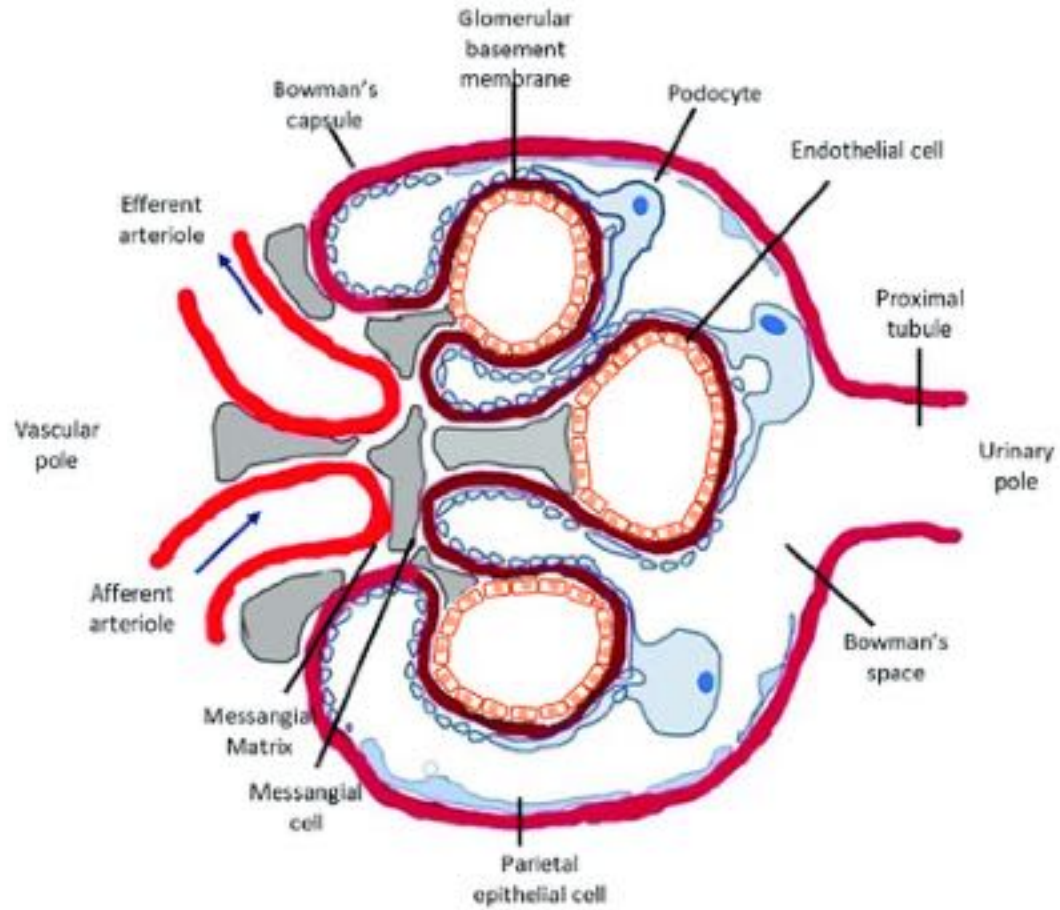
  Capillaries (lumen and capillary wall)

  Mesangial cells and matrix

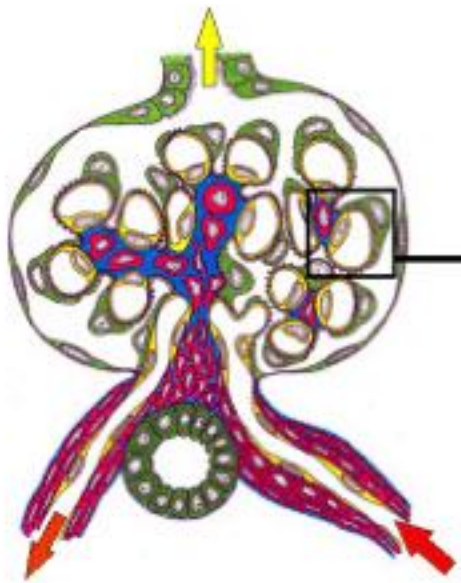




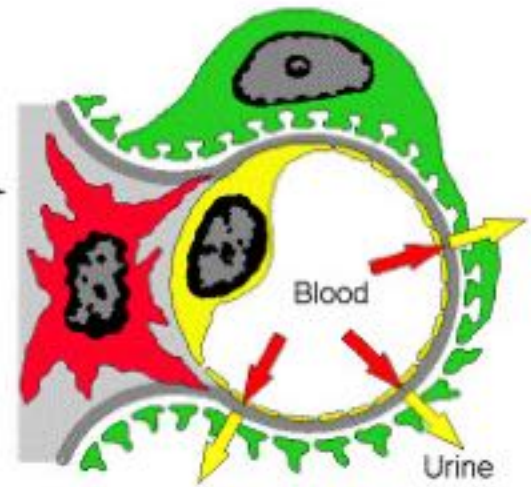



Glomerular basement membrane
Bowman's capsule
Podocyte
Endothelial cell
Efferent arteriole
Proximal tubule
Vascular pole
Urinary pole
Afferent arteriole
Bowman's space
Messangial Matrix
Messangial cell
Parietal epithelial cell

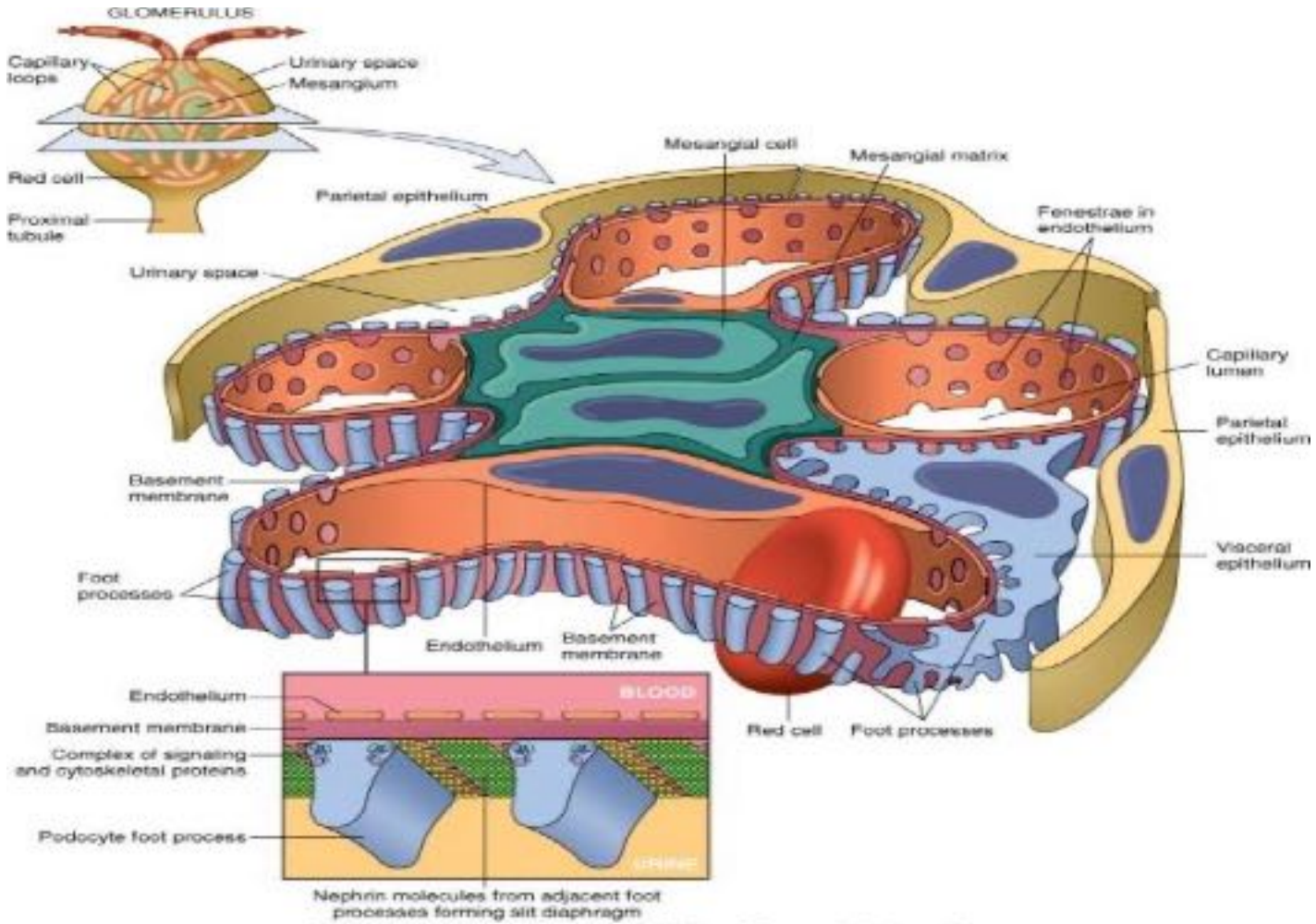
Glomerular Capillaries



Drawing of a cross section of a glomerulus with arrows showing the flow of blood (red) and urine



Drawing of a cross section of one very small blood vessel called a capillary in a glomerulus showing the filtration of blood (red) into urine (yellow)



Capillary

Capillary

lumen ….. blood and blood cells

Glomerular membrane (capillary wall)

