G.U.S.

Pathology

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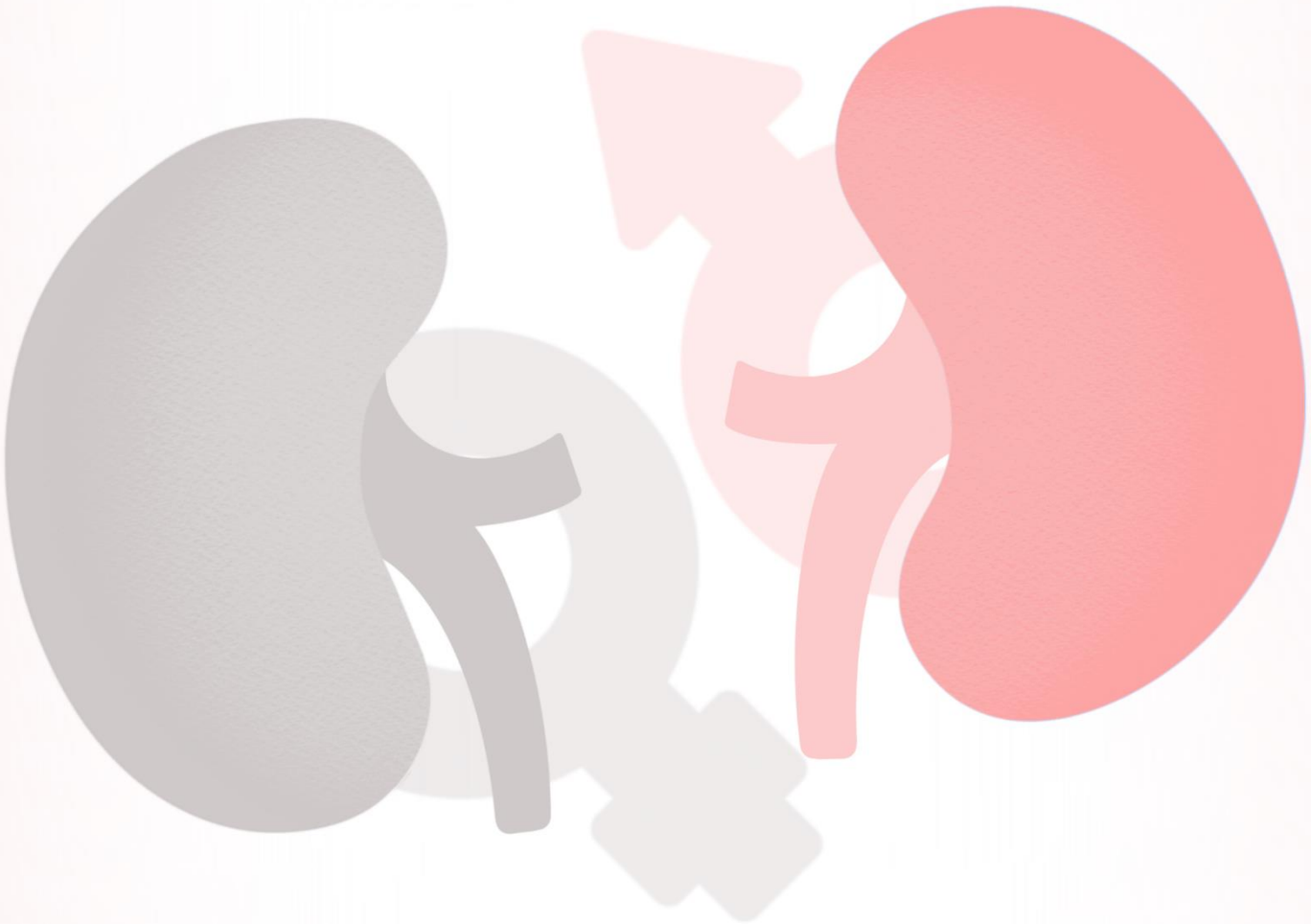

Sheet:
1. Introduction to Renal Pathology
2. Basic Concepts in Glomerular Pathology

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Welcome to GUS Pathology! This sheet will cover pathology lectures 1 and 2. The sheet will include the slides as well as any clarifications or additions mentioned during the lecture videos. *Please note that italicized statements are not mentioned in the slides or lecture videos, and are merely for clarification*

Lecture 1

- ***Introduction to Kidney and Urinary Tract Pathology:*** This lecture includes brief descriptions of some of the headlines of subjects that will be covered throughout this course.

Clinical Manifestations of Renal Diseases & Terminology

- Before we discuss the general clinical manifestations associated with renal diseases, we must first become familiar with some relevant terminology: Azotemia & Uremia

Azotemia

It refers to an elevation of blood urea nitrogen (BUN) & creatinine levels. *Recall that kidneys are primarily involved in elimination and excretion of waste products present in blood such as urea and creatinine through glomerular filtration.* In the case of renal disease, we would expect decreased filtration, and therefore, blood levels of such substances would rise

- It is largely related to a decreased glomerular filtration rate (GFR).
- It has several types, which are outside the scope of this lecture.
- *Extra: Notice that the term "azotemia" merely describes a "biochemical aspect" (i.e., blood levels of BUN and creatinine). The term itself does not reveal whether there are clinical manifestations or not.* If the azotemia progresses and clinical manifestations appear, we collectively call it: Uremia

Uremia

It occurs when the azotemia progresses to clinical manifestations and systemic biochemical abnormalities (Uremia = Azotemia + Clinical manifestations)

- Uremia is **characterized by:**
  - Failure of renal excretory function (logically, since there is elevated BUN and creatinine blood levels, this means the kidneys are failing to get rid of toxic waste products)
  - Metabolic and endocrine alterations
  - Secondary gastrointestinal manifestations (e.g., uremic gastroenteritis)
  - Secondary neuromuscular manifestations (e.g., peripheral neuropathy)
  - Secondary cardiovascular manifestations (e.g., uremic fibrinous pericarditis)

- So, again, the major difference between azotemia and uremia is the presence of clinical manifestations in the case of uremia.
- We will now discuss the clinical manifestations associated with renal diseases.

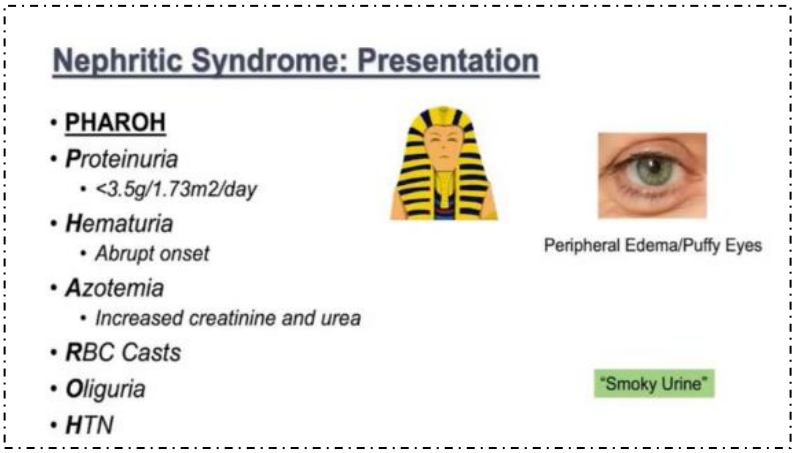
Major Renal Syndromes

- *In general, renal diseases manifest as a group of symptoms/manifestations, or a "syndrome". Since there are various renal diseases with different etiologies, clinical manifestations will somewhat vary and so we have several possible syndromes.* Among the main syndromes that can be seen in renal diseases are Nephr*itic* Syndrome & Nephr*otic* Syndrome. *Which syndrome occurs often depends on the etiology/cause of the disease.* This sheet provides an overview of these syndromes

1. Nephritic Syndrome

It is a glomerular syndrome characterized by:

- Acute onset (usually)
- Gross hematuria (presence of RBCs in urine)
- MILD to MODERATE proteinuria (**< 3.5 gm** of protein/day in adults)
- Azotemia (increased creatinine and urea blood levels)
- Generalized edema
- Hypertension
- RBC casts (will be discussed later). The presence of these RBC casts in urine is why it is described as smoky urine



- The image above helps you recall the characteristics of nephritic syndrome using the acronym "PHAROH". We mentioned most of them. Notice that oliguria is also part of this syndrome

2. Nephrotic Syndrome

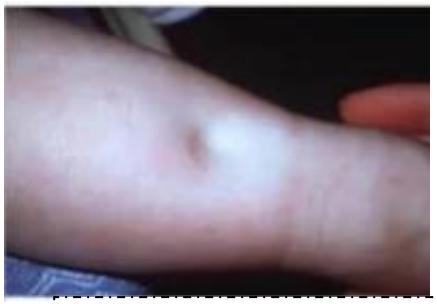
Please pay extra attention to the very subtle difference in spelling.

- It is a glomerular syndrome characterized by:
  - HEAVY proteinuria (excretion of **> 3.5 gm** of protein/day in adults. For children, this value is different, and depends on body weight)
  - Hypoalbuminemia (low concentration of albumin in the blood)
  - Severe edema (which is usually generalized, and involves the eyes (puffy eyes), the face, the abdomen, upper limbs and lower limbs)

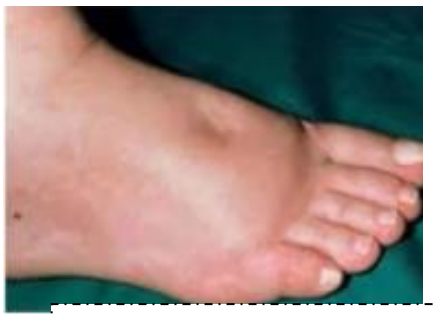
- Hyperlipidemia (elevated concentration of lipids in the blood)
- Lipiduria (presence of lipid in the urine)

- The following images demonstrate generalized edema which is a major clinical manifestation of nephrotic syndrome

Edema that involves the face, lips, and eyes (puffy eyes of nephrotic syndrome)



Pitting edema in the upper limb



Pitting edema in the lower limb

- There are also other possible manifestations/syndromes of renal diseases:

3. Asymptomatic Hematuria or Proteinuria

- It is a manifestation of mild glomerular abnormalities.
- In this case, the patient is completely asymptomatic, but mild hematuria and proteinuria are discovered incidentally (on accident/by chance) during a routine urinalysis.
- This trivial finding should NOT be left without proper evaluation of the patient. Even though this finding is only visible microscopically and insignificant to the patient in terms of symptoms, it should draw your attention to the presence of mild glomerular disease that may progress to something more serious later on if not treated or evaluated correctly.