1-fenestrated endothelium …. endothelial cells

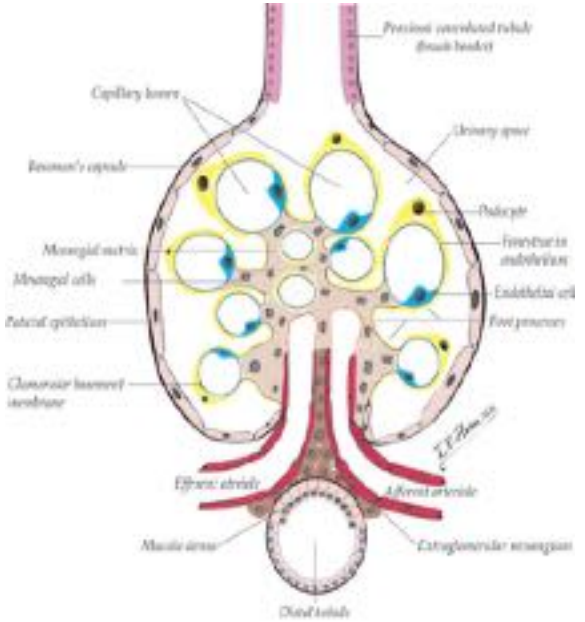
2- basement membrane

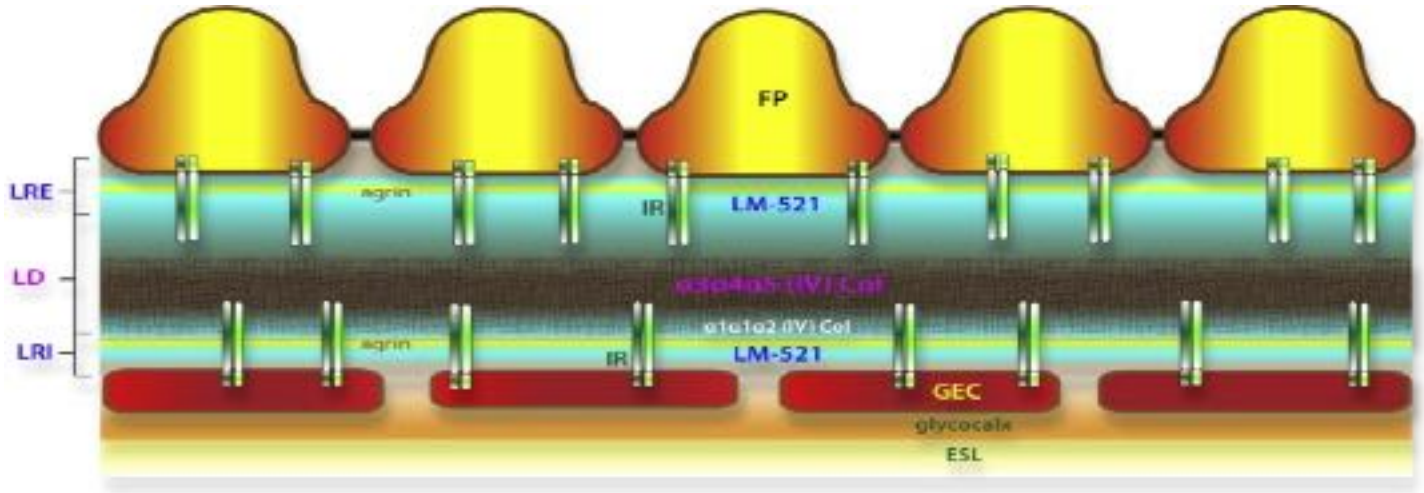
lamina rara interna

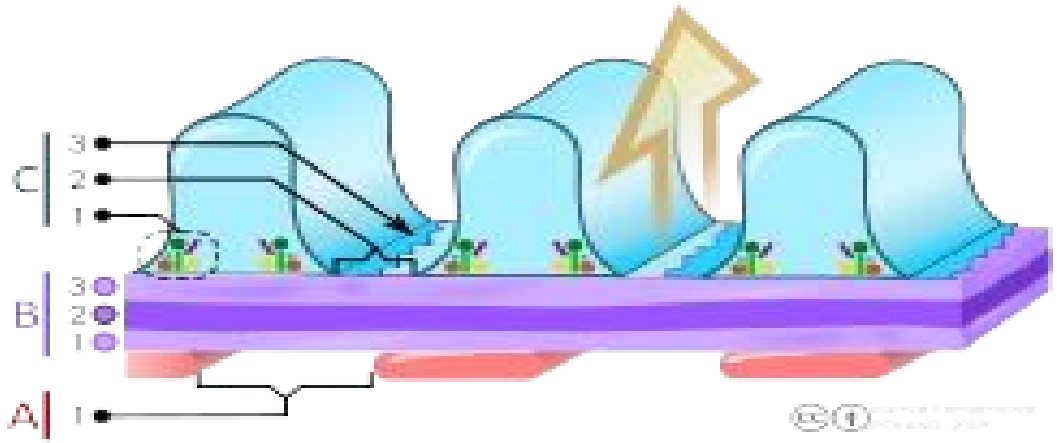
lamina dense

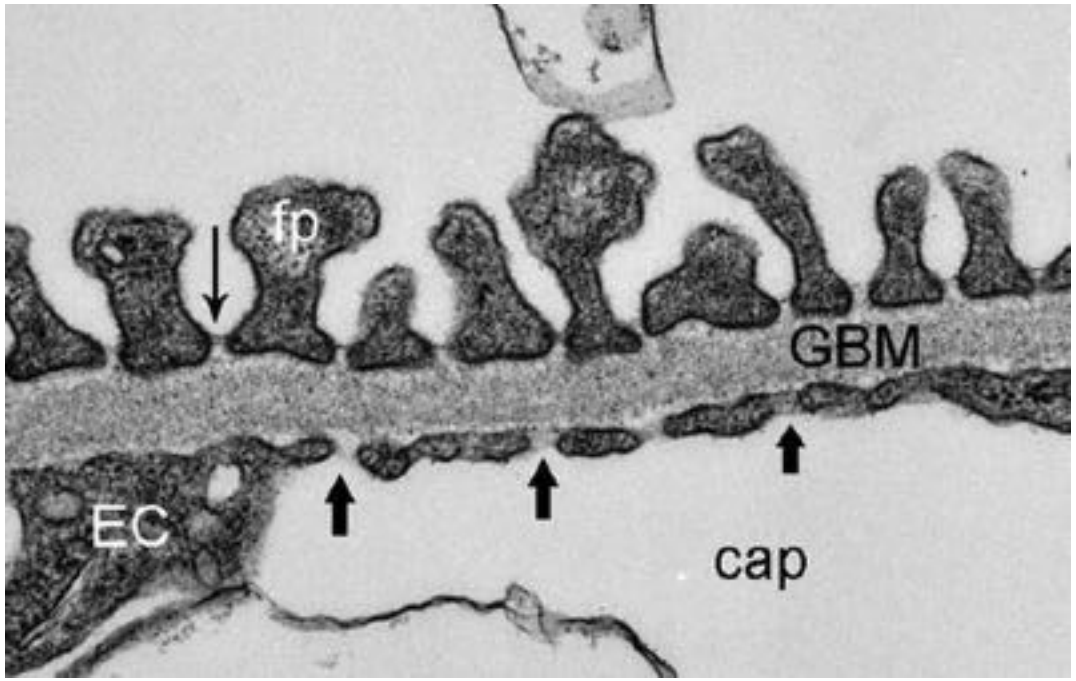
lamina rara externa

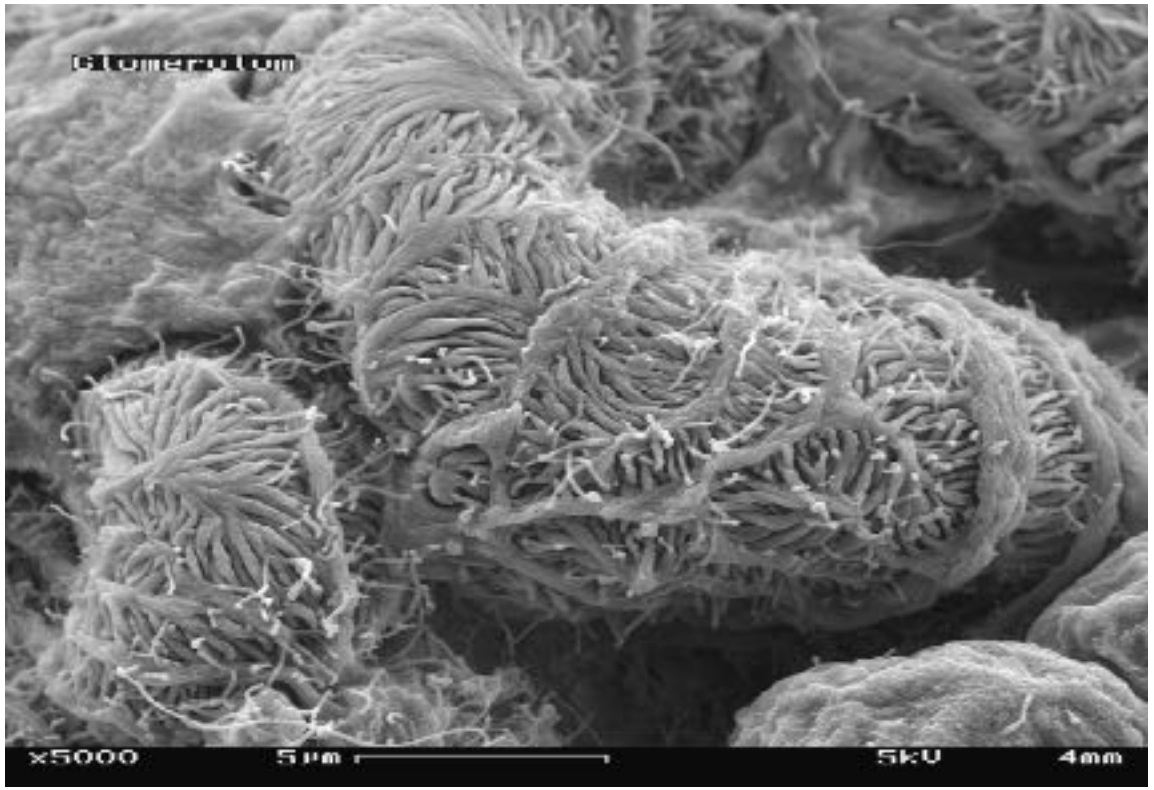
3- visceral epithelium …. podocytes …. foot process

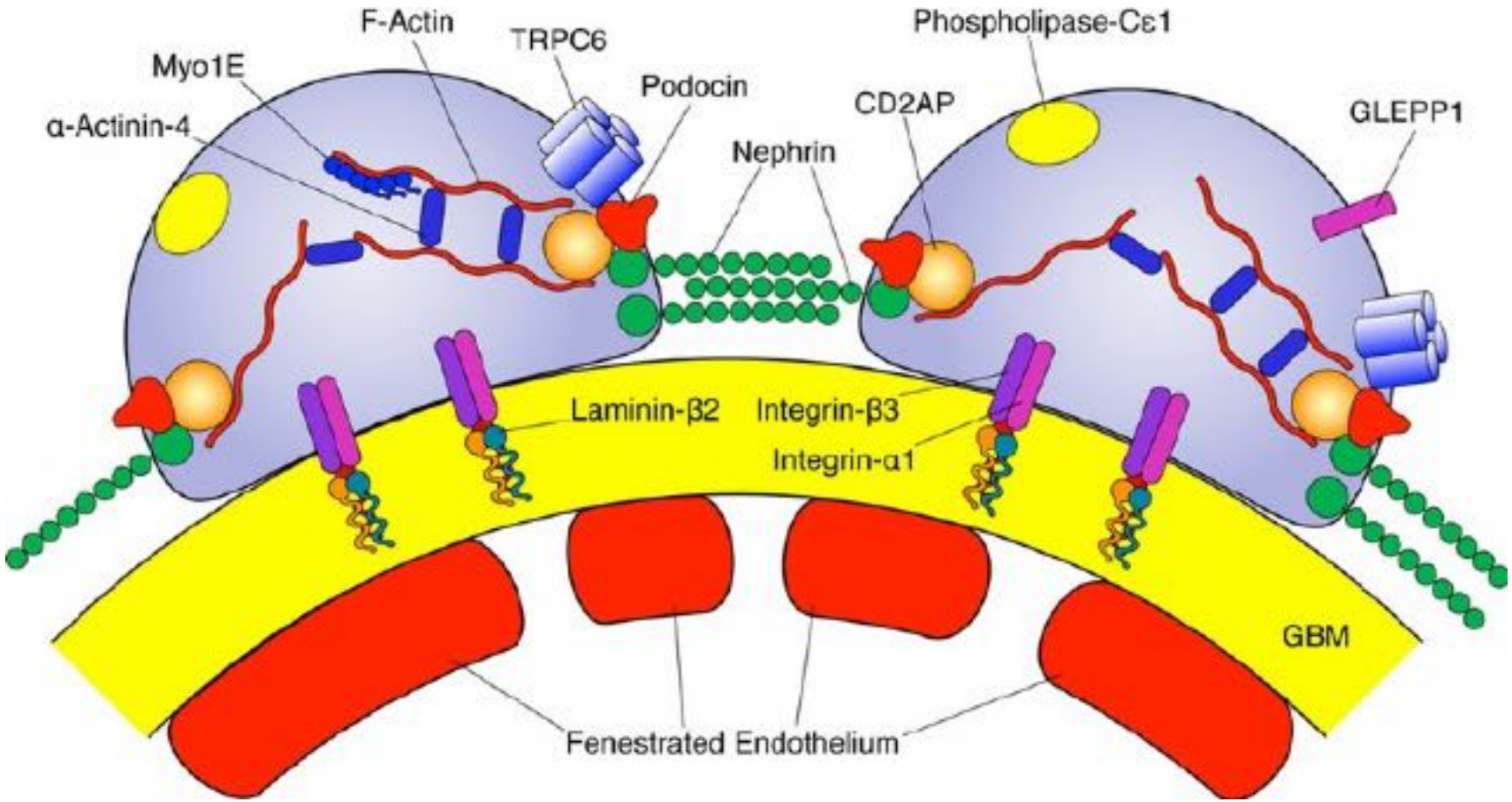



FP
LRE
LD
LRI
agrin
IR
LM-521
α5α4α5 (IV) Col
α1α1α2 (IV) Col
agrin
IR
LM-521
GEC
glycocalix
ESL


C
3
2
1
B
3
2
1
A
1


fp
GBM
EC
cap


Glomerulum
x5000
5µm
5kV
4mm

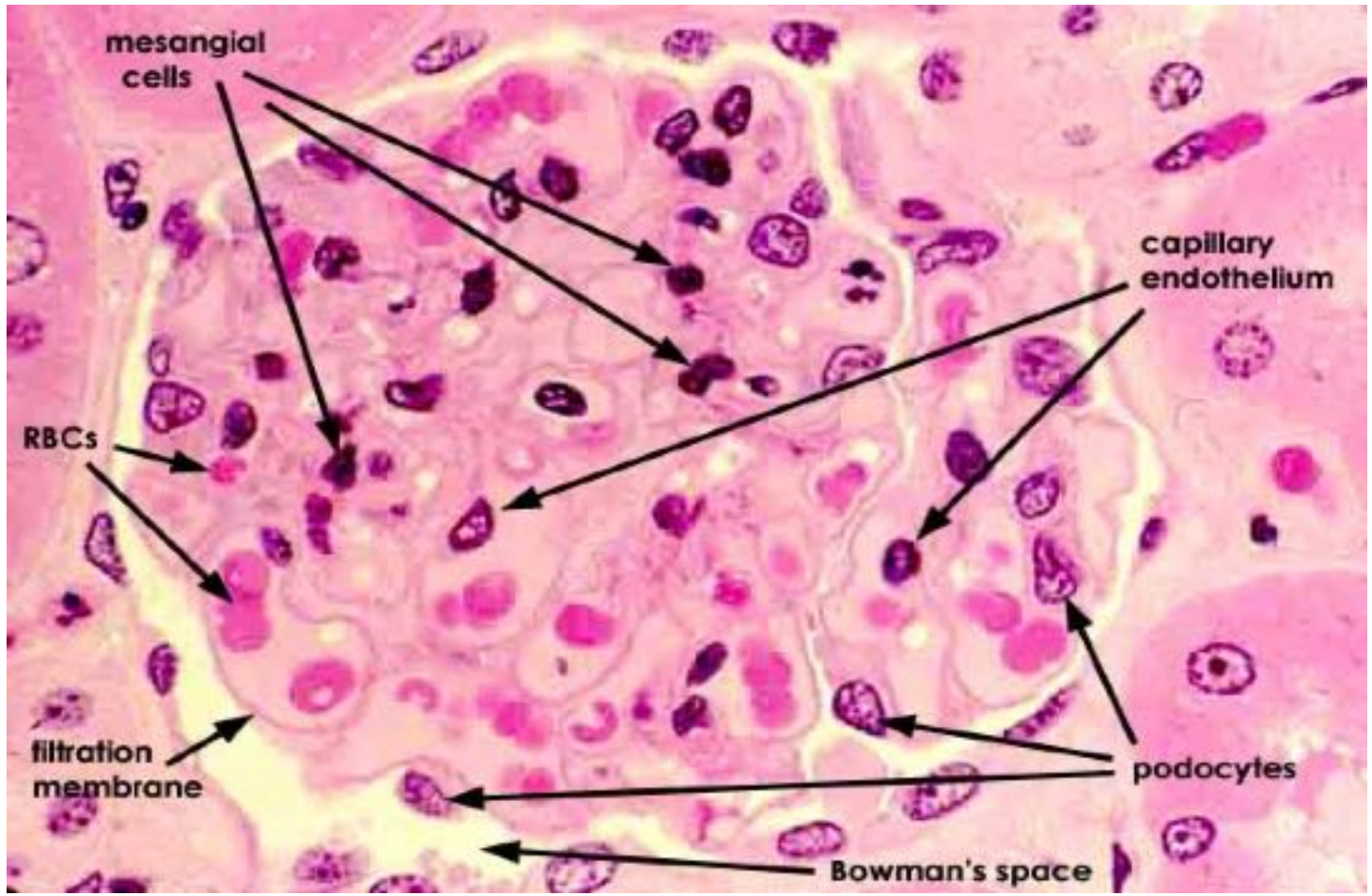

F-Actin
TRPC6
Phospholipase-Cε1
Myo1E
Podocin
CD2AP
α-Actinin-4
Nephrin
GLEPP1
Laminin-β2
Integrin-β3
Integrin-α1
GBM
Fenestrated Endothelium

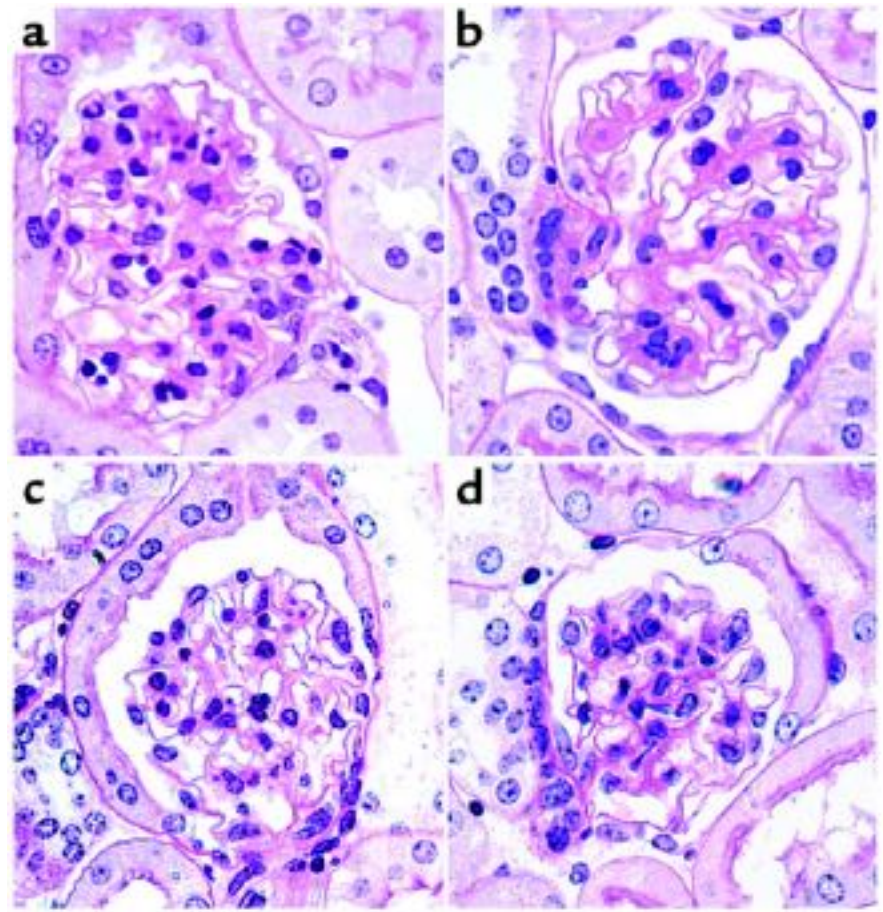
Mesangial cells and matrix

- Mesangial cells
  - Mesenchymal in origin
  - Contractile cells
  - Secret collagen fibers and a number of cytokines

- Mesangial matrix
  - extracellular material mainly of collagen fibers

- Function
  - regulate capillary blood flow
  - immune function
  - mechanical support


mesangial cells
capillary endothelium
RBCs
filtration membrane
podocytes
Bowman's space


a
b
c
d

Etiology

- **Primary**
  - ***Immune mediated***
  - ***Non-immune mediated***
  - ***Unknown***
- **Secondary**
  - ***Infection (bacterial, viral, parasitic, fungal)***
  - ***Systemic autoimmune diseases***
  - ***Neoplastic diseases***
  - ***Hematological diseases***
- **Inherited**

Pathogenesis

- **Mechanism of glomerular immune injury:**
  - **Circulating immune complex**
  - **Immune complex in situ**
  - **Anti glomerular BM antibodies**
  - **Complement dysregulation**
  - **Cell mediated immune response (T-cell)**
  - **Unknown**

Pathogenesis

- **The glomerulus may be injured by several etiologic factors , but it has a limited number of histopathologic responses**
- ***Proliferation*** **of glomerular cells (mesangial, endothelial, epithelial) occurs in most forms of glomerulonephritis.**
- ***Cellular infiltration***

Pathology

- **Non proliferative glomerular diseases**
  - **MCNS**
  - **FSGS**
  - **Membranous GN**

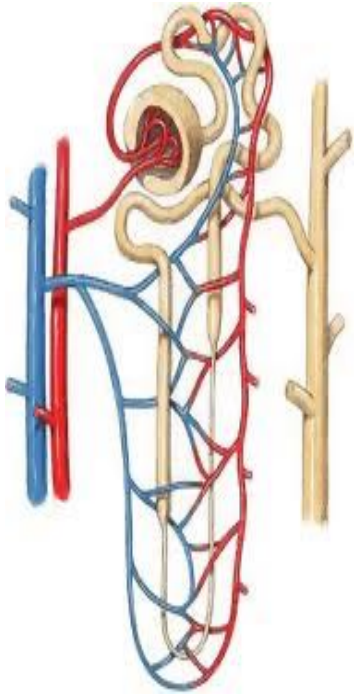
- **Proliferative GN**
  - **Mesangial**
  - **Endocapillary**
  - **Membranoproliferative**
  - **Epithelial crescent**

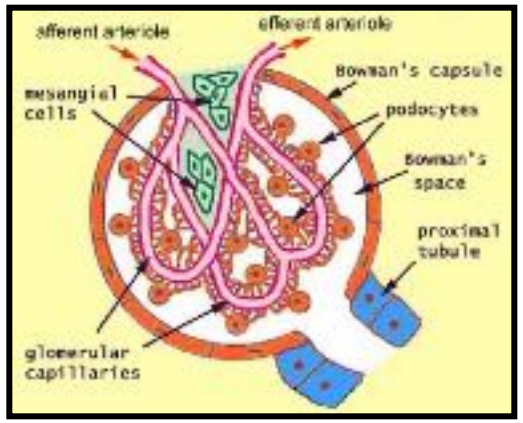
Sequelae

- **Regeneration**
- **Fibrosis**
- **Sclerosis**

Clinical Presentation of Glomerular Diseases

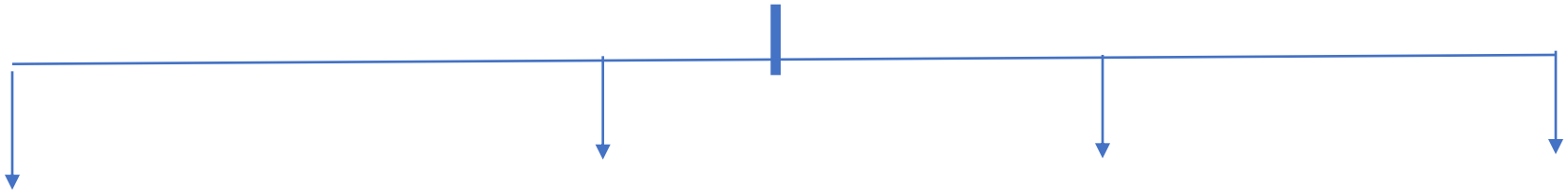
- Isolated hematuria and/or proteinuria
- Glomerulonephritis
- Nephrotic syndrome
- Mixed nephrotic – nephritic





- ***An understanding of the overlapping between clinical diseases, histopathologic lesions and etiologic factors is required to arrive at the proper diagnosis and treatment.***

Diagnosis of Glomerular Diseases



Clinical

- Isolated hematuria and/or proteinuria
- Glomerulonephritis
- Nephrotic syndrome
- Nephrotic - nephritic type

Functional

Normal kidney function

ARF

ARF on top of CRF

CRF

Histopathological

* Normal.
* MCNS.
* FSGS.
* MCGN.
* PGM.
* MGM.
* Crescentic

Etiological

Primary

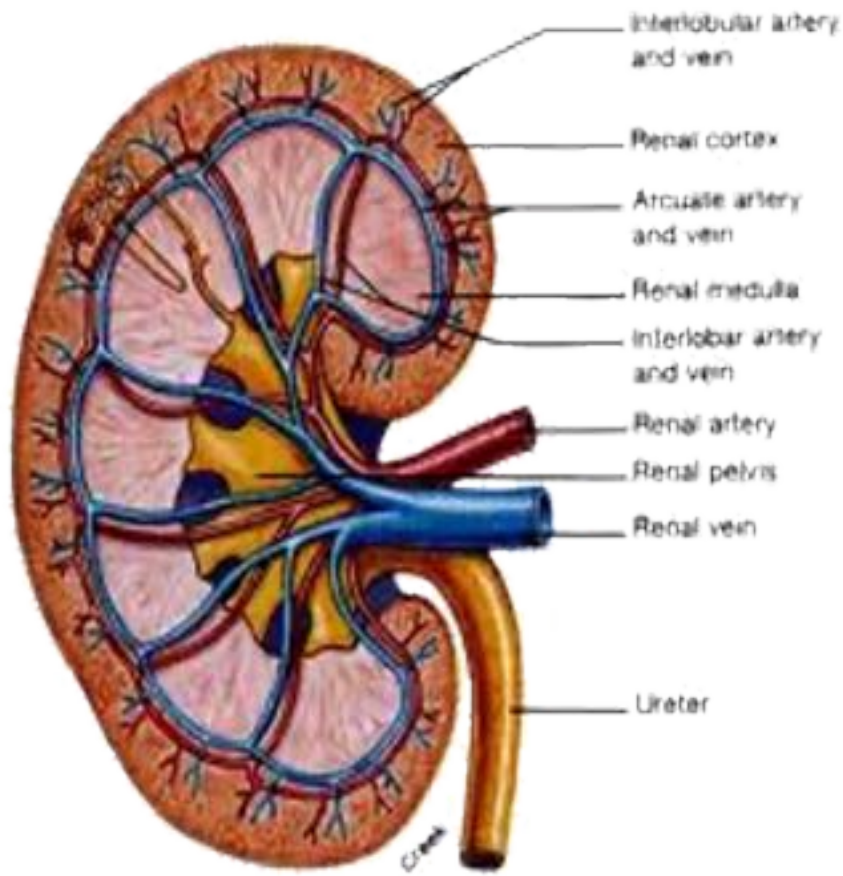
Secondary

- **The same *clinical syndrome* is caused by many etiologic factors and may be associated with different histopathologic lesions.**

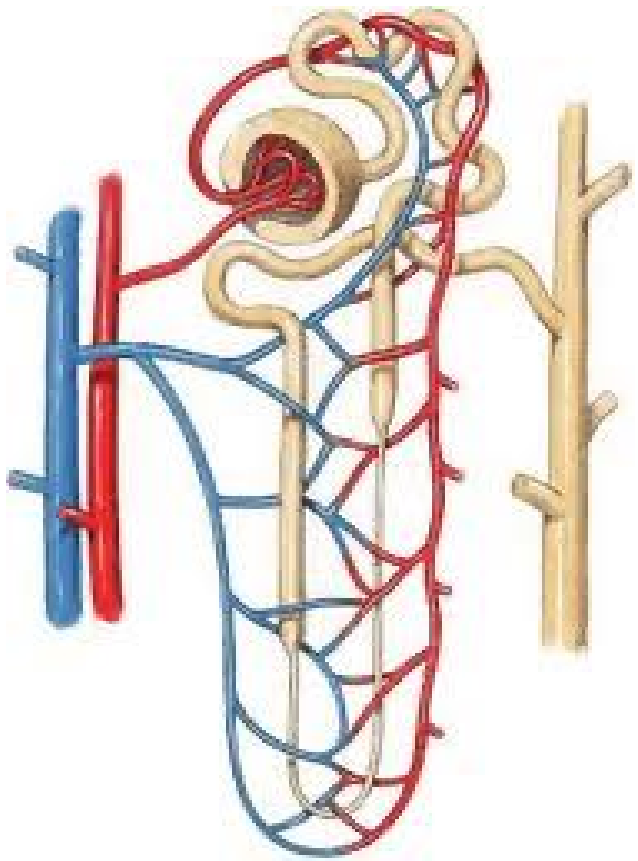
- **Also, the same *histopathologic category* can be caused by different etiologic factors and is presented by different clinical syndromes.**

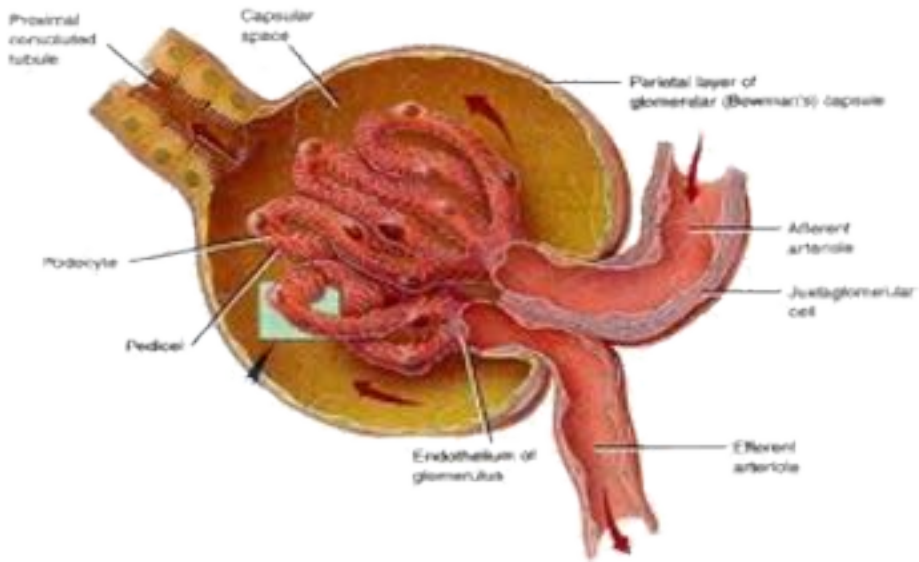
- **Also, the same *disease* can lead to different histopathological lesion and is presented by different clinical syndromes.**

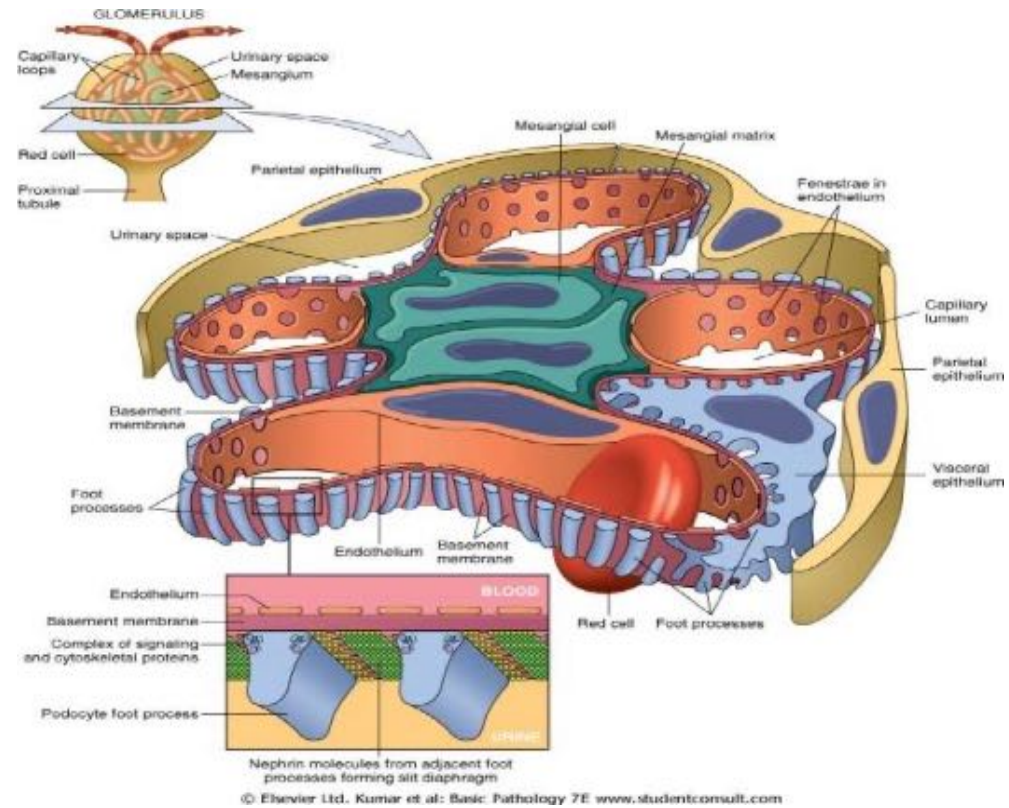
- **Even, the same *patient* can be presented, during the course of his or her illness, by different clinical and pathological categories.**



Source: Fox, S.I., Human Physiology, 6th ed., pg. 526.