4. Rapidly Progressive Glomerulonephritis

- It is also known as crescentic glomerulonephritis (GN)
- In this disease, there is rapid onset of loss of renal function (in a few days or weeks)
- It is manifested by:
    - Microscopic Hematuria
    - Dysmorphic RBC and RBC casts in urine sediment
    - Mild-moderate proteinuria might be present as well

5. Acute Renal Failure

The definition of acute renal failure necessitates the presence of oliguria (decreased urine output, < 400 ml/day in adults) or anuria (no urine flow)

- It is usually associated with recent onset of azotemia
- It can result from:
    - (Severe) Glomerular Injury
    - (Severe) Interstitial injury
    - (Massive) Vascular injury (as occurs in thrombotic microangiopathy)
    - Acute tubular necrosis

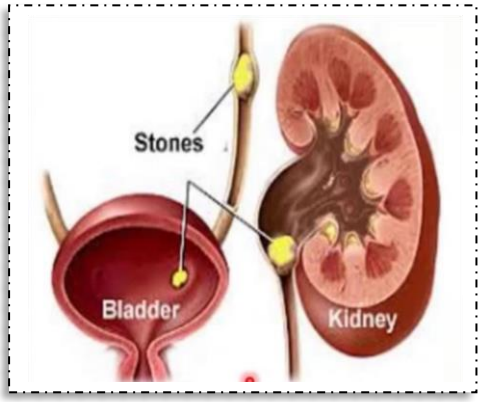
6. Chronic Renal Failure

- It refers to the presence of prolonged signs and symptoms of uremia.
- It is usually the end result of all forms and types of chronic renal diseases.

7. Urinary Tract Infections

- Urinary tract infections will be very briefly discussed in our pathology lectures, as the microbiology lectures will extensively cover this particular disease entity.
- They usually manifest as bacteriuria and pyuria (presence of bacteria and WBCs in urine)
- They can be symptomatic or asymptomatic
- They are classified into types according to the level of infection, and magnitude of urinary tract involvement. So, 1. in its severest form, it can be pyelonephritis (infection of the kidney itself), 2. or it can be limited to the urinary bladder in which case it is called cystitis.

7. Nephrolithiasis



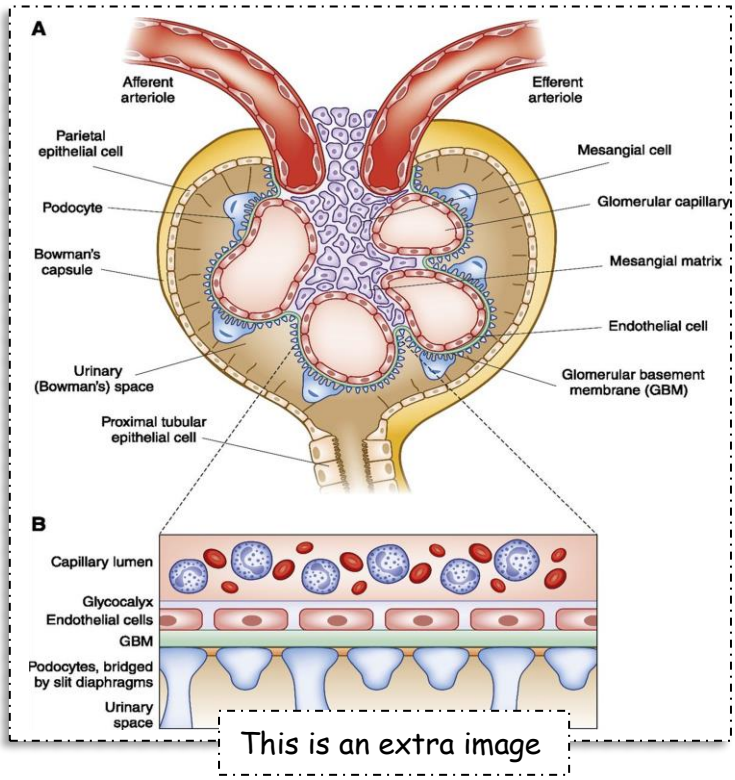
- It refers to the presence of stones anywhere in the urinary tract (the stone could be in the kidney, ureter, urinary bladder or even the urethra)
- It is manifested by
    - Renal colic
    - Hematuria
    - Recurrent stone formation

Lecture 2

- In this lecture, we will discuss basic concepts in glomerular pathology
- Glomerular diseases are among the most common causes of chronic kidney disease. As the name implies, these diseases involve the glomerulus of the kidney. So, we must first understand the structure of the glomerulus. Afterwards, we will demonstrate the possible mechanisms by which the glomerulus can be involved in these diseases.