Proximal convoluted tubule
Capsular space
Parietal layer of glomerular (Bowman's) capsule
Podocyte
Pedicel
Endothelium of glomerulus
Efferent arteriole


GLOMERULUS
Capillary loops
Urinary space
Mesangium
Red cell
Proximal tubule
Mesangial cell
Mesangial matrix
Parietal epithelium
Fenestrae in endothelium
Urinary space
Capillary lumen
Parietal epithelium
Basement membrane
Visceral epithelium
Foot processes
Endothelium
Basement membrane
Endothelium
BLOOD
Basement membrane
Complex of signaling and cytoskeletal proteins
Red cell
Foot processes
Podocyte foot process
URINE
Nephrin molecules from adjacent foot processes forming slit diaphragm
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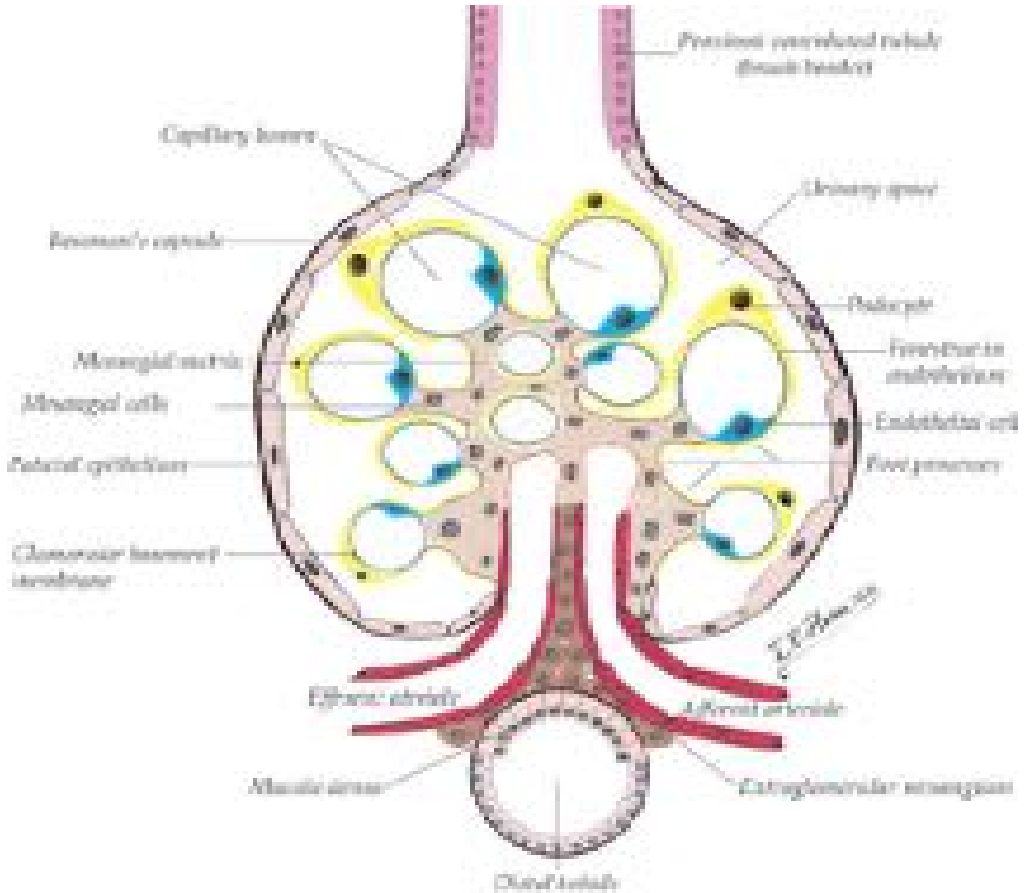
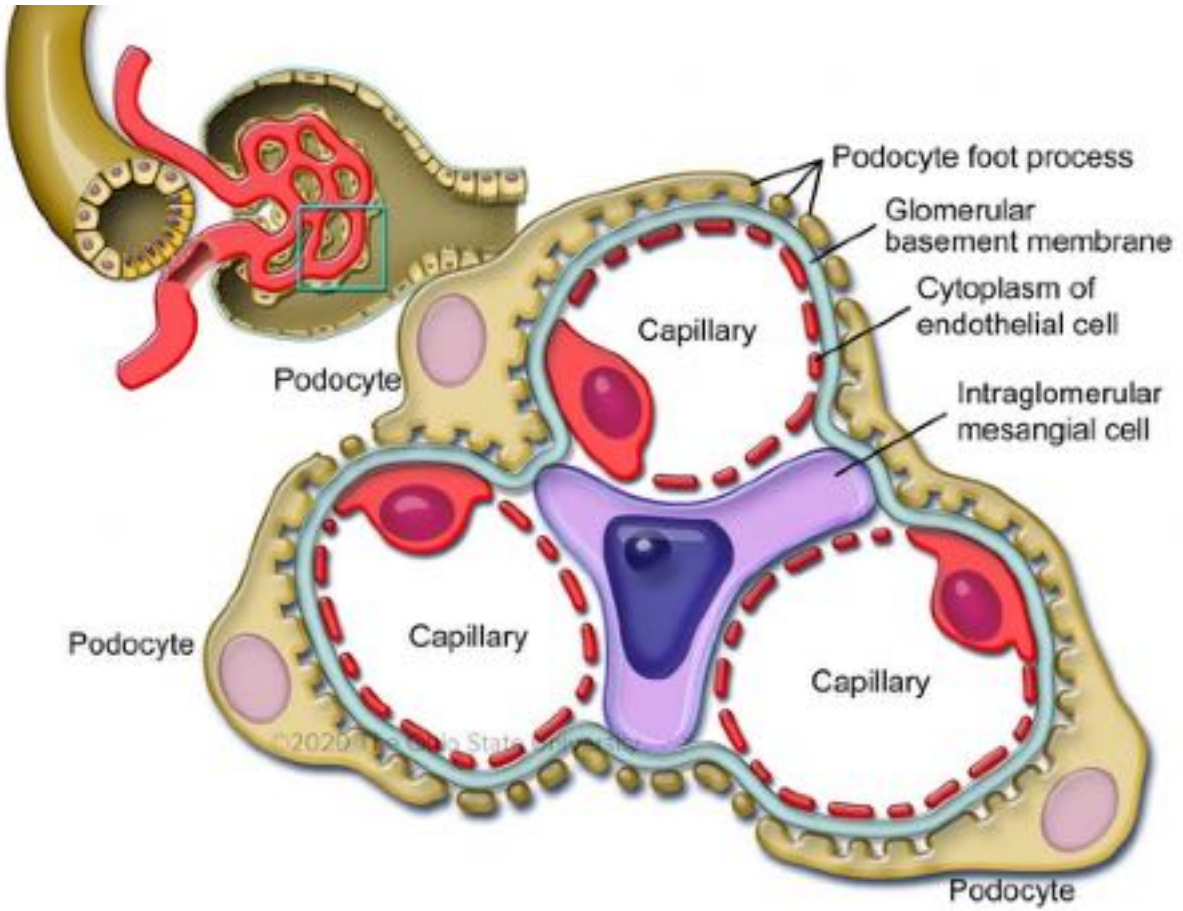
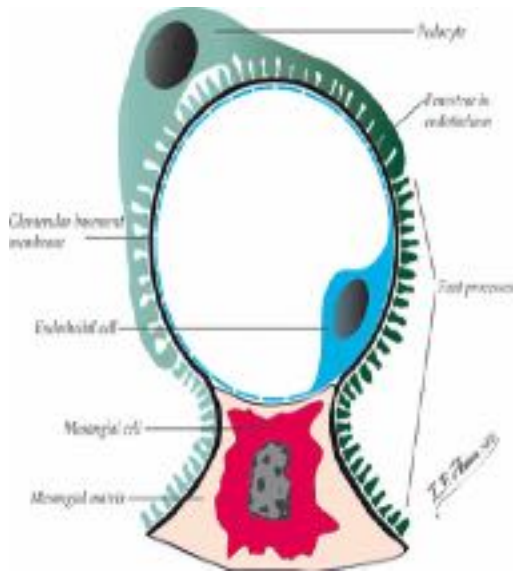
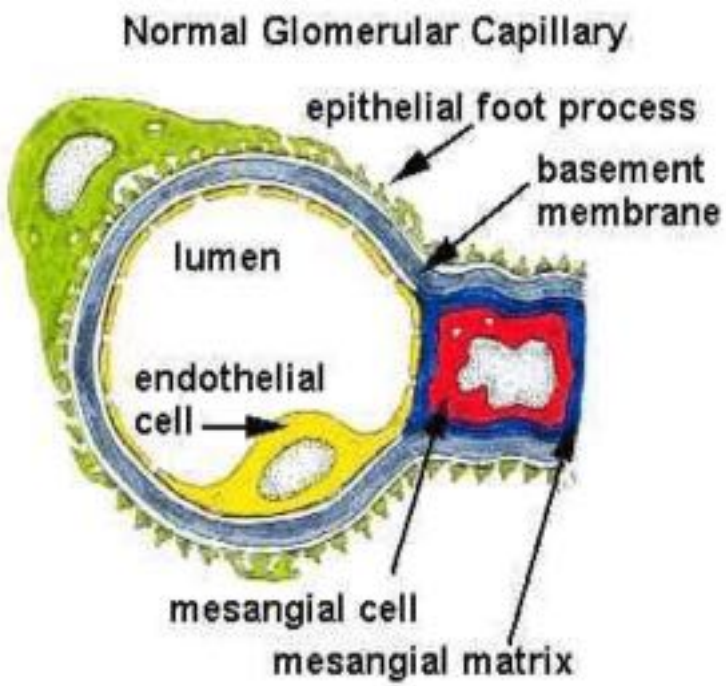
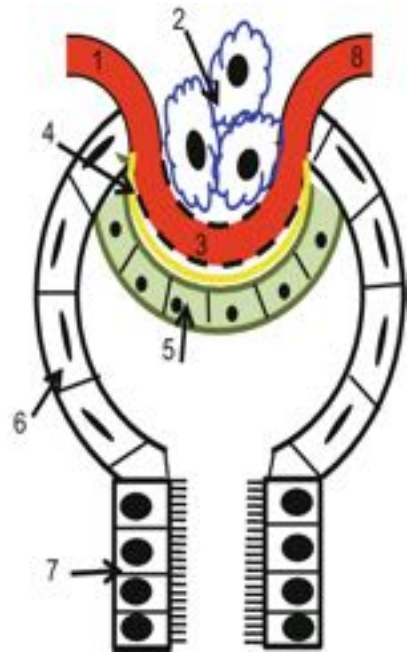


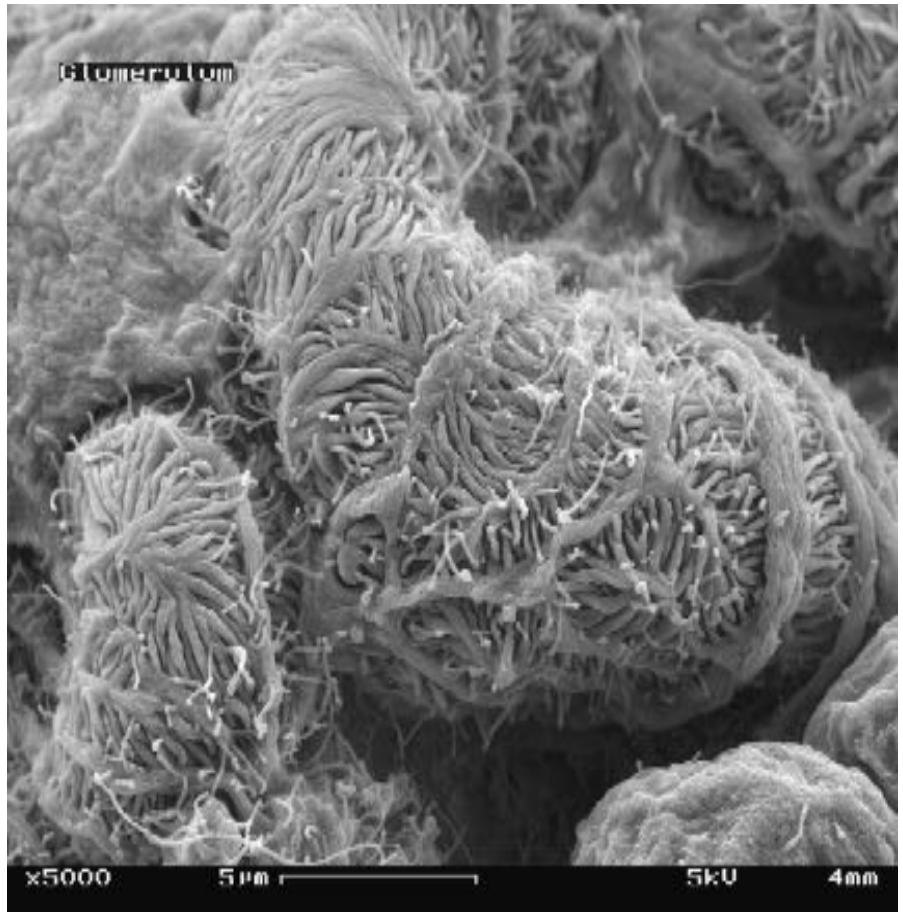

Podocyte foot process
Glomerular basement membrane
Cytoplasm of endothelial cell
Intraglomerular mesangial cell
Capillary
Podocyte
Capillary
Podocyte
Capillary
Podocyte