Structure of the Glomerulus

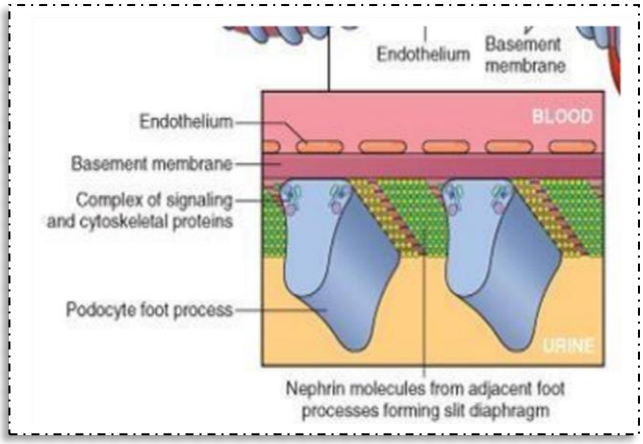
- Refer to the adjacent image as you read
- Blood arrives at the kidney filtration unit through an afferent arteriole
- This afferent arteriole gives off an anastomosing network of capillaries. This capillary network is called the "glomerulus". Its main function is filtration of blood
- Blood then reaches the other end of the capillary network where it leaves through the efferent arteriole
- The glomerulus is invested (surrounded) by two layers of epithelium: Podocytes and Parietal Epithelium.



  - The internal layer is formed by the podocytes (notice how it is attached to the capillary wall),
  - The parietal epithelium forms the outer layer.
  - Between these two layers, there exists a space known as Bowman's space (or urinary space). *Extra: Bowman's capsule vs Bowman's space: Bowman's capsule collectively refers to the two layers (podocytes and parietal epithelium) + the space within. The space alone is called Bowman's space. So, Bowman's capsule = two layers + Bowman's space*

- *Extra Note: You can imagine the podocytes as the visceral layer, and parietal epithelium as the parietal layer, similar to how we used to describe the pleura, peritoneum and pericardium with their visceral and parietal layers, with space in between. But note that Bowman's space is considered "true" space, unlike the pleural, peritoneal and pericardial potential spaces.*
- As we mentioned, this network of capillaries (glomerulus) lies in a space known as Bowman's space (urinary space), which is the cavity in which plasma ultra-filtrate first collects. *So, filtration involves movement of blood components from the glomerular capillary lumen and into Bowman's space through a barrier (capillary wall)*

- The glomerular capillary wall consists of the following:
    - A thin layer of fenestrated endothelial cells
    - Glomerular basement membrane (GBM)
    - Foot processes of podocytes
    - Supportive cells (mesangial cells) These cells lie in the center of the glomerular tuft between the capillaries
- The glomerular capillary wall is considered the filtration unit. That is, it represents the part of the kidney where blood components are filtered so as to be excreted. So, in order for a substance to be filtered, it must cross this barrier: The glomerular capillary wall
- *Filtration occurs as blood components move from the capillary lumen to Bowman's space. During the process, these components will pass through several layers in the capillary wall. These layers are collectively called the glomerular filtration membrane. "Glomerular filtration membrane" and "glomerular capillary wall" are very similar and overlapping terms. Refer to the extra note at the end of the sheet for further distinction.*

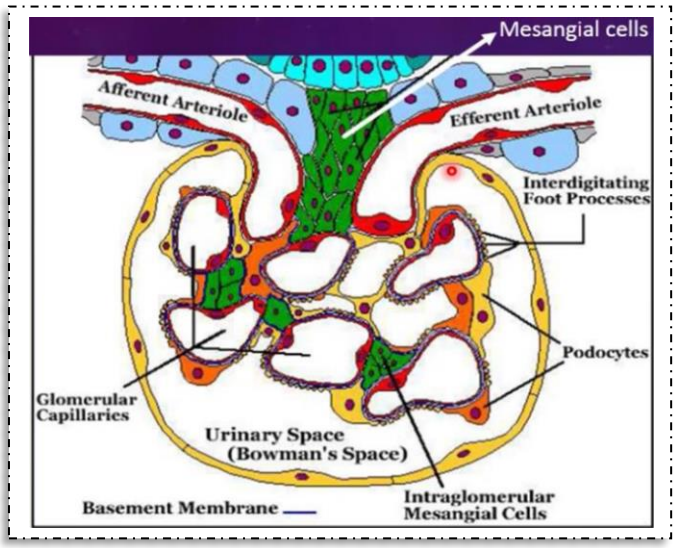


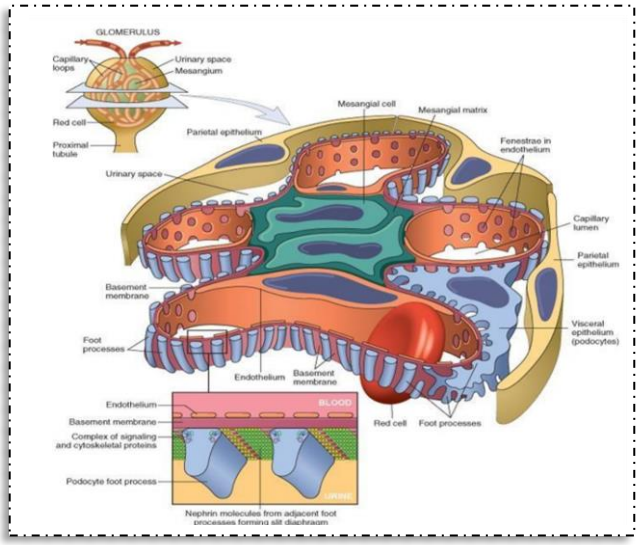
- The glomerular filtration membrane/unit *(between the glomerular capillary lumen and Bowman's space)* of the glomerulus consists of: (notice the adjacent image)
    - Endothelium with its fenestrations
    - Basement membrane (which consists of collagen type 4, laminin, polyanionic proteoglycans, fibronectin and glycoproteins)
    - Complex of signaling and cytoskeletal proteins (many of which come from the foot processes of the podocytes). The most significant of these proteins/molecules is nephrin. Nephrin molecules from adjacent foot processes form a very important structure called the slit diaphragm (important for regulating the permeability of the basement membrane).
- As you might have noticed, there are interdigitating foot processes from visceral epithelial cells (podocytes) embedded in and adherent to the GBM, and thus participate in the glomerular filtration membrane
- Foot processes are separated by filtration slits which are bridged by a thin slit diaphragm composed in large part of nephrin.
- The major characteristics of glomerular filtration:
    - High permeability to water and small solutes
    - Complete impermeability to molecules of large size and molecular charge (e.g., albumin)
    - The larger the molecule, the less permeable the filtration membrane is to it