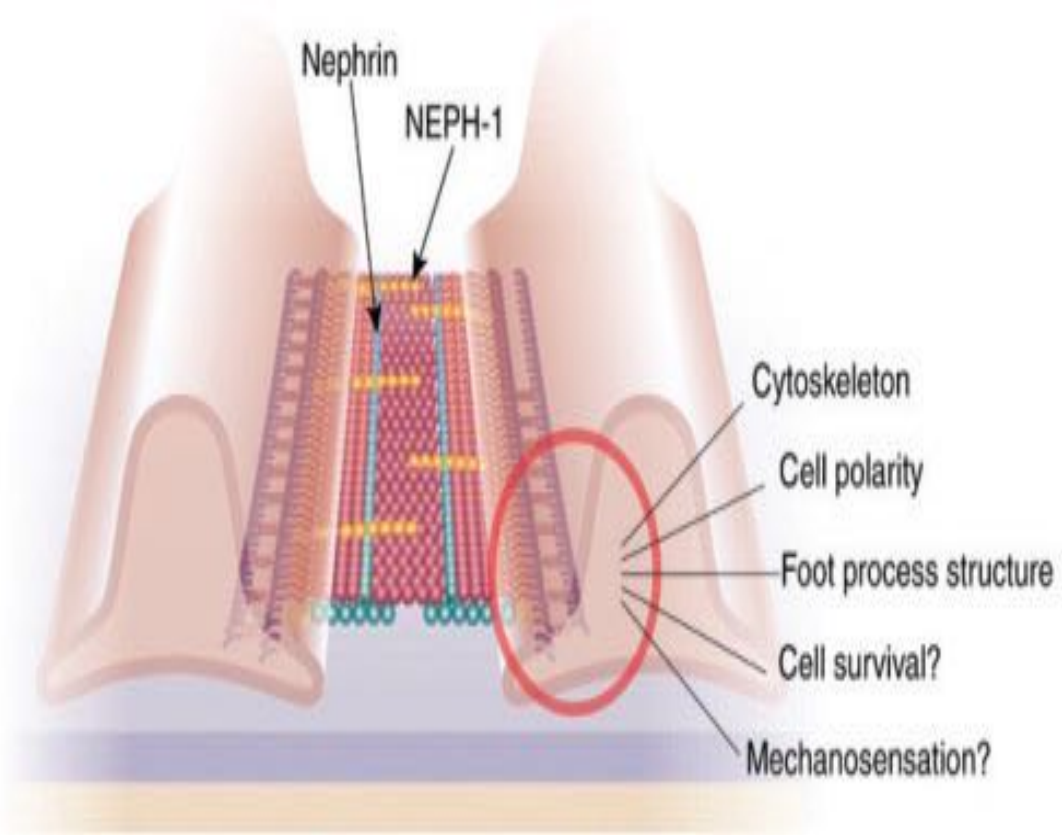


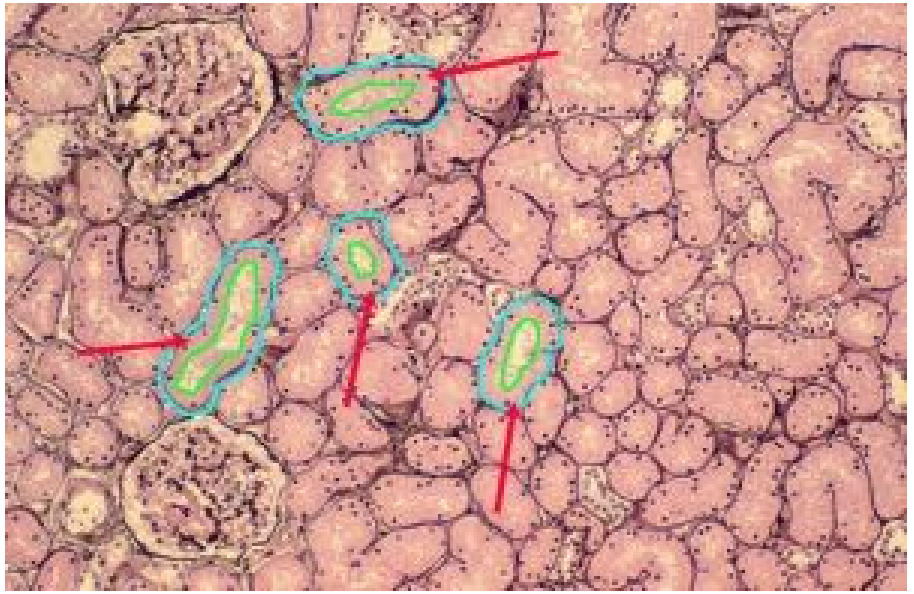


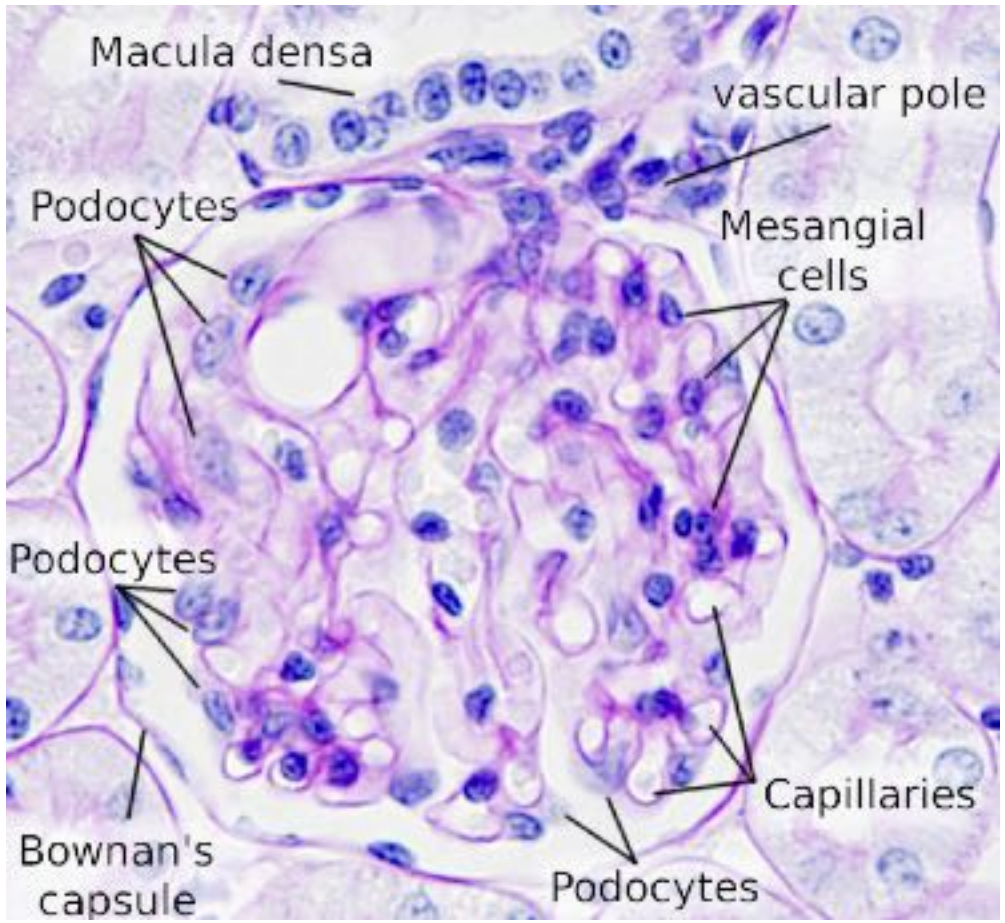
1 - Afferent arteriole
2 - Mesangial Cells
3 - Fenestrated capillaries
4 - Basement membrane
5 - Podocytes
6 - Parietal Cells
7 - Proximal Tubule Cells
8 - Efferent arteriole