- The more cationic (positively charged) the more permeable (Many of the large molecules or proteins in plasma, like albumin, are negatively charged (anionic) which means the GBM is impermeable to such molecules). *Extra*: *This characteristic is probably due to the negatively charged basement membrane, which would preferentially attract cations, and repel anions*

- Nephrin and its associated proteins, including podocin have a crucial role in maintaining the selective permeability of the glomerular filtration barrier

- Below you will find other schematic illustrations of the glomerulus from the slides. Again, notice the different components (in the left image):
  - The mesangial cells (which usually lie in the center of the glomerular tuft) and endothelial cells
  - Notice the parietal epithelial cells which line and form the bowman capsule. Also notice the podocytes (these cells have foot processes)





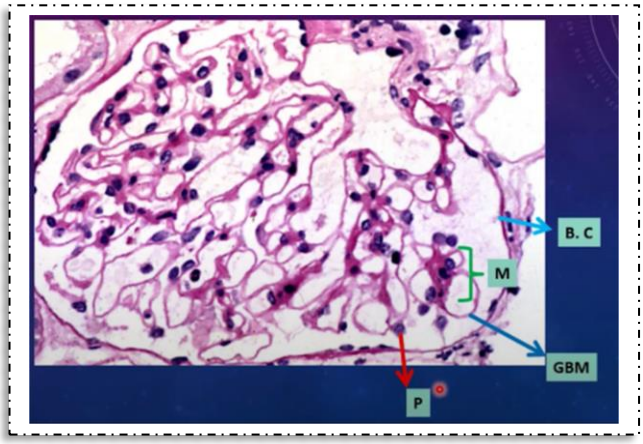
- The right image above also demonstrates the structure of the glomerulus. In this image you can see the foot processes of the podocytes nicely illustrated and how they enclose the capillary
- Also demonstrated in the right image is an RBC, which can help you imagine the relative sizes of the glomerular structures with respect to it

- Let's move from the cartoon world to the real world. We will now view the glomerulus as it appears in: Light microscopy, Immunofluorescent microscopy and Electron microscopy.

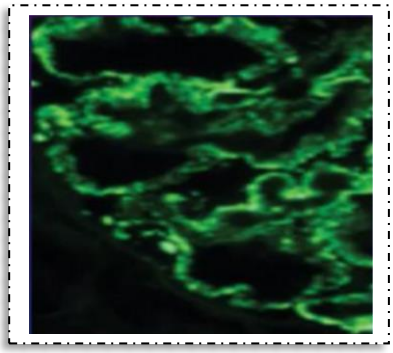
Light Microscopy

- The following image shows how the glomerulus appears under the light microscope (histologic appearance)

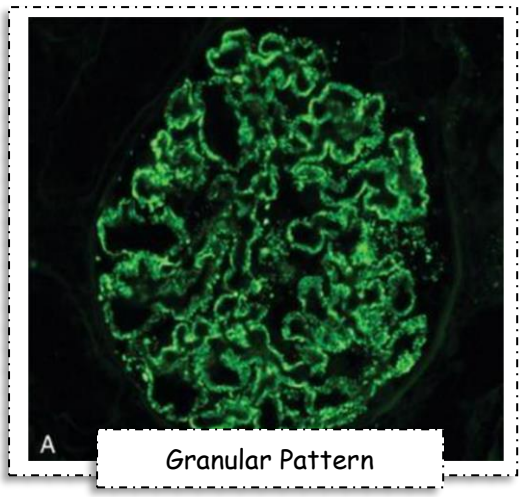
- Notice the following:
    - Bowman's Capsule (labelled B.C.)
    - Mesangial Cell (labelled "M"). Notice how they lie in the center of each glomerular tuft
    - GBM at the periphery of the capillary lumen
    - Podocyte (labelled "P")



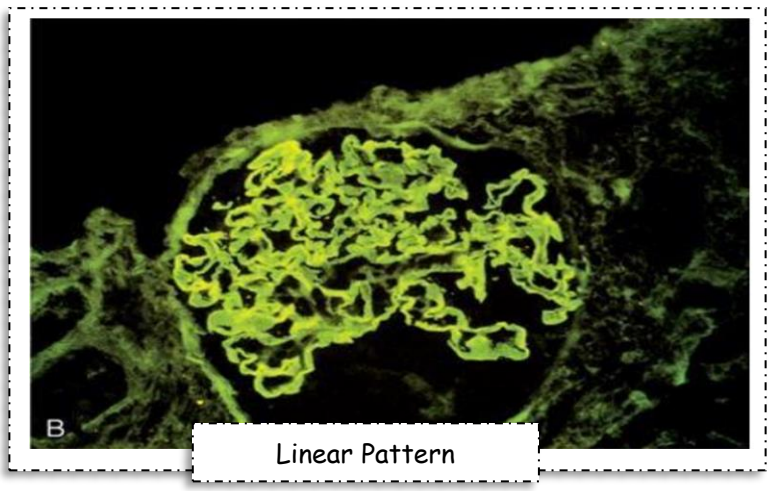
Immunofluorescence Microscopy



- Another tool we use to study kidney biopsies (and in order to study glomerular diseases) is immunofluorescence microscopy
- In this technique, fluorescein-labelled antibodies are used for the antigens that should be routinely examined, including immunoglobulins (primarily IgG, IgM and IgA), complement components (primarily C3, C1q, and C4), fibrin, kappa and lambda light chains.
- The adjacent image shows a kidney biopsy displaying a positive result for immunofluorescence microscopy. The image shows one of the possible patterns in immunofluorescence
- Immunofluorescence microscopy may show different patterns. These patterns are only morphological descriptions. The images below show two possible patterns:
    - One of the patterns is the granular pattern of deposition (that is, the antibodies will deposit in the form of small or large dots, as can be seen in the image below).
    - Another pattern is the linear deposition of immune complexes



Granular Pattern

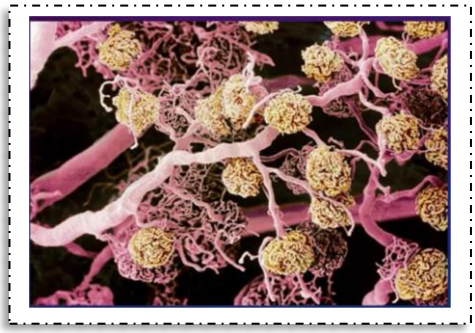


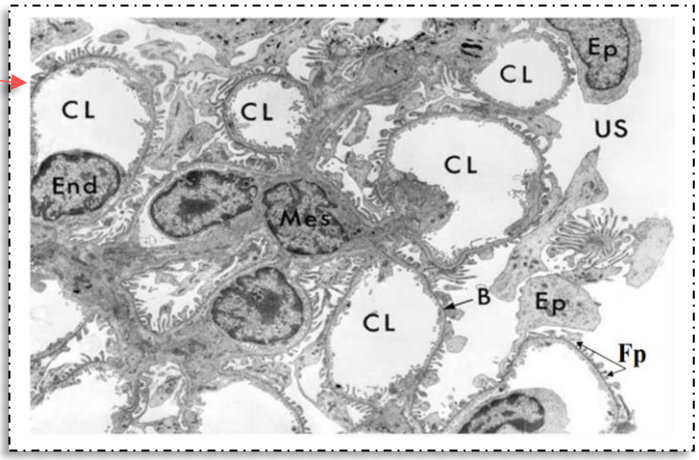
Linear Pattern

- *Extra: The pattern is important as it assists us in determining the etiology of the disease, since certain diseases tend to have certain patterns in immunofluorescence microscopy*

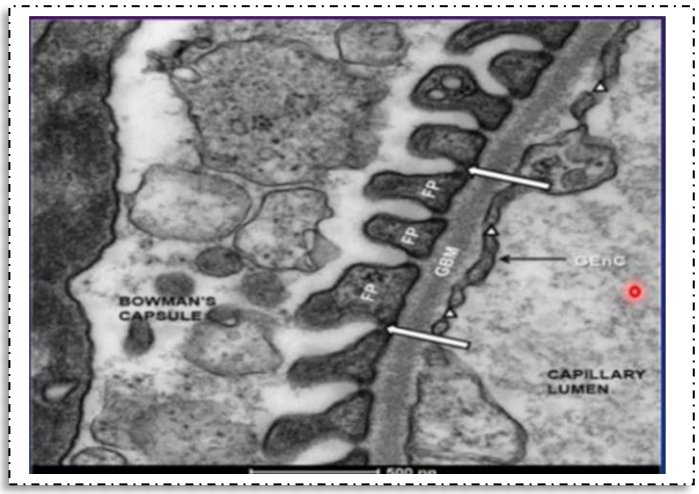
Electron Microscopy

- Electron microscopy also has a role in studying kidney biopsies
- The adjacent image demonstrates a normal glomerulus as it would appear in electron microscopy. It is very similar to the schematic illustrations we displayed in page 7. Notice the following:
    - Mesangial cells "Mes" (supportive cells) in the center of capillary tufts.
    - Capillary lumen (CL), and the capillary wall composed of endothelial cells (End), GBM (labelled "B"), and the overlying podocytes with their foot processes (Fp)
    - Parietal epithelial cells (Ep)
    - Other: US (urinary space)





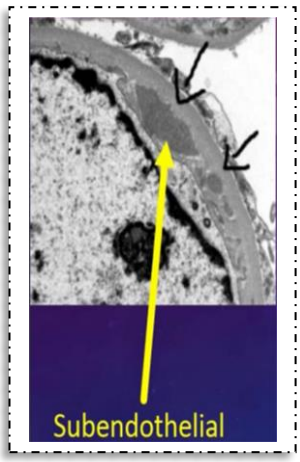
- Let's zoom in even further and examine the normal GBM in electron microscopy:
    - The image to the right shows a section through the GBM
    - Notice the capillary lumen lined by endothelium and their fenestrations, GBM, the foot processes of the podocytes (FP) and Bowman's capsule and space
    - So, the GBM faces the endothelium (& their fenestrations) and capillary lumen from one side, and the foot processes of the podocytes from the other.