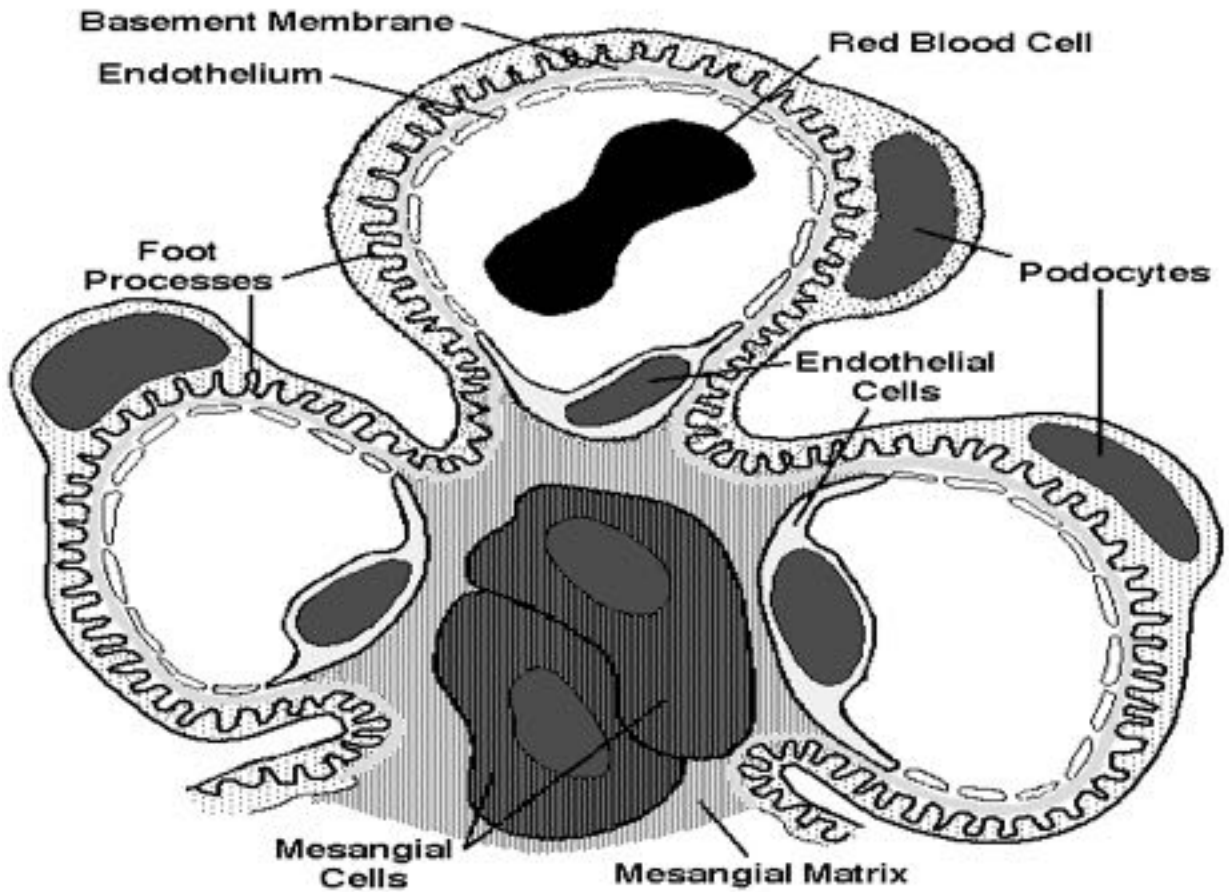


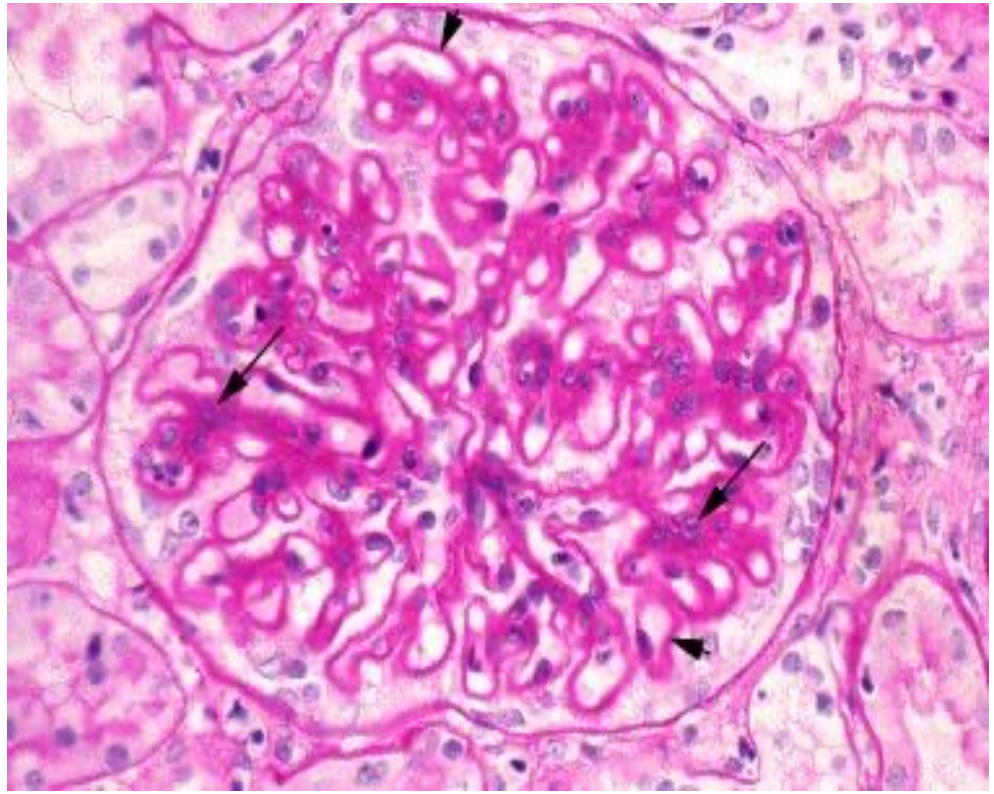

Glomerulum
x5000
5µm
5kV
4mm

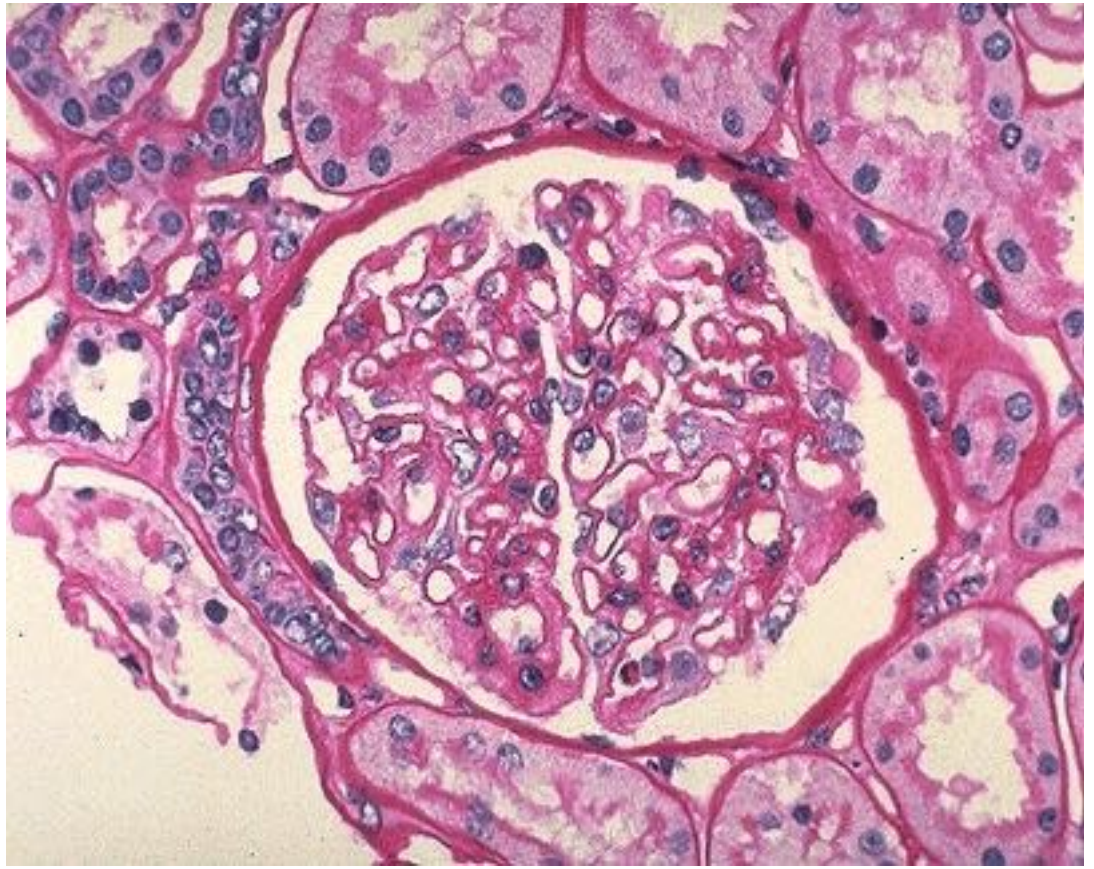

Nephrin
NEPH-1
Cytoskeleton
Cell polarity
Foot process structure
Cell survival?
Mechanosensation?

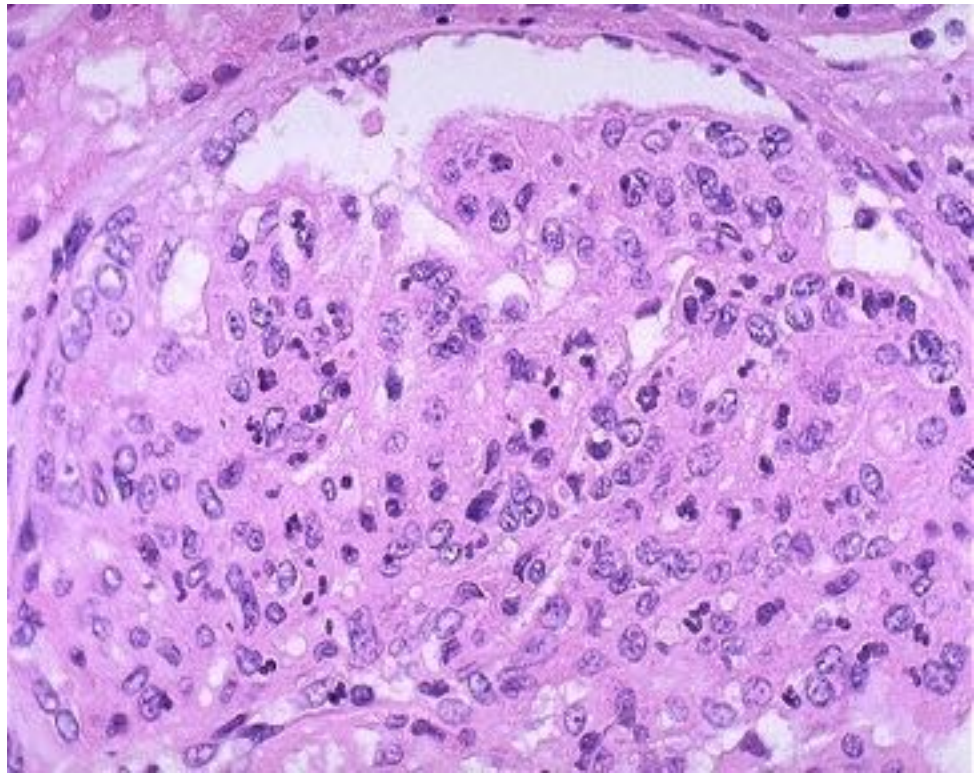


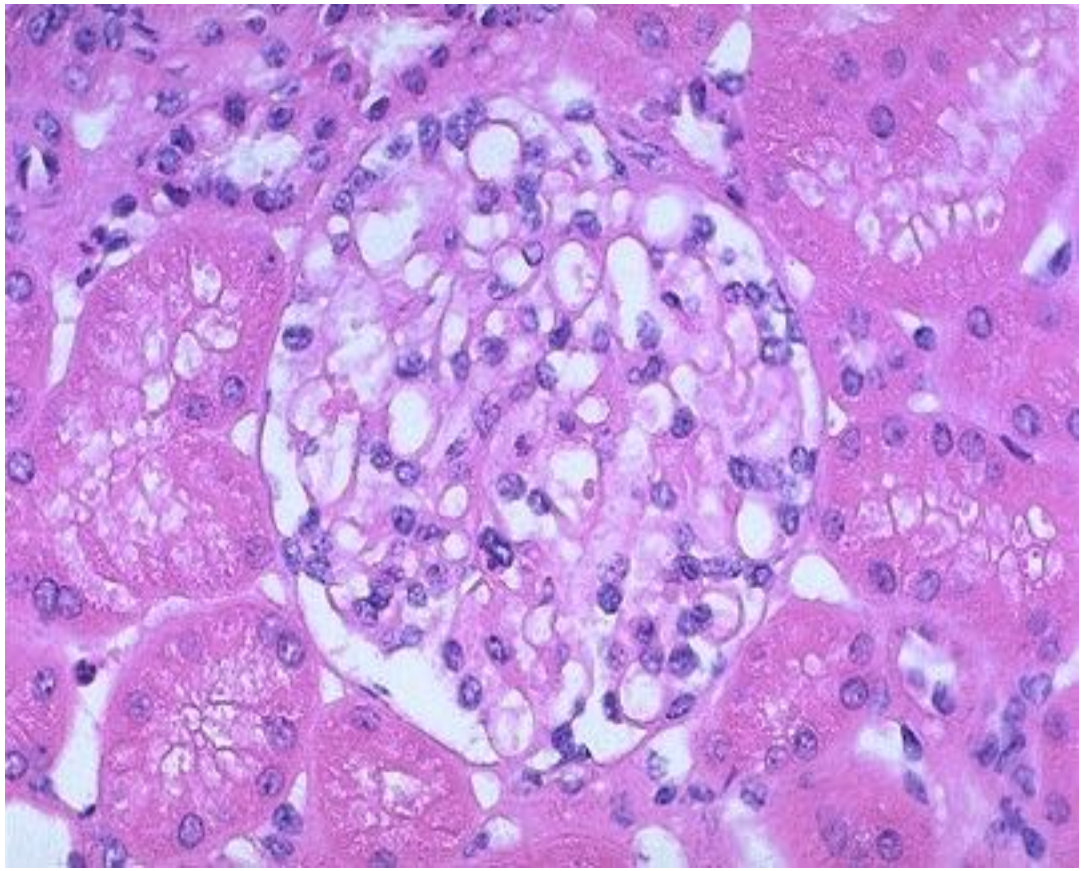

Macula densa
vascular pole
Podocytes
Mesangial cells
Podocytes
Capillaries
Bownan's capsule
Podocytes

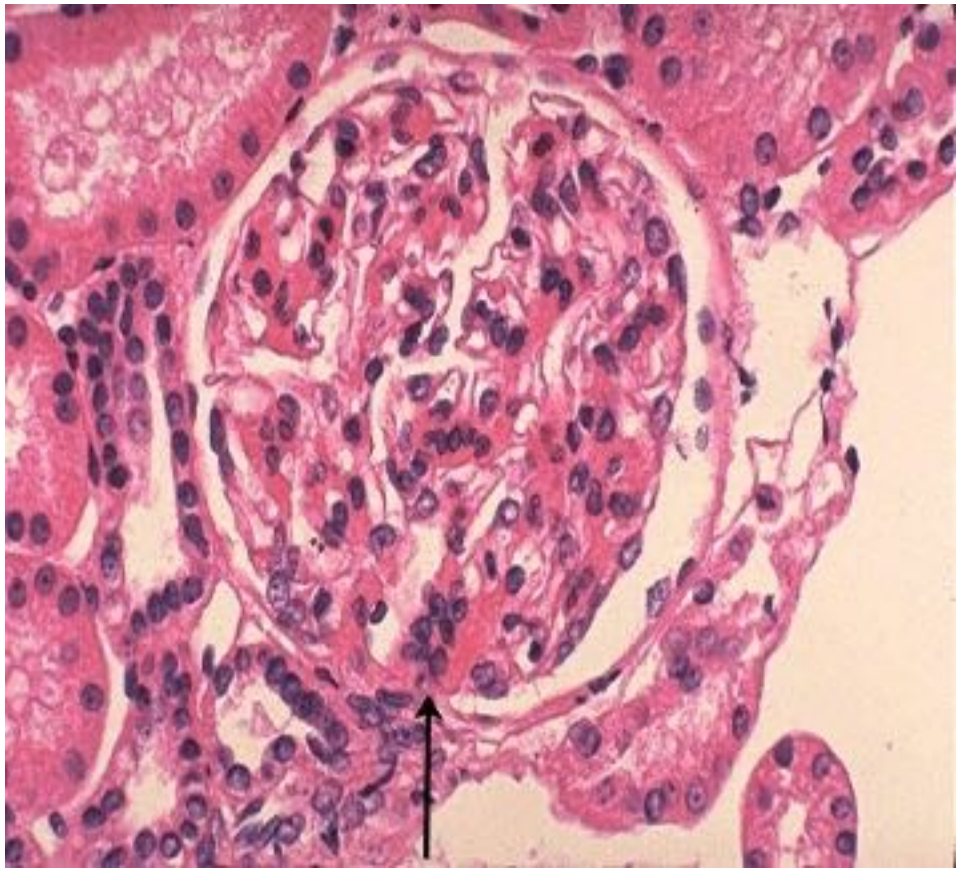

Basement Membrane
Endothelium
Red Blood Cell
Foot Processes
Podocytes
Endothelial Cells
Mesangial Cells
Mesangial Matrix