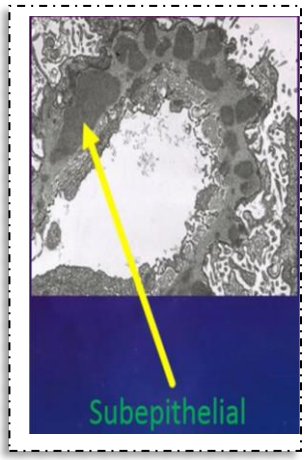


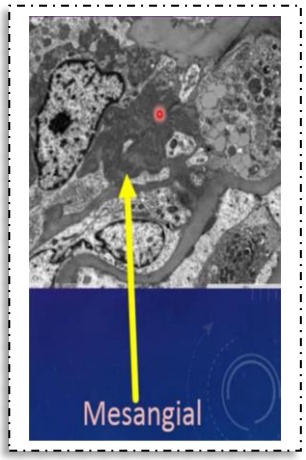
- Immune complex deposition is characteristic of many glomerular diseases. We can use electron microscopy to study/reveal the presence of immune complexes, which appear as electron-dense deposits or clumps that lie in one of three sites:

- In the mesangium
- Between the endothelial cells and GBM (subendothelial deposits)
- Between the outer surface of the GBM and the podocytes (subepithelial deposits)

- The site and pattern of immune complex deposition is helpful in distinguishing various types of glomerulonephritis (GN)
- The electron microscopy images below show the different locations of immune complex deposition:







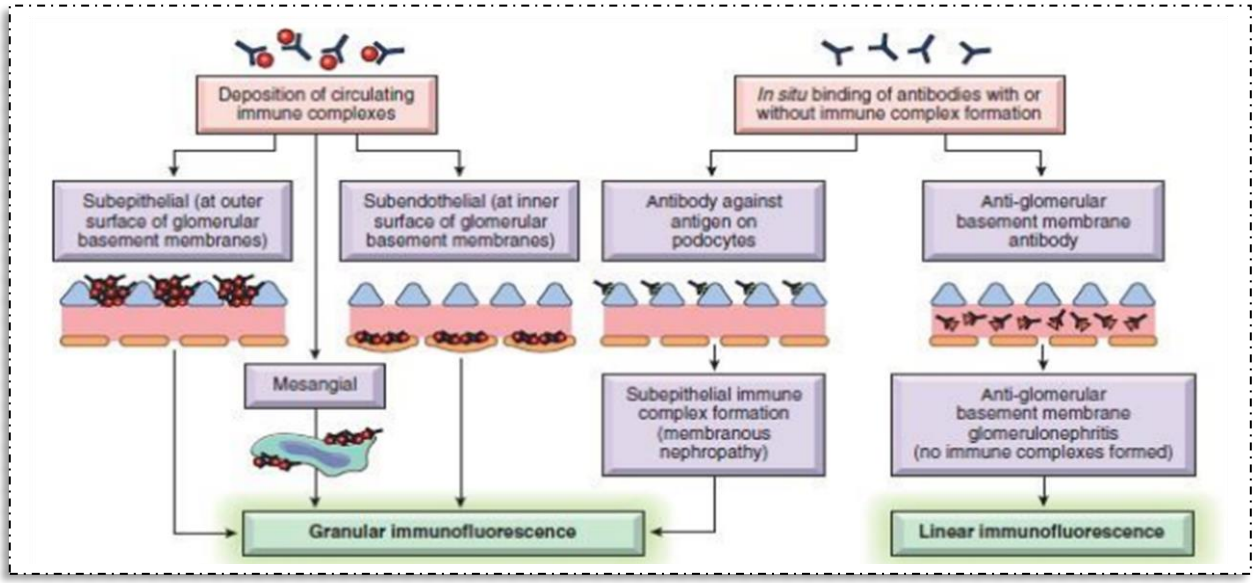
Pathogenesis of Glomerular Diseases

- Now that we have become familiar with the normal structure of the glomerulus, let's discuss how it may be affected in renal diseases.
- There are several mechanisms that underlie the pathogenesis of glomerular diseases (how they occur). These mechanisms may be 1. antibody associated, where the immune response (antibodies) is responsible for the disease, or 2. non-immune, where damage/disease occurs due to non-immune factors, such as toxins

1. Antibody-associated:

- Antibody-associated injury can be detected using immunofluorescence microscopy
- It involves injury to the glomerulus mediated by one of the following mechanisms:
  1. Deposition of soluble **circulating Ag-Ab complexes** in the glomerulus *(Extra: The antigen-antibody complexes were formed outside the kidney, then circulated in the blood, and finally deposited in the glomerulus inducing damage)*
  2. Or it may occur due to **antibodies reacting in situ** within the glomerulus *(Extra: The antibody enters the glomerulus and then reacts with local (in situ) antigens in the glomerulus, causing damage. These local antigens may be intrinsic glomerular antigens, or non-glomerular antigens that first deposited in the glomerulus, and then later reacted with antibodies that entered the glomerulus)*
  3. Abs directed against glomerular cell components *(This is not really a separate point, but it is stated separately in the slides for some reason. It is actually one example of mechanism 2, where the in situ antigens to which Abs react are glomerular cell components. Another example of in situ antigens in the glomerulus is basement membrane antigens)*