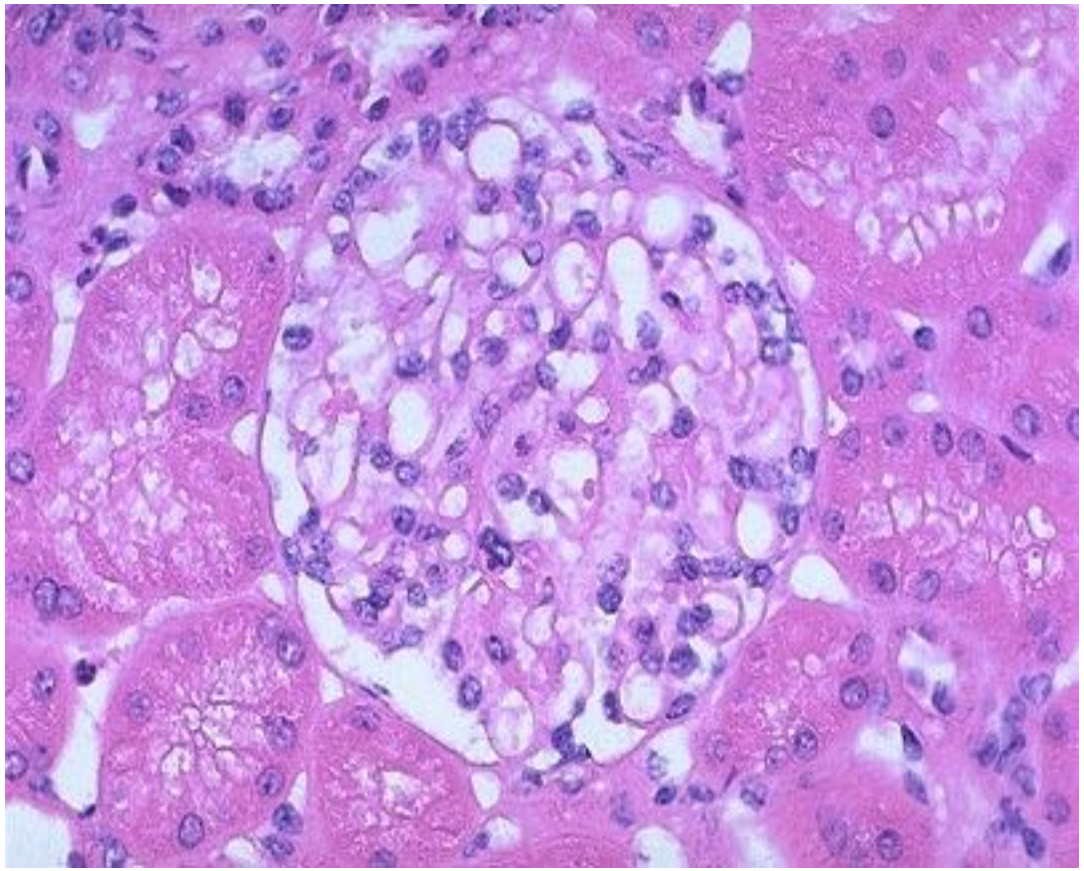


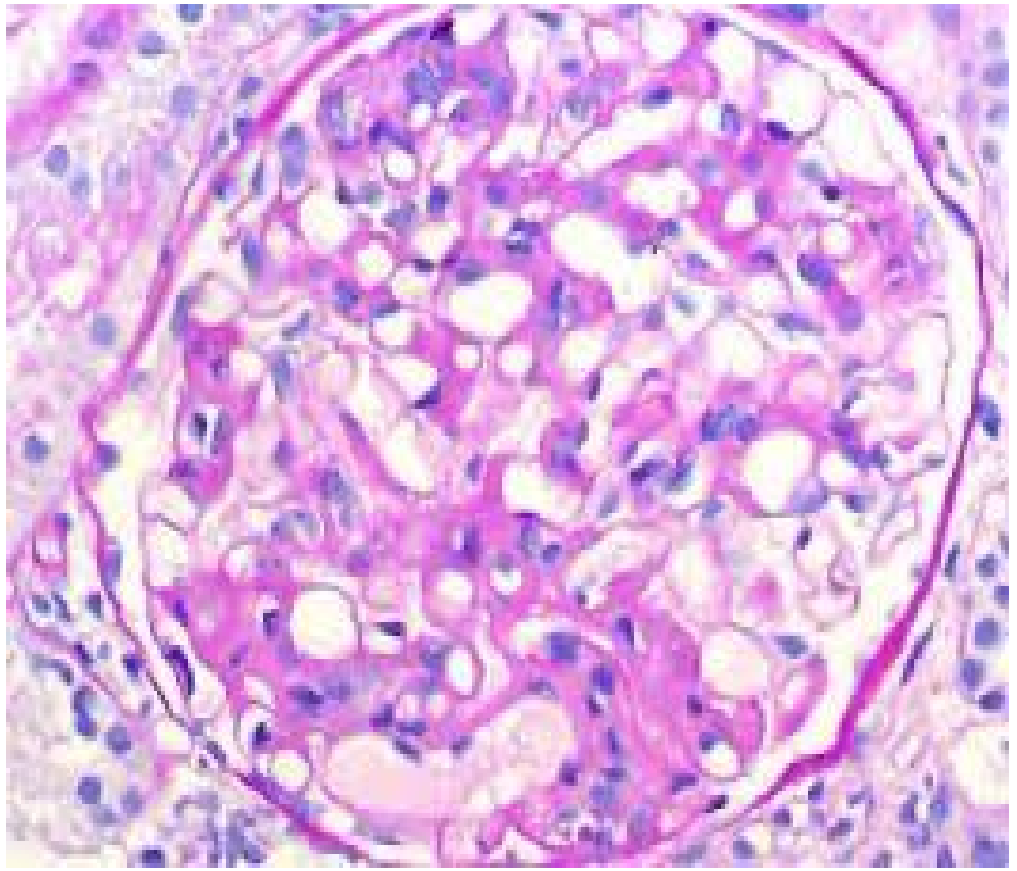


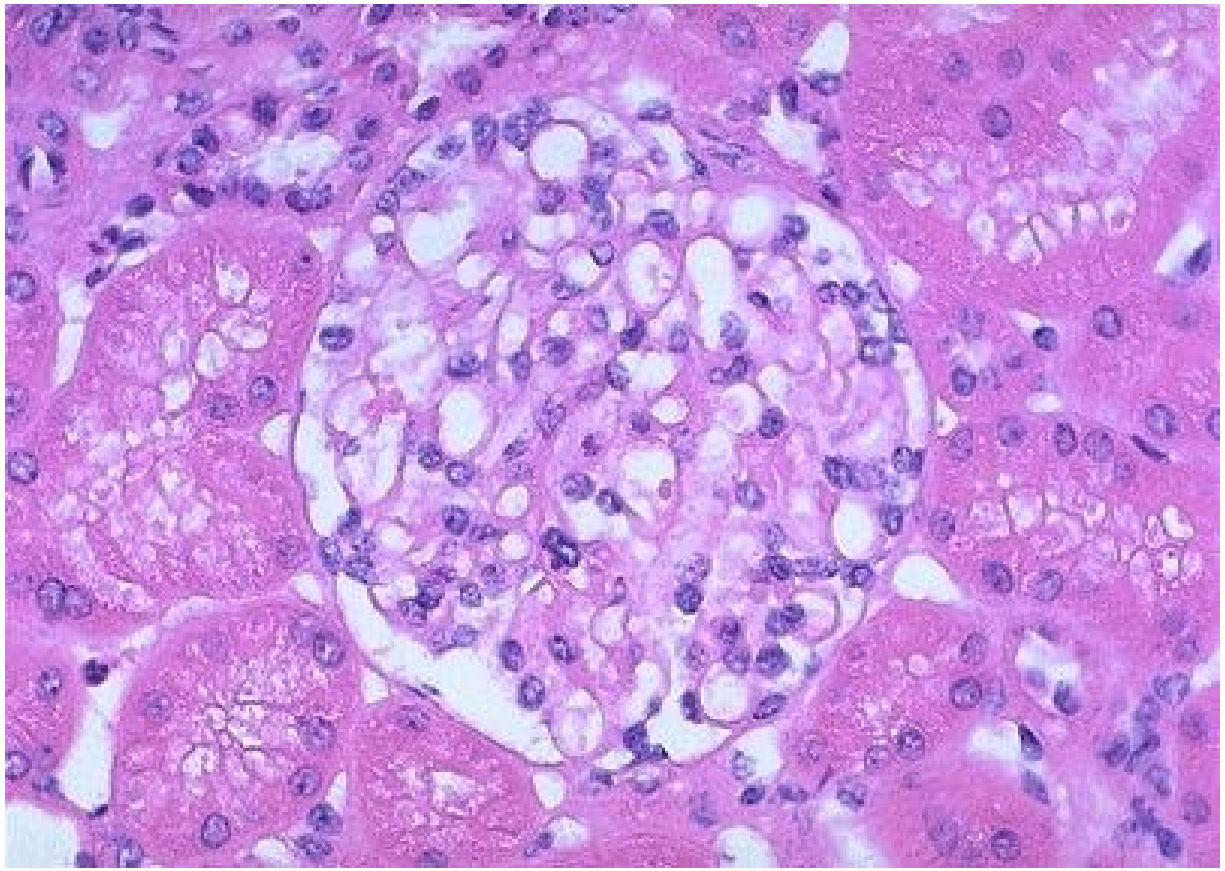


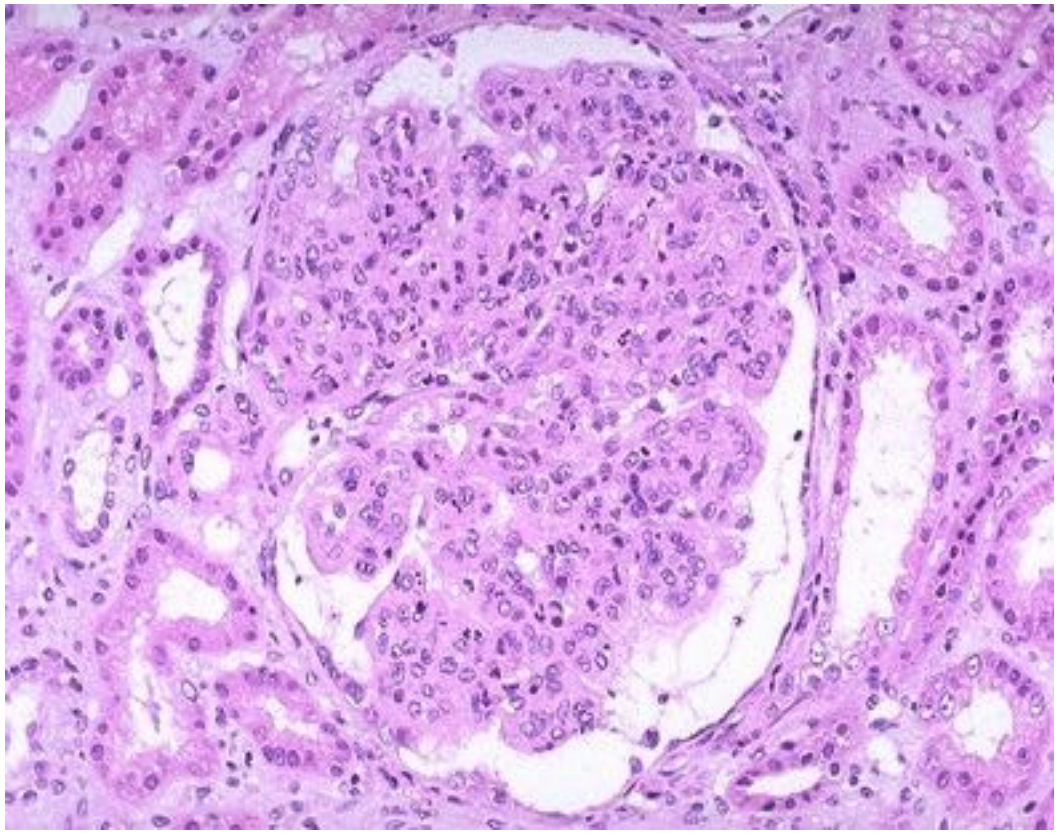


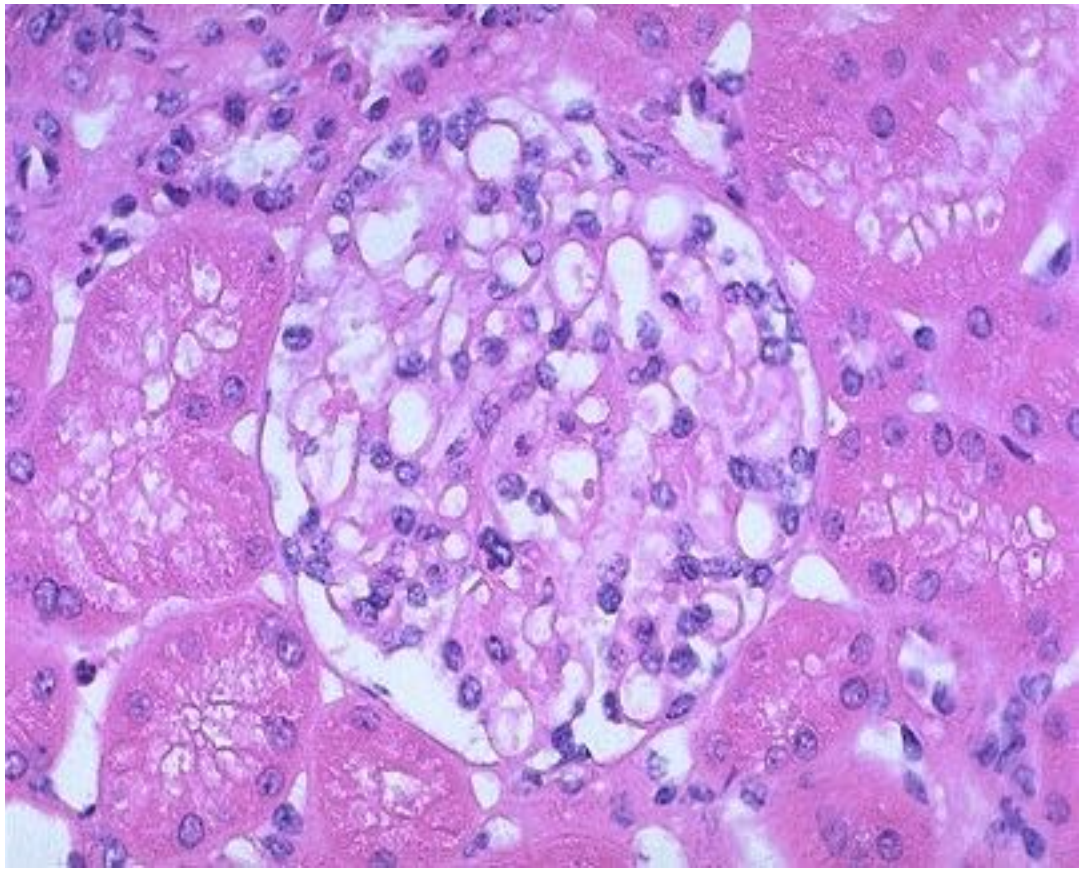


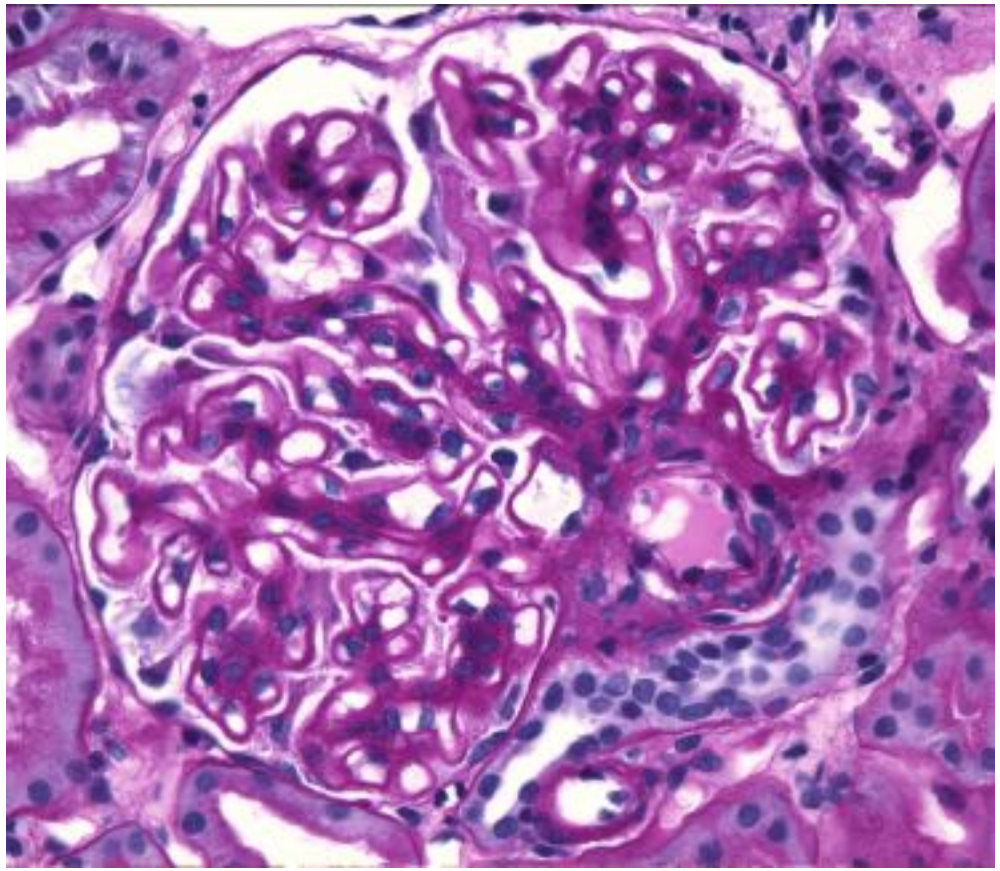