- The following image demonstrates the different patterns of antibody-mediated glomerular injury and clarifies what we previously stated (IMPORTANT! Go through all details of the figure)



- Notes on the previous figure:
  - Notice the different possible sites for immune complex deposition (subepithelial, subendothelial, mesangial)
  - The figure specifies the immunofluorescence microscopy pattern of positivity observed for each mechanism
    - Mechanisms that exhibit Granular Immunofluorescence:
      - Deposition of circulating immune complexes (subendothelial, subepithelial or mesangial)
      - In situ binding of Abs against antigens on podocytes
    - Mechanisms that exhibit Linear Immunofluorescence:
      - In situ binding of anti-glomerular basement membrane Abs

2. Non-immune mechanisms of glomerular injury

A. Podocyte injury:

- Refers to anything that leads to direct/mechanical injury of the podocytes, such as (causes) toxins, inflammatory cytokines, poorly characterized circulating factors or genetic mutations
- Podocyte injury will manifest in the form of effacement of foot processes, and this will result in development of proteinuria (due to loss of normal slit diaphragms)

B. Nephron Loss:

Refers to anything that leads to progressive loss of functional volume of the kidney. This can include many things, like loss of a whole kidney, or other diseases. Eventually, it leads to segmental or global (complete) sclerosis of glomeruli, which causes further reduction in nephron mass, initiating a vicious cycle of progressive glomerulosclerosis. *(Extra: The idea here is that if nephron loss takes place for some reason, sclerosis occurs. In response, the kidney attempts to adapt and compensate for the decreased functional capacity, but the adaptive changes backfire and end up being harmful, leading to further nephron loss. So, nephron loss itself triggers further nephron loss (i.e., vicious cycle))*

Good luck

Extra note (NOT required) for page 6: I could not find sources that clearly define or distinguish between the terms "glomerular capillary wall" and "glomerular filtration membrane", and they sometimes seem to be used interchangeably. A possible distinction may be the following: The glomerular capillary wall refers to the entire circumference of the capillary periphery. Now, you might have noticed that most of the capillary wall faces the Bowman's space, while a relatively smaller portion of the capillary wall faces the mesangial cells in the center. Filtration occurs across the capillary wall and into BOWMAN'S SPACE. So, the portion of the capillary wall that faces the mesangial cells is not really involved in filtration (note, however, that mesangial cells have supportive roles that may secondarily/indirectly affect filtration). Therefore, when we say glomerular filtration membrane, we focus on the portion of the glomerular capillary wall through which filtration occurs, specifically, the part that faces Bowman's space. Hence, you might have noticed that the components of the glomerular capillary wall and glomerular filtration membrane are basically the same, the only difference being the inclusion of mesangial cells as an additional component in the glomerular capillary wall, which is the broader term.

Lecture 1 Summary - Introduction

- Terminology:
  - Azotemia = elevated BUN + creatinine
  - Uremia = Azotemia + clinical manifestations
    - Uremia is characterized by: failure of renal excretory function/ metabolic & endocrine alterations/ Secondary manifestations (uremic gastritis, peripheral neuropathy, uremic fibrinous pericarditis)
- Clinical manifestations: Renal diseases can present with different manifestations (syndromes), such as:
  - Nephritic Syndrome: (acronym for clinical presentation: PHAROH - refer to image (page 2))
  - Nephrotic Syndrome: (heavy proteinuria (>3.5 gm/day), severe edema, lipiduria, hyperlipidemia, hypoalbuminemia)
  - Asymptomatic hematuria or proteinuria: Mild disease. No symptoms. Can progress
  - Rapidly progressive glomerulonephritis (= crescentic glomerulonephritis)
  - Acute renal failure (oliguria (<400 ml/day) or anuria)
  - Chronic Renal failure
  - Nephrolithiasis (Kidney stones). Manifested by renal colic, hematuria, recurrent stones

Lecture 2 Summary – Concepts in Glomerular Pathology

Glomerulus Structure

It is a capillary network invested by podocytes and parietal epithelial cells. Bowman's space between both layers

Glomerular capillary wall:

1. Fenestrated Endothelium
2. Basement Membrane
3. Foot processes of podocytes + slit diagphrams
4. Mesangial cells

Glomerular Filtration Membrane

Nephrin is the main component of the slit diaphragm between foot processes -> Regulates permeability

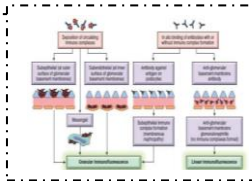
Microscopy

1. Light Microscopy
2. Electron Microscopy
   - Reveals site of immune complex deposition (subendothelial, subepithelial, mesangial)
3. Immunofluorescence Microscopy
   - Reveals pattern of deposition (granular or linear)

Pathogenesis

1. Antibody-mediated

Unless you have super vision and can read the figure below, refer to this figure (page 11) to review it



2. Non-immune: A. Podocyte injury (e.g. toxins, cytokines, mutations). B. Nephron loss (sclerosis)