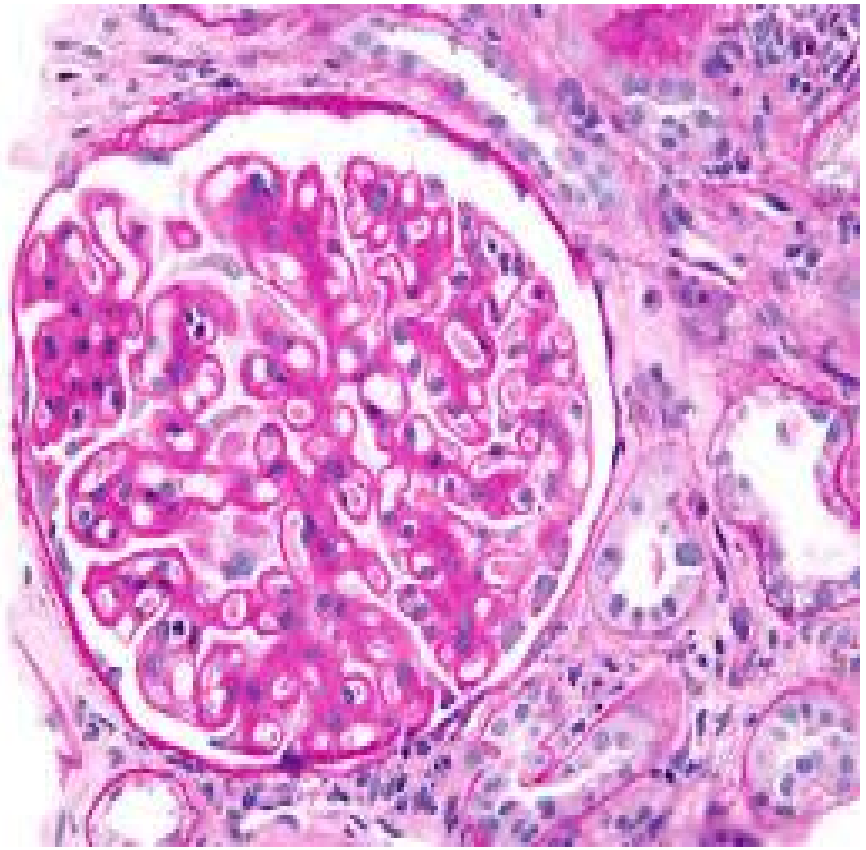


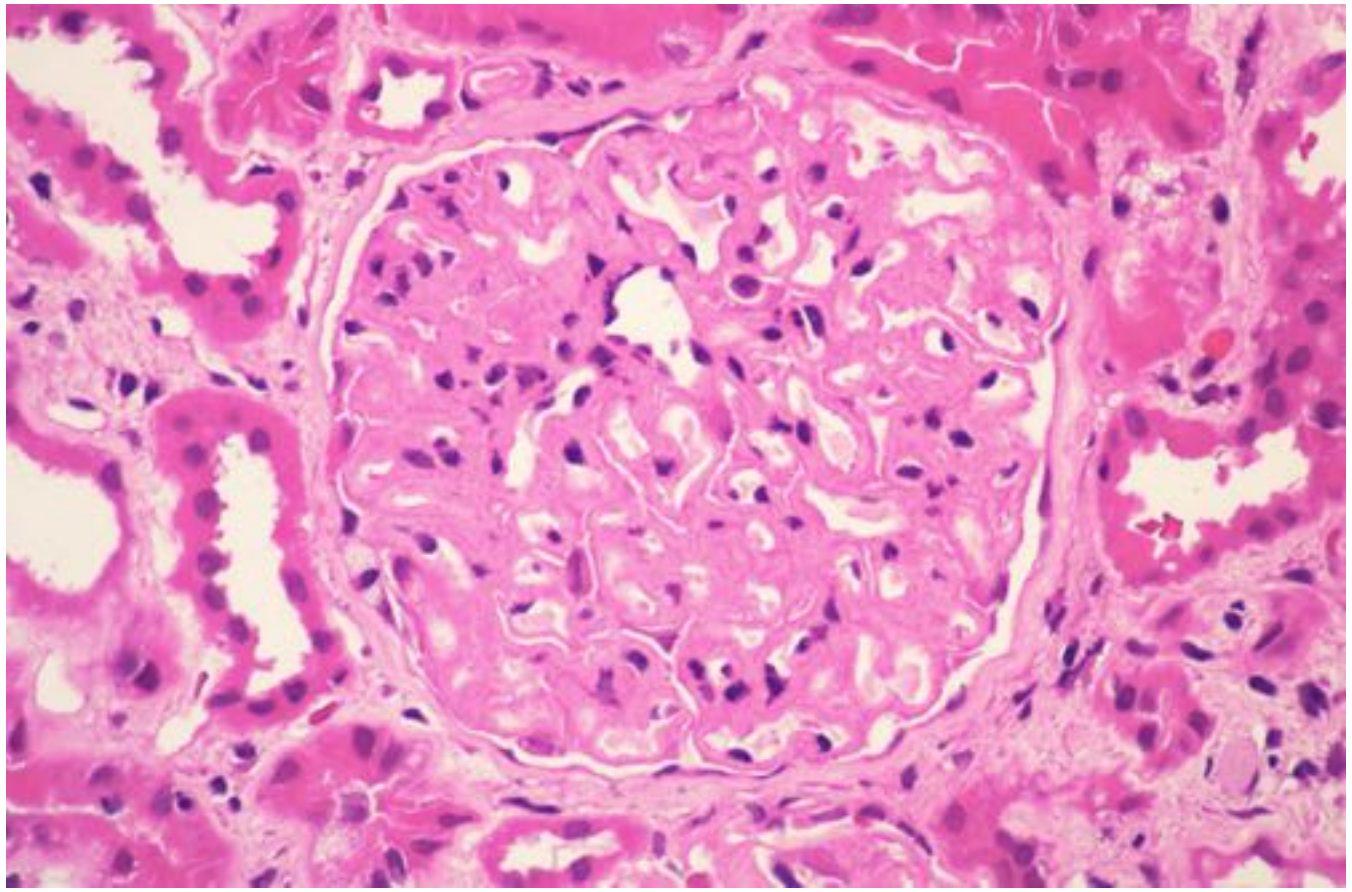


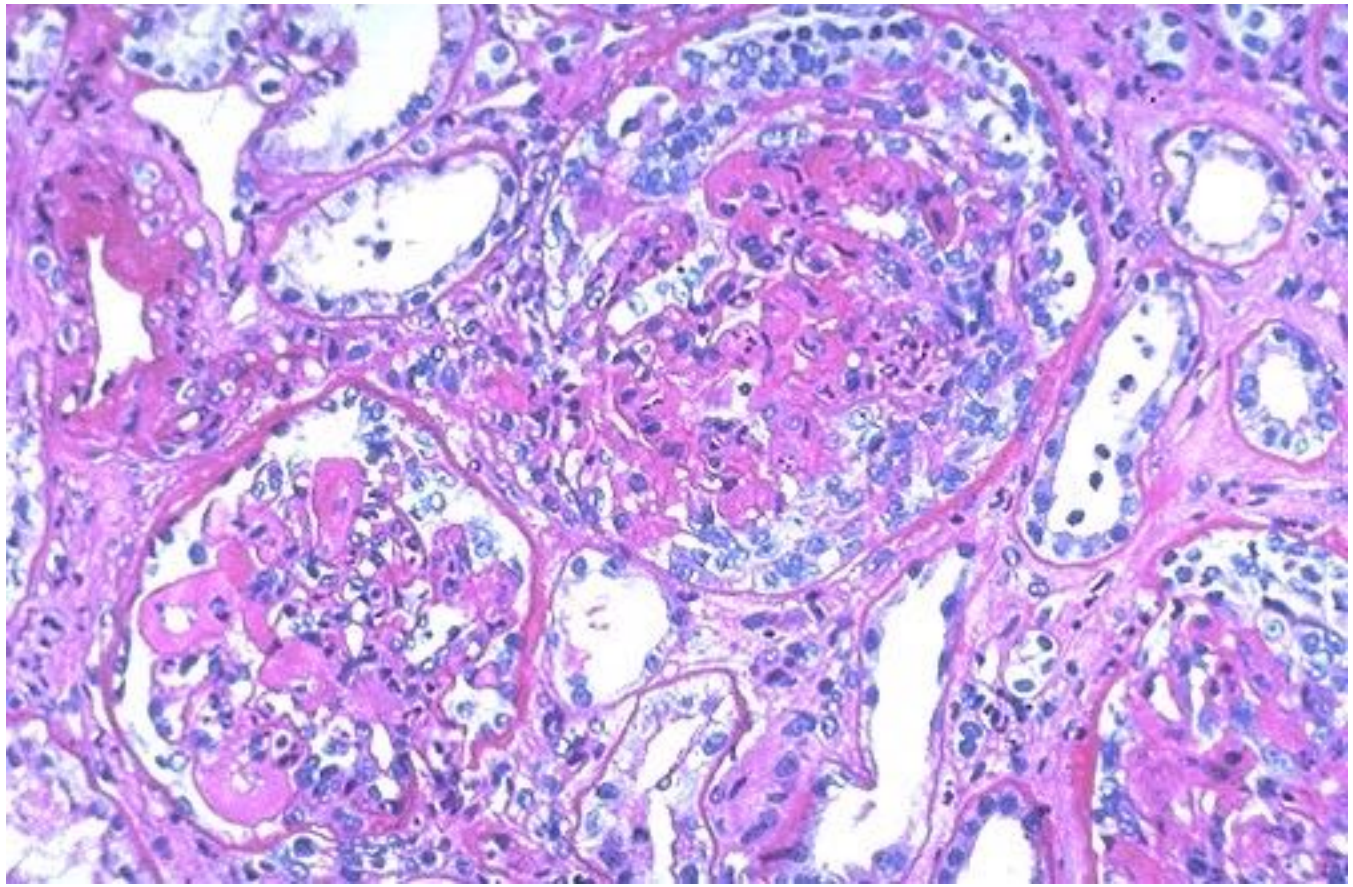


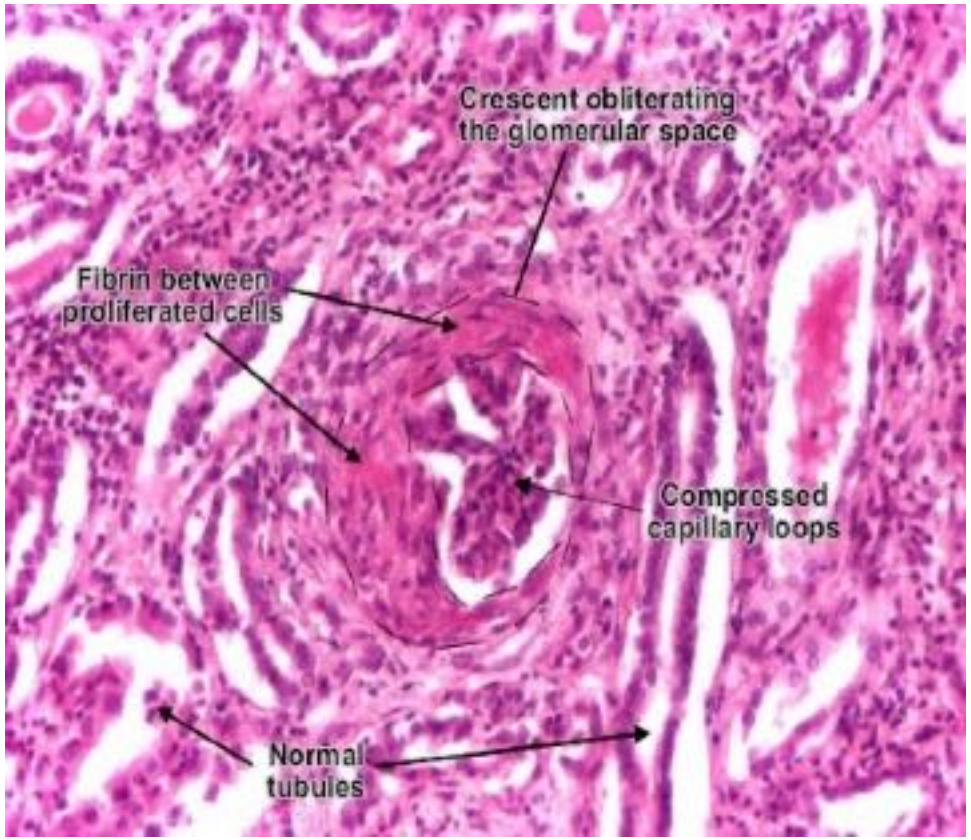











Crescent obliterating the glomerular space
Fibrin between proliferated cells
Compressed capillary loops
Normal tubules